3.Repeated_Measures_ANOVAs

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```
library(here)
library(tidyverse)
library(rstatix)
library(nlme)
library(emmeans)
library(multcomp)
```

Does communities' NE, CE and SE change over time

```
#Net effect, CE, SE
NE_data<-read.csv(here("data", "NE_data.csv"), sep=";")
str(NE_data)
```

```
## 'data.frame':    132 obs. of  8 variables:
##  $ Polyculture: chr  "J0B1LmSp" "J0B1LmWc" "J0B1LmLt" "J0B1SpWc" ...
##  $ Block      : int  1 1 1 1 1 1 1 1 1 1 ...
##  $ Composition: chr  "LmSp" "LmWc" "LmLt" "SpWc" ...
##  $ Days       : int  0 0 0 0 0 0 0 0 0 0 ...
##  $ RYT        : num  1 1 1 1 1 ...
##  $ NE         : num  -1.093 -0.226 -2.291 -0.327 -0.186 ...
##  $ CE         : num  0 0 0 0 0 ...
##  $ SE         : num  -1.093 -0.226 -2.291 -0.327 -0.186 ...
```

```
NE_data<-NE_data[, -1]
NE_data<-NE_data %>%
  mutate(across(c("Composition", "Block", "Days"),
                as.factor)) #transform Composition, Block, Days as factor
```

Do we have outliers in the data?

```
NE_data %>%
  group_by(Days) %>%
  identify_outliers(NE)
```

```
## # A tibble: 1 x 9
##   Days  Block Composition   RYT    NE    CE     SE is.outlier is.extreme
##   <fct> <fct> <fct>       <dbl> <dbl> <dbl>  <dbl> <lgl>      <lgl>
## 1 0     2     LmSpWc       1.04  2.70  3.23 -0.533 TRUE       FALSE
```

```
NE_data %>%
  group_by(Days) %>%
  identify_outliers(CE)
```

```
## [1] Days        Block       Composition RYT         NE          CE
## [7] SE          is.outlier  is.extreme
## <0 rows> (or 0-length row.names)
```

```
NE_data %>%
  group_by(Days) %>%
  identify_outliers(SE)
```

```
## # A tibble: 5 x 9
##   Days  Block Composition   RYT     NE     CE       SE is.outlier is.extreme
##   <fct> <fct> <fct>       <dbl>  <dbl>  <dbl>    <dbl> <lgl>      <lgl>
## 1 0     1     LmLt         1     -2.29    0      -2.29 TRUE       FALSE
## 2 20    3     SpLt         1.15 247.     64.3   183.   TRUE       FALSE
## 3 40    2     WcLt         1.50 855.    251.    604.   TRUE       FALSE
## 4 40    3     WcLt         1.24 884.    274.    611.   TRUE       FALSE
## 5 60    3     LmSpLt       2.29 898.   2435.  -1537.   TRUE       FALSE
```

No extreme outliers

Test normality assumption

```
NE_data %>%
  group_by(Composition, Days) %>%
  shapiro_test(NE) %>%
  ungroup()
```

```
## # A tibble: 44 x 5
##    Composition Days  variable statistic      p
##    <fct>       <fct> <chr>        <dbl>  <dbl>
##  1 LmLt        0     NE           0.779 0.0653
##  2 LmLt        20    NE           0.975 0.699
##  3 LmLt        40    NE           0.971 0.676
##  4 LmLt        60    NE           0.938 0.518
##  5 LmSp        0     NE           1.00  0.961
##  6 LmSp        20    NE           0.967 0.650
##  7 LmSp        40    NE           0.999 0.932
##  8 LmSp        60    NE           0.890 0.353
##  9 LmSpLt      0     NE           0.990 0.807
## 10 LmSpLt      20    NE           0.912 0.424
## # i 34 more rows
```

```
NE_data[NE_data$CE!=0,] %>%
  group_by(Composition, Days) %>%
  shapiro_test(CE)
```

```
## # A tibble: 38 x 5
##    Composition Days  variable statistic      p
##    <fct>       <fct> <chr>        <dbl>  <dbl>
##  1 LmLt        20    CE           0.886 0.344
##  2 LmLt        40    CE           0.992 0.827
##  3 LmLt        60    CE           0.860 0.268
##  4 LmSp        20    CE           1.00  0.965
##  5 LmSp        40    CE           0.999 0.957
##  6 LmSp        60    CE           0.811 0.141
##  7 LmSpLt      0     CE           0.873 0.304
##  8 LmSpLt      20    CE           0.961 0.620
```

```
##  9 LmSpLt      40    CE           0.997 0.889
## 10 LmSpLt      60    CE           0.754 0.00857
## # i 28 more rows
```

