Individual Project Preliminary Analyses

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4/27/2022

This R script is used to clean up and transform soil data to perform regression analysis.

Overview of Goals

My research focuses on how soil health and soil microbiology influence long-term sustainability in semiarid wheat systems. Wheat is one of the most important crops worldwide, yet the semiarid landscapes where it is primarily grown are particularly vulnerable to climate change and land degradation (Asseng et al. 2015; USGCRP 2018). In particular, the traditional wheat-fallow system practiced throughout the High Plains ecoregion inefficiently stores water, depletes soil fertility, and has a high potential for erosion, pushing farmers and researchers to search for economical ways to build long-term soil health (Norton, Mukhwana, and Norton 2012; Kaur et al. 2015). Sustaining agriculture in the High Plains will require alternative farming systems that can rebuild soil health while remaining profitable (Hansen et al. 2012).

Soil health is critical for sustainable and resilient food production, yet evaluating soil health in semiarid climates remains challenging. Agriculture impacts soil health through practices such as crop rotation, fallowing, tillage, and fertilization, though these effects vary depending on environmental factors such as climate and soil type. Soil microorganisms are sensitive indicators of changing soil health, and can help evaluate the long-term sustainability of land management practices (Rodgers, Norton, and Diepen 2021). However, there are gaps in our understanding of how soil microbial properties should be interpreted, especially in semiarid systems.

This project uses soil data from an experiment on the long-term impacts of compost application on soil health and soil microbiology in Wyoming High Plains organic wheat systems. Specifically, four rates of compost was applied to small plots in a randomized complete block design in either 2016 or 2021. In contrast to fresh manure or chemical fertilizers, composted manure is made of concentrated, stabilized nutrients and carbon that release slowly over time and improve soil physical properties (Larney et al. 2006). This includes data on soil physical properties (such as aggregate stability and bulk density), soil chemical properties (such as total organic matter, labile carbon and nitrogen pools, and pH) and soil microbiology (enzyme activity, microbial biomass, and microbial community composition). I aim to use regression analysis to evaluate the relationships between compost rate and soil properties. However, many of the variables do not meet the regression assumptions. Therefore, this project focuses on cleaning and tidying my dataset, testing the regression assumptions on each variable, transforming the data to meet those assumptions, and finally visualizing the data. Ultimately, quantifying soil health benefits over many years could help growers work towards building the long-term health of their soil, and could guide efforts to protect soils and create an agricultural system resistant in the face of climate change and land degradation.

Load Packages

```
library(MASS)
library(readxl)
```

```
library(writexl)
library(car)
library(tidyverse)
```

Import Data

```
#read in data
OREI_soil <- read_excel("OREI_05.2021.xlsx", sheet = "Soil_Data")
OREI_treatments <- read_excel("OREI_05.2021.xlsx", sheet = "Treatment_Data_2021")
OREI_enzymes <- read_excel("OREI_05.2021.xlsx", sheet = "Enzymes")
OREI_PLFA <- read_excel("OREI_05.2021.xlsx", sheet = "PLFAs")
```

Clean Up Data

```
#merge the data
OREI_all <- OREI_treatments %>%
  left_join(OREI_soil, by = 'Sample_ID') %>%
  left_join(OREI_enzymes, by = 'Sample_ID') %>%
  left_join(OREI_PLFA, by = 'Sample_ID')

OREI_all <- OREI_all %>%
#remove plots that are fallow or fertilized
  filter(Rotation == "wheat", Treatment != "fertilizer") %>%

#remove unwanted variables
 dplyr::select(-PER1, -ID, -InorganicC, -Treatment, -Compost_Rate,
         -Crop, -Rotation, -H2O, -BulkDensity)

#separate into two groups by year of compost application
OREI_2016 <- subset(OREI_all, Compost_Year != "2020")
OREI_2020 <- subset(OREI_all, Compost_Year != "2016")
```

Remove Outliers

Outliers can skew a regression, so first I'll remove any outliers from each variable using the outlierKB function.

```
#I ran this function on each variable in both OREI_2016 and OREI_2020 to
#remove outliers.
source("http://goo.gl/UUyEzD")
  #outlierKD(OREI_2020, total_bacteria)

#save no_outlier data
  #write_xlsx(OREI_2020, "OREI_2020_no_outliers.xlsx")
  #write_xlsx(OREI_2016, "OREI_2016_no_outliers.xlsx")

#read in no_outlier data to continue
OREI_2020 <- as.data.frame(read_excel("OREI_2020_no_outliers.xlsx"))
OREI_2016 <- as.data.frame(read_excel("OREI_2016_no_outliers.xlsx"))
```

