

Trends in Giant Kelp Nitrogen Concentration and Surge Uptake

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Rationale and Research Questions

Kelp forests along the United States West Coast are impacted by increasing temperatures as a result of climate change. Since the late 19th century, Santa Barbara County in Southern California has had an increase in average temperature by 4.1 degrees Fahrenheit (Wilson, 2019). Kelp not only provides a healthy ecosystem for other flora and fauna but also provides ecosystem services to humans as well. Kelp assists with human-related environmental issues in two ways. First, kelp can sequester a large amount of carbon, aiding in climate change mitigation (Hurlimann, 2019). Secondly, they absorb excess nitrogen, which can lessen the impacts of agricultural runoff (Conroy, 2023).

A recent study has shown that increased nitrogen uptake by kelp can limit the effect that heat has on them. This is an interesting development because nitrogen and eutrophication are generally seen as negative consequences of human interference. However, in this case, it could assist kelp and help them survive increased climate-change-related temperature increases (Fernández, 2020). Interested in this recent research, data about nitrogen concentration amount and uptake were located in the Long-Term Ecological Research database titled “SBC LTER: Reef: Surge uptake capability in *Macrocystis pyrifera* in response to pulses of three different forms of nitrogen” (Cedeno, 2021) and ” SBC LTER: Reef: *Macrocystis pyrifera* CHN content (carbon, hydrogen, nitrogen), ongoing since 2002” (Santa Barbara, 2021). This data looked simple enough to clean and manipulate, while also providing enough information for a research project.

We are interested in trends in giant kelp nitrogen concentration amount and nitrogen surge uptake. Specific questions include: 1. Has nitrogen concentration in kelp changed over the years (2002-2021)? 2. Does giant kelp nitrogen concentration amount vary by season? 3. Does nitrogen surge uptake vary between the spring and summer seasons? 4. Does giant kelp nitrogen surge uptake vary based on the amount of exposure? 5. Does uptake vary by type of nitrogen (ammonium, nitrate, and urea)?

Github Information: <https://github.com/Tani-ValdezRivas/ValdezKuuskvereAnsbro>

Dataset Information

Data sets were found on the Environmental Data Initiative. All sets were from the Santa Barbara Coastal Long Term Ecological Research Site (SBC LTER) which is based within the University of California, Santa Barbara (UCSB) Marine Science Institute, and is part of the National Science Foundation's LTER Network.

The first data set focuses on one experiment done that examines nitrogen and biomass uptake by kelp during timed nitrogen blasting. The first data set was used to determine if nitrogen uptake varies by time (minutes), season, or nitrogen type. The second data set has dates and percent nitrogen of kelp over almost a twenty year period. This second data set was used to gain a general sense of nitrogen found in kelp in Santa Barbara, CA and to see if that has changed over time.

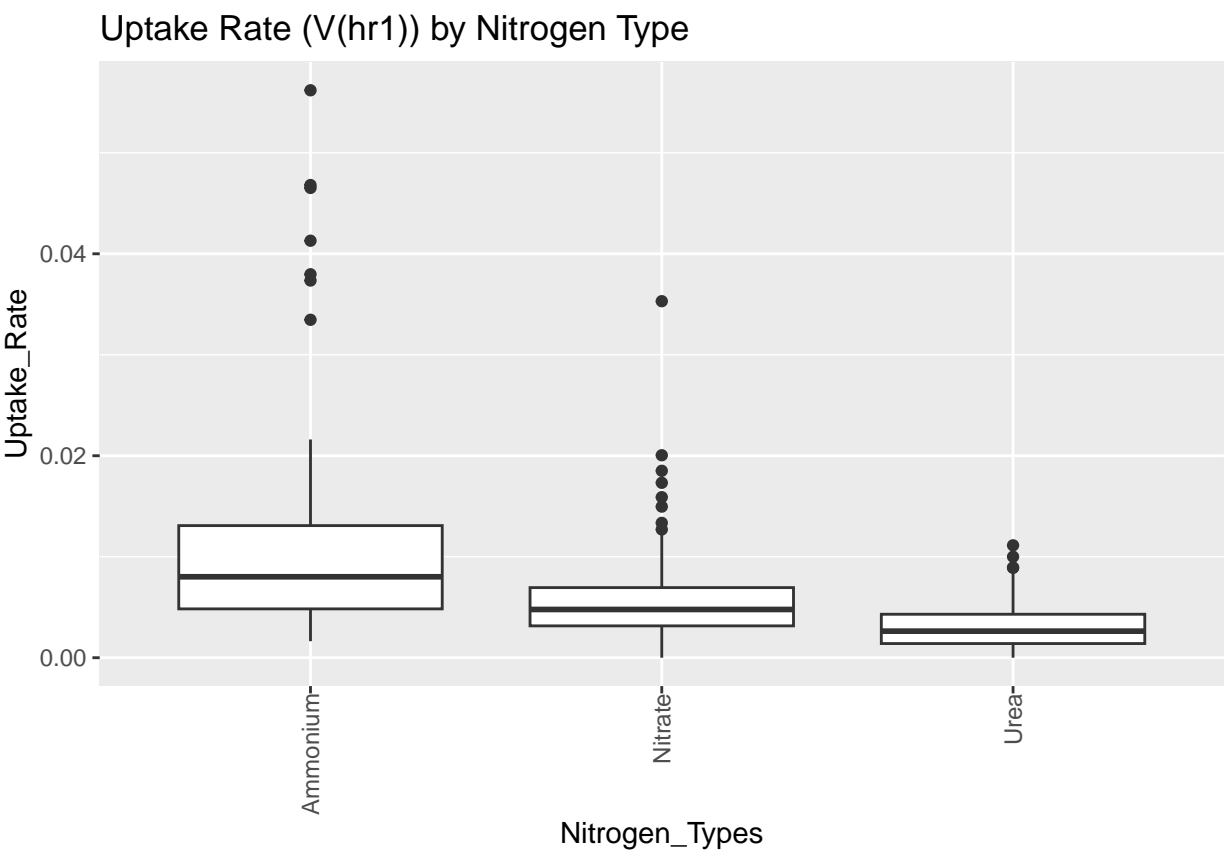
Table 1: Data Set Information

| | Detail | Data Set 1 | Data Set 2 |
|--|----------------|---|---|
| | Source | Santa Barbara Coastal Long Term Ecological Research (LTER) | Santa Barbara Coastal Long Term Ecological Research (LTER) |
| | Retrieved from | https://portal.edirepository.org/nis/mapbrowse?packageid=edi.1022.2 | https://sbclter.msi.ucsb.edu/data/catalog/package/?package=knb-lter-sbc.24 |
| | Variables Used | Nitrogen Types, Season, Day Replicate, Time, Blade Replicate, Uptake Rate, Biomass Uptake Rate | Year, Month, Date, Nitrogen Percent |
| | Date | 2021 | 2002-2021 |

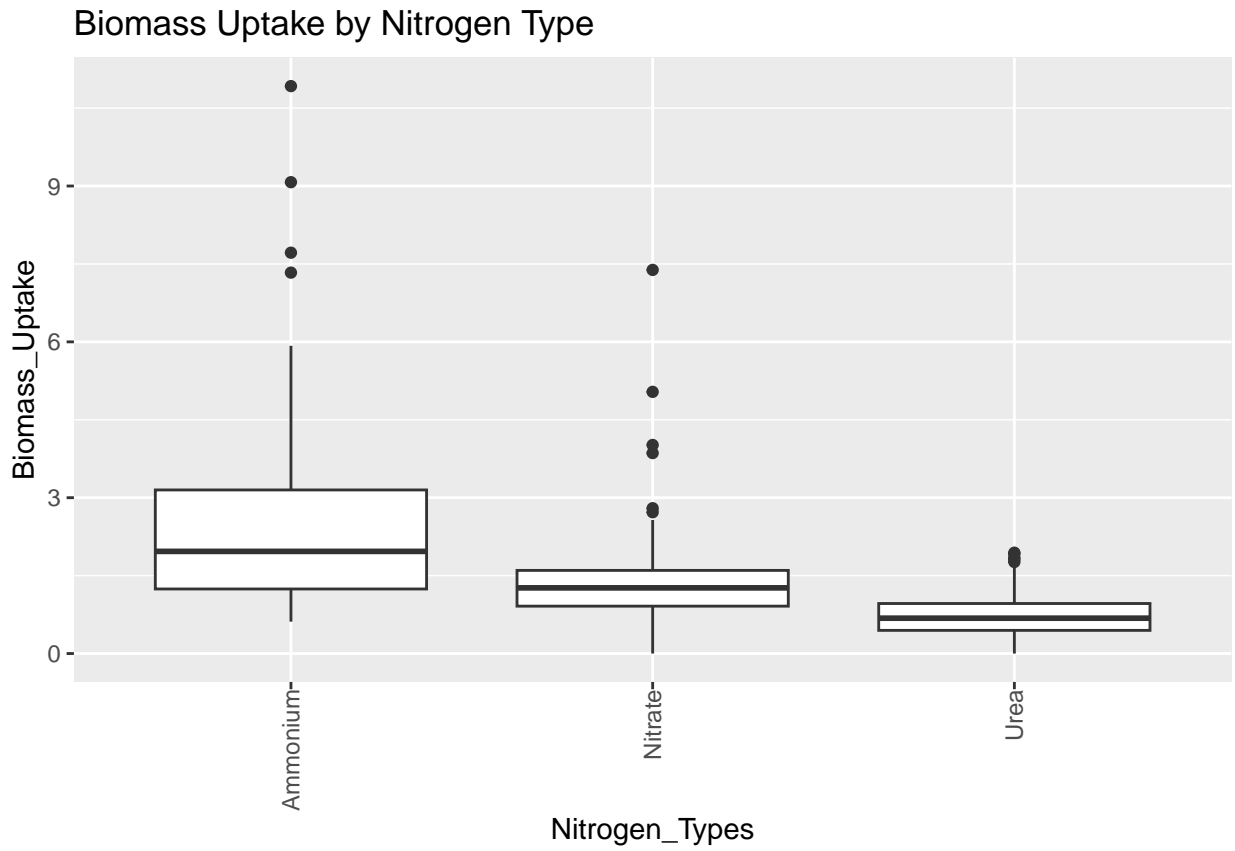
The data for nitrogen uptake was cleaned by renaming the column names to be better understood by the public. Multiple columns including columns for Blade Replicate, Day Replicate, Time, Season, and Nitrogen Type were changed to factors. Season and Nitrogen Type also had labels added to capitalize the names of seasons and nitrogen types. Data cleaning for the second data set entailed renaming column names as well. Date was changed from a factor to a date data type. Sites with missing values were also removed.

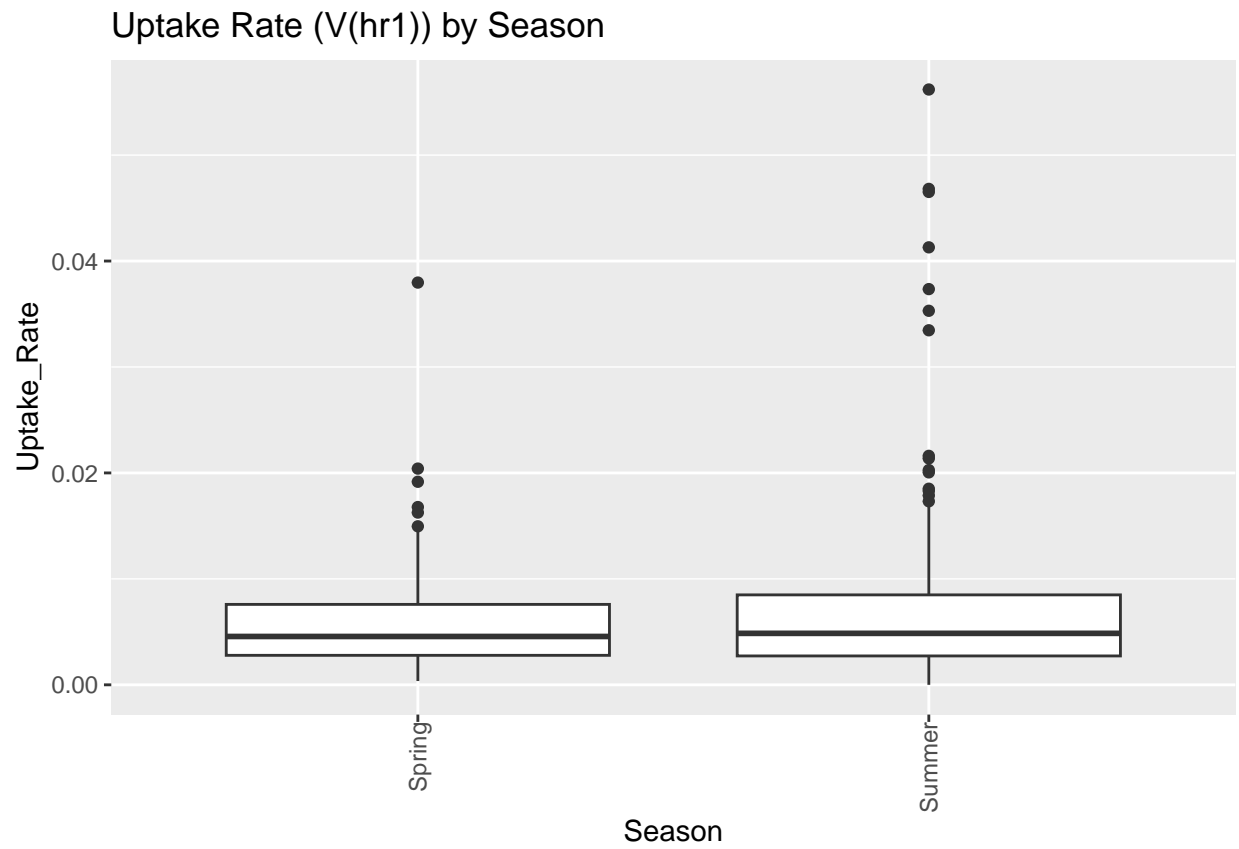
Exploratory Analysis

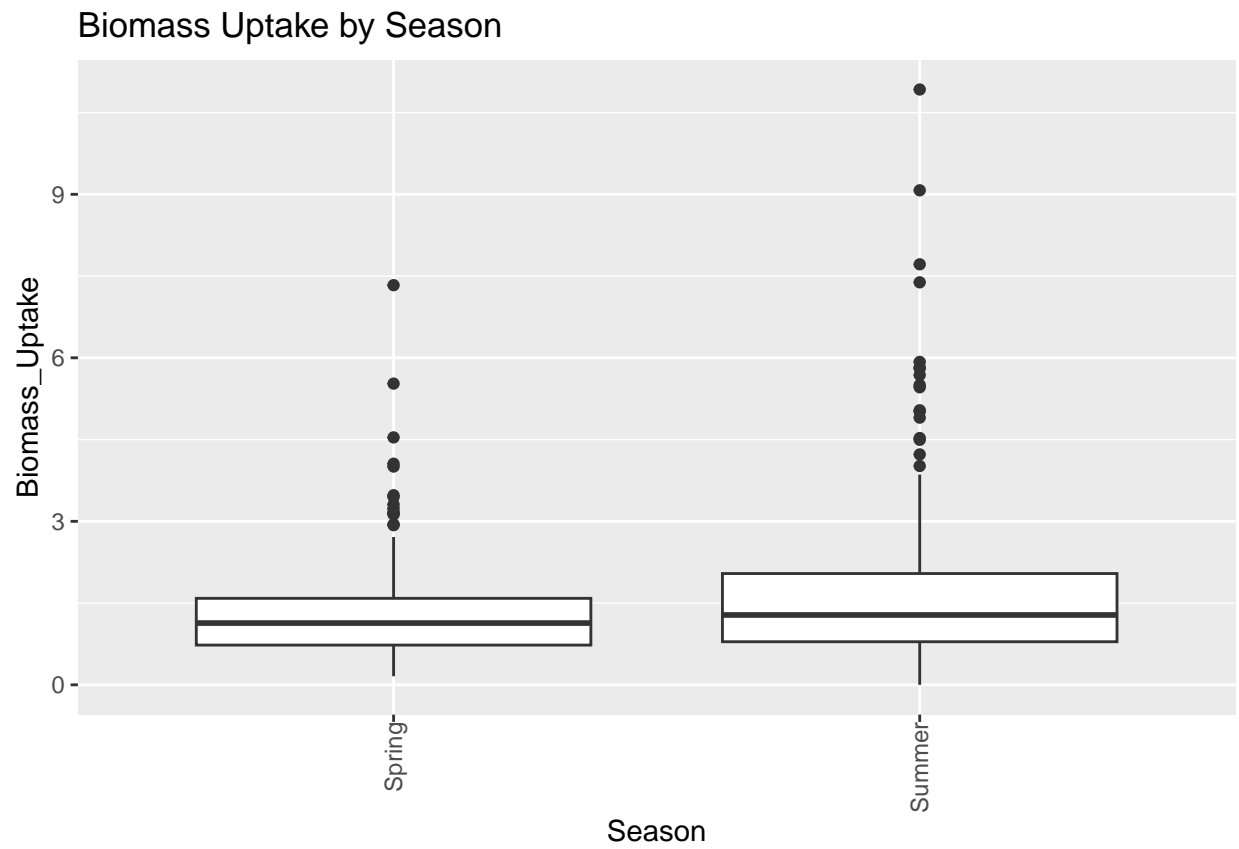
A map was created to gain a sense of location. The Map shows the sampling sites of nitrogen uptake along the coast of California.

