

# Effects of Soil Warming Plus Nitrogen Addition on Plant Richness and Diversity at Harvard Forest

*Roy Moger-Reischer and Katie Beidler, Quantitative Biodiversity, Indiana University*

*09 March, 2017*

## PROJECT OVERVIEW

### ABSTRACT

Contemporary anthropogenically induced ecosystem-level changes can alter nitrogen (N) and carbon (C) cycles.

### 1) INTRODUCTION

Two of the most well-documented outcomes of anthropogenic global change are nitrogen deposition and climate change.

**Research Question:** What are the short-term effects of soil warming plus N additions on plant richness and diversity at Harvard Forest?

**Hypothesis 1:** Richness and diversity will decrease over time in warming and N treatments.

**Hypothesis 2:** Soil respiration will be higher in warmed plots, lower in N-amended plots.

### 2) SETUP

Retrieve and Set Working Directory

### 3) DESCRIPTION OF DATA

The overall objective of this project is to investigate whether soil warming and N fertilization alter plant biodiversity through time (2006-2009). In 2006 a group at the Harvard Forest LTER led by Serita Frey, initiated an experiment to assess the interactive effects of these two environmental changes on plant, arthropod, and soil microbe community composition, and soil respiration (Frey 2009). Six replicate plots for each of four treatments (Control; N deposition; heated; N & heated interaction) were established. N deposition plots received 50 kg N/ha/yr. Heated plots were warmed to 5 degrees C above ambient temperature. The data for this project came from the Harvard Forest LTER site. [link](#)

### B.Install Packages

This analysis will require several packages. The `require()` function in R returns TRUE if the package was successfully loaded or FALSE if the package failed to load. This for loop loads each package and installs the package when `require()` returns FALSE.

```
package.list = c('vegan', 'tidyr', 'dplyr', 'codyn', 'ggplot2', 'betapart', 'splitstackshape', 'cowplot',  
  
for (package in package.list) {  
  if (!require(package, character.only = TRUE, quietly = TRUE)) {  
    install.packages(package, repos='http://cran.us.r-project.org')  
    library(package, character.only = TRUE) }  
}
```

## 4) LOADING DATA

### A. Description of Data Set

1. 'plant': This datasheet is the plot by species abundance (Number of stems) matrix grouped by treatment and year.
2. 'resp': This datasheet includes field season measurements of soil respiration (CO<sub>2</sub> flux (mg C m<sup>-2</sup> h<sup>-1</sup>)))

```
#plant = read.csv("./HF_plants_treat.csv")
#resp = read.csv("./HF_soilresp.csv", stringsAsFactors = FALSE)

plant=read.csv("C:\\Users\\rmoge\\GitHub\\QB2017_DivPro\\Data\\HF_plants_treat.csv")
resp<-read.csv("C:\\Users\\rmoge\\GitHub\\QB2017_DivPro\\Data\\HF_soilresp.csv")
```

### B. Data Wrangling

Subsetting the data by year and producing site by species matrices.

```
# Making the Site by Species Matrix for the plant data set
plant_sbys = plant[,6:43]

# Subsetting the data into the different years
plant_06 = (filter(plant, year == 2006))
plant_07 = (filter(plant, year == 2007))
plant_08 = (filter(plant, year == 2008))
plant_09 = (filter(plant, year == 2009))

# Separating out the treatments from the site by species matrices
plant_06_sbys = plant_06[,6:43]
plant_07_sbys = plant_07[,6:43]
plant_08_sbys = plant_08[,6:43]
plant_09_sbys = plant_09[,6:43]
```

## 5) ANALYSIS: FIGURES AND STATISTICS

```
# Visualizing the Plant data set
str(plant, max.level = 1)

## 'data.frame':   96 obs. of  45 variables:
## $ year      : int  2006 2006 2006 2006 2006 2006 2007 2007 2007 2007 ...
## $ plot      : int   1 12 14 19 20 24 1 12 14 19 ...
## $ treatment : int   1 1 1 1 1 1 1 1 1 1 ...
## $ N_treat   : Factor w/ 2 levels "control","N fertilized": 1 1 1 1 1 1 1 1 1 1 ...
## $ heat_treat: Factor w/ 2 levels "control","heated": 1 1 1 1 1 1 1 1 1 1 ...
## $ acersp    : int   0 1 0 0 0 0 0 0 0 0 ...
## $ acepen    : int   2 4 13 17 8 18 3 5 13 12 ...
## $ acerub    : int   3 2 6 0 1 1 5 5 7 2 ...
## $ aranud    : int  14 0 16 19 9 7 11 0 15 28 ...
## $ aritri    : int   0 0 0 0 0 0 0 0 0 0 ...
## $ betale    : int   0 0 1 0 0 0 1 0 2 0 ...
## $ betlen    : int   1 0 0 0 0 0 0 0 0 0 ...
## $ betsp     : int   0 0 0 0 0 0 1 0 0 0 ...
## $ carpen    : int   2 0 2 139 1 0 32 0 1 65 ...
```

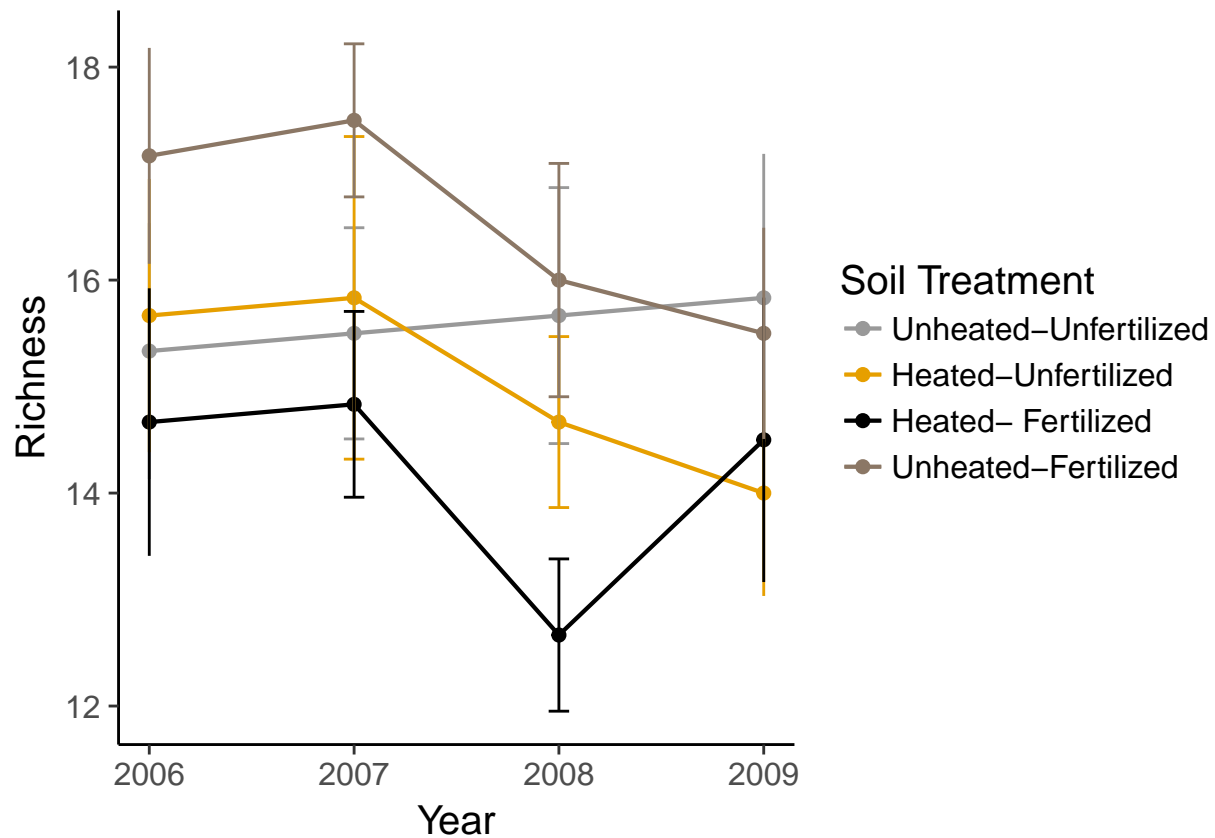
```
## $ casden      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ clibor      : int  0 8 0 0 0 0 0 13 0 0 ...
## $ coptri      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ craspp      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ denobs      : int  0 7 50 28 127 7 0 8 0 31 ...
## $ denpun      : int  0 814 9 324 2 0 3 667 11 275 ...
## $ dipcom      : int  0 0 0 0 16 0 0 0 26 0 ...
## $ dryspp      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ faggra      : int  1 0 0 2 0 3 1 0 0 2 ...
## $ gaupro      : int  117 43 124 275 95 15 87 50 80 269 ...
## $ goopub      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ hupluc      : int  0 21 0 0 152 0 0 5 0 0 ...
## $ lyolig      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ maican      : int  170 334 3 233 27 70 117 236 0 147 ...
## $ medvir      : int  3 0 1 3 1 0 0 0 5 22 ...
## $ mitrep      : int  50 467 142 0 0 0 44 570 185 0 ...
## $ monuni      : int  0 0 4 0 0 0 0 0 1 0 ...
## $ pinstr      : int  0 2 1 0 0 0 0 3 1 0 ...
## $ pruser      : int  0 0 3 1 2 0 2 0 6 2 ...
## $ quealb      : int  0 0 0 0 0 1 0 0 0 0 ...
## $ querub      : int  1 5 0 0 1 0 1 1 0 1 ...
## $ smirac      : int  0 0 0 0 0 0 0 0 2 1 ...
## $ snag        : int  0 0 0 0 0 0 0 0 0 0 ...
## $ tribor      : int  14 1 11 30 36 27 5 1 9 22 ...
## $ tsucan      : int  0 0 0 0 0 0 0 0 0 0 ...
## $ unksp      : int  0 0 0 0 1 0 0 1 0 0 ...
## $ uvuses      : int  27 19 35 24 24 9 31 38 35 32 ...
## $ vaccsp      : int  56 0 48 14 145 31 47 0 46 13 ...
## $ vibace      : int  0 0 0 4 3 18 0 0 0 4 ...
## $ vibden      : int  0 0 5 0 0 0 0 0 3 0 ...
## $ viblen      : int  0 0 57 0 5 0 0 0 55 0 ...
```

```
# Calculate observed richness from time-by-species matrix
p_richness = as.data.frame(rowSums(plant[, -c(1:5)] > 0))
# Create data frame with experimental design and richness data
p_rich.all = data.frame(plant[, 1:5, ], p_richness)
# Rename column
names(p_rich.all)[6] = "richness"

# avg. richness per group
# stand. dev. per group
# num. obs. per group
# calc. std. err. mean.
p_rich.treat.plot = group_by(p_rich.all, treatment, year) %>%
  summarise(mean = mean(richness), sd = sd(richness), n = n(), sem = sd/sqrt(n))

p_rich.plot = ggplot(p_rich.treat.plot, aes(x = year, y = mean, color = as.factor(treatment))) +
  geom_point(size = 2, show.legend = T) +
  geom_line(size = 0.75) +
  geom_errorbar(aes(ymin = mean - sem, ymax = mean + sem), width = .1) +
  scale_color_manual(values = c("#999999", "#E69F00", "#000000", "#8B7765"), name = "Soil Treatment") +
  xlim(2006, 2009) + xlab("Year") + ylab("Richness") +
  theme_classic(base_size = 15) +
  theme(axis.line.x = element_line(color = "black"), axis.line.y = element_line(color = "black"))
```

```
plot(p_rich.plot)
```