I removed outliers from OREI_2016: yield, NO3, PMN, DON, MBC, CBH, PHOS, NAG, actino, gram_pos, AMF, sapro_fungi, total_MB, total_fungi, and from OREI_2020: NO3, protein, MBC, MBN, SOC, N, NAG, BX, AG, SUL

Check Regression Assumptions

The four main assumptions of regression analysis are:

1. Observations are independent.
2. Normality. The residuals are normally distributed.
3. Linearity. The relationship between X and Y is linear.
4. Homoscedasticity. The residuals have constant variance for all values of X.

1. The samples come from randomized plots, so they are independent.

2. Check for normality of residuals.

```
#Create an empty list
p.vals <- list()

#This loop runs a linear model on compost ~ each variable,
#evaluates normality with the Shapiro test, and saves the p value.
for (i in names(OREI_2016[,7:38])) {
  mod <- lm(get(i) ~ compost, data = OREI_2016)
  p.vals[[i]] <- (shapiro.test(mod$residuals))$p.value }
```

The following variables have non-normal residuals ($p > 0.05$): OREI_2020: DOC, DON, PHOS OREI_2016: PHOS

Transform the non-normal variables.

```
#this function performs a box_cox transformation on a variable to make it normal
box_cox_transform <- function(v) {

#calculates the boxcox plot and pulls out lambda
  bc <- boxcox(v ~ compost, data = OREI_2016)
  lambda <- bc$x[which.max(bc$y)]

#transforms the data using lambda and saves it
  v <- (v^lambda-1)/lambda
  return(v) }

#run box_cox_transform on all non-normal variables
OREI_2016$PHOS <- box_cox_transform(OREI_2016$PHOS)
```

```
OREI_2020$PHOS <- box_cox_transform(OREI_2020$PHOS)
```

```
OREI_2020$DOC <- box_cox_transform(OREI_2020$DOC)
```

```
OREI_2020$DON <- box_cox_transform(OREI_2020$DON)
```

```
#test normality of new data using same loop from above
p.vals.trans <- list()
for (i in names(OREI_2016[,7:38])) {
  mod <- lm(get(i) ~ compost, data = OREI_2016)
  p.vals.trans[[i]] <- (shapiro.test(mod$residuals))$p.value }
```

All the variables are normal now! ($p > 0.05$)

3. Check linearity using an F-test for lack of fit.

```
#This function creates a linear and quadratic model for each variable,
#then tests whether the two models differ significantly using ANOVA
F_test <- function(x) {
  mod <- lm(x ~ compost, data = OREI_2016)
  reduced<-lm(x ~ compost, data = OREI_2016)
  full<-lm(x ~ poly(compost,2), data = OREI_2016)
  return(anova(reduced, full)$"Pr(>F)") }

#run this function on each variable
F.test.2016 <- list()
for (i in colnames(OREI_2016[,7:38])) {
  F.test.2016[[i]] <- F_test(OREI_2016[[i]]) }

F.test.2020 <- list()
for (i in colnames(OREI_2020[,7:38])) {
  F.test.2020[[i]] <- F_test(OREI_2020[[i]]) }
```

The regression differs significantly from linear ($p>0.05$) for: OREI_2020: DOC, protein, porosity, WFPS

4. Check constancy of residuals with the Levene test.

```
#Run the levene test (from car package) on each variable,
#then save the p values
levene.2016 <- list()
for (i in colnames(OREI_2016[,7:38])) {
  result <- leveneTest((OREI_2016[[i]]) ~ as.factor(OREI_2016$compost))
  levene.2016[[i]] <- result$`Pr(>F)`[1] }

levene.2020 <- list()
for (i in colnames(OREI_2020[,7:38])) {
  result <- leveneTest((OREI_2020[[i]]) ~ as.factor(OREI_2020$compost))
  levene.2020[[i]] <- result$`Pr(>F)`[1] }
```

All data passes the levene test! ($p < 0.05$)