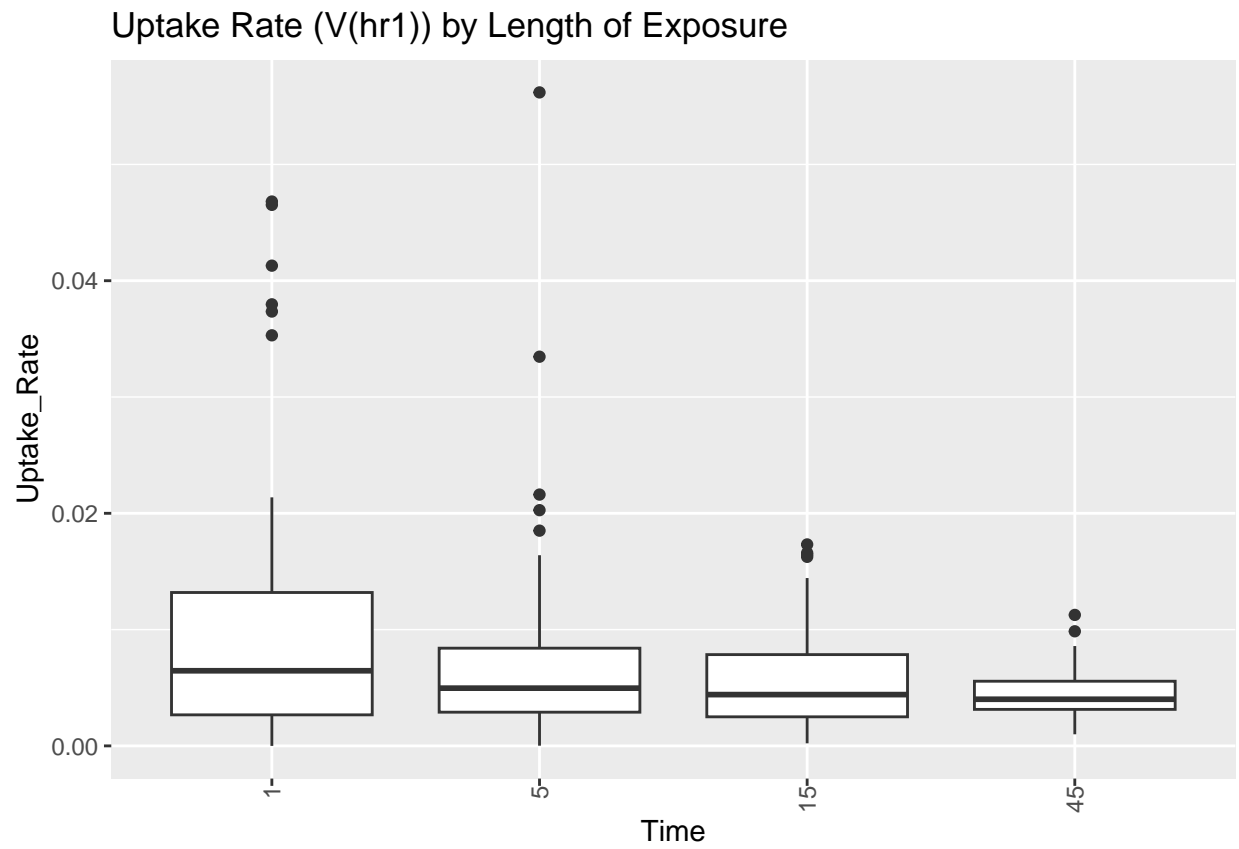


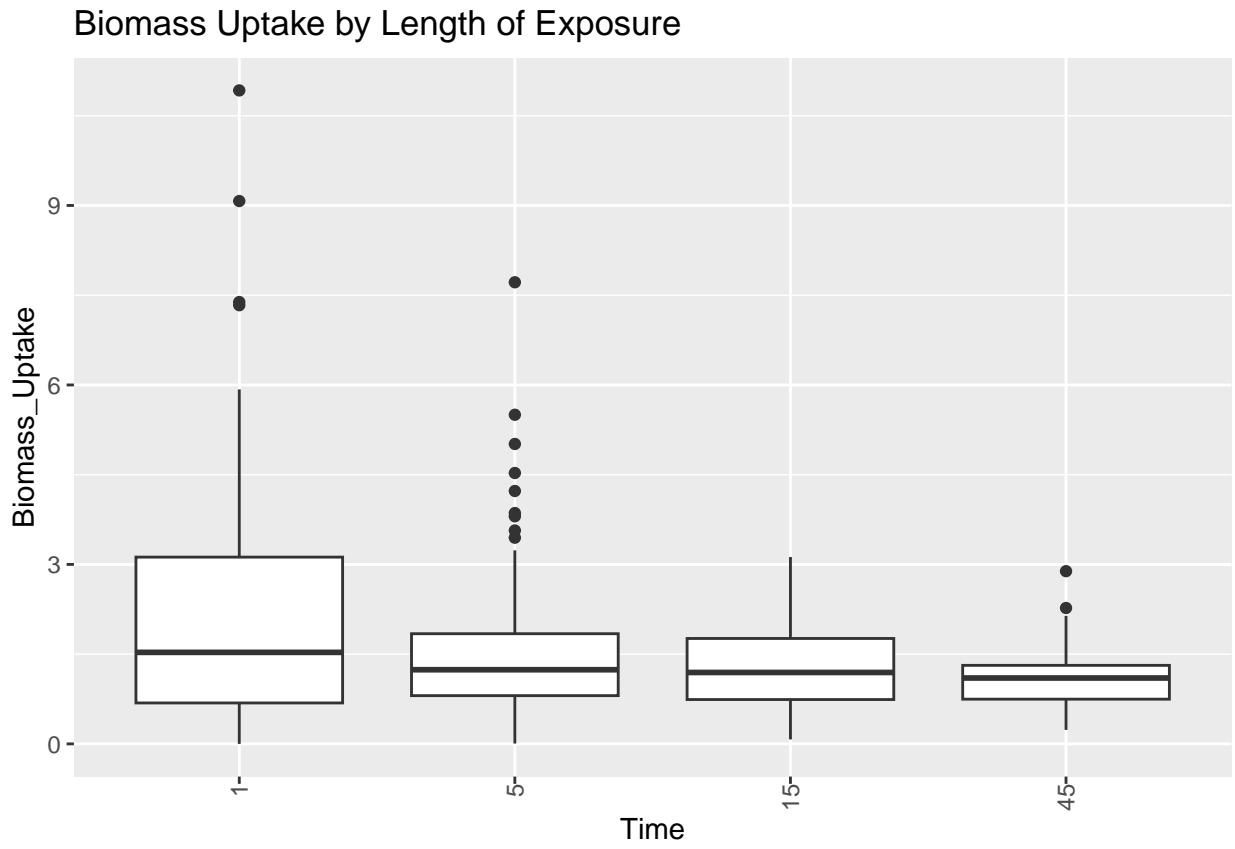
Boxplot text

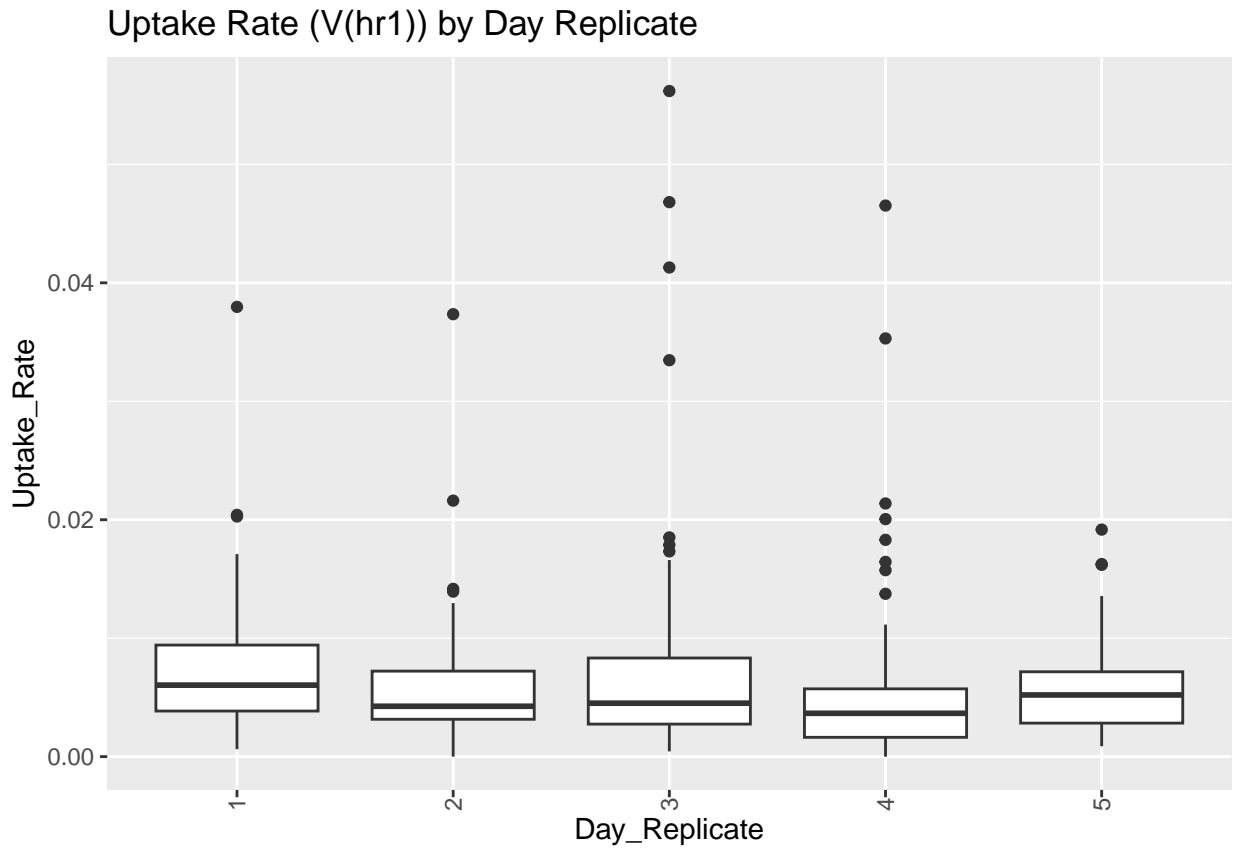


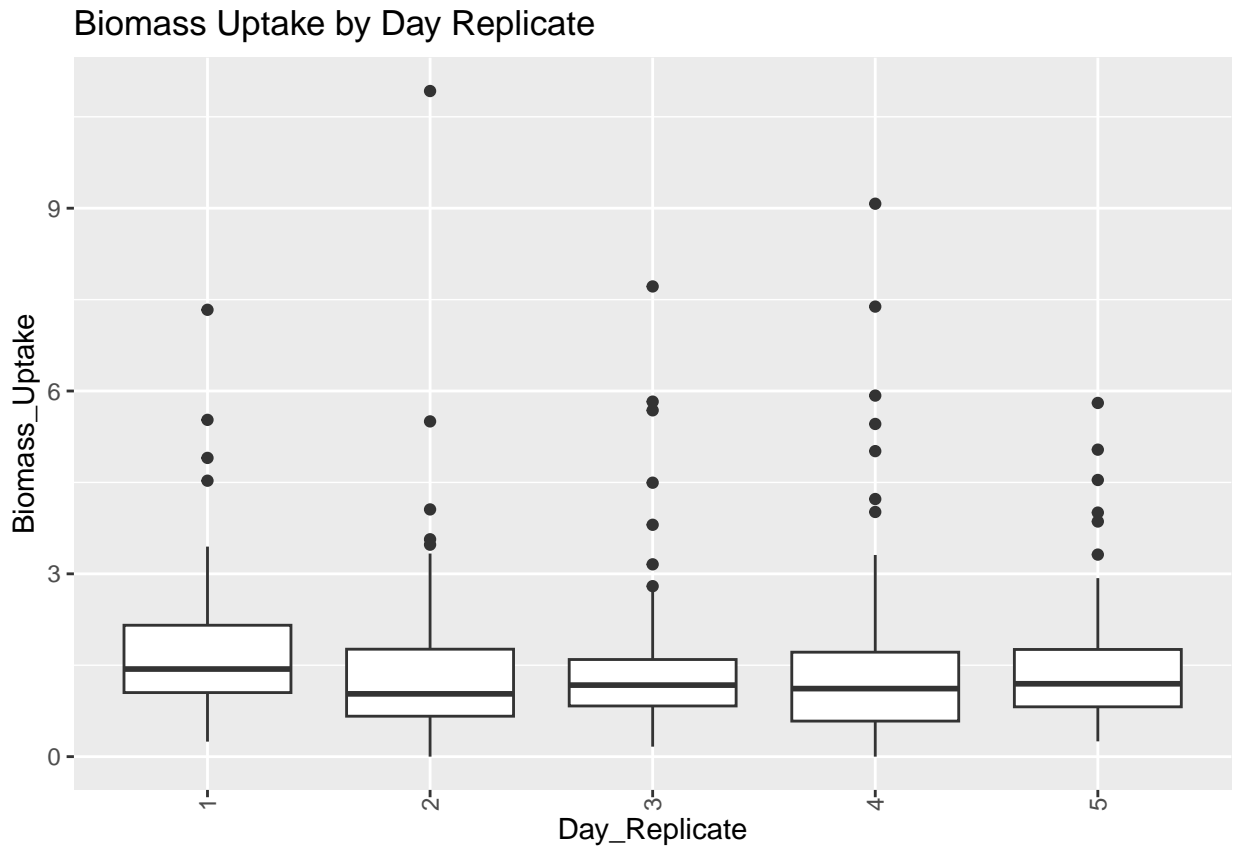


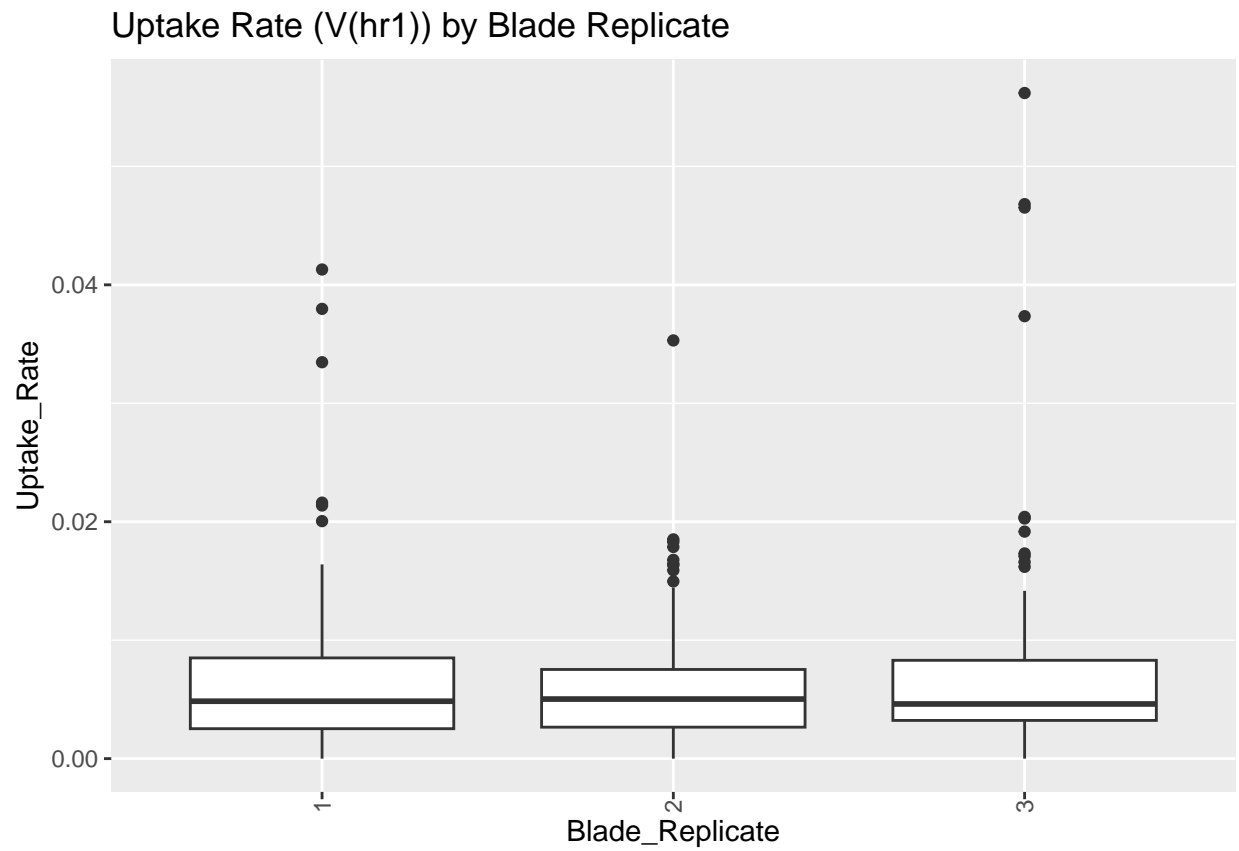


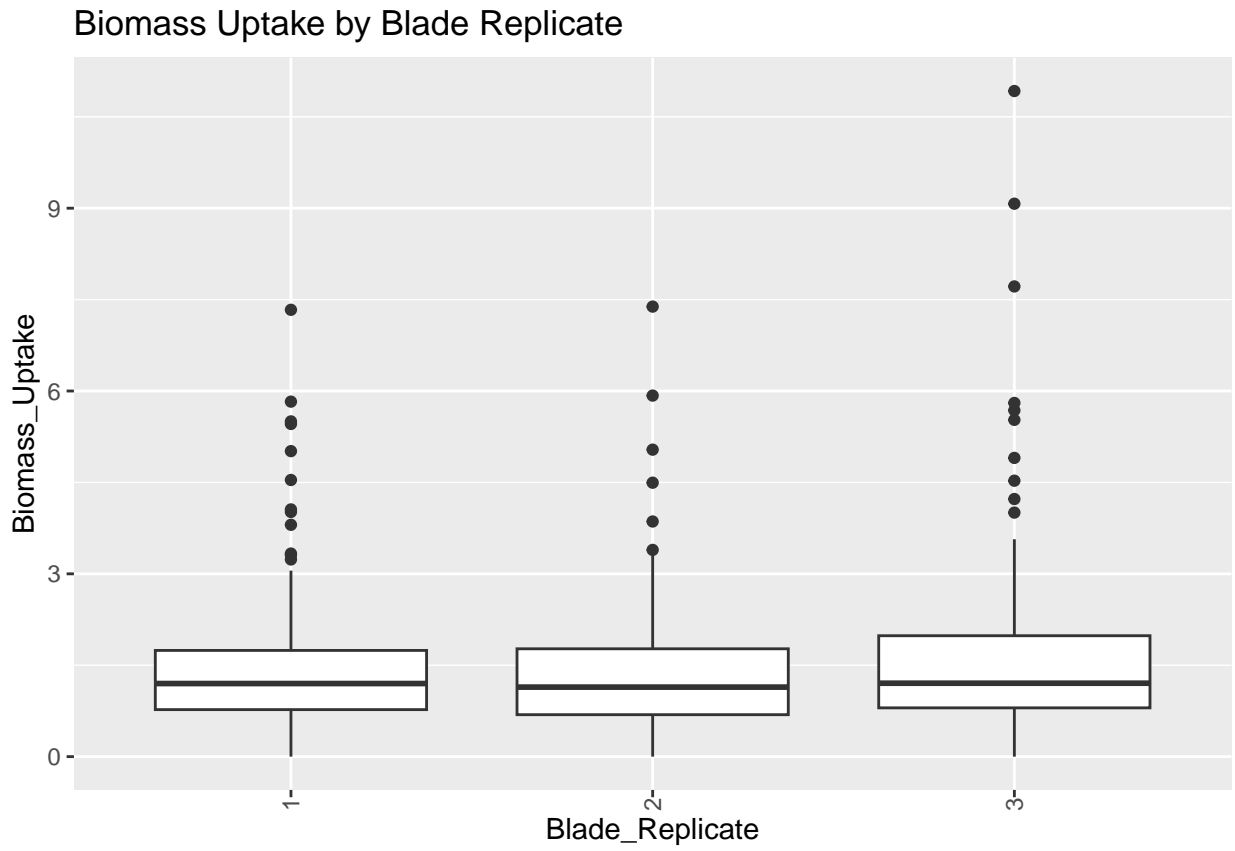




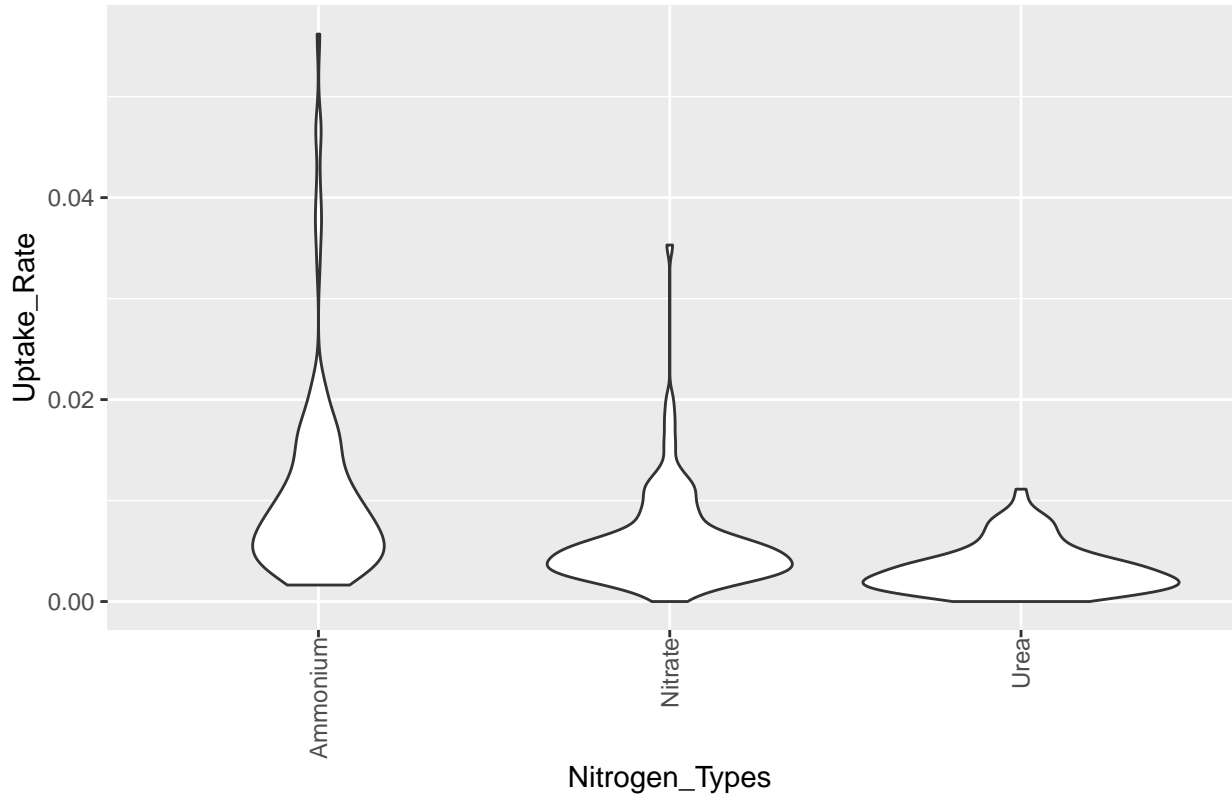




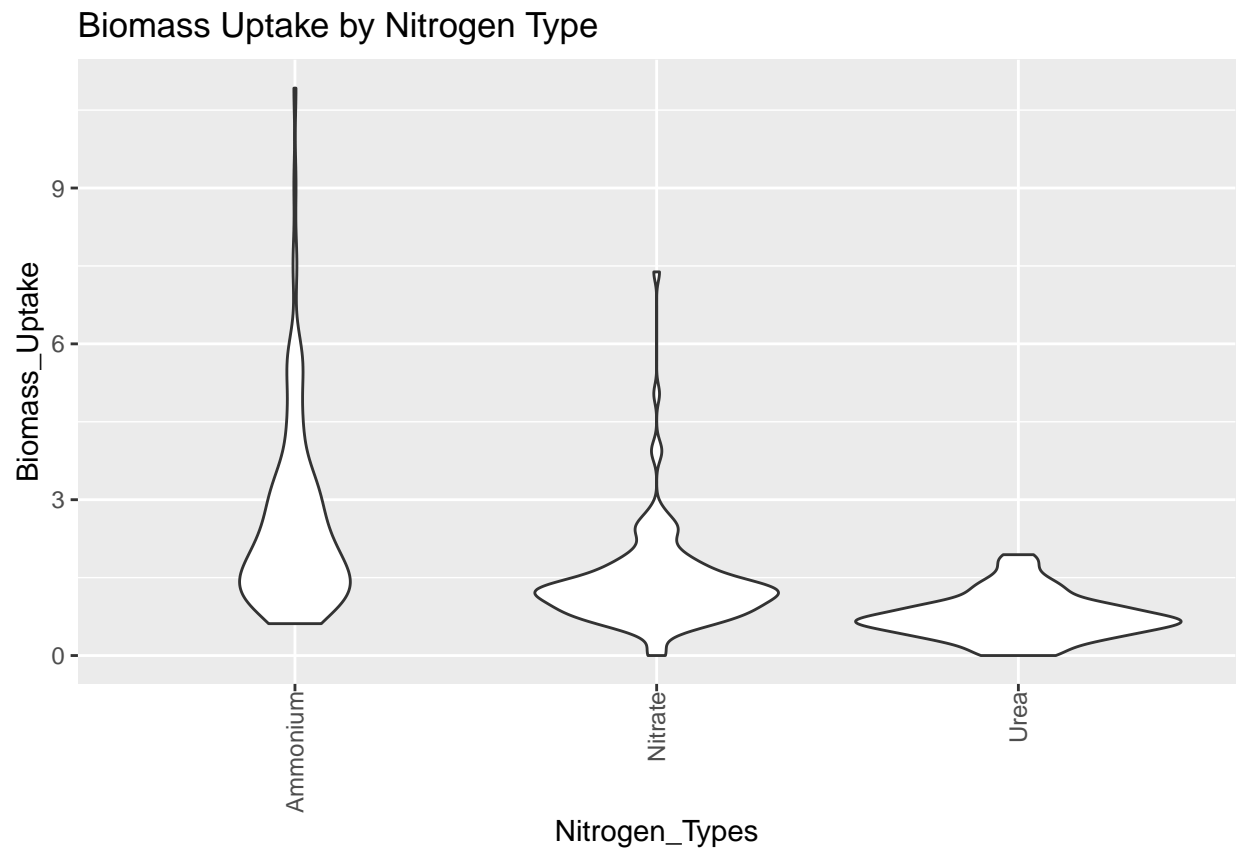


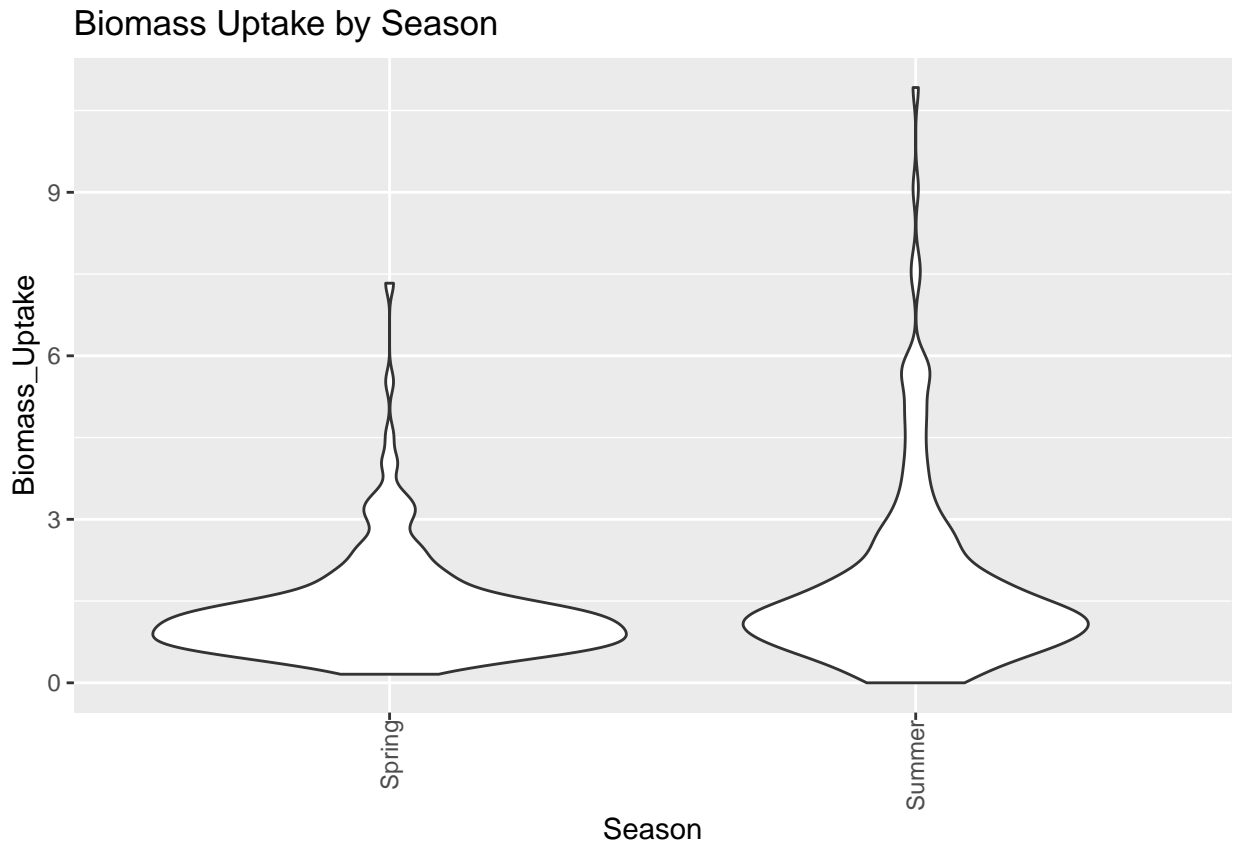


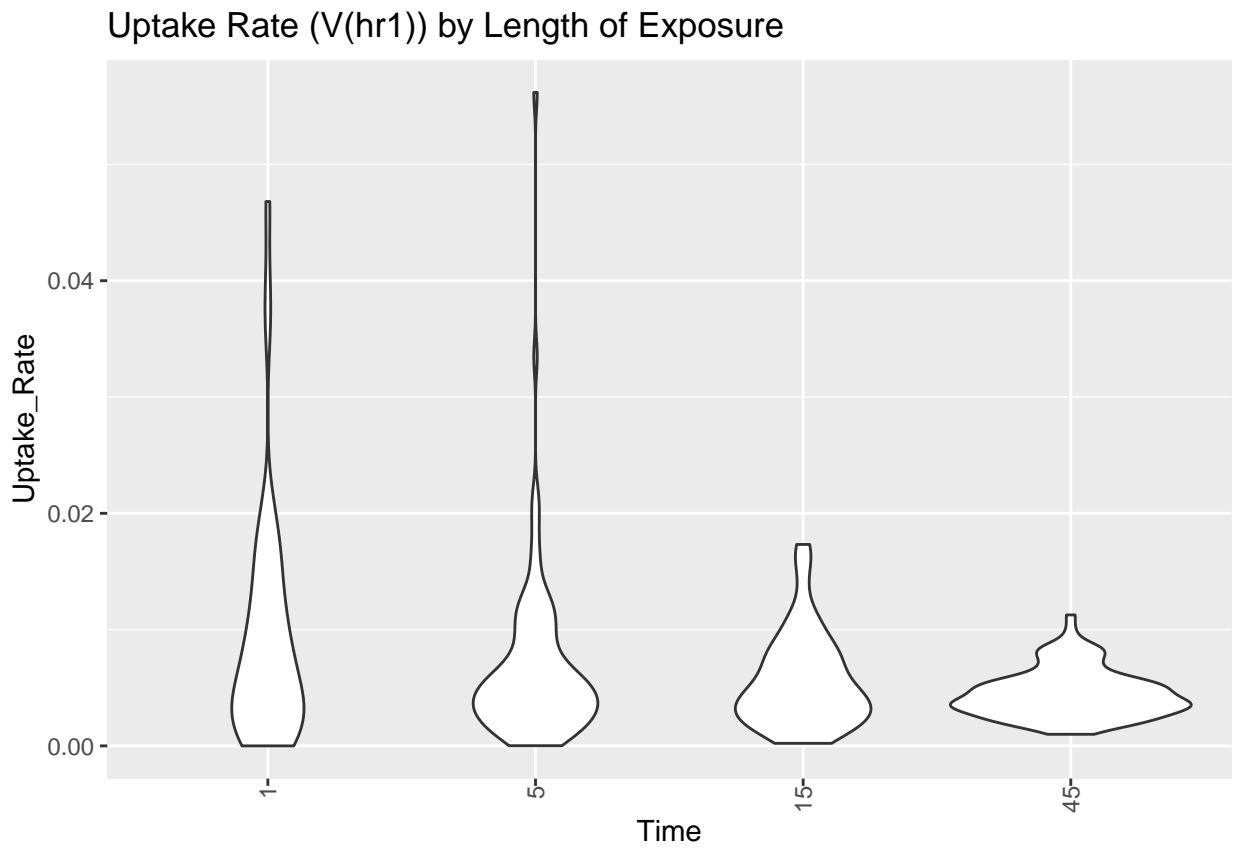
Uptake Rate (V(hr1)) by Nitrogen Type

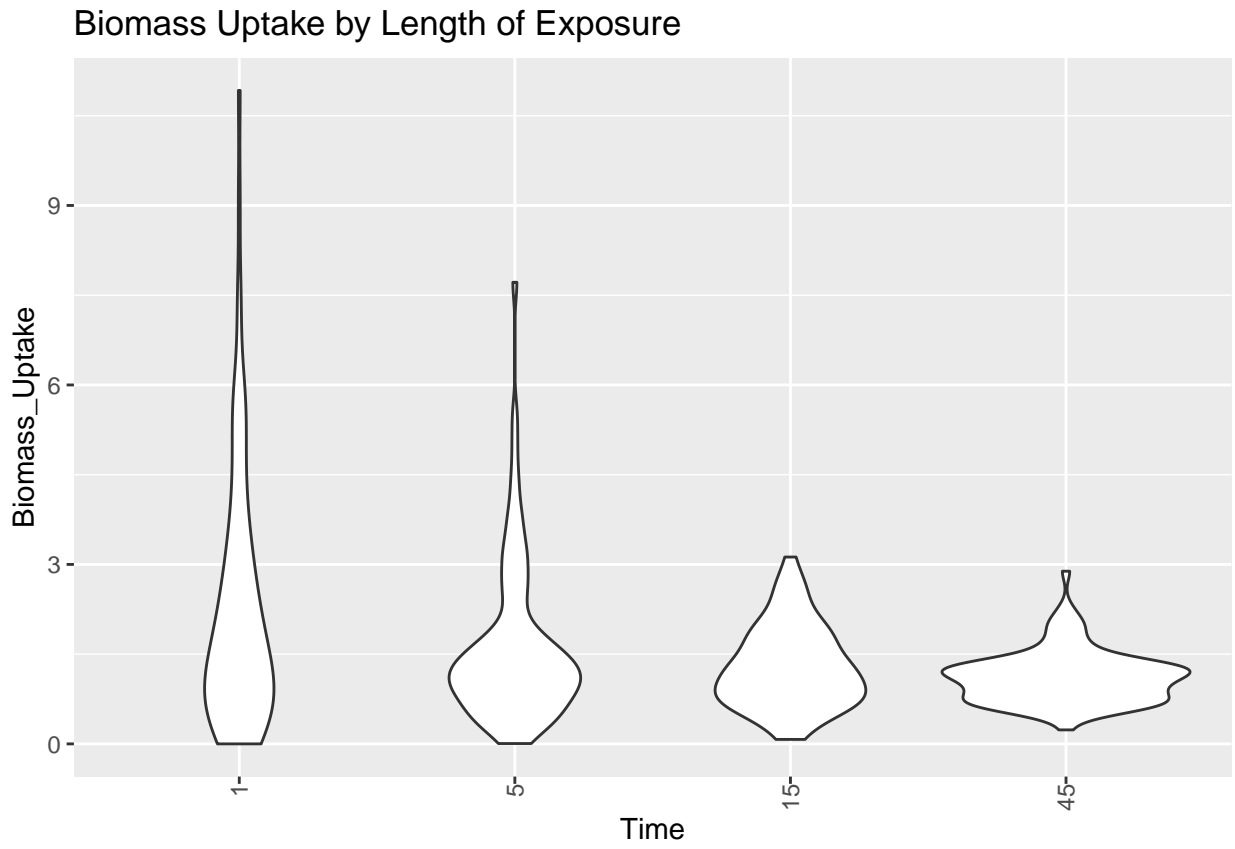


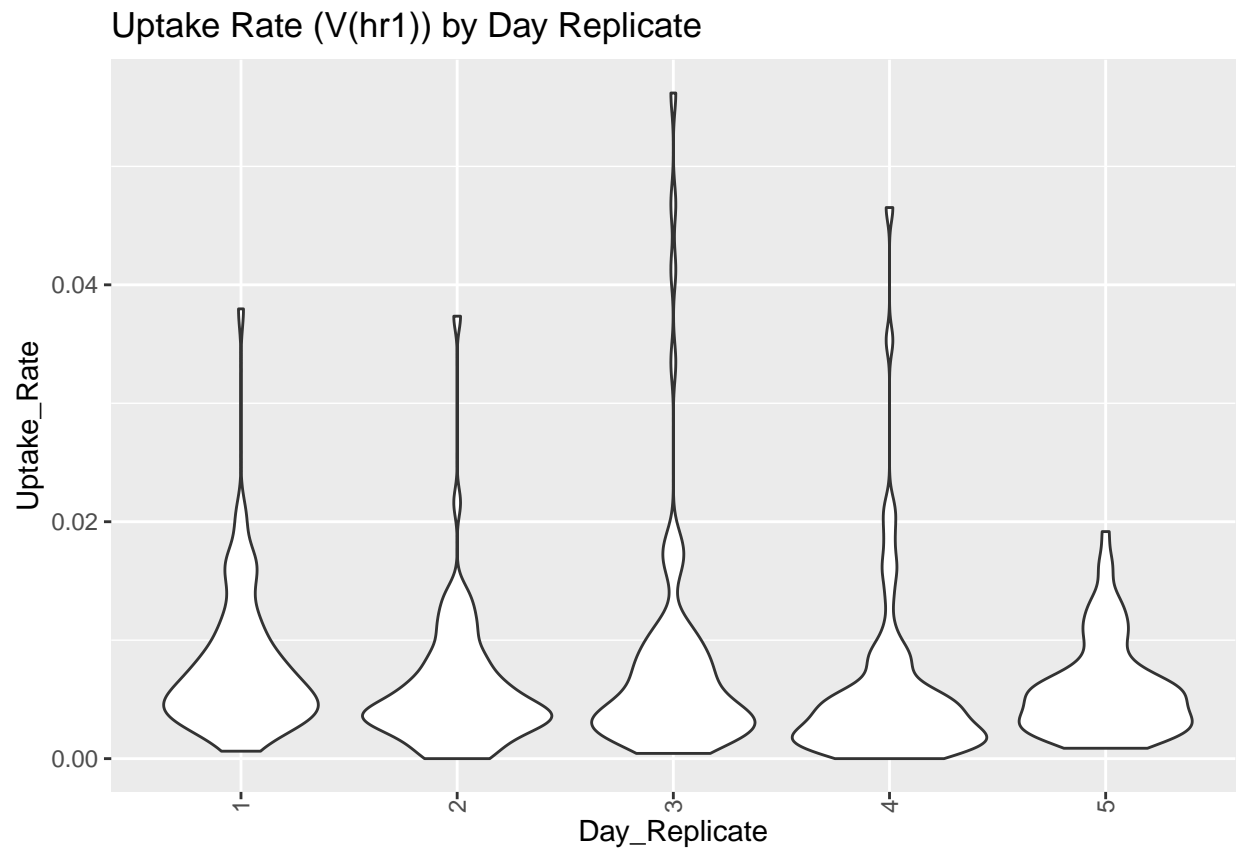
Violin text

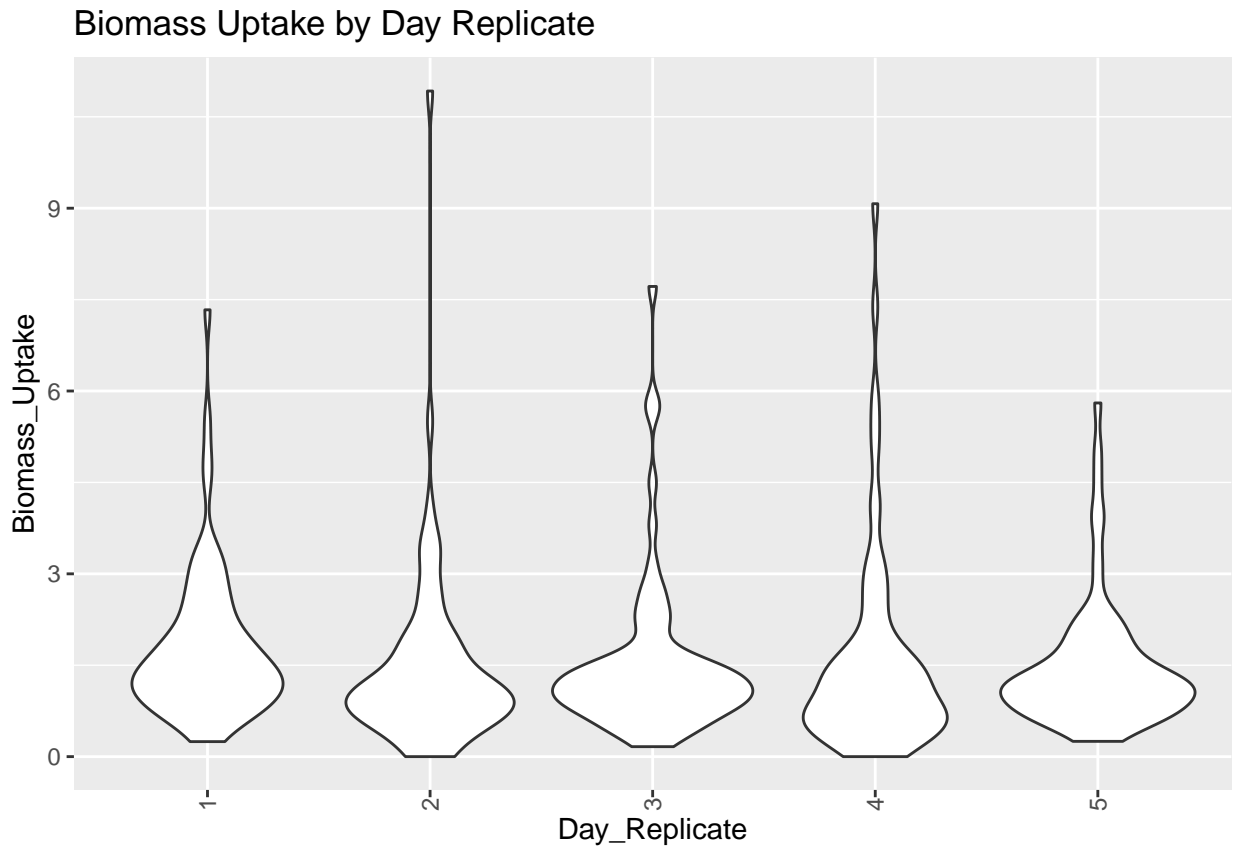


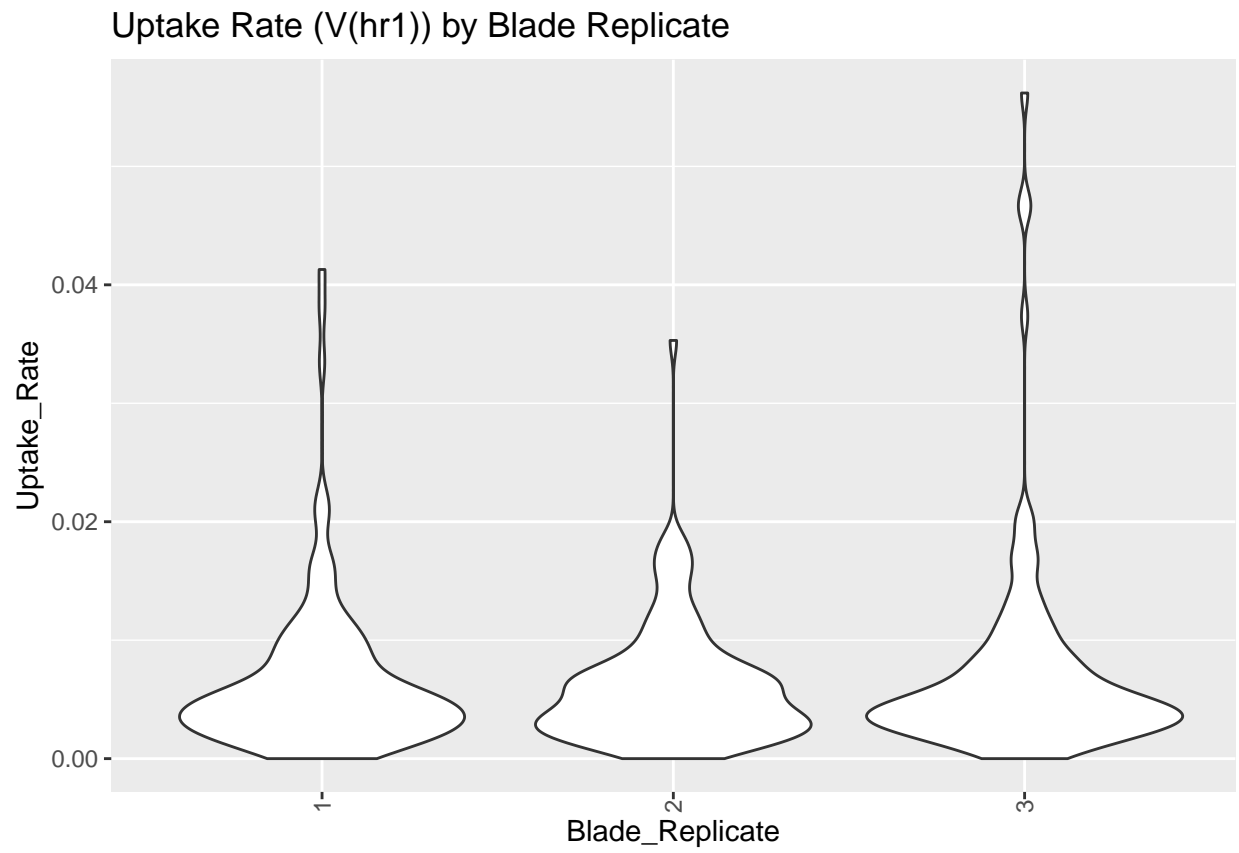


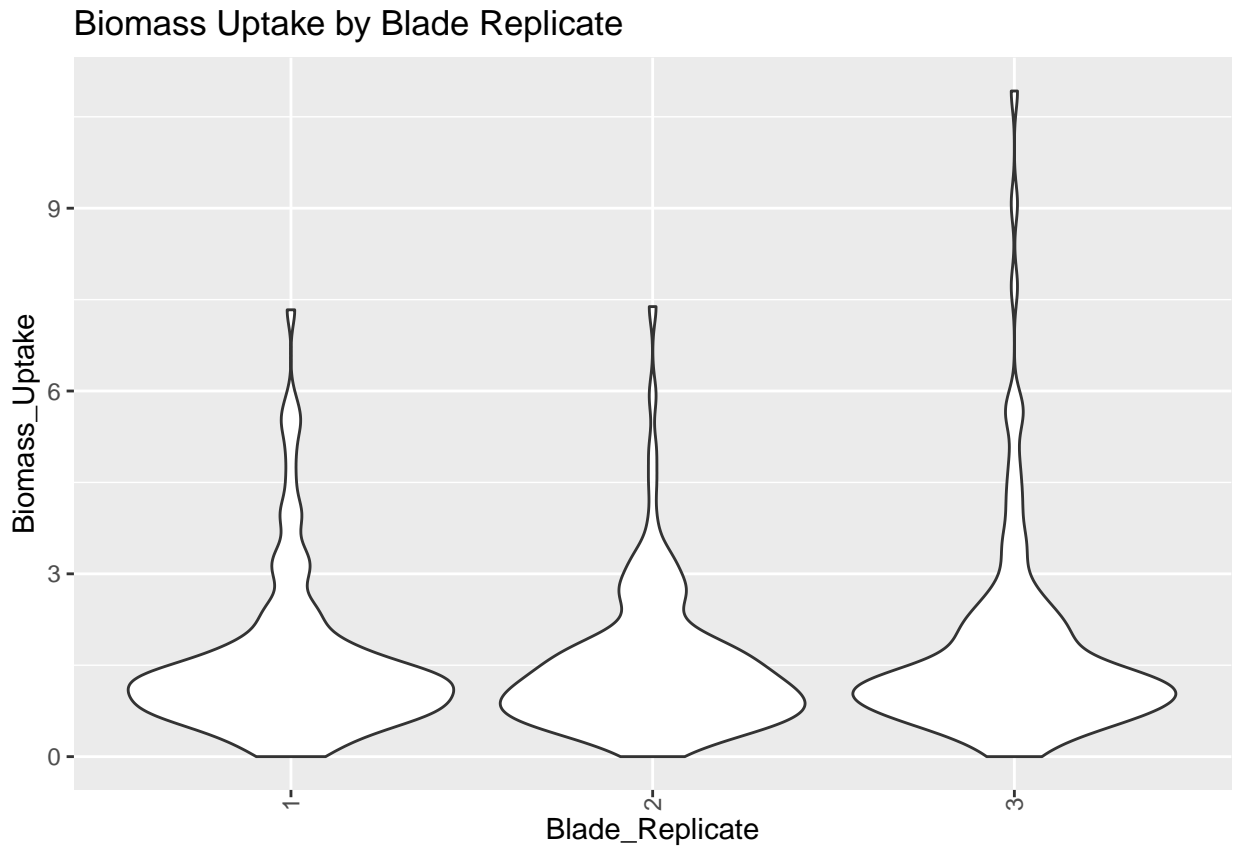












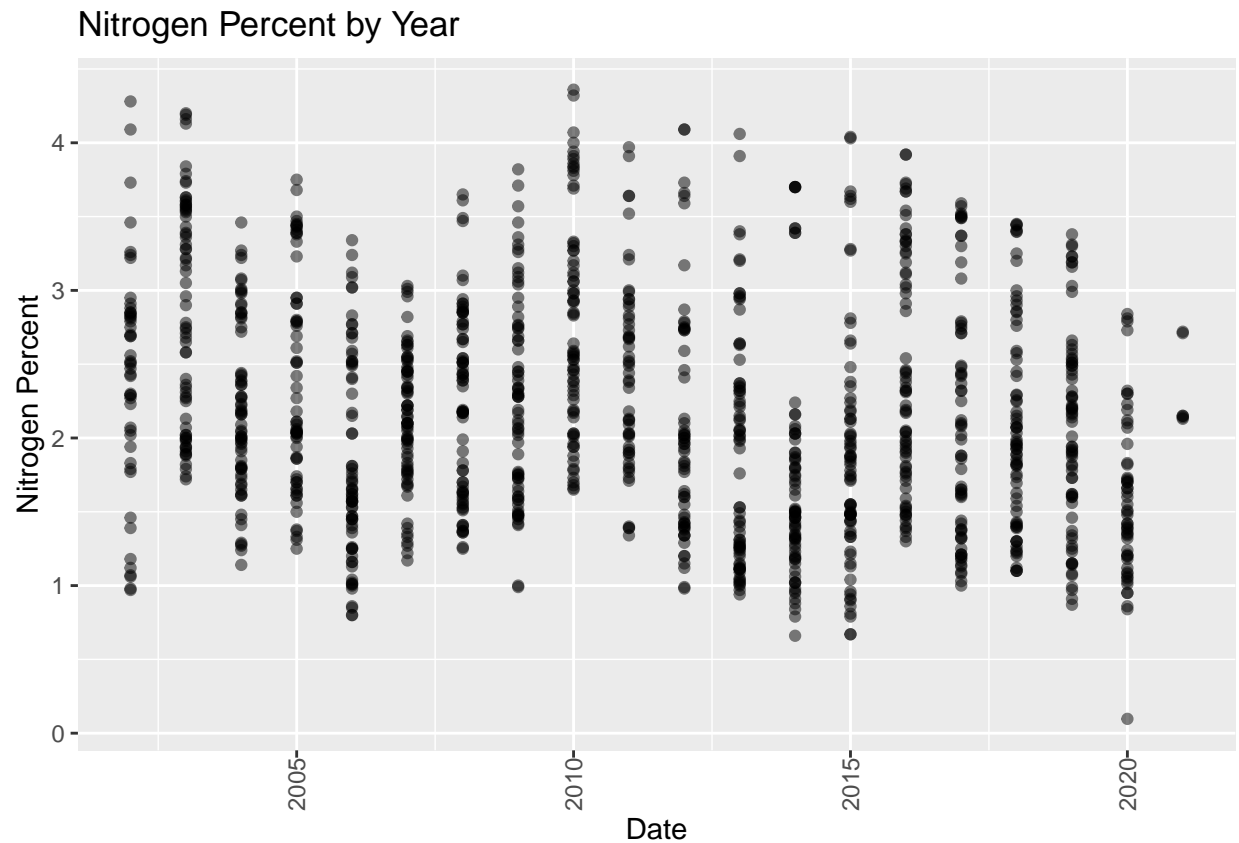
The last part of the exploratory analysis focused on an overview of nitrogen concentration amount over the last twenty years. A summary was used on the entire data set as well as on the nitrogen concentration column to gain a general sense of what amounts were present.

```
##           X           Sample_Number           Year           Month
## Min.      : 3.0      Min.      : 1.0      Min.      :2002      Min.      : 1.000
## 1st Qu.: 337.2      1st Qu.:176.0      1st Qu.:2006      1st Qu.: 4.000
## Median : 665.5      Median :352.0      Median :2011      Median : 7.000
## Mean    : 671.5      Mean    :352.1      Mean    :2011      Mean    : 6.602
## 3rd Qu.:1007.8      3rd Qu.:513.0      3rd Qu.:2016      3rd Qu.:10.000
## Max.    :1334.0      Max.    :910.0      Max.    :2021      Max.    :12.000
##
##           Date           Site           Number_of_Samples Sample_Replicate
## 2014-03-12: 6      ABUR :434      Min.      : 8.00      Min.      :1.0
## 2003-01-16: 4      ABUR : 2      1st Qu.:15.00      1st Qu.:1.0
## 2003-02-18: 4      AQUE :410      Median :15.00      Median :1.5
## 2003-03-05: 4      MOHK :436      Mean    :14.67      Mean     :1.5
## 2007-01-03: 4                        3rd Qu.:15.00      3rd Qu.:2.0
## 2007-02-06: 4                        Max.    :16.00      Max.     :2.0
## (Other)      :1256
##           Wet_Weight           Dry_Weight           Ratio_Dry_to_Wet
## Min.      :-99999.00      Min.      :-99999.00      Min.      :-99999.00
## 1st Qu.: 5.88      1st Qu.: 0.48      1st Qu.: 0.07
## Median : 7.02      Median : 0.66      Median : 0.09
## Mean    : -616.79      Mean    : -779.33      Mean    : -779.93
## 3rd Qu.: 8.53      3rd Qu.: 0.86      3rd Qu.: 0.11
## Max.    : 17.19      Max.    : 2.79      Max.    : 0.38
##
## Analytical_Dry_Weight Carbon_Percent Hydrogen_Percent Nitrogen_Percent
## Min.      :1797      Min.      :20.25      Min.      :-99999.00      Min.      :0.097
## 1st Qu.:2155      1st Qu.:28.39      1st Qu.: 4.01      1st Qu.:1.570
## Median :2310      Median :31.33      Median : 4.72      Median :2.063
## Mean    :2394      Mean    :32.17      Mean    : -4675.62      Mean    :2.174
## 3rd Qu.:2517      3rd Qu.:35.27      3rd Qu.: 5.27      3rd Qu.:2.710
## Max.    :4770      Max.    :44.52      Max.    : 7.57      Max.    :4.360
##
## Carbon_Nitrogen_Ratio
## Min.      : 6.64
## 1st Qu.:11.43
## Median :14.94
## Mean    :16.89
## 3rd Qu.:20.86
## Max.    :48.16
##
##
## Notes
## :1247
## dry_wet ratio very large, possible processing error : 6
## reproducibility and replicate data noisy. Will reanalyze.: 6
## missing dry wt : 4
## used average of individual plants : 4
## Average of 10 composite samples : 2
## (Other) : 13
##           NewSites
## Arroyo Burro :436
```

```
## Arroyo Quemado:410
## Mohawk      :436
##
##
##
##
```

```
##   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
## 0.097  1.570   2.063   2.174  2.710   4.360
```