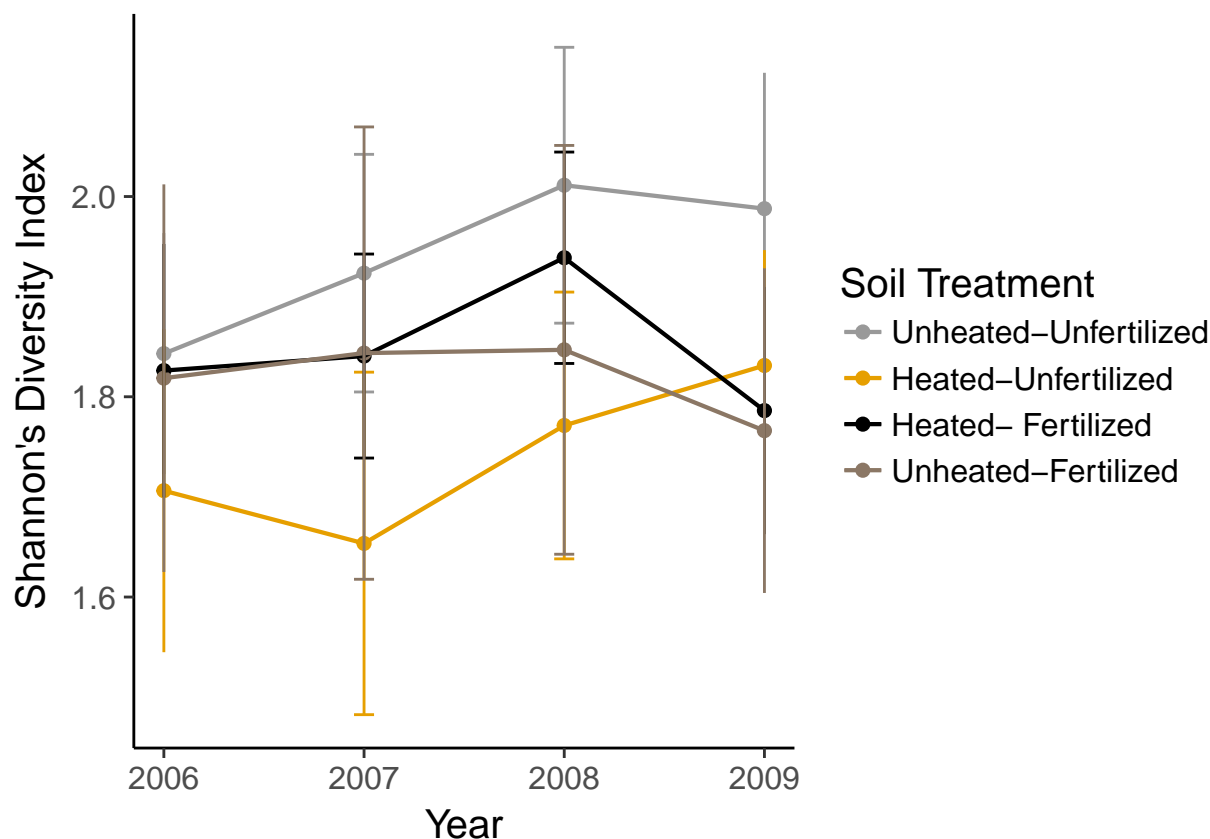


```
# Calculate diversity from time-by-species matrix
p_diversity = diversity(plant[, -c(1:5)], "shannon")

# Create data frame with experimental design and diversity data
p_div.all = data.frame(plant[, 1:5, ], p_diversity)
# Rename column
names(p_div.all)[6] = "diversity"

p_div.treat.plot = group_by(p_div.all, treatment, year) %>%
  summarise(mean = mean(diversity), sd = sd(diversity), n = n(), sem = sd/sqrt(n))

p_div.plot = ggplot(p_div.treat.plot, aes(x = year, y = mean, color = as.factor(treatment))) +
  geom_point(size = 2, show.legend = T) +
  geom_line(size = 0.75) +
  geom_errorbar(aes(ymin = mean - sem, ymax = mean + sem), width = .1) +
  scale_color_manual(values = c("#999999", "#E69F00", "#000000", "#8B7765"), name = "Soil Treatment") +
  xlim(2006, 2009) + xlab("Year") + ylab("Shannon's Diversity Index") +
  theme_classic(base_size = 15) +
  theme(axis.line.x = element_line(color = "black"), axis.line.y = element_line(color = "black"))
plot(p_div.plot)
```



### BIODIVERSITY HYPOTHESIS TESTING

```
p_rich.rm = lme(richness ~ year + heat_treat + N_treat * heat_treat * N_treat * year, random = ~ 1 | plot, data = p_rich.all)
summary(p_rich.rm)
```

## Linear mixed-effects model fit by REML

## Data: p\_rich.all

## AIC BIC logLik

## 418.1614 445.4121 -198.0807

##

## Random effects:

## Formula: ~1 | plot

## (Intercept) Residual

## StdDev: 2.253468 1.497991

##

## Correlation Structure: AR(1)

## Formula: ~1 | plot

## Parameter estimate(s):

## Phi

## -0.09584397

## Fixed effects: richness ~ year + heat\_treat + N\_treat \* heat\_treat \* N\_treat \* year

## Value Std.Error DF

## (Intercept) -319.0000 533.6371 68

## year 0.1667 0.2658 68

## heat\_treatheated 1589.7925 754.6768 20

## N\_treatN fertilized 1667.9090 754.6768 20

## heat\_treatheated:N\_treatN fertilized -2327.7884 1067.2742 20

```

## year:N_treatN fertilized          -0.8304    0.3759 68
## year:heat_treatheated             -0.7922    0.3759 68
## year:heat_treatheated:N_treatN fertilized    1.1586    0.5316 68
##                                t-value p-value
## (Intercept)                     -0.5977845  0.5520
## year                           0.6269876  0.5328
## heat_treatheated                2.1065871  0.0480
## N_treatN fertilized              2.2100970  0.0389
## heat_treatheated:N_treatN fertilized -2.1810593  0.0413
## year:N_treatN fertilized         -2.2088181  0.0306
## year:heat_treatheated            -2.1072957  0.0388
## year:heat_treatheated:N_treatN fertilized  2.1793094  0.0328
## Correlation:
##                                (Intr) year   ht_trt N_trNf
## year                          -1.000
## heat_treatheated              -0.707  0.707
## N_treatN fertilized            -0.707  0.707  0.500
## heat_treatheated:N_treatN fertilized  0.500 -0.500 -0.707 -0.707
## year:N_treatN fertilized        0.707 -0.707 -0.500 -1.000
## year:heat_treatheated           0.707 -0.707 -1.000 -0.500
## year:heat_treatheated:N_treatN fertilized -0.500  0.500  0.707  0.707
##                                h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized        0.707
## year:heat_treatheated           0.707  0.500
## year:heat_treatheated:N_treatN fertilized -1.000 -0.707 -0.707
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -2.5089111 -0.5625465  0.1048975  0.6703417  1.6948990
##
## Number of Observations: 96
## Number of Groups: 24

```

```
anova(p_rich.rm)
```

```

##                                numDF denDF   F-value p-value
## (Intercept)                   1      68 1014.4761 <.0001
## year                           1      68   7.1322  0.0095
## heat_treat                     1      20   2.3242  0.1430
## N_treat                        1      20   0.0011  0.9737
## heat_treat:N_treat             1      20   0.9445  0.3427
## year:N_treat                   1      68   0.8920  0.3483
## year:heat_treat                1      68   0.6414  0.4260
## year:heat_treat:N_treat        1      68   4.7494  0.0328

```

```
# Make cleaner ANOVA table
```

```

set.caption("RMANOVA for Plant Richness Data")
pander(anova(p_rich.rm))

```

Table 1: RMANOVA for Plant Richness Data

	numDF	denDF	F-value	p-value
(Intercept)	1	68	1014	0
year	1	68	7.132	0.009464
heat_treat	1	20	2.324	0.143
N_treat	1	20	0.001114	0.9737
heat_treat:N_treat	1	20	0.9445	0.3427
year:N_treat	1	68	0.892	0.3483
year:heat_treat	1	68	0.6414	0.426
year:heat_treat:N_treat	1	68	4.749	0.03278

*# Perform an RM-ANOVA and construct a F-test using the AR(1)*

```
p_div.rm = lme(diversity ~year + heat_treat + N_treat * heat_treat * N_treat * year, random = ~ 1 | plot,
summary(p_div.rm)
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: p_div.all
```

```
##      AIC      BIC    logLik
```

```
## 45.91417 73.16488 -11.95709
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | plot
```

```
##      (Intercept) Residual
```

```
## StdDev: 0.2416783 0.2710299
```

```
##
```

```
## Correlation Structure: AR(1)
```

```
## Formula: ~1 | plot
```

```
## Parameter estimate(s):
```

```
##      Phi
```

```
## 0.6311864
```

```
## Fixed effects: diversity ~ year + heat_treat + N_treat * heat_treat * N_treat *      year
```

```
##      Value Std.Error DF
```

```
## (Intercept) -96.42080 90.53669 68
```

```
## year 0.04899 0.04510 68
```

```
## heat_treatheated 11.63861 128.03821 20
```

```
## N_treatN fertilized 132.51317 128.03821 20
```

```
## heat_treatheated:N_treatN fertilized -23.36506 181.07338 20
```

```
## year:N_treatN fertilized -0.06607 0.06378 68
```

```
## year:heat_treatheated -0.00589 0.06378 68
```

```
## year:heat_treatheated:N_treatN fertilized 0.01174 0.09020 68
```

```
##      t-value p-value
```

```
## (Intercept) -1.0649915 0.2906
```

```
## year 1.0863041 0.2812
```

```
## heat_treatheated 0.0908995 0.9285
```

```
## N_treatN fertilized 1.0349501 0.3130
```

```
## heat_treatheated:N_treatN fertilized -0.1290364 0.8986
```

```
## year:N_treatN fertilized -1.0359107 0.3039
```

```
## year:heat_treatheated -0.0922735 0.9268
```

```
## year:heat_treatheated:N_treatN fertilized 0.1301299 0.8968
```

```
## Correlation:
```

```
##      (Intr) year ht_trt N_trNf
```

```
## year -1.000
```

```
## heat_treatheated -0.707 0.707
## N_treatN fertilized -0.707 0.707 0.500
## heat_treatheated:N_treatN fertilized 0.500 -0.500 -0.707 -0.707
## year:N_treatN fertilized 0.707 -0.707 -0.500 -1.000
## year:heat_treatheated 0.707 -0.707 -1.000 -0.500
## year:heat_treatheated:N_treatN fertilized -0.500 0.500 0.707 0.707
## h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized 0.707
## year:heat_treatheated 0.707 0.500
## year:heat_treatheated:N_treatN fertilized -1.000 -0.707 -0.707
##
## Standardized Within-Group Residuals:
## Min Q1 Med Q3 Max
## -2.0663335 -0.2975686 0.1382110 0.5149486 1.6528550
##
## Number of Observations: 96
## Number of Groups: 24
```

```
anova(p_div.rm)
```

```
## numDF denDF F-value p-value
## (Intercept) 1 68 786.2574 <.0001
## year 1 68 0.5002 0.4818
## heat_treat 1 20 0.3474 0.5622
## N_treat 1 20 0.0334 0.8568
## heat_treat:N_treat 1 20 0.5754 0.4569
## year:N_treat 1 68 1.7819 0.1864
## year:heat_treat 1 68 0.0000 0.9997
## year:heat_treat:N_treat 1 68 0.0169 0.8968
```

```
set.caption("RMANOVA for Plant Diversity Data")
pander(anova(p_div.rm))
```