```
NE_data %>%
  group_by(Composition, Days) %>%
  shapiro_test(SE)
```

```
## # A tibble: 44 x 5
##    Composition Days  variable statistic      p
##    <fct>       <fct> <chr>        <dbl>  <dbl>
##  1 LmLt        0     SE           0.779 0.0653
##  2 LmLt        20    SE           0.923 0.461
##  3 LmLt        40    SE           1.00  0.973
##  4 LmLt        60    SE           0.791 0.0924
##  5 LmSp        0     SE           1.00  0.961
##  6 LmSp        20    SE           0.904 0.399
##  7 LmSp        40    SE           0.781 0.0698
##  8 LmSp        60    SE           0.950 0.571
##  9 LmSpLt      0     SE           0.999 0.937
## 10 LmSpLt      20    SE           0.896 0.372
## # i 34 more rows
```

```
#All response vars follow a normal distribution when considered by every level of the factor Days
```

Test

```
#Compute many repeated measures ANOVAs, one per different Composition for NE, CE and SE seperately
NE_Anova <- NE_data %>%
  group_by(Composition) %>%
  anova_test(dv = NE, wid = Block, within = Days) %>%
  adjust_pvalue(method="fdr") %>%
  add_significance("p.adj") %>%
  get_anova_table()
data.frame(NE_Anova)
```

```
##    Composition Effect DFn DFd      F        p p..05   ges       p.adj
## 1         LmLt   Days   3   6 87.501 2.42e-05     * 0.977 0.000133100
## 2         LmSp   Days   3   6  0.359 7.85e-01       0.120 0.785000000
## 3       LmSpLt   Days   3   6 38.394 2.61e-04     * 0.919 0.000797500
## 4       LmSpWc   Days   3   6  1.056 4.34e-01       0.300 0.477400000
## 5     LmSpWcLt   Days   3   6  8.597 1.40e-02     * 0.776 0.019250000
## 6         LmWc   Days   3   6  2.179 1.91e-01       0.494 0.233444444
## 7       LmWcLt   Days   3   6 16.994 2.00e-03     * 0.856 0.004400000
## 8         SpLt   Days   3   6 36.975 2.90e-04     * 0.930 0.000797500
## 9         SpWc   Days   3   6  9.208 1.20e-02     * 0.798 0.018857143
## 10      SpWcLt   Days   3   6 13.346 5.00e-03     * 0.839 0.009166667
## 11        WcLt   Days   3   6 95.060 1.90e-05     * 0.973 0.000133100
##    p.adj.signif
## 1           ***
## 2            ns
## 3           ***
## 4            ns
## 5             *
## 6            ns
```

```
## 7            **
## 8           ***
## 9             *
## 10            **
## 11          ***
```

```
result<-anova_test(data=NE_data[NE_data$Composition=="LmLt", ], dv = NE, wid = Block, within = Days)

CE_Anova <- NE_data %>%
  group_by(Composition) %>%
  anova_test(dv = CE, wid = Block, within = Days) %>%
  adjust_pvalue(method="fdr") %>%
  add_significance("p.adj") %>%
  get_anova_table()
data.frame(CE_Anova)
```

```
##    Composition Effect DFn DFd       F        p p..05   ges       p.adj
## 1         LmLt   Days   3   6 172.461 3.28e-06     * 0.978 0.000036080
## 2         LmSp   Days   3   6   0.966 4.68e-01       0.245 0.468000000
## 3       LmSpLt   Days   3   6  15.609 3.00e-03     * 0.850 0.006600000
## 4       LmSpWc   Days   3   6   1.430 3.24e-01       0.397 0.356400000
## 5     LmSpWcLt   Days   3   6  22.448 1.00e-03     * 0.871 0.002750000
## 6         LmWc   Days   3   6   2.220 1.87e-01       0.500 0.228555556
## 7       LmWcLt   Days   3   6   6.028 3.00e-02     * 0.665 0.041250000
## 8         SpLt   Days   3   6  62.179 6.54e-05     * 0.962 0.000359700
## 9         SpWc   Days   3   6  12.104 6.00e-03     * 0.832 0.011000000
## 10      SpWcLt   Days   3   6   9.463 1.10e-02     * 0.811 0.017285714
## 11        WcLt   Days   3   6  31.134 4.70e-04     * 0.893 0.001723333
##    p.adj.signif
## 1          ****
## 2            ns
## 3            **
## 4            ns
## 5            **
## 6            ns
## 7             *
## 8           ***
## 9             *
## 10            *
## 11           **
```