A scatter plot was used to visualize the relationship between year and nitrogen concentration amount.



Lastly, a boxplot, see below, was used to visualize the relationship between sites and nitrogen concentration. Looking at this data, it appeared concentration amount was not too different by site. Looking at the scatter plot for year, it looked like nitrogen decreased slightly. This was then explored further in the Data Analysis section.

#Data Exploration Part 3

```
NitrogenYears_Box <- ggplot(NitrogenYears, aes(x=NewSites, y=Nitrogen_Percent))+
  geom_boxplot()+
  labs(title="Nitrogen Percent by Site", x="Site", y="Nitrogen Percent")

print(NitrogenYears_Box)
```

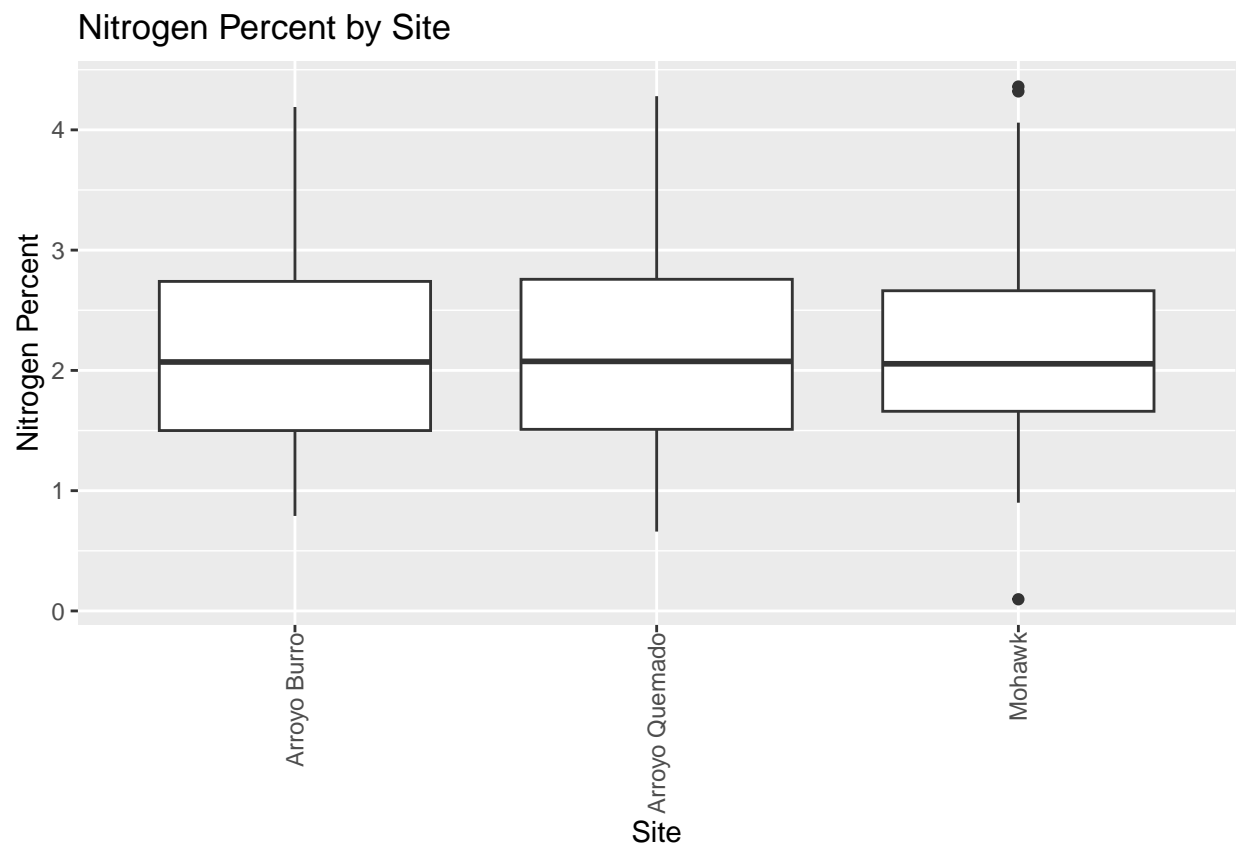


Figure 1: Exploratory Analysis for Nitrogen Concentration by Site

Analysis

Question 1: Does giant kelp nitrogen percent vary over year or season?

For the first question, we wanted to understand the relationship between nitrogen concentration and time. Linear regression and normal distribution tests were run for both the year and the specific date of collection. The box plot below shows the nitrogen concentrations over the years, starting with 2002.

H0 : There is no significant difference of nitrogen percent over the past twenty years. HA : There is significant difference of nitrogen percent over the past twenty years.

The interaction between nitrogen concentration and year is significant ($p < 2.2e-16$). The interaction between nitrogen concentration and collection date is significant ($p < 0.001$). We can reject the null hypothesis and accept that there is significant difference of nitrogen percent from 2002 to 2021. The R-squared is .974, so a high amount of nitrogen concentration variance is in response to date change. Below is a box plot showing change in nitrogen concentration over time.

```
#2. LM for Date
NitrogenRegressionDate <- lm(NitrogenYears$Nitrogen_Percent ~ NitrogenYears$Date)

#Linear regression on the nitrogen percent amount by year. P-value is less than .05. Which means there
summary(NitrogenRegressionDate)

##
## Call:
## lm(formula = NitrogenYears$Nitrogen_Percent ~ NitrogenYears$Date)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.965 -0.030  0.000  0.030  0.985
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    2.800e+00  1.165e-01  24.033 < 2e-16 ***
## NitrogenYears$Date2002-05-10 -2.450e-01  1.648e-01  -1.487 0.137461
## NitrogenYears$Date2002-05-15  7.950e-01  1.648e-01   4.825 1.71e-06 ***
## NitrogenYears$Date2002-06-10 -1.020e+00  1.648e-01  -6.191 1.00e-09 ***
## NitrogenYears$Date2002-06-12  2.150e-01  1.648e-01   1.305 0.192348
## NitrogenYears$Date2002-06-13  1.385e+00  1.648e-01   8.406 2.23e-16 ***
## NitrogenYears$Date2002-07-10 -3.100e-01  1.648e-01  -1.881 0.060310 .
## NitrogenYears$Date2002-07-11 -1.735e+00  1.648e-01 -10.530 < 2e-16 ***
## NitrogenYears$Date2002-07-12 -6.750e-01  1.648e-01  -4.097 4.66e-05 ***
## NitrogenYears$Date2002-08-07 -9.150e-01  1.648e-01  -5.553 3.93e-08 ***
## NitrogenYears$Date2002-08-08 -7.400e-01  1.648e-01  -4.491 8.23e-06 ***
## NitrogenYears$Date2002-08-09  3.500e-02  1.648e-01   0.212 0.831838
## NitrogenYears$Date2002-09-04 -1.650e+00  1.648e-01 -10.014 < 2e-16 ***
## NitrogenYears$Date2002-09-05 -5.200e-01  1.648e-01  -3.156 0.001666 **
## NitrogenYears$Date2002-09-10 -3.050e-01  1.648e-01  -1.851 0.064560 .
## NitrogenYears$Date2002-10-09 -1.375e+00  1.648e-01  -8.345 3.57e-16 ***
## NitrogenYears$Date2002-10-10 -1.825e+00  1.648e-01 -11.076 < 2e-16 ***
## NitrogenYears$Date2002-10-11  5.000e-02  1.648e-01   0.303 0.761627
## NitrogenYears$Date2002-11-20  4.500e-01  1.648e-01   2.731 0.006465 **
## NitrogenYears$Date2002-11-21 -7.500e-02  1.648e-01  -0.455 0.649109
## NitrogenYears$Date2002-11-22  1.000e-01  1.648e-01   0.607 0.544095
```

```

## NitrogenYears$Date2002-12-06 -2.900e-01 1.648e-01 -1.760 0.078817 .
## NitrogenYears$Date2002-12-09 -5.050e-01 1.648e-01 -3.065 0.002258 **
## NitrogenYears$Date2002-12-10 2.000e-02 1.648e-01 0.121 0.903421
## NitrogenYears$Date2003-01-14 -2.200e-01 1.648e-01 -1.335 0.182222
## NitrogenYears$Date2003-01-16 -4.550e-01 1.427e-01 -3.189 0.001490 **
## NitrogenYears$Date2003-02-18 -8.475e-01 1.427e-01 -5.939 4.43e-09 ***
## NitrogenYears$Date2003-02-21 -9.950e-01 1.648e-01 -6.039 2.47e-09 ***
## NitrogenYears$Date2003-03-05 -6.250e-02 1.427e-01 -0.438 0.661512
## NitrogenYears$Date2003-03-06 -7.550e-01 1.648e-01 -4.582 5.41e-06 ***
## NitrogenYears$Date2003-04-08 5.350e-01 1.648e-01 3.247 0.001220 **
## NitrogenYears$Date2003-04-10 2.900e-01 1.648e-01 1.760 0.078817 .
## NitrogenYears$Date2003-04-15 1.380e+00 1.648e-01 8.375 2.82e-16 ***
## NitrogenYears$Date2003-05-06 8.300e-01 1.648e-01 5.037 5.96e-07 ***
## NitrogenYears$Date2003-05-12 7.400e-01 1.648e-01 4.491 8.23e-06 ***
## NitrogenYears$Date2003-05-13 3.950e-01 1.648e-01 2.397 0.016766 *
## NitrogenYears$Date2003-06-03 9.350e-01 1.648e-01 5.675 2.01e-08 ***
## NitrogenYears$Date2003-06-10 -6.000e-01 1.648e-01 -3.642 0.000290 ***
## NitrogenYears$Date2003-06-11 1.360e+00 1.648e-01 8.254 7.18e-16 ***
## NitrogenYears$Date2003-07-02 1.015e+00 1.648e-01 6.160 1.20e-09 ***
## NitrogenYears$Date2003-07-07 -1.070e+00 1.648e-01 -6.494 1.55e-10 ***
## NitrogenYears$Date2003-07-15 -5.200e-01 1.648e-01 -3.156 0.001666 **
## NitrogenYears$Date2003-08-05 -1.200e-01 1.648e-01 -0.728 0.466663
## NitrogenYears$Date2003-08-06 6.450e-01 1.648e-01 3.915 9.90e-05 ***
## NitrogenYears$Date2003-08-08 7.800e-01 1.648e-01 4.734 2.65e-06 ***
## NitrogenYears$Date2003-09-02 7.850e-01 1.648e-01 4.764 2.29e-06 ***
## NitrogenYears$Date2003-09-04 7.500e-01 1.648e-01 4.552 6.23e-06 ***
## NitrogenYears$Date2003-09-05 5.700e-01 1.648e-01 3.459 0.000573 ***
## NitrogenYears$Date2003-10-01 1.300e-01 1.648e-01 0.789 0.430373
## NitrogenYears$Date2003-11-06 4.600e-01 1.648e-01 2.792 0.005379 **
## NitrogenYears$Date2003-11-13 -7.900e-01 1.648e-01 -4.795 1.98e-06 ***
## NitrogenYears$Date2003-11-14 -8.150e-01 1.648e-01 -4.946 9.40e-07 ***
## NitrogenYears$Date2003-12-03 -9.000e-01 1.648e-01 -5.462 6.46e-08 ***
## NitrogenYears$Date2003-12-05 6.650e-01 1.648e-01 4.036 6.01e-05 ***
## NitrogenYears$Date2003-12-08 -9.150e-01 1.648e-01 -5.553 3.93e-08 ***
## NitrogenYears$Date2004-01-07 7.000e-02 1.648e-01 0.425 0.671077
## NitrogenYears$Date2004-01-09 4.450e-01 1.648e-01 2.701 0.007078 **
## NitrogenYears$Date2004-02-02 8.000e-02 1.648e-01 0.486 0.627444
## NitrogenYears$Date2004-02-06 1.950e-01 1.648e-01 1.183 0.237001
## NitrogenYears$Date2004-02-12 1.950e-01 1.648e-01 1.183 0.237001
## NitrogenYears$Date2004-03-08 -3.700e-01 1.648e-01 -2.246 0.025029 *
## NitrogenYears$Date2004-03-10 -8.200e-01 1.648e-01 -4.977 8.08e-07 ***
## NitrogenYears$Date2004-03-12 -5.400e-01 1.648e-01 -3.277 0.001098 **
## NitrogenYears$Date2004-04-05 -1.335e+00 1.648e-01 -8.102 2.27e-15 ***
## NitrogenYears$Date2004-04-07 -1.185e+00 1.648e-01 -7.192 1.59e-12 ***
## NitrogenYears$Date2004-04-12 -6.250e-01 1.648e-01 -3.793 0.000161 ***
## NitrogenYears$Date2004-05-07 -6.250e-01 1.648e-01 -3.793 0.000161 ***
## NitrogenYears$Date2004-05-15 -4.150e-01 1.648e-01 -2.519 0.011992 *
## NitrogenYears$Date2004-05-19 3.000e-02 1.648e-01 0.182 0.855574
## NitrogenYears$Date2004-06-02 -1.065e+00 1.648e-01 -6.464 1.87e-10 ***
## NitrogenYears$Date2004-06-16 2.750e-01 1.648e-01 1.669 0.095543 .
## NitrogenYears$Date2004-06-21 5.500e-01 1.648e-01 3.338 0.000887 ***
## NitrogenYears$Date2004-07-07 -5.500e-01 1.648e-01 -3.338 0.000887 ***
## NitrogenYears$Date2004-07-09 -2.600e-01 1.648e-01 -1.578 0.115003
## NitrogenYears$Date2004-07-16 -5.500e-01 1.648e-01 -3.338 0.000887 ***