Visualize Data

This section needs some work, but for now I'll just print some plots.

```
p1 <- ggplot(data= OREI_2016, aes(x = compost, y = DOC))+
  geom_point() +
  geom_smooth(method = 'lm') +
  labs (title = 'One Year After Compost Application',
        x = "Compost Rate (Mg/ha)", y = "DOC (mg/kg)")

p2 <- ggplot(data= OREI_2020, aes(compost, DOC)) +
  geom_point() +
  geom_smooth(method='lm') +
  labs (title = 'Five Years After Compost Application',
        x = "Compost Rate (Mg/ha)", y = "DOC (mg/kg)")

library(patchwork)
```

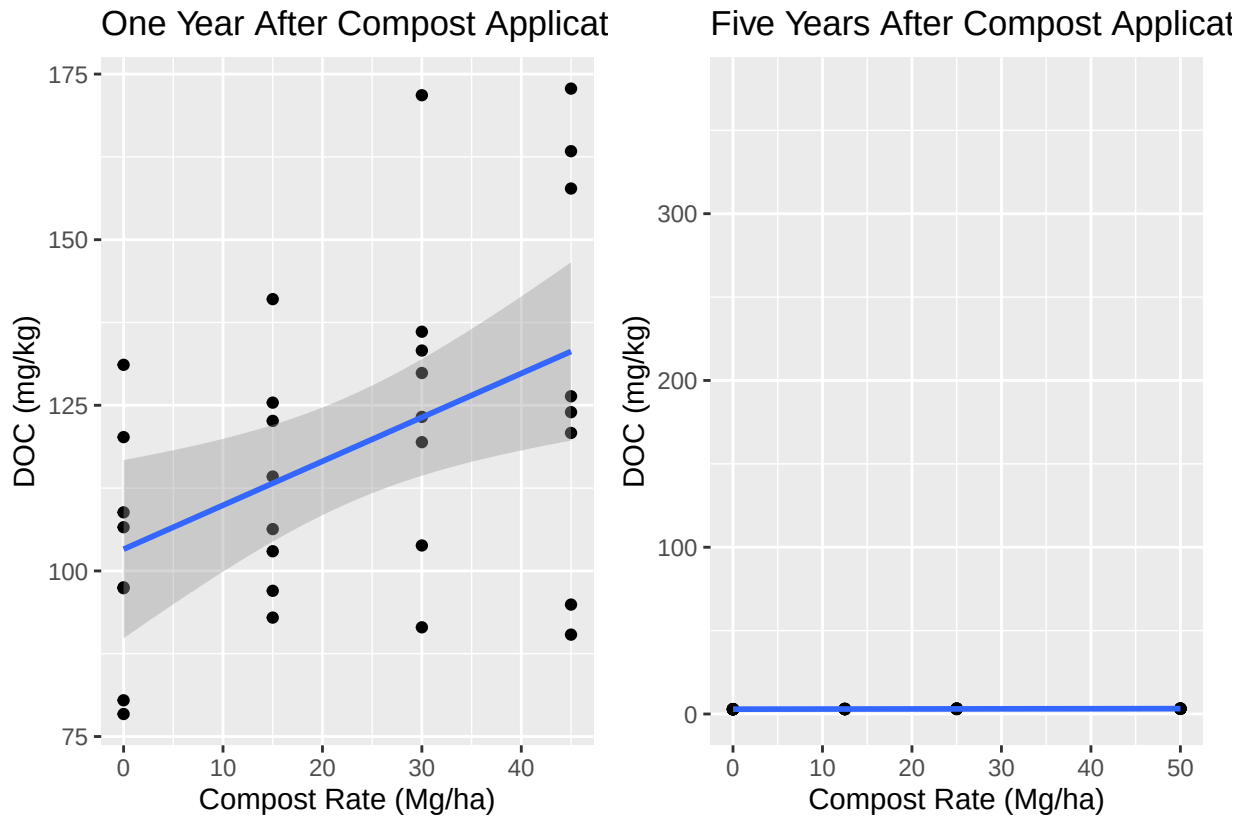
```
##
## Attaching package: 'patchwork'
```

```
## The following object is masked from 'package:MASS':
##
##     area
```

```
p1 + p2 + scale_y_continuous(limits = c(0, 375))
```

```
## `geom_smooth()` using formula 'y ~ x'
```

```
## `geom_smooth()` using formula 'y ~ x'
```



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