```
SE_Anova <- NE_data %>%
  group_by(Composition) %>%
  anova_test(dv = SE, wid = Block, within = Days) %>%
  adjust_pvalue(method="fdr") %>%
  add_significance("p.adj") %>%
  get_anova_table()
data.frame(SE_Anova)
```

```
##    Composition Effect DFn DFd       F        p p..05   ges       p.adj
## 1         LmLt   Days   3   6  31.466 4.56e-04     * 0.892 0.002508000
## 2         LmSp   Days   3   6   6.452 2.60e-02     * 0.742 0.057200000
## 3       LmSpLt   Days   3   6   9.996 9.00e-03     * 0.799 0.024750000
## 4       LmSpWc   Days   3   6   0.229 8.73e-01       0.074 0.873000000
## 5     LmSpWcLt   Days   3   6   0.986 4.60e-01       0.284 0.632500000
```

```
## 6         LmWc   Days   3   6   0.252 8.57e-01       0.098 0.873000000
## 7       LmWcLt   Days   3   6   1.897 2.31e-01       0.400 0.363000000
## 8         SpLt   Days   3   6  21.095 1.00e-03     * 0.894 0.003666667
## 9         SpWc   Days   3   6   0.435 7.36e-01       0.162 0.873000000
## 10      SpWcLt   Days   3   6   3.158 1.07e-01       0.547 0.196166667
## 11        WcLt   Days   3   6 145.264 5.45e-06     * 0.986 0.000059950
##    p.adj.signif
## 1            **
## 2            ns
## 3             *
## 4            ns
## 5            ns
## 6            ns
## 7            ns
## 8            **
## 9            ns
## 10           ns
## 11         ****
```

Plot the relative contribution of species to the average biomass on day 60 of the experiment

```
Biom_data<-read.csv(here("data", "Relativebiomass_data.csv"), sep=";")
#info on data
#individual mass : in mg
#mean LA = mean leaf area : in mg
#mean SLA = mean specific leaf area : in mm2/mg
#initial relbiomass : in mg
#final relbiomass: in mg

#Mean biomass per species data
Plot_species<-Biom_data[Biom_data$Days==60,] %>%
  group_by(Mixture, Species) %>%
  mutate(mean_fin_relbiomass=mean(Final_relbiomass)) %>%
  mutate(standard_error=sd(Final_relbiomass)/sqrt(n())) %>%  #calculates standard error in biomass of e
  dplyr::select(., 3:5, 11, 12) %>%
  ungroup() %>%
  unique()

Plot_species$Mixture<-fct_relevel(Plot_species$Mixture, c("Lm", "Sp", "Wc", "Lt", "LmSp", "LmWc", "LmLt

