Impacts of Regenerative Agricultural Practices on Soil Arthropods in Midwestern Crop Fields

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Introduction

Agricultural soil is a vital natural resource that can be greatly impacted by management practices. Although fertile topsoil takes considerable time to regenerate, much of the focus in modern agricultural research has been directed toward ever-increasing yields rather than long-term sustainability. Consequently, high yields have often come at the cost of soil health, with soils becoming eroded, depleted of nutrients, compromised in structure, and lacking the microfauna necessary to maintain a balanced soil ecosystem (Schreefel et al., 2020). Conventional tillage practices allow for rapid loss of soil organic matter and soil erosion; likewise, the overuse of fertilizer inputs allows for leaching nutrients into groundwater supplies (Doran, 2002). Additionally, each of these conventional field practices comes with high input costs, including tillage equipment, chemicals, machinery, and labor (Al-Kaisi & Lal, 2020).

As it relates to the soil microfauna, conventional agricultural practices have been found to reduce soil biodiversity (Wagg et al., 2014). However, soil organisms are important in regulating soil health in agricultural fields (Chamorro-Martínez et al., 2022; Parisi & Menta, 2008). A plethora of microorganisms perform various tasks, from decomposing pollutants to recycling nutrients and augmenting root efficiency (Dobrovol'skaya et al., 2015). Earthworms can increase soil aeration and water transport, while arthropods play a large role in breaking down organic matter (Culliney, 2013). Collectively, soil organisms play a considerable role in sustaining the long-term productivity and health of agricultural soils.

Collembola are an order of hexapods colloquially known as springtails. As abundant and ubiquitous members of the soil microfauna, collembolans are key participants in the soil food web, which drives carbon and nitrogen cycling in soil (Coulibaly et al., 2019). They are also often used to indicate overall soil health (de Oliveira Filho et al., 2020). Nonetheless, the impacts of regenerative agricultural practices on Collembola in Midwestern agricultural fields remain poorly understood, as most research on the topic has been conducted in European countries (Dulaurent et al., 2023). In this research, I therefore chose to focus on collembolan and microfaunal biodiversity here in the Midwestern United States' agricultural systems.

In this study, I collected data on the diversity and abundance of collembolan families, as well as the abundance of Acari (mite) individuals. This research was carried out to provide a better understanding of regenerative practices' impact on microfaunal diversity and abundance, with the hope that the knowledge gained will aid farmers, land managers, and regulatory agencies in determining which regenerative practices show the most promise for lasting impact and implementation. I hypothesized that in Midwestern crop fields, Collembola and Acari would show a higher level of diversity and abundance in regeneratively managed fields compared to conventionally managed fields as a result of their regenerative score, crop diversity, cover crop use, tillage regime, synthetic fertility amendments, and pesticide use. Furthermore, I expected

some regenerative practices to be more impactful than others on collembolan populations due to varying levels of ecosystem disruption between practices.

Materials and Methods

Field Sampling

Nine commercial agricultural fields were selected based on a point-system scoring of regenerative practices, including evaluations of crop diversity, cover crop use, tillage, fertility, pesticide use, livestock integration, in-field perennialization, and extra-field perennialization. A score of 14 was the maximum possible regenerative ranking, with a higher score indicating an increased use of regenerative practices. Each regenerative practice was given a rank number based on the extent of their implementation, generally low, medium, or high (see Table 1. A.)). The selected fields ranged in regenerative scores from 2 to 11.

These fields were located across central Indiana in Benton, Delaware, Fountain, Henry, and Tippecanoe counties and ranged from 25 to 200 acres (see Table 1. B.)). I collected six soil subsamples from each field using a 4" diameter hole cutter to a depth of 4", yielding a subsample volume of approximately 50.27 cubic inches. The six sampling locations in each field were selected semi-randomly, using a randomized number of steps (0-200) from the field edge or from established collection sites for a preexisting study. Using the GPS Tracks app, I recorded each sample location and attached it to a unique sample ID number. I then placed each subsample in an individual airtight plastic freezer bag and brought them to the lab for arthropod extraction within six hours of field collection.

Scoring Procedure			
Crop diversity	Low >2, 1 pt	Medium 3-4, pts	High <5, 3 pts
Cover crop use	Never, 0 pts	Low/sporadic use, 1 pt	High/always used, 2 pts
Tillage	No 6 yrs, 2 pts	Low, 1 pt	High, 0 pts
Fertility ammendments	No, 2 pts	Low, 1 pt	High, 0 pts
Pesticides	No, 2 pts	Low, 1 pt	High, 0 pts
Livestock integration	No, 0 pts	Low, 1 pt	High, 2 pts
In-field perennialization	No, 0 pts	Yes, 1 pt	
Extra-field perennialization	No, 0 pts	Yes, 1 pt	

A.