Table 2: RMANOVA for Plant Diversity Data ### TEMPORAL  
BIODIVERSITY ### A. Turnover

	numDF	denDF	F-value	p-value
(Intercept)	1	68	786.3	0
year	1	68	0.5002	0.4818
heat_treat	1	20	0.3474	0.5622
N_treat	1	20	0.03342	0.8568
heat_treat:N_treat	1	20	0.5754	0.4569
year:N_treat	1	68	1.782	0.1864
year:heat_treat	1	68	1.329e-07	0.9997
year:heat_treat:N_treat	1	68	0.01693	0.8968

```
# Making the wide site by species format into a long format- to get abundances for each species
plant$row_id = 1:nrow(plant)
p.id = dplyr::select(plant, row_id, year, treatment)
plant.m = as.matrix(plant_sbys)
```



```

m1 = setNames(melt(plant.m), c('row_id','species','count'))
m2 = dplyr::filter(m1, count > 0)
m3 = expandRows(m2, "count")
plant_long = left_join(p.id,m3, by = "row_id")
p_long = dplyr::select(plant_long, -row_id)

# Calculate species abundances for each taxonomic group
plant.sp.abunds = p_long %>%
  group_by(year,treatment) %>%
  count(species)
write.csv(plant.sp.abunds, file = "plant abundances")
# Calculate total turnover
plant.total = turnover(df = plant.sp.abunds, time.var = "year",
  species.var = "species",
  abundance.var = "n",
  replicate.var = "treatment",
  metric = "total")

# Calculate species gained
plant.appearance = turnover(df = plant.sp.abunds, time.var = "year",
  species.var = "species",
  abundance.var = "n",
  replicate.var = "treatment",
  metric = "appearance")

# Calculate species lost
plant.disappearance = turnover(df = plant.sp.abunds, time.var = "year",
  species.var = "species",
  abundance.var = "n",
  replicate.var = "treatment",
  metric = "disappearance")

plant.turnover = full_join(plant.total, plant.disappearance) %>%
  full_join(plant.appearance)

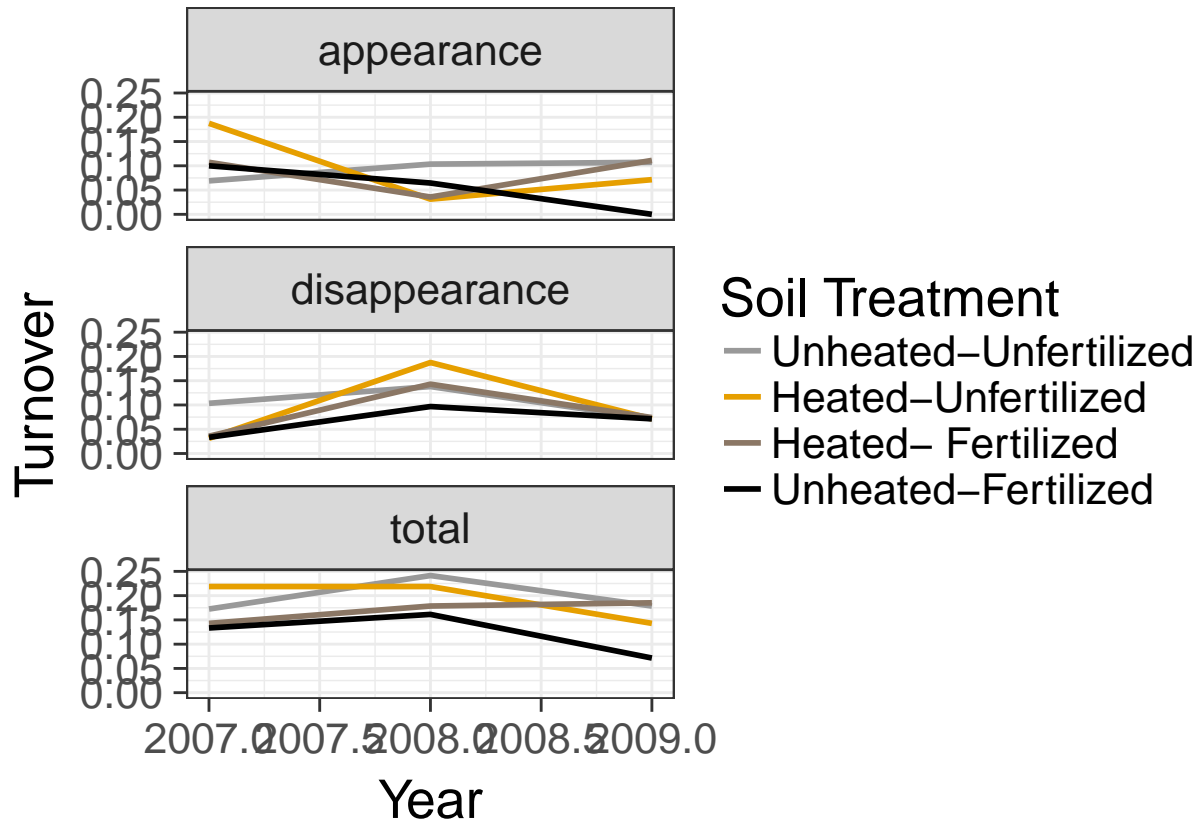
## Joining, by = c("year", "treatment")
## Joining, by = c("year", "treatment")

plant.turnover = gather(plant.turnover, key = metric, value = turnover, total, appearance, disappearance)
View(plant.turnover)

# 3. Visualize turnover within each group

plant.turn.plot = ggplot(plant.turnover, aes(x = year, y = turnover, color = treatment)) +
  geom_line(size = 1, show.legend = T) + facet_wrap(~metric, ncol = 1) +
  xlim(2007, 2009) +
  xlab("Year") +
  ylab("Turnover") +
  scale_color_manual(values = c("#999999", "#E69F00", "#8B7765", "#000000"),name="Soil Treatment",labels=
  theme_bw(base_size=20)
plot(plant.turn.plot)

```



*# Low turnover is indicative of a stable community and high turnover is indicative of a dynamic community*

*# Abundance Plot treatment by year*

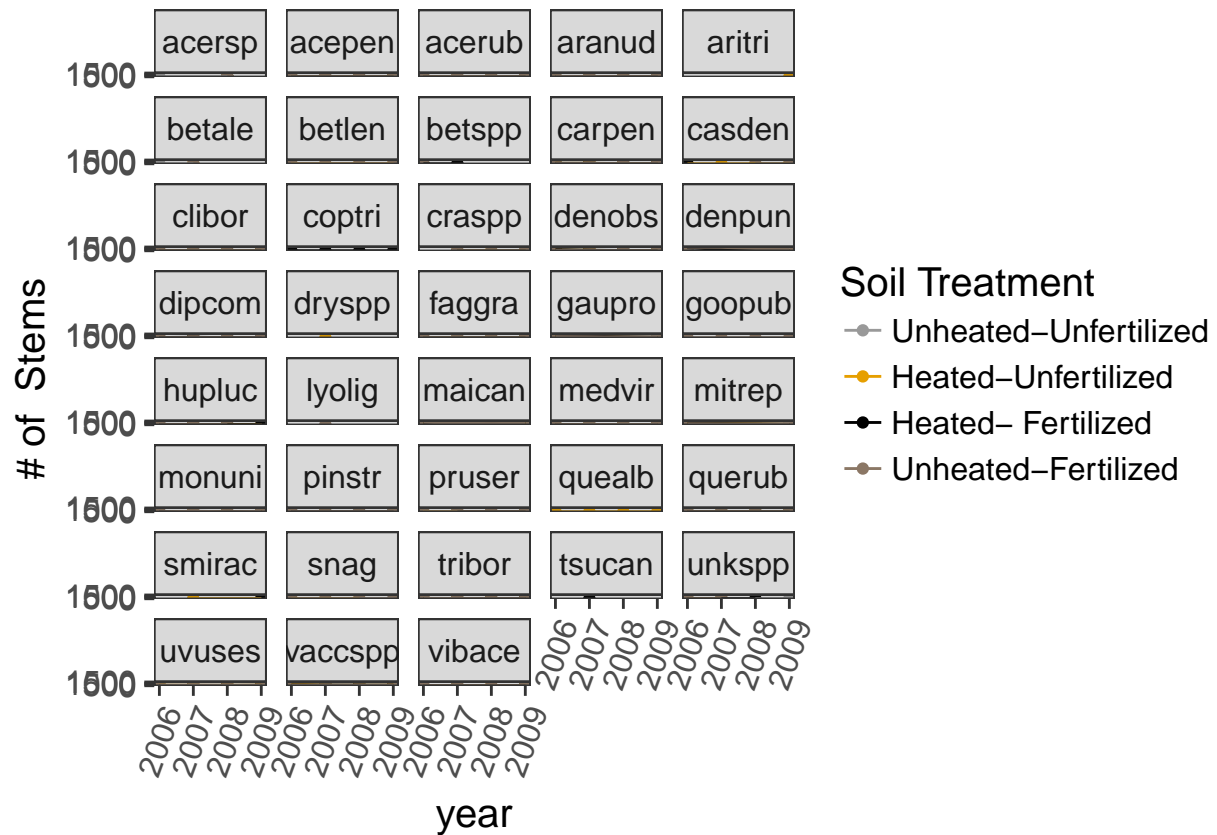
```
p_abun.plot1 = ggplot(plant.sp.abunds, aes(year, n, color= as.factor(treatment))) +
  geom_point() +
  geom_line(aes(color = as.factor(treatment))) +
  facet_wrap(~species, nrow = 8) +
  scale_color_manual(values = c("#999999", "#E69F00", "#000000", "#8B7765"),name="Soil Treatment") +
  ylab("# of Stems") +
  theme_bw(base_size=15) +
  theme(axis.text.x = element_text(angle=70, vjust=0.5))
plot(p_abun.plot1)
```