```

```

## NitrogenYears$Date2004-08-03 -1.500e-02 1.648e-01 -0.091 0.927488
## NitrogenYears$Date2004-08-05 -8.050e-01 1.648e-01 -4.886 1.27e-06 ***
## NitrogenYears$Date2004-08-06 -7.000e-01 1.648e-01 -4.248 2.43e-05 ***
## NitrogenYears$Date2004-09-01 -4.800e-01 1.648e-01 -2.913 0.003687 **
## NitrogenYears$Date2004-09-02 -8.000e-01 1.648e-01 -4.855 1.47e-06 ***
## NitrogenYears$Date2004-09-07 -1.450e+00 1.648e-01 -8.800 < 2e-16 ***
## NitrogenYears$Date2004-10-06 -1.525e+00 1.648e-01 -9.255 < 2e-16 ***
## NitrogenYears$Date2004-10-07 -9.600e-01 1.648e-01 -5.826 8.50e-09 ***
## NitrogenYears$Date2004-10-14 -8.350e-01 1.648e-01 -5.068 5.11e-07 ***
## NitrogenYears$Date2004-11-03 -1.000e+00 1.648e-01 -6.069 2.07e-09 ***
## NitrogenYears$Date2004-11-04 -8.900e-01 1.648e-01 -5.402 8.95e-08 ***
## NitrogenYears$Date2004-11-10 -1.610e+00 1.648e-01 -9.771 < 2e-16 ***
## NitrogenYears$Date2004-12-01 -1.170e+00 1.648e-01 -7.101 2.95e-12 ***
## NitrogenYears$Date2004-12-02 -1.030e+00 1.648e-01 -6.251 6.93e-10 ***
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## NitrogenYears$Date2012-07-02 -1.360e+00 1.648e-01 -8.254 7.18e-16 ***
## NitrogenYears$Date2012-07-03 -8.300e-01 1.648e-01 -5.037 5.96e-07 ***
## NitrogenYears$Date2012-07-10 -9.900e-01 1.648e-01 -6.008 2.96e-09 ***
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## NitrogenYears$Date2012-08-08 -1.200e+00 1.648e-01 -7.283 8.50e-13 ***
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## NitrogenYears$Date2013-05-09 -7.700e-01 1.648e-01 -4.673 3.53e-06 ***
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## NitrogenYears$Date2013-06-05 -1.290e+00 1.648e-01 -7.829 1.73e-14 ***
## NitrogenYears$Date2013-06-10 -1.425e+00 1.648e-01 -8.649 < 2e-16 ***
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## NitrogenYears$Date2013-08-06 -7.700e-01 1.648e-01 -4.673 3.53e-06 ***
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## NitrogenYears$Date2013-09-30 -1.560e+00 1.427e-01 -10.933 < 2e-16 ***
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## NitrogenYears$Date2014-02-04 -6.400e-01 1.648e-01 -3.884 0.000112 ***
## NitrogenYears$Date2014-02-06 -1.100e+00 1.648e-01 -6.676 4.87e-11 ***
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## NitrogenYears$Date2014-04-09 6.050e-01 1.648e-01 3.672 0.000258 ***
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## NitrogenYears$Date2014-05-09 6.050e-01 1.648e-01 3.672 0.000258 ***
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## NitrogenYears$Date2015-06-02 -7.750e-01 1.648e-01 -4.704 3.06e-06 ***
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## NitrogenYears$Date2017-04-11 7.050e-01 1.648e-01 4.279 2.13e-05 ***
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## NitrogenYears$Date2019-03-05 -2.750e-01 1.648e-01 -1.669 0.095543 .
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## NitrogenYears$Date2019-11-04 -1.195e+00 1.648e-01 -7.253 1.05e-12 ***
## NitrogenYears$Date2019-11-06 -8.800e-01 1.648e-01 -5.341 1.24e-07 ***
## NitrogenYears$Date2019-12-03 -6.000e-01 1.648e-01 -3.642 0.000290 ***
## NitrogenYears$Date2019-12-05 -2.700e-01 1.648e-01 -1.639 0.101712
## NitrogenYears$Date2019-12-06 -8.700e-01 1.648e-01 -5.280 1.71e-07 ***
## NitrogenYears$Date2020-01-07 -5.350e-01 1.648e-01 -3.247 0.001220 **
## NitrogenYears$Date2020-01-14 -1.478e+00 1.427e-01 -10.354 < 2e-16 ***
## NitrogenYears$Date2020-02-04 -1.410e+00 1.648e-01 -8.558 < 2e-16 ***
## NitrogenYears$Date2020-02-06 -9.950e-01 1.427e-01 -6.973 6.97e-12 ***
## NitrogenYears$Date2020-03-02 -1.780e+00 1.648e-01 -10.803 < 2e-16 ***
## NitrogenYears$Date2020-03-03 -1.590e+00 1.648e-01 -9.650 < 2e-16 ***
## NitrogenYears$Date2020-03-05 -2.227e+00 1.648e-01 -13.513 < 2e-16 ***
## NitrogenYears$Date2020-07-08 -4.900e-01 1.648e-01 -2.974 0.003038 **
## NitrogenYears$Date2020-07-10 2.500e-02 1.648e-01 0.152 0.879442
## NitrogenYears$Date2020-07-13 -1.765e+00 1.648e-01 -10.712 < 2e-16 ***
## NitrogenYears$Date2020-08-04 -1.415e+00 1.648e-01 -8.588 < 2e-16 ***
## NitrogenYears$Date2020-08-06 -1.275e+00 1.648e-01 -7.738 3.37e-14 ***
## NitrogenYears$Date2020-08-07 -1.075e+00 1.648e-01 -6.524 1.28e-10 ***
## NitrogenYears$Date2020-09-01 -1.140e+00 1.648e-01 -6.919 9.99e-12 ***
## NitrogenYears$Date2020-09-02 -1.353e+00 1.427e-01 -9.478 < 2e-16 ***
## NitrogenYears$Date2020-10-05 -1.483e+00 1.427e-01 -10.389 < 2e-16 ***
## NitrogenYears$Date2020-10-06 -1.095e+00 1.648e-01 -6.646 5.91e-11 ***
## NitrogenYears$Date2020-11-02 -1.715e+00 1.648e-01 -10.409 < 2e-16 ***
## NitrogenYears$Date2020-11-04 -1.200e+00 1.427e-01 -8.410 < 2e-16 ***
## NitrogenYears$Date2020-12-03 -7.050e-01 1.648e-01 -4.279 2.13e-05 ***
## NitrogenYears$Date2020-12-04 -9.200e-01 1.427e-01 -6.447 2.07e-10 ***
## NitrogenYears$Date2021-01-06 -6.550e-01 1.648e-01 -3.975 7.73e-05 ***
## NitrogenYears$Date2021-01-12 -3.725e-01 1.427e-01 -2.611 0.009227 **
## ---
## Signif. codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.1648 on 728 degrees of freedom
## Multiple R-squared: 0.974, Adjusted R-squared: 0.9542
## F-statistic: 49.28 on 553 and 728 DF, p-value: < 2.2e-16

```



```

NitrogenYears$Year <- as.factor(NitrogenYears$Year)

NitrogenPlot <- ggplot(NitrogenYears, aes(x=Year, y=Nitrogen_Percent))+
  geom_boxplot()+
  labs(title="Nitrogen Concentration Percent by Year", x="Year", y="Nitrogen Concentration Percent")
print(NitrogenPlot)

```

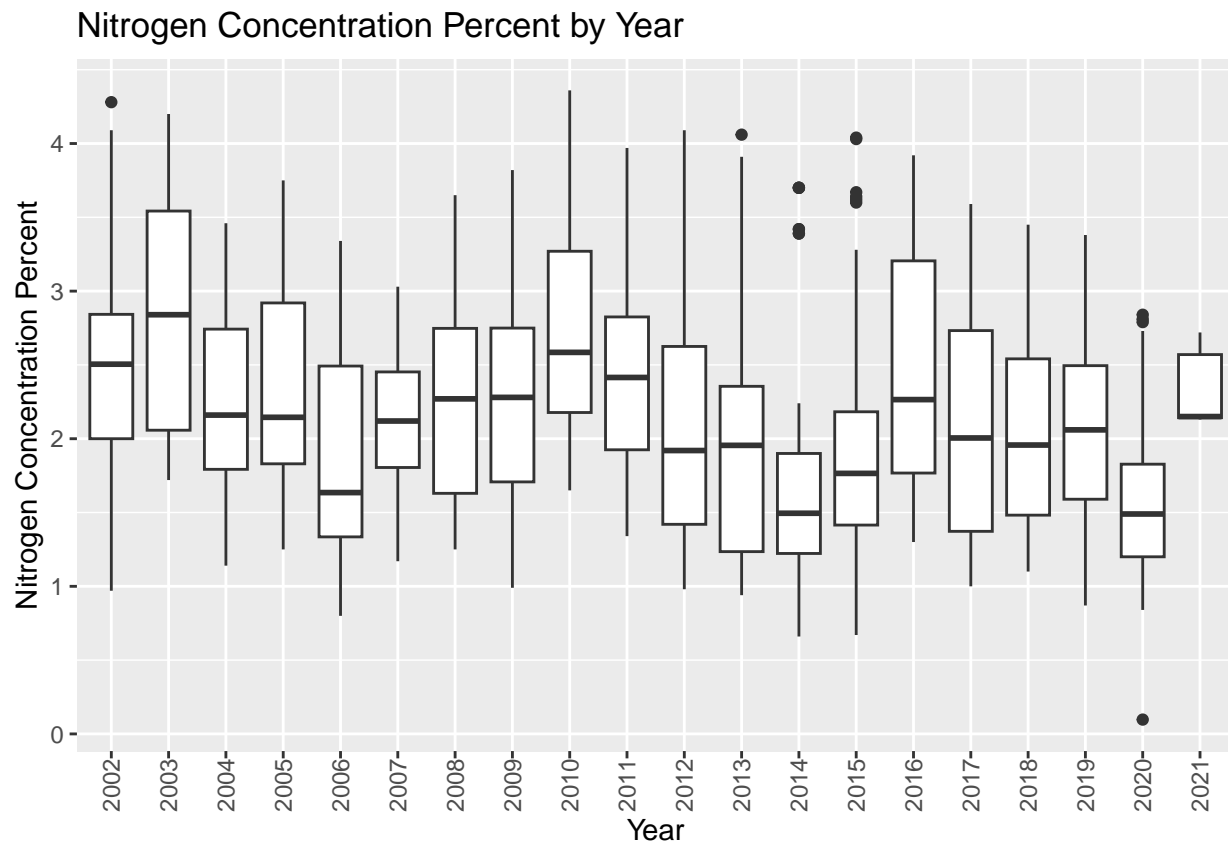


Figure 2: Nitrogen Concentration over the Years

Below are the normal distribution residuals charts for the linear regression of nitrogen concentration by date.

```

par(mfrow = c(2,2), mar=c(2,2,2,2))
plot(NitrogenRegressionDate)

```

```

par(mfrow = c(1,1))

```

A time series analysis was also completed to see how nitrogen concentration changes over time. The following plot displays the decomposed nitrogen concentration components of the time series run. As you can see, there is seasonality, which confirms our rejecting of the null hypothesis.

```

#6. Season Plot #average the months
Nitrogen.monthly.preliminary <- NitrogenYears %>%
  mutate(CleanDate = my(paste0(Month,"-",Year)))

```

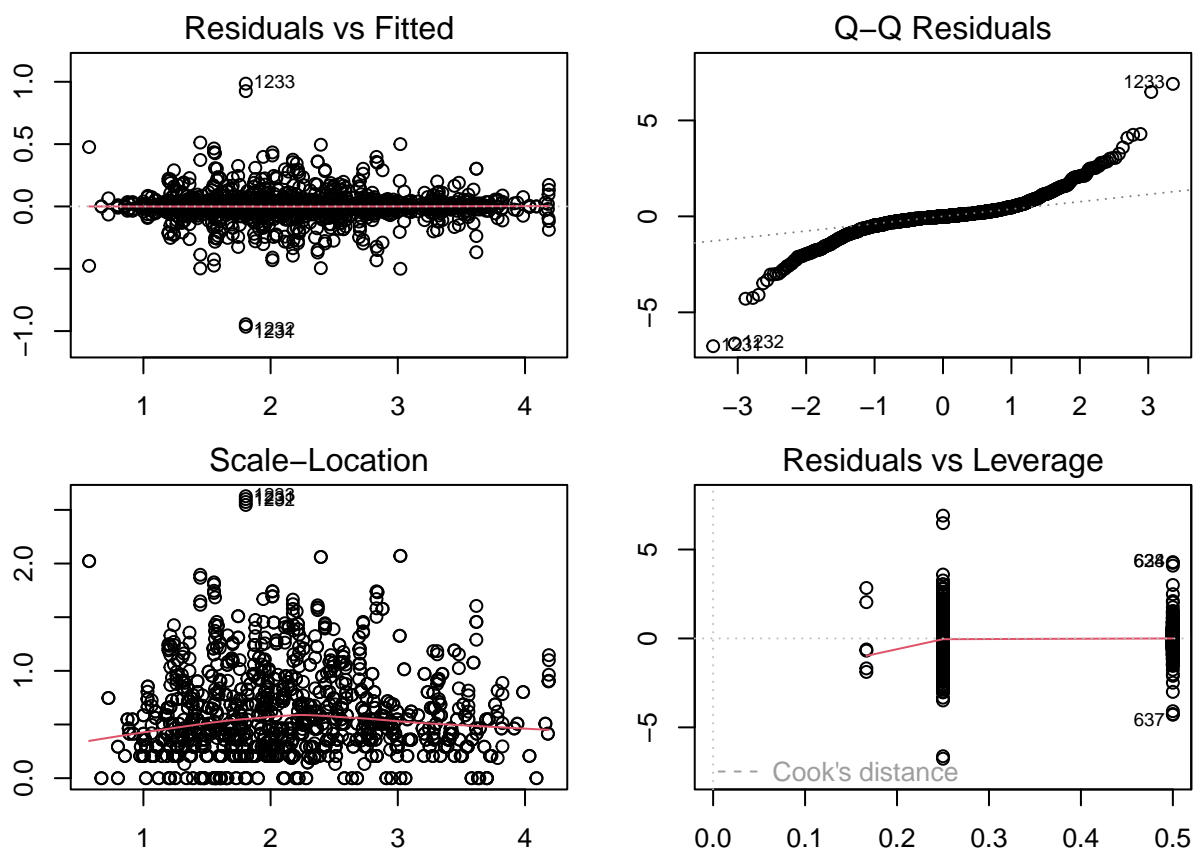


Figure 3: Nitrogen Concentration Residual Plots by Date

```

Nitrogen.monthly <- aggregate(Nitrogen.monthly.preliminary$Nitrogen_Percent,
                             by=list(Nitrogen.monthly.preliminary$CleanDate),
                             FUN=mean)

colnames(Nitrogen.monthly) <- c("Date", "Mean_NitrogenPercent")

#7. Generate time series monthly
f_monthmonthly <- month(first(Nitrogen.monthly$Date))
f_yearmonthly <- year(first(Nitrogen.monthly$Date))

Nitrogen.monthly.ts <- ts(Nitrogen.monthly$Mean_NitrogenPercent,
                         start=c(f_yearmonthly,f_monthmonthly),
                         frequency=12)

#Decompose monthly
Nitrogen.monthly.ts.decomp <- stl(Nitrogen.monthly.ts,s.window = "periodic")
plot(Nitrogen.monthly.ts.decomp)

```

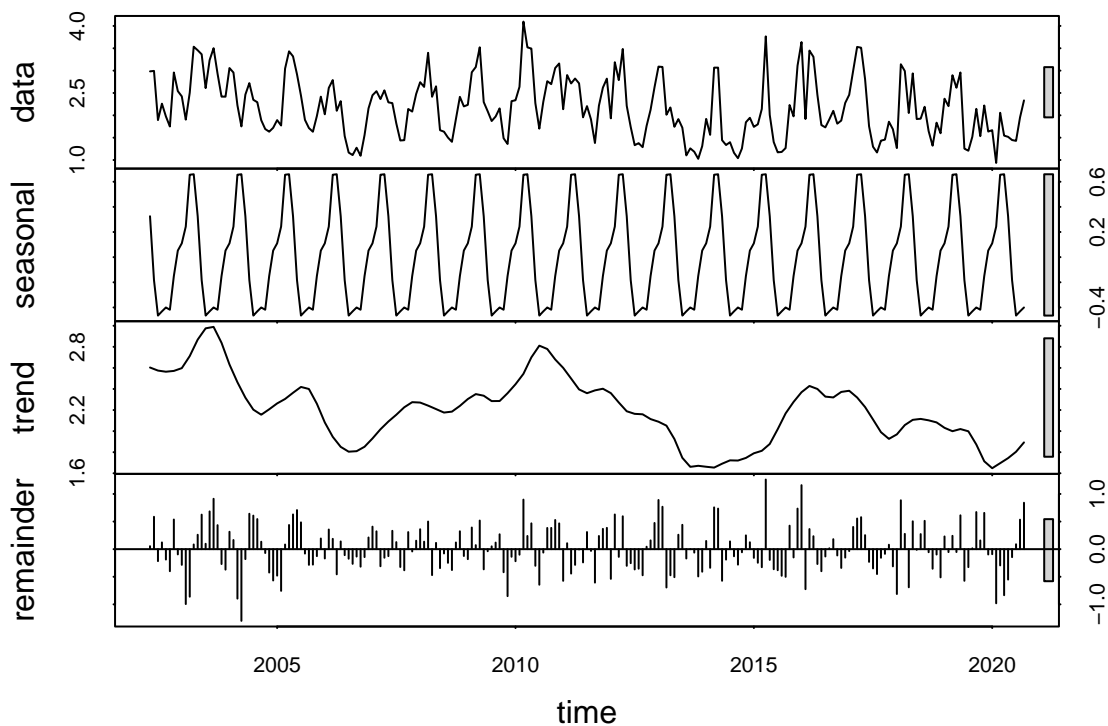


Figure 4: Nitrogen Concentration Residual Plots

The Mann-Kendall analysis performed on the nitrogen concentration non-seasonal time series produced a p-value of less than 0.05 ($<.001$, tau value of -0.19). As a result, the data displays a significant, decreasing trend for nitrogen concentration. The plot below displays the non-seasonal nitrogen concentration amount in the time series which was produced by removing the seasonal component from the time series.

```
#12 Run SMK test monthly
Nitrogen_Monthly_SMK <- Kendall::SeasonalMannKendall(Nitrogen.monthly.ts)
```

```
Nitrogen_Monthly_SMK
```

```
## tau = -0.19, 2-sided pvalue =0.00011555
```

```
summary(Nitrogen_Monthly_SMK)
```

```
## Score = -365 , Var(Score) = 8963
## denominator = 1925.499
## tau = -0.19, 2-sided pvalue =0.00011555
```

```
# 13 Visualization
```

```
Nitrogen_Plot <-
ggplot(Nitrogen.monthly, aes(x = Date, y = Mean_NitrogenPercent)) +
  geom_point() +
  geom_line() +
  labs("Mean Nitrogen (percent)", title="Nitrogen Concentration Percent over Time") +
  geom_smooth( method = lm )

print(Nitrogen_Plot)
```

```
#taking out seasonal
```

```
NitrogenComponents <- as.data.frame(Nitrogen.monthly.ts.decomp$time.series[,1:3])
```

```
Nitrogen_NoSeasonal <-NitrogenComponents$trend+NitrogenComponents$remainder
```

```
#16 Run MK test monthly
```

```
Nitrogen_Monthly_MK <- Kendall::MannKendall(Nitrogen_NoSeasonal)
```

```
Nitrogen_Monthly_MK
```

```
## tau = -0.188, 2-sided pvalue =3.0712e-05
```

```
summary(Nitrogen_Monthly_MK)
```

```
## Score = -4581 , Var(Score) = 1207396
## denominator = 24309.5
## tau = -0.188, 2-sided pvalue =3.0712e-05
```