Crop	Sample date	Sample stage	Acres	Crop div	CC use	Tillage	Fertility	Pesticides	Regen Score
Soybeans	9/18/23	R6-R7	50	1	2	0	0	1	5
Corn	9/18/23	R5-R6	80	1	2	0	0	1	5
Soybeans	9/18/23	R8	140	2	1	1	0	0	6
Corn	9/11/23	R5	200	2	2	1	1	1	9
Soybeans	9/11/23	R6-R7	150	1	1	2	1	0	6
Soybeans	9/11/23	R6-R7	150	1	1	0	0	0	2
Soybeans	9/25/23	R8	50	2	2	1	2	2	11
Hort	9/25/23	Fruiting	25	1	1	0	0	0	3
Hort	9/25/23	Fruiting	140	2	0	0	0	0	4

B.

Table 1. A.) Rubric determining regenerative scoring number for each field management practice. 1. B.) Regenerative data and score for fields included in analysis.

Berlese Funnel Extraction

I used Berlese-Tullgren funnels for arthropod extraction following common practice in soil research (de Oliveira et al., 2021; Ustinova et al., 2021). Soil samples (n=54) were placed individually in mesh-bottomed canisters with funnels underneath, leading to collection vials filled with 70% ethanol (see Fig. 1). Lamps were placed above the samples to drive soil arthropods downward due to the heat. I left these samples in the Berlese-Tullgren funnels for approximately 72 hours. I then labeled each collection vial with location details, time of collection, and its unique subsample ID number.

Figure 1. Berlese-Tullgren funnels used for arthropod extraction.

Data Collection and Analysis

Vials were drained into grid-bottomed Petri dishes and rinsed out with 70% ethanol to ensure none of the specimens or soil remained in the vials. Using a dissection microscope, I counted Collembola and identified them to family according to a dichotomous key (Borror et al., 1989). Additionally, I utilized supplemental identification materials from *The Collembola of North America North of the Rio Grande* (Christiansen & Bellinger, 1980). Acari were also identified, counted, and recorded.

Data were analyzed using Microsoft Excel (v. 2402 Build 16.0.17328.20124) and R (v. 4.3.1) (see Appendix X). To visualize clustering of soil microfaunal communities, I used non-metric multidimensional scaling (NMDS). The metaMDS function in the R package Vegan was used for this analysis, utilizing Bray-Curtis dissimilarity, which is common in invertebrate community analyses. I specified five dimensions in this analysis as I expected regenerative score, crop diversity, cover crop use, tillage regime, synthetic fertility amendments, and pesticide use to strongly influence the resulting dissimilarity matrices. I visualized the NMDS results using the R package ggplot2 according to regenerative score. Stress was measured using the function stressplot in Vegan. To assess the extent to which the variation of assemblage structure could be

related to regenerative score and the four listed environmental factors, I performed a permutational multivariate analysis of variation (PERMANOVA) using the function adonis2 in the R package Vegan with one thousand permutations to adjust for limited sample size.

Results

My analysis showed a significant relationship between the abundance of mites collected and the field's regenerative score. Collembolan abundance and diversity both showed non-significant relationships (see Fig. 2).

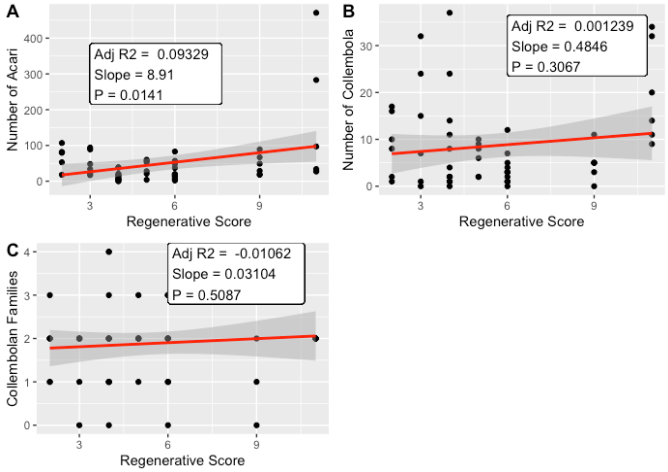

Figure 2. Relationship between mite and collembolan abundance and collembolan diversity with field regenerative score.