## geom\_path: Each group consists of only one observation. Do you need to  
## adjust the group aesthetic?

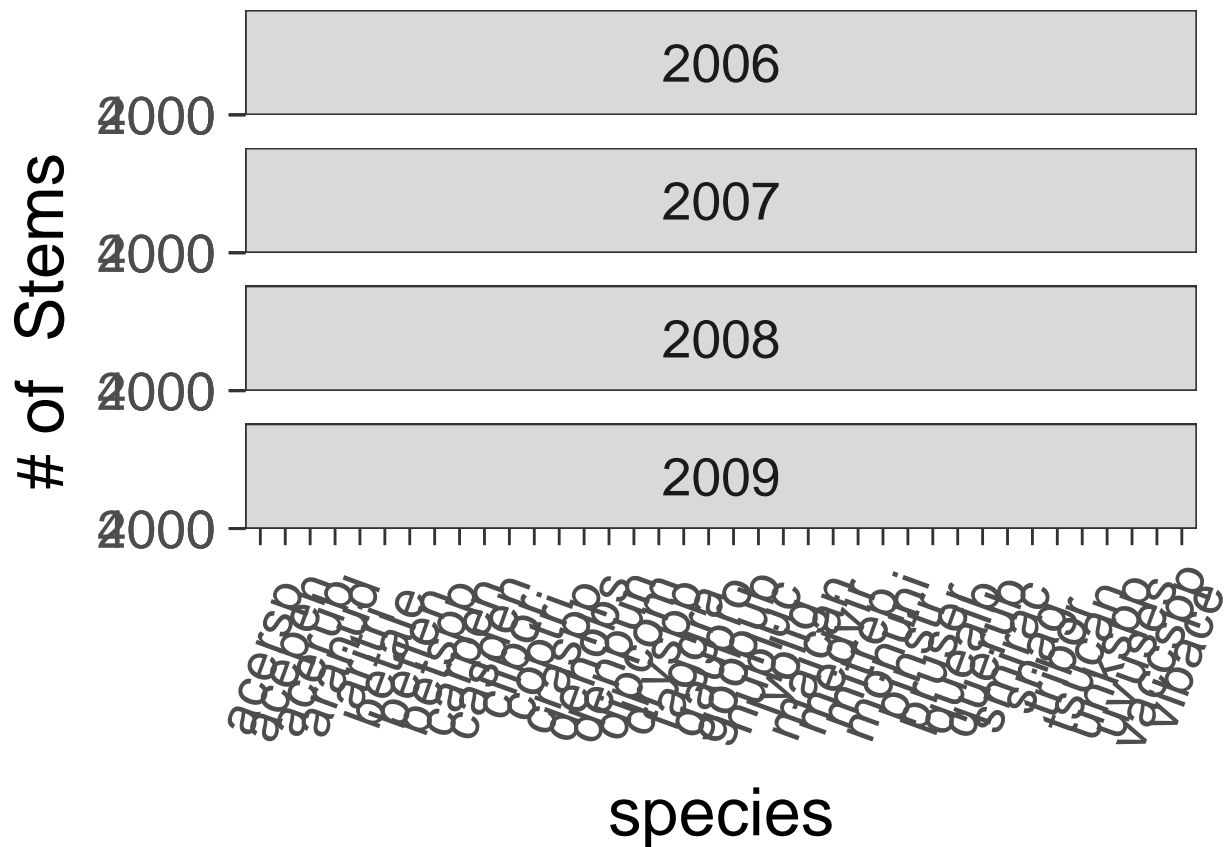
## geom\_path: Each group consists of only one observation. Do you need to  
## adjust the group aesthetic?

## geom\_path: Each group consists of only one observation. Do you need to  
## adjust the group aesthetic?

## geom\_path: Each group consists of only one observation. Do you need to  
## adjust the group aesthetic?



```
# Abundances across years
p_abun.plot2 = ggplot(plant.sp.abunds , aes(x = species, y = n)) +
  geom_bar(stat = "identity") +
  facet_wrap(~year, ncol = 1) +
  ylab("# of Stems") +
  theme_bw(base_size=25) +
  theme(axis.text.x = element_text(angle=70, vjust=0.5))
plot(p_abun.plot2)
```



### B. Rank Shift

*# Calculate species abundances for each treatment group*

```
plant.sp.abunds = p_long %>%
  group_by(year, treatment) %>%
  count(species)
```

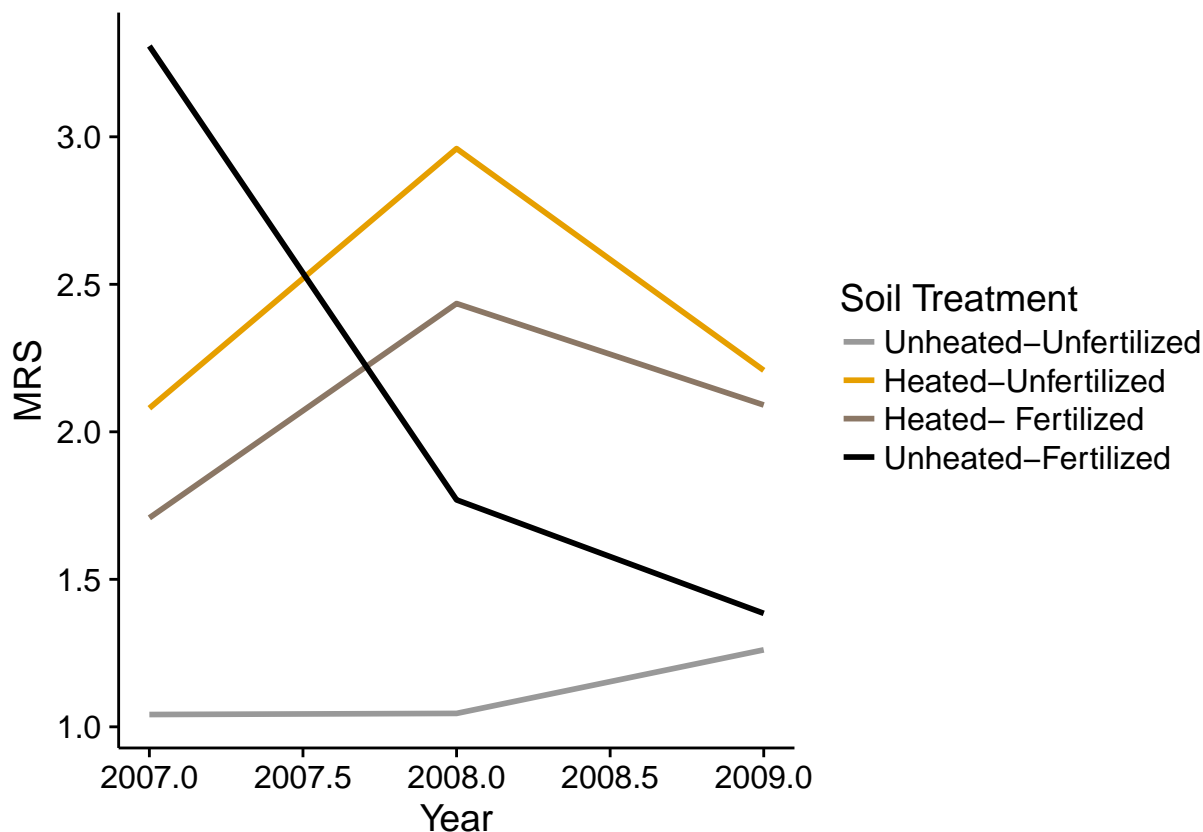
*# Calculate MRS*

```
plant.rankshift = rank_shift(df = as.data.frame(plant.sp.abunds),
  time.var = "year",
  species.var = "species",
  abundance.var = "n",
  replicate.var = "treatment")
```

```
plant.rankshift$year = as.numeric(substr(plant.rankshift$year_pair, 6, 9))
```

*# Create ggplot*

```
rankshift.plot = ggplot(plant.rankshift, aes(x = year, y = MRS, color = treatment)) + geom_line(size =
xlim(2007, 2009) +
xlab("Year") +
scale_color_manual(values = c("#999999", "#E69F00", "#8B7765", "#000000"), name="Soil Treatment", labels=
plot(rankshift.plot)
```

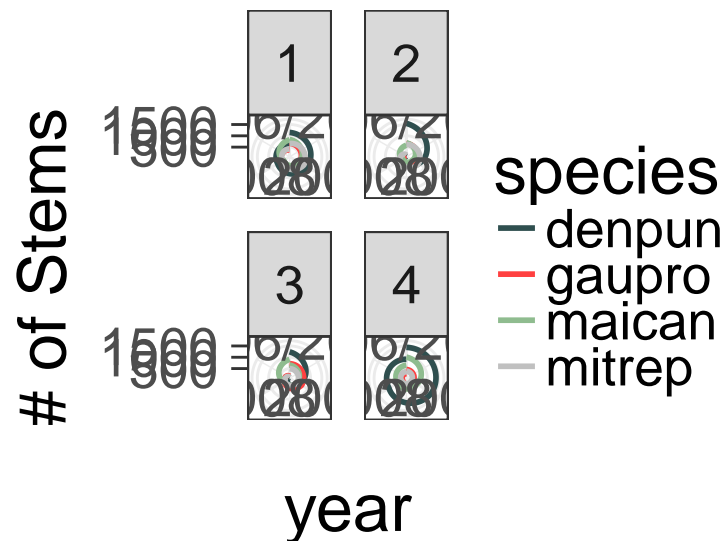


```
plant.rankshift %>%
  group_by (treatment) %>%
  summarise(mean = mean(MRS), cv = sd(MRS)/mean)
```

```
## # A tibble: 4 × 3
##   treatment    mean    cv
##   <chr>      <dbl>  <dbl>
## 1         1  1.115997 0.1124355
## 2         2  2.416111 0.1967510
## 3         3  2.078008 0.1748773
## 4         4  2.153846 0.4724556
```

```
# Rank Clock
ag_plant.dat = aggregate(n ~ species * year * treatment,
  data = subset(plant.sp.abunds,
    species == "denpun" |
    species == "maican" |
    species == "mitrep" |
    species == "gaupro"), FUN = mean)
write.csv(ag_plant.dat, file = "dominant plant abundances.csv")
rclock_plot = ggplot(ag_plant.dat, aes(year, n, color = species)) +
  geom_line(size = 1) + coord_polar() + theme_bw(base_size = 25) +
  facet_wrap(~treatment) +
  ylab("# of Stems") +
  scale_color_manual(values = c("#2F4F4F", "#FF4040", "#8FBC8F", "#C0C0C0")) +
  ggtitle("Dominant species abundances \n for the different soil treatments, Harvard Forest \n")
plot(rclock_plot)
```