#Total biomass & standard error data
Errorbar_data_totalbiom<-Biom_data[Biom_data$Days==60,] %>%
  group_by(Block, Mixture) %>%
  mutate(tot_biomass_per_blockmix=sum(Final_relbiomass)) %>%
  ungroup() %>%
  dplyr::select(., 2, 3, 11) %>%
  unique() %>%
  group_by(Mixture) %>%
  mutate(mean_tot_biomass=mean(tot_biomass_per_blockmix)) %>%
  mutate(sd_tot_biomass=sd(tot_biomass_per_blockmix)/sqrt(n())) %>%
  dplyr::select(., 2, 4, 5) %>%
  unique() %>%
  ungroup
```

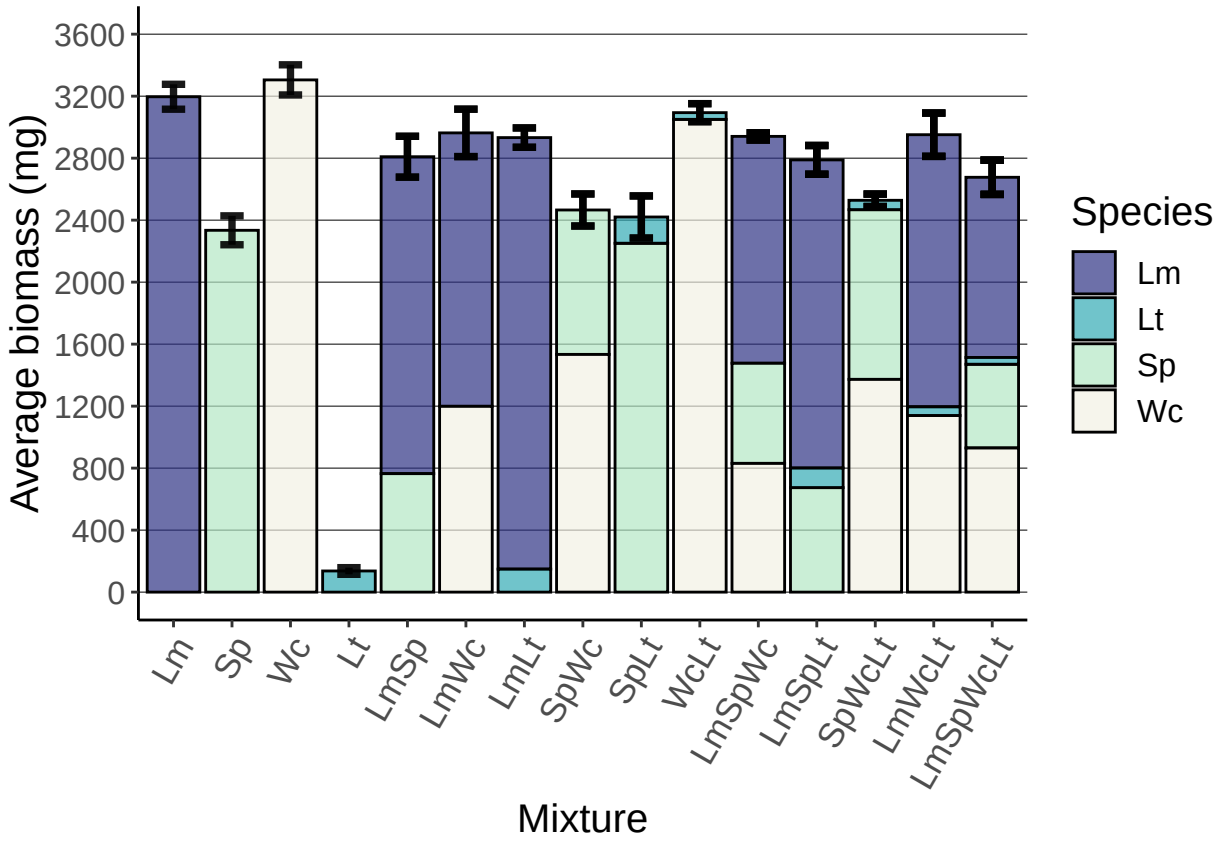
```r
#Significance in net effect data
Plotdat_fin<-merge(Plot_species, Errorbar_data_totalbiom, by="Mixture") %>%
  unique()
Plotdat_fin$mean_errorbar_sum<-Plotdat_fin$mean_tot_biomass+Plotdat_fin$sd_tot_biomass

p<-ggplot(data=Plotdat_fin, aes(fill=Species, y=mean_fin_relbiomass, x=Mixture)) +
    geom_bar(position="stack", stat="identity", colour="black") +
    scale_fill_manual(values=alpha(c("#2D3184", "#32AAB5", "#B3E7C5", "#F3F1E4"), 0.7)) +
    theme_classic() +
    theme(text=element_text(size=15), axis.text.x = element_text(size = 13, angle = 60, hjust=1), panel
    labs(x="Mixture", y="Average biomass (mg)", pch=8) +
    scale_y_continuous(limits=c(0, 3600), breaks=seq(0, 3600, by=400)) +
    geom_errorbar(aes(x=Mixture, ymin=mean_tot_biomass-sd_tot_biomass, ymax=mean_tot_biomass+sd_tot_bio
```

```
## Warning: The `size` argument of `element_line()` is deprecated as of ggplot2 3.4.0.
## i Please use the `linewidth` argument instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```
## Warning: Using `size` aesthetic for lines was deprecated in ggplot2 3.4.0.
## i Please use `linewidth` instead.
## This warning is displayed once every 8 hours.
## Call `lifecycle::last_lifecycle_warnings()` to see where this warning was
## generated.
```

```r
  # Add stars based on significance levels; LmLt, SpWc, SpLt, WcLt, LmSpLt, SpWcLt, LmWcLt, LmSpWcLt
  #annotate(
    #"text",
    #x = c(7, 8, 9, 10, 12, 13, 14, 15),  # x-coordinates where stars should be placed
    #y = c(3105.0551, 2668.9939, 2656.2304, 3251.6792, 2981.5882, 2668.1739, 3191.0420, 2887.1803),
    #label = c("***", "*", "***", "***", "***", "**", "**", "*"),  # significance labels
    #size = 6,  # adjust the size of the stars
    #color = "black"  # color of the stars
  #)
p
```


Average biomass (mg)
3600
3200
2800
2400
2000
1600
1200
800
400
0
Lm
Sp
Wc
Lt
LmSp
LmWc
LmLt
SpWc
SpLt
WcLt
LmSpWc
LmSpLt
SpWcLt
LmWcLt
LmSpWcLt
Mixture
Species
Lm
Lt
Sp
Wc