NMDS analysis of the entire data set showed some clustering, especially at the extreme ends of the regenerative spectrum (see Fig. 3). The analysis had a stress level of 0.085 and a linear-fit R^2 value of 0.923, indicating a suitable model. Given the shallower classification of mites and their super-abundance compared to all non-mite taxa (see Fig. 4), I also assessed soil microfauna using NMDS analysis of the data set excluding mites (see Fig. 3. B.)). Three samples had only mites, resulting in zero values after their exclusion. Since NMDS cannot run with zero-value samples, these three were omitted out of necessity. This analysis had a comparatively lower stress level of 0.076 and a higher linear R^2 value of 0.936, indicating a better model fit.

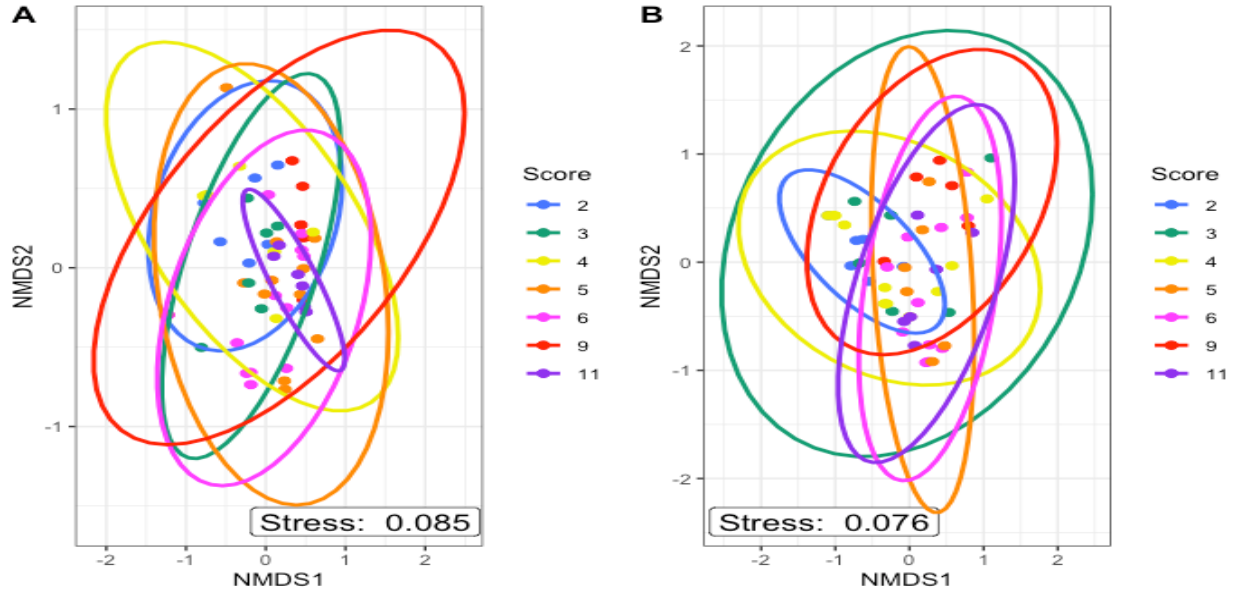

Figure 3. A.) NMDS analysis of the entire data set. B.) NMDS analysis of data set excluding mites.