# Dominant species abundance for the different soil treatments



# Species Codes: Denpun = *Dennstaedtia punctilobula*, Gauopro = *Gaultheria procumbens*, maican = *Maianthe*

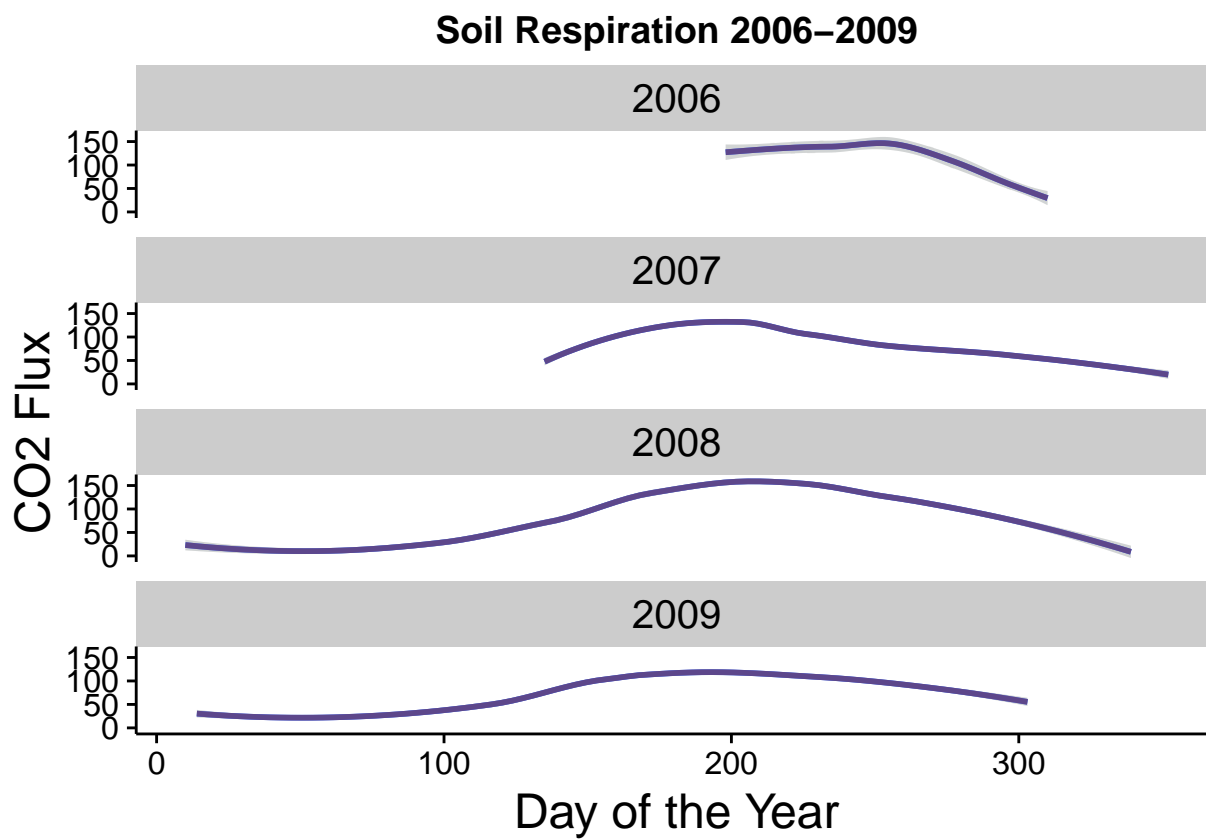
## SOIL RESPIRATION

### A. Plots

```
# Soil respiration
resp = resp %>%
  filter(year %in% c("2006", "2007", "2008", "2009"))
resp$date = as.Date(resp$date, format = "%m/%d/%Y")
resp$doy = yday(resp$date)
resp = na.exclude(resp)

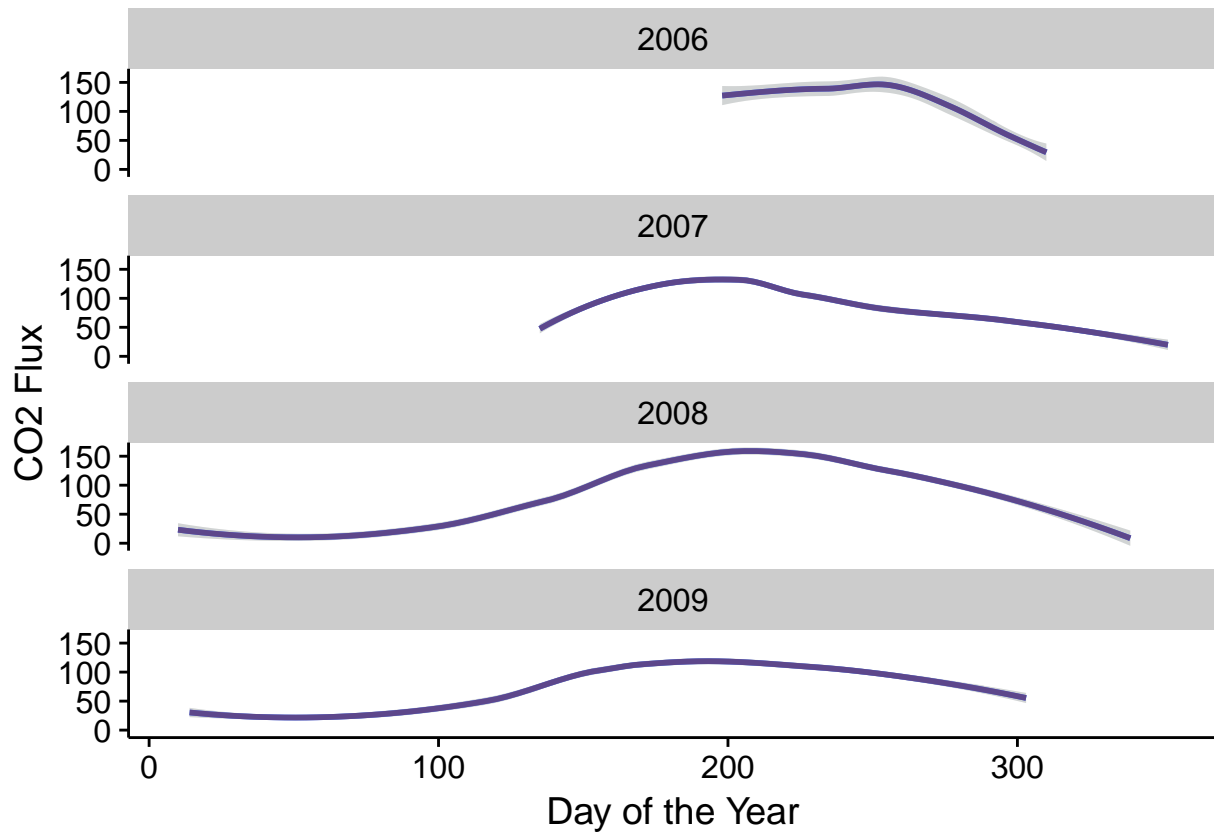
resp_yr.plot = qplot(doy, co2flux, data = resp, geom="smooth") +
  stat_smooth(fill="azure3", colour="mediumpurple4", size=1, alpha = 0.2) +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black")) +
  facet_wrap(~year, ncol = 1) +
  xlab("Day of the Year") +
  ylab("CO2 Flux")
resp_yr.plot + theme(text = element_text(size=18), plot.title = element_text(lineheight=.8, face="bold"))

## `geom_smooth()` using method = 'loess'
## `geom_smooth()` using method = 'loess'
```



```
plot(resp_yr.plot)
```

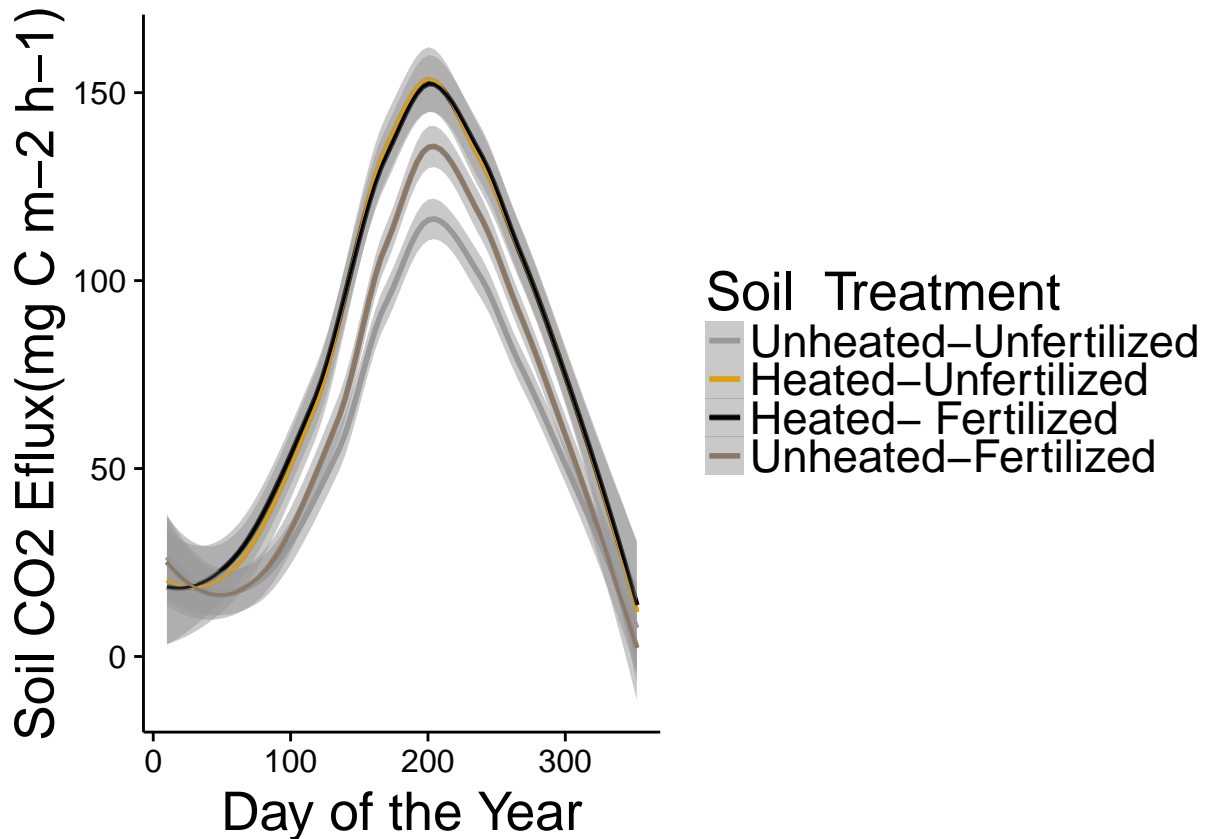
```
## `geom_smooth()` using method = 'loess'  
## `geom_smooth()` using method = 'loess'
```



```
resp_trt.plot = qplot(doy, co2flux, data = resp, colour = as.factor(trt), geom="smooth") +
  stat_smooth(method="auto", se = TRUE, formula = y ~ x, size = 0.5, alpha = 0.2) +
  theme(panel.grid.major = element_blank(), panel.grid.minor = element_blank(),
        panel.background = element_blank(), axis.line = element_line(colour = "black")) +
  scale_color_manual(values = c("#999999", "#E69F00", "#000000", "#8B7765"), name="Soil Treatm") +
  xlab("Day of the Year") +
  ylab("Soil CO2 Eflux(mg C m-2 h-1)") +
  theme(text = element_text(size=20))
plot(resp_trt.plot)
```

```
## `geom_smooth()` using method = 'loess'
## `geom_smooth()` using method = 'loess'
```





### B. Hypothesis Testing

*# Soil Respiration*

```
resp.treat.mean = group_by(resp, trt) %>%
  summarise(mean = mean(co2flux), sd = sd(co2flux), n = n(), sem = sd/sqrt(n))
```

*# Trying different variance - covariance structures*

*# AR(1) covariance structure*

```
soil.rm1 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year, random = ~ 1 | plot,
  summary(soil.rm1) # Obtain F-test
```

## Linear mixed-effects model fit by REML

## Data: resp

```
##      AIC      BIC  logLik
## 12193.8 12249.96 -6085.9
```

##

## Random effects:

## Formula: ~1 | plot

## (Intercept) Residual

```
## StdDev: 0.04868632 54.29218
```

##

## Correlation Structure: AR(1)

## Formula: ~1 | plot

## Parameter estimate(s):

## Phi

```
## 0.7650751
```

```
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year
```

```

##                               Value Std.Error   DF
## (Intercept)                 9685.752 12014.206 1197
## year                       -4.791    5.984 1197
## heat_treatheated           5707.207 16882.156 1197
## N_treatN fertilized        -5761.977 16796.061 1197
## heat_treatheated:N_treatN fertilized 3653.012 23965.367   22
## year:N_treatN fertilized     2.875    8.366 1197
## year:heat_treatheated       -2.833    8.409 1197
## year:heat_treatheated:N_treatN fertilized -1.822   11.937 1197
##                               t-value p-value
## (Intercept)                0.8061917 0.4203
## year                     -0.8005555 0.4235
## heat_treatheated         0.3380615 0.7354
## N_treatN fertilized      -0.3430553 0.7316
## heat_treatheated:N_treatN fertilized 0.1524288 0.8802
## year:N_treatN fertilized 0.3436185 0.7312
## year:heat_treatheated    -0.3369009 0.7363
## year:heat_treatheated:N_treatN fertilized -0.1526663 0.8787
## Correlation:
##                               (Intr) year   ht_trt N_trNf
## year                       -1.000
## heat_treatheated          -0.712  0.712
## N_treatN fertilized       -0.715  0.715  0.526
## heat_treatheated:N_treatN fertilized 0.501 -0.501 -0.716 -0.713
## year:N_treatN fertilized 0.715 -0.715 -0.526 -1.000
## year:heat_treatheated     0.712 -0.712 -1.000 -0.526
## year:heat_treatheated:N_treatN fertilized -0.501 0.501 0.716 0.713
##                               h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized      0.713
## year:heat_treatheated        0.716 0.526
## year:heat_treatheated:N_treatN fertilized -1.000 -0.713 -0.716
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -1.4855614 -0.8707739 -0.2082458  0.6043130  5.7170426
##
## Number of Observations: 1227
## Number of Groups: 24