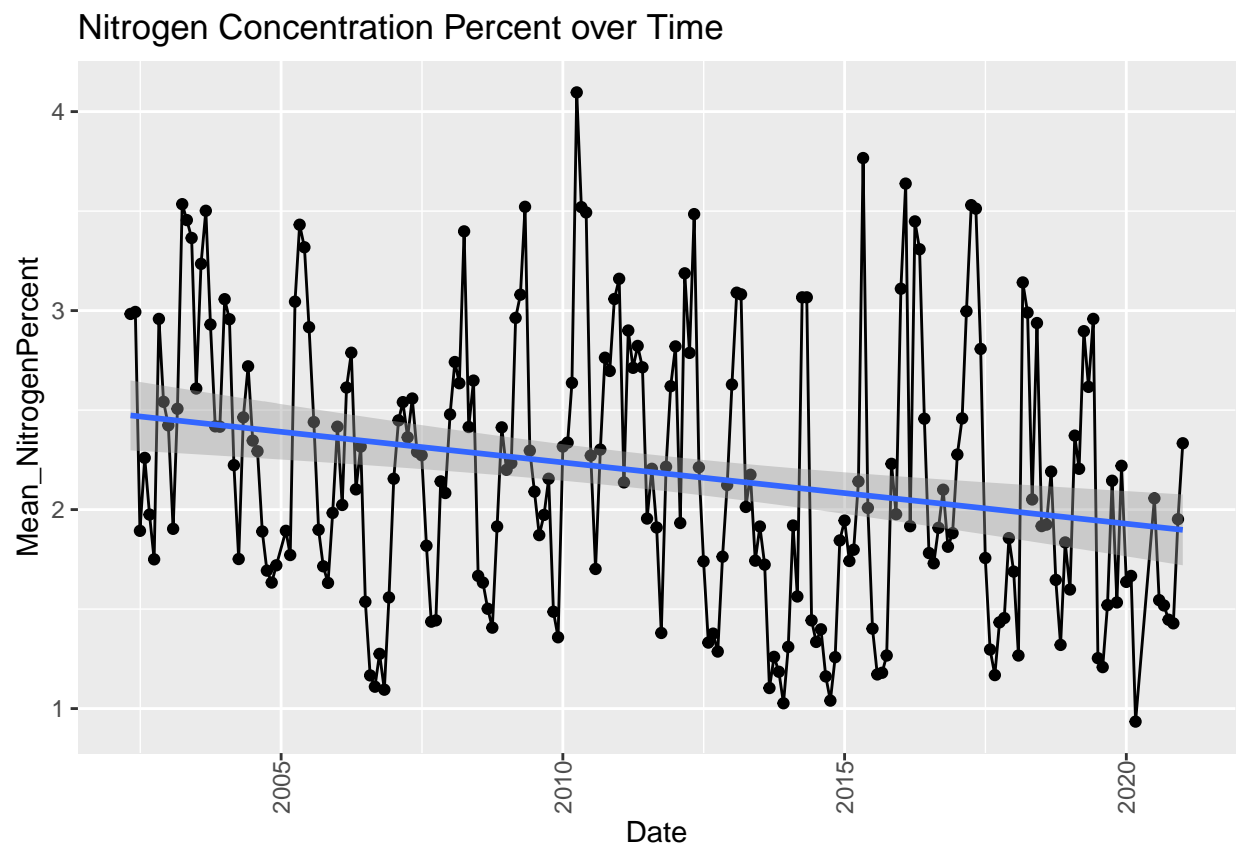


Figure 5: Time Series Analysis for Nitrogen Concentration

Question 2: Does giant kelp nitrogen concentration amount vary by season?

For the second question, we wanted to know the relationship between nitrogen concentration and season. Winter is defined as beginning of December through February, Spring is beginning of March through May, Summer is June through August, and Fall is September through November.

H0 : There is no significant difference of nitrogen concentration by season. HA : There is significant difference of nitrogen concentration by season.

The interaction between nitrogen concentration and season is significant because the p-value is $< .05$ ($p < 2.2e-16$). We can reject the null hypothesis and accept that there is significant difference of nitrogen concentration by season. The box plot below shows the change in nitrogen concentration over seasons.

```
##
## Call:
## lm(formula = NitrogenYears$Nitrogen_Percent ~ NitrogenYears$SeasonNames)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -2.64975 -0.49742 -0.05865  0.43835  2.19235
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      2.18584    0.03784  57.764  <2e-16 ***
## NitrogenYears$SeasonNamesSpring  0.56091    0.05430  10.330  <2e-16 ***
## NitrogenYears$SeasonNamesSummer -0.09819    0.05335  -1.840   0.0659 .
## NitrogenYears$SeasonNamesFall   -0.46618    0.05319  -8.765  <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.679 on 1278 degrees of freedom
## Multiple R-squared:  0.2242, Adjusted R-squared:  0.2224
## F-statistic: 123.1 on 3 and 1278 DF,  p-value: < 2.2e-16
```

Below are the normal distribution residuals charts for the linear regression of nitrogen concentration

```
#3. Normal Distribution
```

```
par(mfrow = c(2,2), mar=c(2,2,2,2))
plot(NitrogenRegressionSeason)
```

```
par(mfrow = c(1,1))
```

```
NitrogenSeasonPlot <- ggplot(NitrogenYears, aes(x=SeasonNames, y=Nitrogen_Percent))+
  geom_boxplot()+
  labs(title="Nitrogen Concentration Percent by Season", x="Season", y="Nitrogen Concentration Percent")
print(NitrogenSeasonPlot)
```

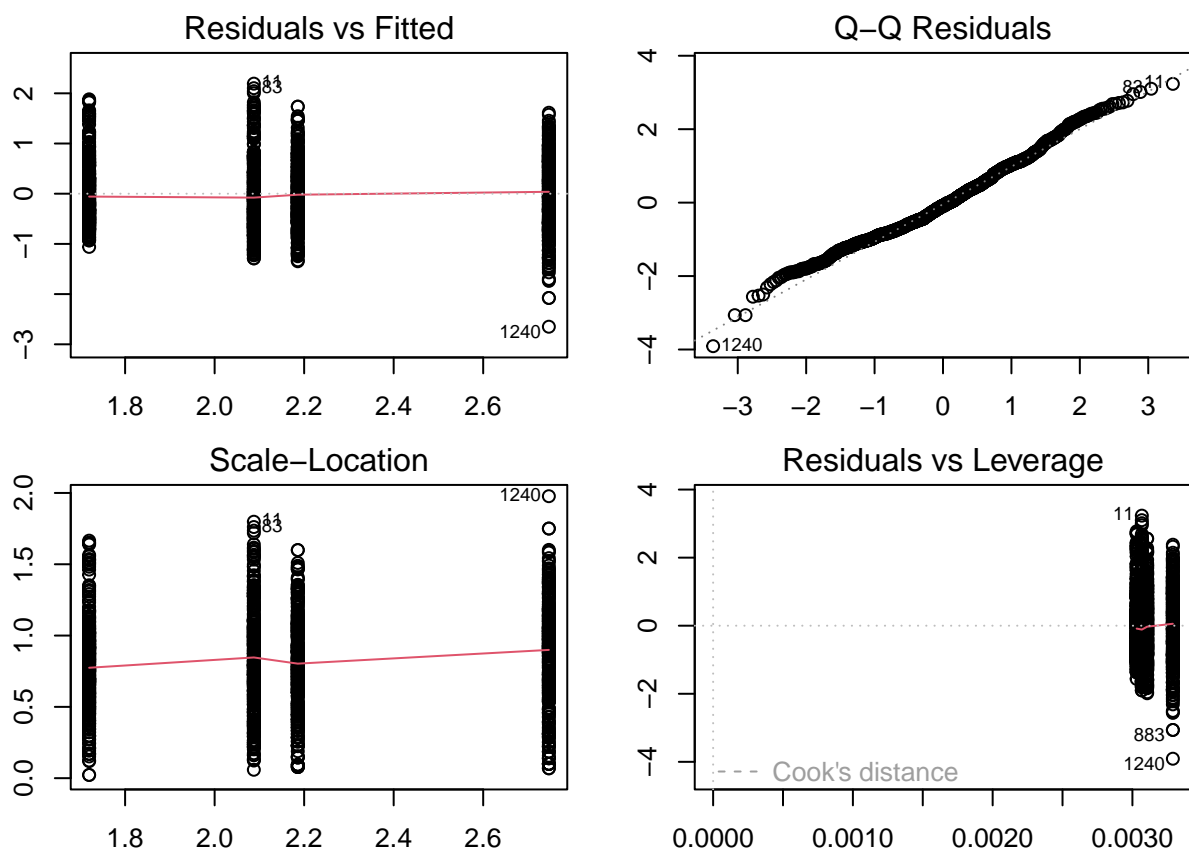


Figure 6: Nitrogen Concentration Residuals Plots for Season

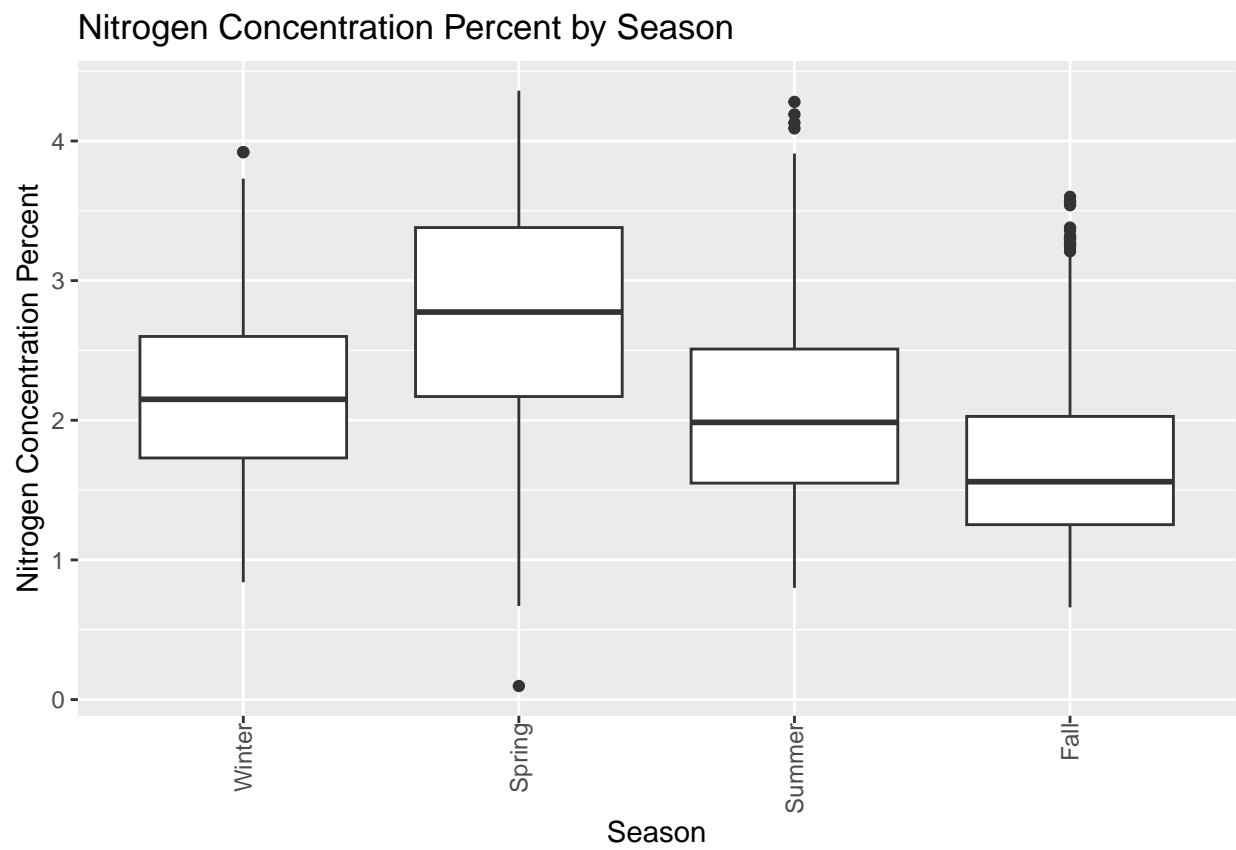


Figure 7: Nitrogen Concentration over Seasons

Question 3: Does nitrogen surge uptake vary between the spring and summer seasons?

The last three questions focus on the experiment completed over five days in the spring and summer of 2019. Nitrogen was blasted for 45 minutes and data on nitrogen and biomass uptake was recorded.

The metadata indicates that the giant kelp blades studied were collected during the nitrate replete spring and the nitrate-deplete summer. The five replicate experiments were conducted in both spring and summer of 2019, which allows us to find potential differences in the nitrogen surge uptake of the two populations. The experimenters calculate uptake rate using a modified version of an equation by Legendre and Grosselin (1997) $V = (nt - n0) / [t(ds - d0)]$ where nt is the atom percentage of Nitrogen-15 in the blade after incubation; $n0$ is the average concentration of isotope in the control blades; ds is the atom percentage of Nitrogen-15 in the seawater at the start of the incubation; $d0$ is the atom percentage of Nitrogen-15 in the nitrate pool before isotope tracer was added; and t is the length of the incubation time in minutes.

H0 : There is no significant difference of nitrogen surge uptake between spring and summer seasons. HA : There is significant difference of nitrogen surge uptake between spring and summer seasons.

The interaction between nitrogen uptake and season is significant because the p-value is $< .05$ ($p < .0216$). We can reject the null hypothesis and accept that there is significant difference of nitrogen surge uptake between spring and summer seasons.. The R-squared is .014, so a low amount of nitrogen uptake variance is in response to data being collected in the spring or summer. The box plot below shows the change in nitrogen concentration over seasons.

```
NitrogenSeasonBox <-  
  ggplot(SurgeUptakeNitrogen, aes(x = Season, y = Uptake_Rate)) +  
  geom_boxplot() +  
  labs(title="Nitrogen Uptake Rate by Season", x="Season", y="Nitrogen Uptake Rate (V(hr1))")  
  
print(NitrogenSeasonBox)
```

```
#ggsave(file=here('Output', 'Analysis', 'NitrogenBoxSeason.png'), width = 10, height = 10, units = "#cm")
```

The scatter plot below shows the change in nitrogen concentration over seasons by testing day replicate.

```
NitrogenSeasonPoint <-  
  ggplot(SurgeUptakeNitrogen, aes(x = Day_Replicate, y = Uptake_Rate, color=Season)) +  
  geom_point(alpha=.5) +  
  labs(title="Nitrogen Uptake Rate by Day and Season", x="Days", y="Nitrogen Uptake Rate (V(hr1))")  
  
print(NitrogenSeasonPoint)
```

```
#ggsave(file=here('Output', 'Analysis', 'NitrogenPointSeason.png'), width = 10, height = 10, units = "#cm")
```

```
##  
## Call:  
## lm(formula = SurgeUptakeNitrogen$Uptake_Rate ~ SurgeUptakeNitrogen$Season)  
##  
## Residuals:  
##      Min      1Q   Median      3Q      Max   
## -0.007419 -0.003936 -0.001798  0.001434  0.048773
```