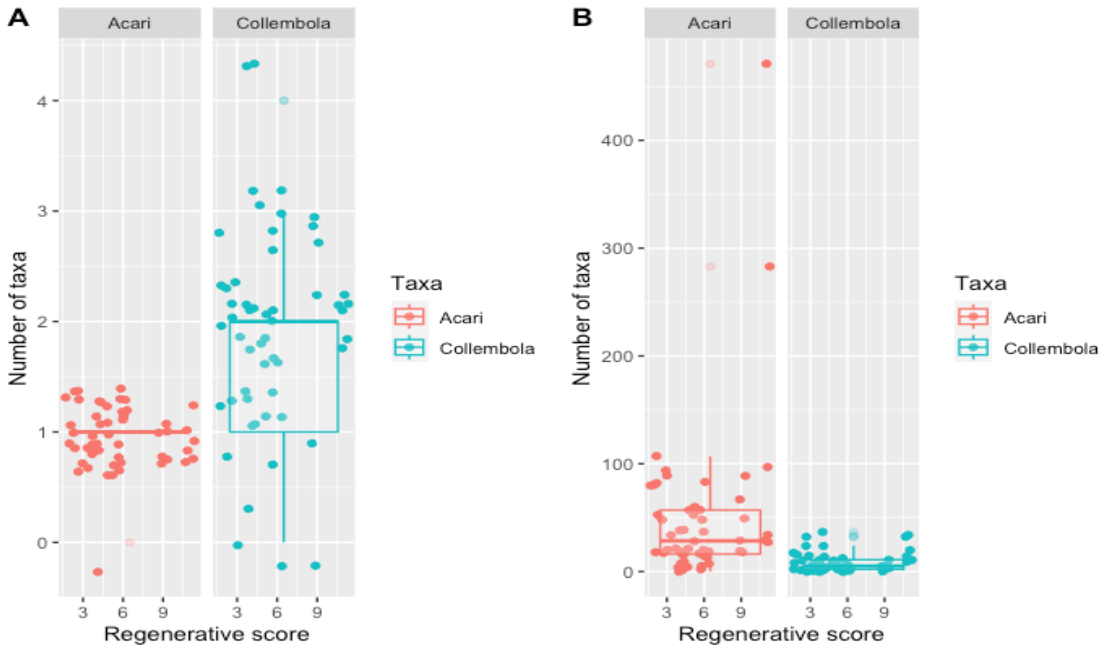

Figure 4. Box plot of sampling data showing differences in identification depth and abundance between mites and collembolans.

Permanova tests were applied to the data set exclusive of mites following the indications of fit. Regenerative score, cover crop use, synthetic fertilizer use, and pesticide use were all statistically significant in influencing community composition (see Table 2). Crop diversity and tillage were non-significant, as was the crop growing in each field at the time of sampling.

Factor	R^2 value	F value	Df
Regenerative Score	0.2228	0.002**	6
Crop Diversity	0.0095	0.869	1
Cover Crop	0.0963	0.004**	2
Tillage	0.0487	0.252	2
Synthetic Fertilizer	0.0896	0.004**	2
Pesticide	0.09565	0.003**	2

*Table 2. Results from Adonis Permutational Multivariate Analysis of Variation on Collembola data; ** Indicates statistical significance at $p<0.005$.*

Discussion and Conclusions

Mite abundance was shown to have a statistically significant positive relationship with regenerative score. As expected, this shows regenerative practices benefit mite populations. Conversely, both collembolan abundance and diversity showed no significant relationship with overall field regenerative score. This may be due to taxonomic resolution – because collembolans were only identified to family, there may have been greater diversity at the genus or species levels that remained undiscovered. However, the soil microfauna is challenging to identify, given their small size, their cryptic morphology, and the current lack of subject-matter expertise, making finer taxonomic resolution fall beyond the scope of this project (Young & Hebert, 2022). Collembola may also be less sensitive than Acari to the regenerative practices

included in this study, meaning that mites would more readily show a significant difference in abundance between regenerative and conventional management practices.

NDMS analysis of the entire dataset indicated some grouping according to regenerative score (see Fig. 3. A.)). However, there was significant overlap in communities, suggesting they were not discrete and shared many characteristics, i.e., taxa. As before, the observed overlap may have been due to identifying collembolans to family and other soil arthropods only to subclass. This resolution level may not have detected some of the community differences that would have been apparent at the genus or species level. Additionally, I found unequal variation between numbers of Acari and Collembola at both the diversity and abundance levels. Mites displayed abundance levels that were typically one to two orders of magnitude larger than collembolans, which may have obscured some community differences (see Fig. 4). To investigate whether mite super-abundance was masking community differences I ran a second NMDS on the data set with mites omitted (see Fig. 3. B.)). This analysis resulted in a better model and slightly better separation, though there was still less than I expected. The continued community convergence could indicate that collembolan diversity was, in fact, obscured by insufficient taxonomic resolution.

The PERMANOVA test, applied to investigate which environmental factors were driving community composition, indicated that regenerative score was the strongest driver of variance in collembolan community structure, followed by cover crops and agrochemical usage. The R^2 values for all statistically significant environmental factors are low, though this is not surprising in ecological or agroecological studies. The low values emphasize the complexity of factors driving community assemblage within agroecosystems, such as variations in soil biotic and abiotic factors, crop species, and penology.

Of the factors evaluated with PERMANOVA, regenerative score was found to have the highest R^2 value, indicating that it explained the most variation among the communities studied. This suggests that a holistic view of regenerative agriculture may be the most accurate; instead of one factor being a sufficient explanation of variance, it seems that soil microfauna responds to the interactions of many different stimuli. Consequently, it is reasonable to infer that no single alteration to agriculture practices will be a ‘silver bullet’ solution to improve soil biodiversity. Rather, a concerted effort across multiple field management strategies may be needed to improve long-term soil health.