```

```
anova(soil.rm1)
```

```

##           numDF denDF  F-value p-value
## (Intercept)      1  1197 407.3546 <.0001
## year             1  1197  3.0510 0.0809
## heat_treat       1  1197  4.2642 0.0391
## N_treat          1  1197  0.7549 0.3851
## heat_treat:N_treat 1    22  0.1341 0.7177
## year:N_treat     1  1197  0.1261 0.7226
## year:heat_treat  1  1197  0.4087 0.5227
## year:heat_treat:N_treat 1  1197  0.0233 0.8787

```

```
# corARMA autoregressive moving average process
soil.rm2 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot
summary(soil.rm2) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
## Data: resp
##      AIC      BIC    logLik
## 13224.39 13280.56 -6601.197
##
## Random effects:
## Formula: ~1 | plot
##      (Intercept) Residual
## StdDev:    12.33154  52.8103
##
## Correlation Structure: Compound symmetry
## Formula: ~1 | plot
## Parameter estimate(s):
## Rho
## 0
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat *      year
##
##              Value Std.Error   DF
## (Intercept) 19008.191  6343.268 1197
## year        -9.436    3.159 1197
## heat_treatheated 6260.049  8976.043 1197
## N_treatN fertilized -5509.047  8960.614 1197
## heat_treatheated:N_treatN fertilized 5272.973 12624.286   22
## year:N_treatN fertilized 2.750    4.463 1197
## year:heat_treatheated -3.107    4.471 1197
## year:heat_treatheated:N_treatN fertilized -2.631    6.288 1197
##
##              t-value p-value
## (Intercept) 2.9965929  0.0028
## year        -2.9865468  0.0029
## heat_treatheated 0.6974174  0.4857
## N_treatN fertilized -0.6148069  0.5388
## heat_treatheated:N_treatN fertilized 0.4176849  0.6802
## year:N_treatN fertilized 0.6161744  0.5379
## year:heat_treatheated -0.6949731  0.4872
## year:heat_treatheated:N_treatN fertilized -0.4184200  0.6757
##
## Correlation:
##
##              (Intr) year   ht_trt N_trNf
## year        -1.000
## heat_treatheated -0.707  0.707
## N_treatN fertilized -0.708  0.708  0.499
## heat_treatheated:N_treatN fertilized 0.502 -0.502 -0.710 -0.709
## year:N_treatN fertilized 0.708 -0.708 -0.499 -1.000
## year:heat_treatheated 0.707 -0.707 -1.000 -0.499
## year:heat_treatheated:N_treatN fertilized -0.502  0.502  0.710  0.709
##
##              h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized 0.709
## year:heat_treatheated 0.710  0.499
```

```
## year:heat_treatheated:N_treatN fertilized -1.000 -0.709 -0.710
```

```
##
```

```
## Standardized Within-Group Residuals:
```

```
##      Min      Q1      Med      Q3      Max
## -1.8753475 -0.8002387 -0.1344678  0.6750015  5.8030273
```

```
##
```

```
## Number of Observations: 1227
```

```
## Number of Groups: 24
```

```
anova(soil.rm2)
```

```
##              numDF denDF  F-value p-value
## (Intercept)          1  1197 711.9576 <.0001
## year                1  1197  43.1190 <.0001
## heat_treat          1  1197   8.4465 0.0037
## N_treat             1  1197   1.8246 0.1770
## heat_treat:N_treat   1    22   0.6421 0.4315
## year:N_treat         1  1197   0.1942 0.6595
## year:heat_treat      1  1197   1.9862 0.1590
## year:heat_treat:N_treat 1  1197   0.1751 0.6757
```

```
# cirCAR1 cnt AR process
```

```
soil.rm3 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot
```

```
summary(soil.rm3) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: resp
```

```
##      AIC      BIC  logLik
```

```
## 12193.8 12249.96 -6085.9
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | plot
```

```
##      (Intercept) Residual
```

```
## StdDev:  0.0061929 54.29221
```

```
##
```

```
## Correlation Structure: Continuous AR(1)
```

```
## Formula: ~1 | plot
```

```
## Parameter estimate(s):
```

```
##      Phi
```

```
## 0.7650753
```

```
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year
```

```
##              Value Std.Error   DF
## (Intercept)  9685.736 12014.212 1197
## year        -4.791    5.984 1197
## heat_treatheated 5707.204 16882.164 1197
## N_treatN fertilized -5761.970 16796.069 1197
## heat_treatheated:N_treatN fertilized 3653.006 23965.379 22
## year:N_treatN fertilized 2.875    8.366 1197
## year:heat_treatheated -2.833    8.409 1197
## year:heat_treatheated:N_treatN fertilized -1.822    11.937 1197
```

```
##              t-value p-value
## (Intercept)  0.8061899 0.4203
## year        -0.8005537 0.4235
## heat_treatheated 0.3380612 0.7354
## N_treatN fertilized -0.3430547 0.7316
## heat_treatheated:N_treatN fertilized 0.1524285 0.8802
```

```
## year:N_treatN fertilized          0.3436180  0.7312
## year:heat_treatheated             -0.3369006  0.7363
## year:heat_treatheated:N_treatN fertilized -0.1526659  0.8787
## Correlation:
##                               (Intr) year   ht_trt N_trNf
## year                         -1.000
## heat_treatheated             -0.712  0.712
## N_treatN fertilized           -0.715  0.715  0.526
## heat_treatheated:N_treatN fertilized  0.501 -0.501 -0.716 -0.713
## year:N_treatN fertilized         0.715 -0.715 -0.526 -1.000
## year:heat_treatheated            0.712 -0.712 -1.000 -0.526
## year:heat_treatheated:N_treatN fertilized -0.501  0.501  0.716  0.713
##                               h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized          0.713
## year:heat_treatheated              0.716  0.526
## year:heat_treatheated:N_treatN fertilized -1.000 -0.713 -0.716
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.4855628 -0.8707742 -0.2082468  0.6043113  5.7170404
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm3)
```

```
##               numDF denDF  F-value p-value
## (Intercept)         1  1197 407.3562 <.0001
## year                 1  1197  3.0510  0.0809
## heat_treat           1  1197  4.2643  0.0391
## N_treat              1  1197  0.7549  0.3851
## heat_treat:N_treat    1   22  0.1341  0.7177
## year:N_treat          1  1197  0.1261  0.7226
## year:heat_treat       1  1197  0.4087  0.5227
## year:heat_treat:N_treat 1  1197  0.0233  0.8787
```

```
# corARMA autoregressive moving average process, with arbitrary components
```

```
soil.rm4 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot)
summary(soil.rm4) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
## Data: resp
##      AIC      BIC    logLik
## 12184.46 12245.73 -6080.23
##
## Random effects:
## Formula: ~1 | plot
##      (Intercept) Residual
## StdDev: 0.009913942 54.36617
##
## Correlation Structure: ARMA(1,1)
## Formula: ~1 | plot
```

```
## Parameter estimate(s):
##      Phi1      Theta1
## 0.7206300 0.1122147
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat *      year
##                               Value Std.Error   DF
## (Intercept)                9310.932 11713.010 1197
## year                     -4.604      5.834 1197
## heat_treatheated          3942.018 16465.233 1197
## N_treatN fertilized        -6992.177 16384.659 1197
## heat_treatheated:N_treatN fertilized    5245.718 23358.892   22
## year:N_treatN fertilized         3.487      8.161 1197
## year:heat_treatheated         -1.953      8.201 1197
## year:heat_treatheated:N_treatN fertilized -2.616     11.635 1197
##                               t-value p-value
## (Intercept)                0.7949222 0.4268
## year                     -0.7891874 0.4302
## heat_treatheated          0.2394147 0.8108
## N_treatN fertilized        -0.4267515 0.6696
## heat_treatheated:N_treatN fertilized    0.2245705 0.8244
## year:N_treatN fertilized         0.4273296 0.6692
## year:heat_treatheated         -0.2381811 0.8118
## year:heat_treatheated:N_treatN fertilized -0.2248353 0.8221
## Correlation:
##                               (Intr) year   ht_trt N_trNf
## year                        -1.000
## heat_treatheated            -0.711  0.711
## N_treatN fertilized          -0.715  0.715  0.524
## heat_treatheated:N_treatN fertilized    0.501 -0.501 -0.716 -0.712
## year:N_treatN fertilized         0.715 -0.715 -0.524 -1.000
## year:heat_treatheated           0.711 -0.711 -1.000 -0.524
## year:heat_treatheated:N_treatN fertilized -0.501  0.501  0.716  0.712
##                               h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized         0.712
## year:heat_treatheated           0.716  0.524
## year:heat_treatheated:N_treatN fertilized -1.000 -0.712 -0.716
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.4830352 -0.8680755 -0.2048938  0.6109948  5.6997263
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm4)
```

```
##                               numDF denDF  F-value p-value
## (Intercept)                  1   1197 457.5664 <.0001
## year                         1   1197   2.3703  0.1239
## heat_treat                   1   1197   5.1306  0.0237
## N_treat                      1   1197   0.7796  0.3774
## heat_treat:N_treat           1    22   0.1771  0.6779
```

```

## year:N_treat          1  1197   0.1579  0.6911
## year:heat_treat       1  1197   0.3266  0.5678
## year:heat_treat:N_treat 1  1197   0.0506  0.8221

#corExp exponential spatial correlation.
soil.rm5 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot
summary(soil.rm5) # Obtain F-test

## Linear mixed-effects model fit by REML
## Data: resp
##      AIC      BIC  logLik
## 12193.8 12249.96 -6085.9
##
## Random effects:
## Formula: ~1 | plot
##      (Intercept) Residual
## StdDev:  0.00874299 54.29221
##
## Correlation Structure: Exponential spatial correlation
## Formula: ~1 | plot
## Parameter estimate(s):
##      range
## 3.734394
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat *      year
##
##              Value Std.Error   DF
## (Intercept)  9685.738 12014.211 1197
## year        -4.791    5.984 1197
## heat_treatheated 5707.204 16882.163 1197
## N_treatN fertilized -5761.970 16796.068 1197
## heat_treatheated:N_treatN fertilized 3653.007 23965.378 22
## year:N_treatN fertilized 2.875    8.366 1197
## year:heat_treatheated -2.833    8.409 1197
## year:heat_treatheated:N_treatN fertilized -1.822    11.937 1197
##
##              t-value p-value
## (Intercept)  0.8061901 0.4203
## year        -0.8005539 0.4235
## heat_treatheated 0.3380612 0.7354
## N_treatN fertilized -0.3430547 0.7316
## heat_treatheated:N_treatN fertilized 0.1524285 0.8802
## year:N_treatN fertilized 0.3436180 0.7312
## year:heat_treatheated -0.3369006 0.7363
## year:heat_treatheated:N_treatN fertilized -0.1526660 0.8787
## Correlation:
##
##              (Intr) year  ht_trt N_trNf
## year        -1.000
## heat_treatheated -0.712  0.712
## N_treatN fertilized -0.715  0.715  0.526
## heat_treatheated:N_treatN fertilized 0.501 -0.501 -0.716 -0.713
## year:N_treatN fertilized 0.715 -0.715 -0.526 -1.000
## year:heat_treatheated 0.712 -0.712 -1.000 -0.526
## year:heat_treatheated:N_treatN fertilized -0.501 0.501 0.716 0.713
##
##              h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized

```

```
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized 0.713
## year:heat_treatheated 0.716 0.526
## year:heat_treatheated:N_treatN fertilized -1.000 -0.713 -0.716
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.4855629 -0.8707742 -0.2082468  0.6043114  5.7170408
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm5)
```

```
##              numDF denDF  F-value p-value
## (Intercept)      1  1197 407.3563 <.0001
## year             1  1197  3.0510 0.0809
## heat_treat       1  1197  4.2643 0.0391
## N_treat          1  1197  0.7549 0.3851
## heat_treat:N_treat 1   22  0.1341 0.7177
## year:N_treat     1  1197  0.1261 0.7226
## year:heat_treat  1  1197  0.4087 0.5227
## year:heat_treat:N_treat 1 1197 0.0233 0.8787
```

```
# CorGaus Gaussian spatial correlation.
```

```
soil.rm6 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot)
summary(soil.rm6) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: resp
```

```
##      AIC      BIC    logLik
```

```
## 12437.69 12493.86 -6207.846
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | plot
```

```
##      (Intercept) Residual
```

```
## StdDev: 10.69376 48.74015
```

```
##
```

```
## Correlation Structure: Gaussian spatial correlation
```

```
## Formula: ~1 | plot
```

```
## Parameter estimate(s):
```

```
## range
```

```
## 1.28212
```

```
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year
```

```
##              Value Std.Error   DF
```

```
## (Intercept) 15691.395 8146.750 1197
```

```
## year        -7.783    4.058 1197
```

```
## heat_treatheated 3854.306 11506.838 1197
```

```
## N_treatN fertilized -8425.135 11470.732 1197
```

```
## heat_treatheated:N_treatN fertilized 7756.017 16218.984 22
```

```
## year:N_treatN fertilized 4.202    5.713 1197
```

```
## year:heat_treatheated -1.909    5.731 1197
```

```
## year:heat_treatheated:N_treatN fertilized -3.867    8.078 1197
```

```
##              t-value p-value
```

```
## (Intercept) 1.9260926 0.0543
```

```
## year        -1.9181601 0.0553
```



```
## heat_treatheated          0.3349579  0.7377
## N_treatN fertilized       -0.7344898  0.4628
## heat_treatheated:N_treatN fertilized  0.4782061  0.6372
## year:N_treatN fertilized   0.7354333  0.4622
## year:heat_treatheated     -0.3330064  0.7392
## year:heat_treatheated:N_treatN fertilized -0.4787347  0.6322
## Correlation:
##                               (Intr) year   ht_trt N_trNf
## year                        -1.000
## heat_treatheated            -0.708  0.708
## N_treatN fertilized          -0.710  0.710  0.506
## heat_treatheated:N_treatN fertilized  0.502 -0.502 -0.712 -0.710
## year:N_treatN fertilized      0.710 -0.710 -0.506 -1.000
## year:heat_treatheated         0.708 -0.708 -1.000 -0.506
## year:heat_treatheated:N_treatN fertilized -0.502  0.502  0.712  0.710
##                               h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized      0.710
## year:heat_treatheated        0.712  0.506
## year:heat_treatheated:N_treatN fertilized -1.000 -0.710 -0.712
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -1.8645120 -0.8903077 -0.1563755  0.7280396  6.2992236
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm6)
```

```
##               numDF denDF  F-value p-value
## (Intercept)         1  1197 687.2837 <.0001
## year                 1  1197  14.2721  0.0002
## heat_treat           1  1197   8.9255  0.0029
## N_treat              1  1197   1.2587  0.2621
## heat_treat:N_treat    1    22   0.5210  0.4780
## year:N_treat          1  1197   0.3178  0.5731
## year:heat_treat       1  1197   0.9208  0.3375
## year:heat_treat:N_treat 1  1197   0.2292  0.6322
```

```
# corLin    linear spatial correlation.
```

```
soil.rm7 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot)
summary(soil.rm7) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
## Data: resp
##      AIC      BIC    logLik
## 12326.72 12382.88 -6152.358
##
## Random effects:
## Formula: ~1 | plot
##      (Intercept) Residual
## StdDev:  0.03468838 170.535
```

```
##
## Correlation Structure: Linear spatial correlation
## Formula: ~1 | plot
## Parameter estimate(s):
##   range
## 42.34558
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat *      year
##                                     Value Std.Error   DF
## (Intercept)                    -7989.840  17084.36 1197
## year                           4.020      8.51 1197
## heat_treatheated                11977.917  24017.96 1197
## N_treatN fertilized              1319.883  24063.30 1197
## heat_treatheated:N_treatN fertilized -13180.793  34176.83   22
## year:N_treatN fertilized         -0.658    11.98 1197
## year:heat_treatheated            -5.961    11.96 1197
## year:heat_treatheated:N_treatN fertilized 6.571    17.02 1197
##                                     t-value p-value
## (Intercept)                   -0.4676699  0.6401
## year                          0.4724529  0.6367
## heat_treatheated              0.4987067  0.6181
## N_treatN fertilized            0.0548505  0.9563
## heat_treatheated:N_treatN fertilized -0.3856646  0.7034
## year:N_treatN fertilized       -0.0548892  0.9562
## year:heat_treatheated          -0.4983656  0.6183
## year:heat_treatheated:N_treatN fertilized 0.3859872  0.6996
## Correlation:
##                                     (Intr) year   ht_trt N_trNf
## year                             -1.000
## heat_treatheated                 -0.711  0.711
## N_treatN fertilized               -0.710  0.710  0.516
## heat_treatheated:N_treatN fertilized 0.500 -0.500 -0.711 -0.712
## year:N_treatN fertilized          0.710 -0.710 -0.516 -1.000
## year:heat_treatheated             0.711 -0.711 -1.000 -0.516
## year:heat_treatheated:N_treatN fertilized -0.500 0.500 0.711 0.712
##                                     h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized          0.712
## year:heat_treatheated             0.711 0.516
## year:heat_treatheated:N_treatN fertilized -1.000 -0.712 -0.711
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -0.5555369 -0.3332354 -0.1174624  0.1434040  1.7858305
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm7)
```

```
##               numDF denDF   F-value p-value
## (Intercept)         1  1197 13.884319 0.0002
## year                 1  1197 0.305597 0.5805
```

```

## heat_treat          1  1197  0.071048  0.7899
## N_treat             1  1197  0.041809  0.8380
## heat_treat:N_treat  1    22  0.013099  0.9099
## year:N_treat        1  1197  0.102430  0.7490
## year:heat_treat     1  1197  0.101428  0.7502
## year:heat_treat:N_treat 1  1197  0.148986  0.6996

# corRatio Rational quadratics spatial correlation.
soil.rm8 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot
summary(soil.rm8) # Obtain F-test

## Linear mixed-effects model fit by REML
## Data: resp
##      AIC      BIC    logLik
## 12258.98 12315.14 -6118.489
##
## Random effects:
## Formula: ~1 | plot
##      (Intercept) Residual
## StdDev:      4.022598 50.52358
##
## Correlation Structure: Rational quadratic spatial correlation
## Formula: ~1 | plot
## Parameter estimate(s):
##      range
## 1.465046
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat *      year
##
##              Value Std.Error   DF
## (Intercept) 13057.695 10084.786 1197
## year        -6.471    5.023 1197
## heat_treatheated 3055.431 14211.590 1197
## N_treatN fertilized -8211.315 14141.609 1197
## heat_treatheated:N_treatN fertilized 7307.039 20096.611 22
## year:N_treatN fertilized 4.095    7.044 1197
## year:heat_treatheated -1.511    7.079 1197
## year:heat_treatheated:N_treatN fertilized -3.643    10.010 1197
##
##              t-value p-value
## (Intercept) 1.2947915 0.1956
## year        -1.2882423 0.1979
## heat_treatheated 0.2149957 0.8298
## N_treatN fertilized -0.5806493 0.5616
## heat_treatheated:N_treatN fertilized 0.3635956 0.7196
## year:N_treatN fertilized 0.5813504 0.5611
## year:heat_treatheated -0.2134664 0.8310
## year:heat_treatheated:N_treatN fertilized -0.3639631 0.7159
## Correlation:
##
##              (Intr) year  ht_trt N_trNf
## year        -1.000
## heat_treatheated -0.710  0.710
## N_treatN fertilized -0.713  0.713  0.516
## heat_treatheated:N_treatN fertilized 0.502 -0.502 -0.714 -0.711
## year:N_treatN fertilized 0.713 -0.713 -0.516 -1.000
## year:heat_treatheated 0.710 -0.710 -1.000 -0.516
## year:heat_treatheated:N_treatN fertilized -0.502 0.502 0.714 0.711
##
##              h_:N_f y:N_Nf yr:ht_