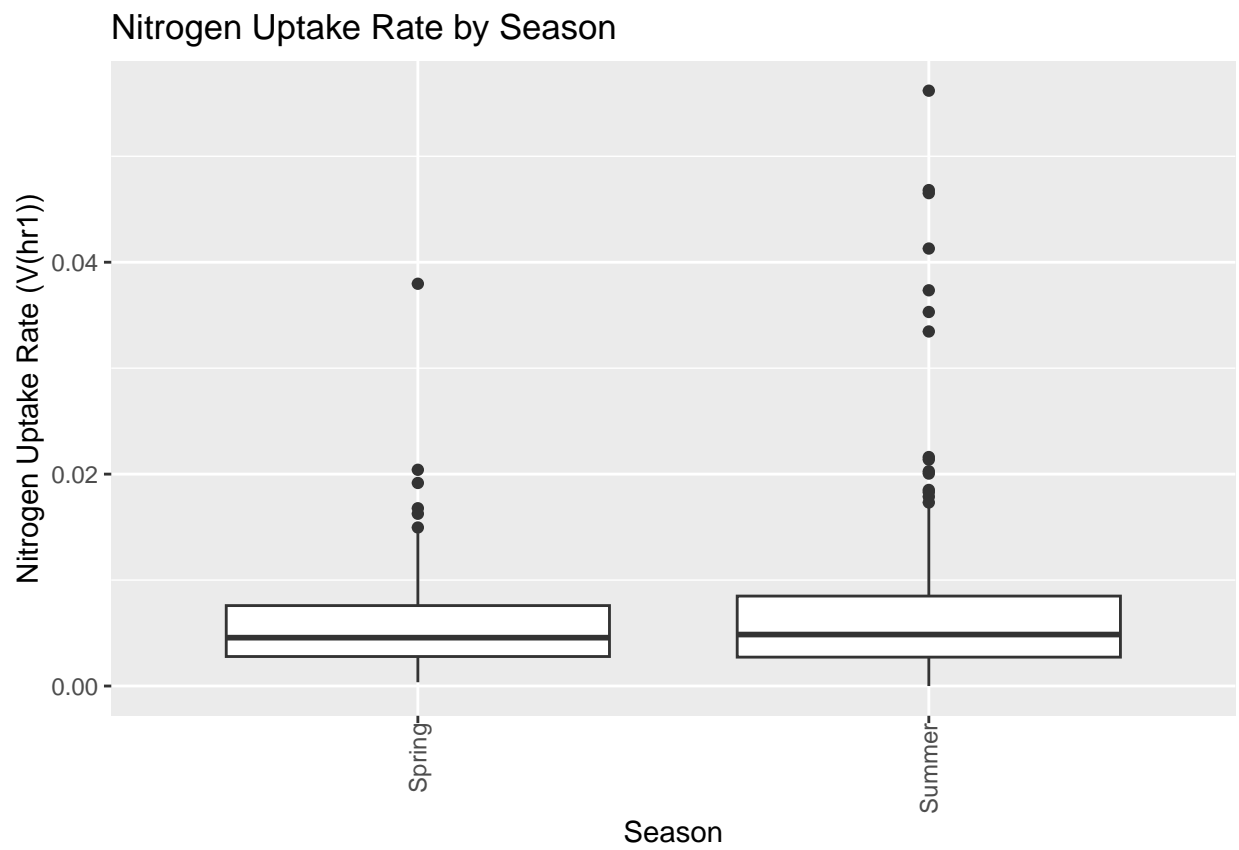


Figure 8: Nitrogen Uptake Rate by Season Box Plot

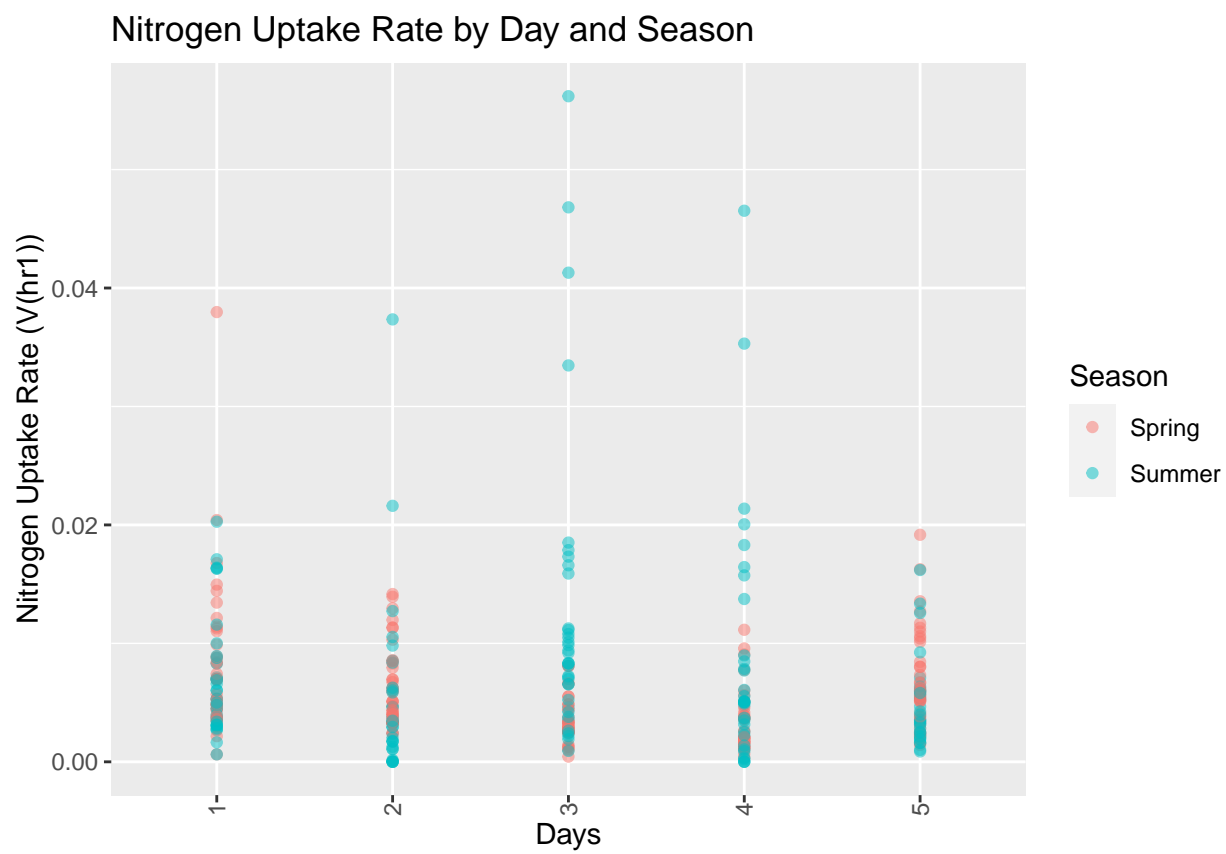


Figure 9: Nitrogen Uptake by Season Scatter Plot

```
##
## Coefficients:
##               Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.0057806  0.0005195  11.127  <2e-16 ***
## SurgeUptakeNitrogen$SeasonSummer 0.0016387  0.0007336   2.234   0.0261 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.00695 on 357 degrees of freedom
## Multiple R-squared:  0.01378,    Adjusted R-squared:  0.01102
## F-statistic: 4.989 on 1 and 357 DF,  p-value: 0.02612
```

Question 4: Does giant kelp nitrogen surge uptake vary based on the amount of exposure?

H0 : There is no significant difference of nitrogen surge uptake between different exposure lengths. HA : There is significant difference of nitrogen surge uptake between different exposure lengths.

#can only put one plot at a time because of figure titles

newpage ## Question 5: Does uptake vary by type of nitrogen (ammonium, nitrate, and urea)? H0 : There is no significant difference of nitrogen surge uptake of different nitrogen types. HA : There is significant difference of nitrogen surge uptake between different nitrogen types.

The interaction between nitrogen uptake and season is significant because the p-value is $< .05$ ($p < 2.2e-16$). We can reject the null hypothesis and accept that there is significant difference of nitrogen surge uptake between spring and summer seasons.. The R-squared is .192, so a moderate amount of nitrogen uptake variance is in response to the nitrogen type being blasted. The box plot below shows the change in nitrogen uptake rate by nitrogen type.

Creating a ggplot to compare uptake of various types of nitrogen

```
NitrogenUptaketype <-  
  ggplot(SurgeUptakeNitrogen, aes(x = Nitrogen_Types, y = Uptake_Rate)) +  
  geom_boxplot() +  
  labs(title="Uptake Rate by Nitrogen Type", x="Nitrogen Types", y="Nitrogen Uptake Rate (V(hr1))")  
print(NitrogenUptaketype)
```

```
ggsave(file=here('Output', 'Analysis', 'NitrogenUptakeBoxtype.png'), width = 10, height = 10, units = "cm")  
# Creating a ggplot to compare biomass uptake of various types of nitrogen
```

The jitter plot below shows the change in biomass uptake rate by nitrogen type with day replicate added.

Creating a ggplot to compare how nitrogen uptake varies per day per nitrogen type

```
NitrogenDayUptake <-  
  ggplot(SurgeUptakeNitrogen, aes(x = Nitrogen_Types, y = Uptake_Rate, color = Day_Replicate)) +  
  geom_jitter(alpha = 0.2) +  
  scale_color_manual(values = c("#FF0000", "#BF003F", "#7F007F", "#3F00BF", "#0000FF")) +  
  labs(title="Nitrogen Uptake per Day for Each Nitrogen Type",  
       x="Nitrogen Types",  
       y="Nitrogen Uptake Rate (V(hr1))", color = "Day")  
print(NitrogenDayUptake)
```

```
ggsave(file=here('Output', 'Analysis', 'NitrogenUptakeJitterType.png'), width = 10, height = 10, units = "cm")
```

Generalized Linear Model (GLM)

```
NitorgenLM <-lm(data = SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types)  
summary(NitorgenLM)
```

##

Call:

lm(formula = Uptake_Rate ~ Nitrogen_Types, data = SurgeUptakeNitrogen)

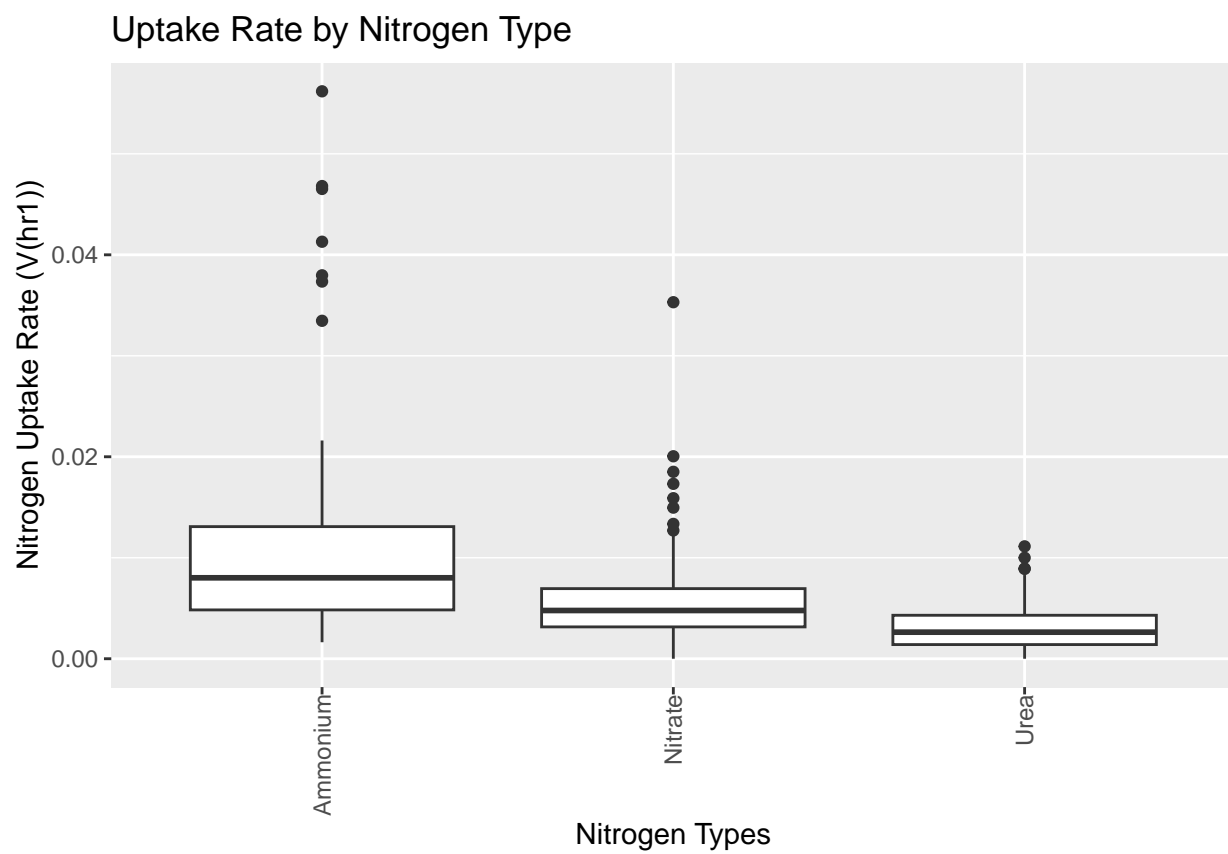


Figure 10: Nitrogen Uptake by Type Box Plot

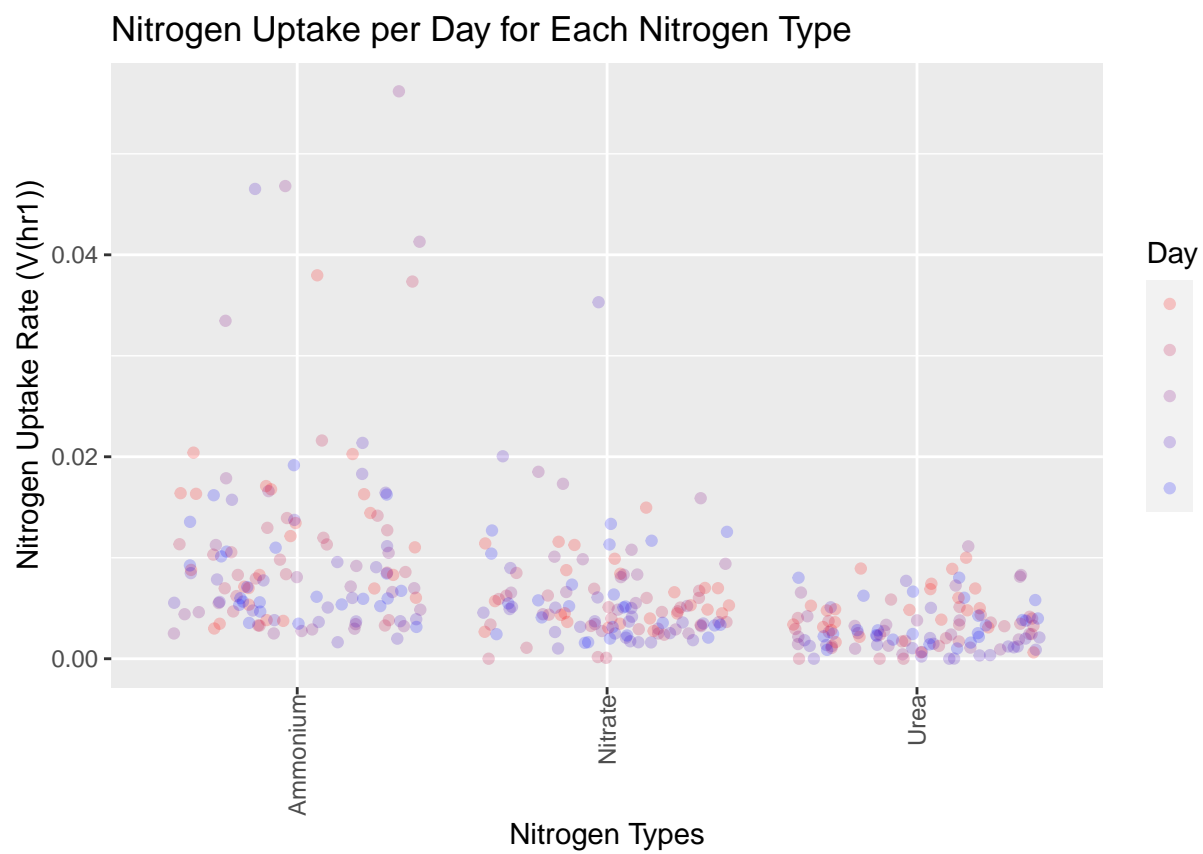


Figure 11: Nitrogen Uptake by Type Jigger Plot

```
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.009010 -0.002975 -0.001082  0.001395  0.045547
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)      0.0106449  0.0005750  18.512 < 2e-16 ***
## Nitrogen_TypesNitrate -0.0047592  0.0008132  -5.852 1.1e-08 ***
## Nitrogen_TypesUrea   -0.0073967  0.0008149  -9.077 < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.006299 on 356 degrees of freedom
## Multiple R-squared:  0.1922, Adjusted R-squared:  0.1877
## F-statistic: 42.36 on 2 and 356 DF,  p-value: < 2.2e-16
```

```
# One-way ANOVA
#NitrogenAOV <- aov(data = SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types)
#summary(NitrogenAOV)
#TukeyHSD(NitrogenAOV)

# AIC-based Model Selection:

NitrogenTypeAIC <- lm(data=SurgeUptakeNitrogen, Uptake_Rate~Nitrogen_Types+Season+Time)
step(NitrogenTypeAIC)
```

```
## Start: AIC=-3667.7
## Uptake_Rate ~ Nitrogen_Types + Season + Time
##
##              Df Sum of Sq      RSS      AIC
## <none>                0.012625 -3667.7
## - Season              1 0.0002483 0.012873 -3662.7
## - Time                 3 0.0012540 0.013879 -3639.7
## - Nitrogen_Types      2 0.0033740 0.015998 -3586.7

##
## Call:
## lm(formula = Uptake_Rate ~ Nitrogen_Types + Season + Time, data = SurgeUptakeNitrogen)
##
## Coefficients:
##      (Intercept) Nitrogen_TypesNitrate Nitrogen_TypesUrea
##           0.012720          -0.004759          -0.007413
##      SeasonSummer              Time5              Time15
##           0.001663          -0.002679          -0.003956
##           Time45
##          -0.004991
```

```
summary(NitrogenTypeAIC)
```

```
##
## Call:
```



```
## lm(formula = Uptake_Rate ~ Nitrogen_Types + Season + Time, data = SurgeUptakeNitrogen)
##
## Residuals:
##      Min       1Q   Median       3Q      Max
## -0.009974 -0.003295 -0.000467  0.001954  0.044488
##
## Coefficients:
##              Estimate Std. Error t value Pr(>|t|)
## (Intercept)    0.0127198  0.0008351  15.232 < 2e-16 ***
## Nitrogen_TypesNitrate -0.0047592  0.0007731  -6.156 2.04e-09 ***
## Nitrogen_TypesUrea    -0.0074125  0.0007748  -9.567 < 2e-16 ***
## SeasonSummer      0.0016633  0.0006322   2.631  0.00888 **
## Time5             -0.0026791  0.0008927  -3.001  0.00288 **
## Time15            -0.0039562  0.0008953  -4.419 1.32e-05 ***
## Time45            -0.0049909  0.0008927  -5.590 4.55e-08 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 0.005989 on 352 degrees of freedom
## Multiple R-squared:  0.278, Adjusted R-squared:  0.2657
## F-statistic: 22.59 on 6 and 352 DF, p-value: < 2.2e-16

# For biomass
#BiomassTypeAIC <- lm(data=SurgeUptakeNitrogen, Biomass_Uptake~Nitrogen_Types+Season+Time)
#step(NitrogenTypeAIC)
#summary(NitrogenTypeAIC)
```

Summary and Conclusions

Akaike information criterion (AIC) was performed for the final three questions to see if those three variables all contributed to the variation of nitrogen uptake. All three variables (season, nitrogen exposure lengths, and nitrogen type), contributed to the variance of nitrogen uptake rate.

What does these pvalues about the first data set tell us about the intro

What does the second set tell us about the intro as well as the data set (aka, more testing needs to be done, was a short experiment) Table 2: Significance Values

| Research Question | P-Value |
|-------------------|-----------|
| Question 1 | < 2.2e-16 |
| Question 2 | < 1.16e-4 |
| Question 3 | .0216 |
| Question 4 | |
| Question 5 | < 2.2e-16 |

References

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- Fernández, P.A., Gaitán-Espitia, J.D., Leal, P.P. et al. (2020). Nitrogen sufficiency enhances thermal tolerance in habitat-forming kelp: implications for acclimation under thermal stress. *Scientific Reports* 10, 3186.<https://doi.org/10.1038/s41598-020-60104-4>
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