```

```
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized          0.711
## year:heat_treatheated            0.714  0.516
## year:heat_treatheated:N_treatN fertilized -1.000 -0.711 -0.714
##
## Standardized Within-Group Residuals:
##      Min      Q1      Med      Q3      Max
## -1.5780993 -0.9142183 -0.2011923  0.6785817  6.1249494
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm8)
```

```
##              numDF denDF  F-value p-value
## (Intercept)         1  1197 689.1108 <.0001
## year                 1  1197  5.9050  0.0152
## heat_treat           1  1197  8.6477  0.0033
## N_treat              1  1197  1.0978  0.2950
## heat_treat:N_treat    1    22  0.3813  0.5432
## year:N_treat         1  1197  0.2213  0.6382
## year:heat_treat       1  1197  0.4577  0.4988
## year:heat_treat:N_treat 1  1197  0.1325  0.7159
```

```
# corRatioSpher spherical spatial correlation
```

```
soil.rm9 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | plot
```

```
summary(soil.rm9) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: resp
```

```
##      AIC      BIC    logLik
```

```
## 12150.61 12206.77 -6064.304
```

```
##
```

```
## Random effects:
```

```
## Formula: ~1 | plot
```

```
##      (Intercept) Residual
```

```
## StdDev: 0.007654081 57.45838
```

```
##
```

```
## Correlation Structure: Spherical spatial correlation
```

```
## Formula: ~1 | plot
```

```
## Parameter estimate(s):
```

```
##      range
```

```
## 7.346906
```

```
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year
```

```
##              Value Std.Error   DF
```

```
## (Intercept)    6019.759 11810.144 1197
```

```
## year           -2.966    5.882 1197
```

```
## heat_treatheated 3128.548 16605.481 1197
```

```
## N_treatN fertilized -5393.838 16527.472 1197
```

```
## heat_treatheated:N_treatN fertilized 2688.769 23545.206 22
```

```
## year:N_treatN fertilized 2.691    8.232 1197
```

```
## year:heat_treatheated -1.548    8.271 1197
```

```
## year:heat_treatheated:N_treatN fertilized -1.342 11.727 1197
```

```
##                                t-value p-value
## (Intercept)                   0.5097109 0.6103
## year                         -0.5041373 0.6143
## heat_treatheated             0.1884046 0.8506
## N_treatN fertilized          -0.3263559 0.7442
## heat_treatheated:N_treatN fertilized 0.1141960 0.9101
## year:N_treatN fertilized      0.3269319 0.7438
## year:heat_treatheated        -0.1871986 0.8515
## year:heat_treatheated:N_treatN fertilized -0.1144603 0.9089
## Correlation:
##                                (Intr) year   ht_trt N_trNf
## year                         -1.000
## heat_treatheated            -0.711  0.711
## N_treatN fertilized          -0.715  0.715  0.523
## heat_treatheated:N_treatN fertilized 0.502 -0.502 -0.715 -0.712
## year:N_treatN fertilized      0.715 -0.715 -0.523 -1.000
## year:heat_treatheated         0.711 -0.711 -1.000 -0.523
## year:heat_treatheated:N_treatN fertilized -0.502 0.502 0.715 0.712
##                                h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized      0.712
## year:heat_treatheated         0.715 0.523
## year:heat_treatheated:N_treatN fertilized -1.000 -0.712 -0.715
##
## Standardized Within-Group Residuals:
##           Min           Q1           Med           Q3           Max
## -1.3893606 -0.7948937 -0.1631757  0.6170542  5.4097825
##
## Number of Observations: 1227
## Number of Groups: 24
```

```
anova(soil.rm9)
```

```
##                                numDF denDF F-value p-value
## (Intercept)                   1 1197 450.8057 <.0001
## year                         1 1197 0.8567 0.3548
## heat_treat                   1 1197 5.0976 0.0241
## N_treat                     1 1197 0.7912 0.3739
## heat_treat:N_treat           1 22 0.1802 0.6753
## year:N_treat                 1 1197 0.1297 0.7188
## year:heat_treat              1 1197 0.1484 0.7002
## year:heat_treat:N_treat      1 1197 0.0131 0.9089
```

```
# corSymm general correlation matrix, with no additional structure
```

```
soil.rm10 = lme(co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year , random = ~ 1 | pl
```

```
summary(soil.rm10) # Obtain F-test
```

```
## Linear mixed-effects model fit by REML
```

```
## Data: resp
```

```
##           AIC           BIC       logLik
```

```
## 12150.61 12206.77 -6064.304
```

```
##
```

```
## Random effects:
```

```

## Formula: ~1 | plot
## (Intercept) Residual
## StdDev: 0.007654081 57.45838
##
## Correlation Structure: Spherical spatial correlation
## Formula: ~1 | plot
## Parameter estimate(s):
## range
## 7.346906
## Fixed effects: co2flux ~ year + heat_treat + N_treat * heat_treat * N_treat * year
##
## Value Std.Error DF
## (Intercept) 6019.759 11810.144 1197
## year -2.966 5.882 1197
## heat_treatheated 3128.548 16605.481 1197
## N_treatN fertilized -5393.838 16527.472 1197
## heat_treatheated:N_treatN fertilized 2688.769 23545.206 22
## year:N_treatN fertilized 2.691 8.232 1197
## year:heat_treatheated -1.548 8.271 1197
## year:heat_treatheated:N_treatN fertilized -1.342 11.727 1197
##
## t-value p-value
## (Intercept) 0.5097109 0.6103
## year -0.5041373 0.6143
## heat_treatheated 0.1884046 0.8506
## N_treatN fertilized -0.3263559 0.7442
## heat_treatheated:N_treatN fertilized 0.1141960 0.9101
## year:N_treatN fertilized 0.3269319 0.7438
## year:heat_treatheated -0.1871986 0.8515
## year:heat_treatheated:N_treatN fertilized -0.1144603 0.9089
## Correlation:
## (Intr) year ht_trt N_trNf
## year -1.000
## heat_treatheated -0.711 0.711
## N_treatN fertilized -0.715 0.715 0.523
## heat_treatheated:N_treatN fertilized 0.502 -0.502 -0.715 -0.712
## year:N_treatN fertilized 0.715 -0.715 -0.523 -1.000
## year:heat_treatheated 0.711 -0.711 -1.000 -0.523
## year:heat_treatheated:N_treatN fertilized -0.502 0.502 0.715 0.712
## h_:N_f y:N_Nf yr:ht_
## year
## heat_treatheated
## N_treatN fertilized
## heat_treatheated:N_treatN fertilized
## year:N_treatN fertilized 0.712
## year:heat_treatheated 0.715 0.523
## year:heat_treatheated:N_treatN fertilized -1.000 -0.712 -0.715
##
## Standardized Within-Group Residuals:
## Min Q1 Med Q3 Max
## -1.3893606 -0.7948937 -0.1631757 0.6170542 5.4097825
##
## Number of Observations: 1227
## Number of Groups: 24

```

```

anova(soil.rm10)

##               numDF denDF  F-value p-value
## (Intercept)         1  1197  450.8057 <.0001
## year                1  1197   0.8567  0.3548
## heat_treat          1  1197   5.0976  0.0241
## N_treat             1  1197   0.7912  0.3739
## heat_treat:N_treat   1    22   0.1802  0.6753
## year:N_treat         1  1197   0.1297  0.7188
## year:heat_treat      1  1197   0.1484  0.7002
## year:heat_treat:N_treat 1  1197   0.0131  0.9089

# Compare the AICs
AIC(soil.rm1, soil.rm2, soil.rm3,soil.rm4, soil.rm5, soil.rm6,soil.rm7,soil.rm8,soil.rm9,soil.rm10)

##           df      AIC
## soil.rm1  11 12193.80
## soil.rm2  11 13224.39
## soil.rm3  11 12193.80
## soil.rm4  12 12184.46
## soil.rm5  11 12193.80
## soil.rm6  11 12437.69
## soil.rm7  11 12326.72
## soil.rm8  11 12258.98
## soil.rm9  11 12150.61
## soil.rm10 11 12150.61

# The unstructured covariance matrix has the lowest AIC score

```

## 6) DISCUSSION AND CONCLUSION

Rising temperatures and N deposition are occurring globally and can influence plant species composition through altered competition between neighboring species, increased environmental stress sensitivity, and changes in edaphic factors. Plant species richness and diversity have been shown to decline with increasing temperature and N deposition in a number of different ecosystems including: heathlands, grasslands, arctic tundra, and boreal and temperate forests (Strengbom et al. 2001; Gilliam and Roberts 2003; Clark and Tilman 2008; Southon et al. 2013). We found that soil warming and N fertilization did not influence plant diversity in the short term (2006-2009). However, average species richness differed among years and there was a significant year by nitrogen by warming interaction ( $P < 0.05$ ). This interaction was likely driven by a significant decrease in richness in the warming x N treatment in 2008. Additionally, species turnover increased between 2007 and 2008 due to a greater number of species disappearances relative to appearances at that time. Abundances of the four most dominant species, comprising primarily understory plants, declined from 2006-2009, most notably in the warming treatments. These temporal shifts in richness and abundance may be related to changes in belowground dynamics and microbial community response to warming and N additions.

Soil respiration was significantly higher in warmed soils ( $P < 0.01$ ). This finding is consistent with the results from a previous soil warming experiment conducted at Harvard Forest. In the short-term (first 12 years) rates of soil respiration were higher in heated plots compared to control plots (Melillo et al. 2002; DeAngelis et al. 2015). Increases in microbial activity and soil respiration can result in greater nutrient mineralization (Davidson et al. 2006). Furthermore, increases in soil nitrogen either from enhanced microbial activity or nitrogen fertilization can alter beneficial plant-microbial interactions (Classen et al. 2015). Once nitrogen is freely available, plants may invest less in relationships with symbiotic mycorrhizal fungi (Johnson et al. 2015). It has been demonstrated that warming and N fertilization can alter mycorrhizal community composition and abundance (Frey et al. 2004; Compant et al. 2013). Changes in plant-microbe interactions could influence plant performance and ultimately plant community composition (Classen et al. 2015).

## 7) REFERENCES

- Arend, M., Kuster, T., Guenthardt-Goerg, M.S. and Dobbertin, M., 2011. Provenance-specific growth responses to drought and air warming in three European oak species (*Quercus robur*, *Q. petraea* and *Q. pubescens*). *Tree Physiology*, 31(3), pp.287-297.
- Bowden, R.D., Davidson, E., Savage, K., Arabia, C. and Steudler, P., 2004. Chronic nitrogen additions reduce total soil respiration and microbial respiration in temperate forest soils at the Harvard Forest. *Forest Ecology and Management*, 196(1), pp.43-56.
- Clark C.M., Tilman D., 2008. Loss of plant species after chronic low-level nitrogen deposition to prairie grasslands. *Nature*, 451, pp.712-715.
- Classen, A. T., M. K. Sundqvist, J. A. Henning, G. S. Newman, J. A. M. Moore, M. A. Cregger, L. C. Moorhead, and C. M. Patterson. 2015. Direct and indirect effects of climate change on soil microbial and soil microbial-plant interactions: What lies ahead?, *Ecosphere* 6(8), pp 130.
- Compant, S., Van der Heijden, M. and Sessitsch, A., 2013. Soil Warming Effects on Beneficial Plant-Microbe Interactions. *Molecular Microbial Ecology of the Rhizosphere*, 1, pp.1047-1054
- Davidson, E.A., and Janssens, I. A., 2006. Temperature Sensitivity of Soil Carbon Decomposition and Feedbacks to Climate Change. *Nature*, 440, pp.165-73.
- DeAngelis, K.M., Pold, G., Beguem, D., van Diepen, L.T., Varney, R.M., Blanchard, J.L., Melillo, J. and Frey, S.D., 2015. Long-term forest soil warming alters microbial communities in temperate forest soils. *Frontiers in Microbiology*, 6 (140).
- Frey, S.D., Knorr, M., Parrent, J.L. and Simpson, R.T., 2004. Chronic nitrogen enrichment affects the structure and function of the soil microbial community in temperate hardwood and pine forests. *Forest Ecology and Management*, 196(1), pp.159-171.
- Frey, Serita (2009): Soil warming plus nitrogen addition experiment at Harvard Forest since 2006. Long Term Ecological Research Network. [link]<http://dx.doi.org/10.6073/pasta/08505f370bbcf98b7237434e41c78bc8>
- Gilliam F.S. and Roberts M.E., 2003. The herbaceous layer in forests of eastern North America. Oxford University Press, New York, USA.
- Hesse, C.N., Mueller, R.C., Vuyisich, M., Gallegos-Graves, L.V., Gleasner, C.D., Zak, D.R. and Kuske, C.R., 2015. Forest floor community metatranscriptomes identify fungal and bacterial responses to N deposition in two maple forests. *Frontiers in microbiology*, 6, p.337.
- Johnson N.C., Wilson G.W.T., Wilson J.A., Miller R.M., and Bowker M.A. 2015. Mycorrhizal phenotypes and the law of the minimum. *New Phytologist*, 205(4), pp. 1473-1484.
- Melillo, J. M., Steudler, P. A., Aber, J. D., Newkirk, K., Lux, H., and Bowles, F. P., et al. (2002). Soil warming and carbon-cycle feedbacks to the climate system. *Science*, 298, pp. 2173-2176.
- Ramirez, K.S., Craine, J.M. and Fierer, N., 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology*, 18(6), pp.1918-1927
- Strengbom J.A., Nordin A., and Nasholm T. and Ericson L , 2001. Slow recovery of a boreal forest ecosystem following decreased nitrogen input. *Functional Ecology*, 15, pp.454-457.
- Schindlbacher, A., Rodler, A., Kuffner, M., Kitzler, B., Sessitsch, A. and Zechmeister-Boltenstern, S., 2011. Experimental warming effects on the microbial community of a temperate mountain forest soil. *Soil Biology and Biochemistry*, 43(7), pp.1417-1425.
- Thakur, M.P., Reich, P.B., Eddy, W.C., Stefanski, A., Rich, R., Hobbie, S.E. and Eisenhauer, N., 2014. Some plants like it warmer: Increased growth of three selected invasive plant species in soils with a history of experimental warming. *Pedobiologia*, 57(1), pp.57-60.



Xu, Z., Hu, T. and Zhang, Y., 2012. Effects of experimental warming on phenology, growth and gas exchange of treeline birch (*Betula utilis*) saplings, Eastern Tibetan Plateau, China. *European Journal of Forest Research*, 131(3), pp.811-819.

Zhao, C. and Liu, Q., 2009. Growth and physiological responses of *Picea asperata* seedlings to elevated temperature and to nitrogen fertilization. *Acta physiologiae plantarum*, 31(1), p.163.