Pesticide use was also a statistically significant driver of collembolan community structure. This is unsurprising, given that many pesticides are applied as a liquid broadcast spray and prone to overspray onto the soil, impacting soil arthropod populations. Non-target effects of insecticides have long been under scrutiny. While the primary focus of pesticide research is reducing mammalian, and, consequently, human toxicity, recent awareness of their adverse effects on pollinators has resulted in a push to minimize insecticides’ impact on non-pest species. Chemistries are becoming increasingly specific to pest taxon levels, and current research increasingly advocates for integrated pest management, only suggesting pesticide application when it is necessary, such as when pest populations exceed an economic threshold. Pesticides’ significance suggests that integrated pest management may be a key practice in improving soil

biodiversity. Nonetheless, the relationship between pesticide use and microfaunal communities should be further articulated. For instance, future research could be conducted specifically to determine pesticide impact on soil microfauna, focusing on comparison of 1. different pesticide chemistries, and 2. varying application regimes (i.e., scheduled application vs application at economic threshold).

I also found synthetic fertilizer amendments to inform collembolan community structure. Fertilizers affect soil fauna both directly and indirectly. As these agrochemicals are applied directly to the soil, soil arthropods are also physically exposed. Furthermore, synthetic fertilizer use alters the abiotic and biotic conditions of the soil, and microfauna are sensitive to these alterations (Fratello et al., 1989; Gbarakoro & Abajue, 2023). As with pesticide inputs, this relationship could benefit from further analysis. For example, the nutrient requirements in corn and soybean fields are quite different due to differences in the plant biology of these two crops. Therefore, future research could focus on different fertilizer practices in a single crop type and later compare those data with other crops and their unique nutrient requirements. It could also be useful to determine the impacts of different application methods on microfaunal biodiversity (e.g., broadcasting vs. soil injection).

The last significant driver of collembolan community variation was cover crop use. In non-agricultural systems, the importance and interrelatedness of biodiversity is often stressed, with greater plant diversity often leading to greater biodiversity throughout an ecosystem's food web. This aphorism has been successfully applied to agroecosystems as well, with diverse cover crops leading to increases in arthropod diversity and abundance (Jabbour et al., 2016). However, the exact relationship between cover crop management and soil arthropod diversity remains elusive (Inveninato Carmona et al., 2021). In the future, research could clarify the relationship between cover crop diversity and collembolan biodiversity. While this study offers valuable insight into real-world interactions, future research could also focus on fields that only vary in the use of cover crops as opposed to the multivariate differences in management practices investigated herein. Alternatively, sufficiently large fields could be divided in half and used to compare between cover crop and non-cover crop usage.

The findings of this study emphasize the importance of regenerative agriculture's impact on soil arthropod abundance and underscore the utility of regenerative scoring. Multiple agricultural practices were shown to significantly influence community composition, and viewing them as a whole yielded the most biologically applicable information. The ability to assign a numerical value for the regenerative nature of a field that is ecologically relevant facilitates future evaluation and comparison. While varied regenerative scoring schemes exist, they remain largely theoretical (Fenster et al., 2021). This study provides some proof of utility for the scoring scheme used.

Collectively, each significant driver in this study (regenerative score, pesticide use, synthetic fertilizer amendments, and cover crop use) presents itself as a tremendous opportunity for future research. Although the body of research on regenerative agriculture continues to grow, there is still much that remains uncertain, particularly in relation to actual costs and benefits to commercial farmers. On a practical level, regenerative agricultural practices are difficult to

implement if they are not clearly defined and do not provide tangible benefits to the farmer (Thompson et al., 2021). Because of the often-narrow profit margins in farming, implementing these practices may not be immediately feasible due to economic constraints. Nonetheless, the more research is conducted and knowledge is gained, the more farmers and land managers can be certain of these management practices' benefits. Continued research into these drivers of soil biodiversity and their resulting ecosystem services would do much to streamline the decision-making process for farmers and potentially bolster adoption of regenerative agriculture, increasing sustainability and prosperity for farmers and rural communities.

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Appendix A

R code used for all analyses is included here. Associated data will be provided upon request.

```
##########---------##########
###     Capstone Stats    ###
##########----------#########

#open libraries
library(vegan)
library(ggplot2)
library(dplyr)

#input file
regen <- read.csv("Dane Capstone Tidy LM.csv", na.strings = c("","NA"), header=TRUE)

#look at variables
str(mites)
#format variables as factors
regen$Site <- as.factor(regen$Site)
regen$Taxa <- as.factor(regen$Taxa)
#rename Regen.Score for brevity
names(regen)[names(regen) == "Regen.Score"] <- "Regen"
#check to make sure everything actually changed
str(regen)

#graph data for taxa
taxa<- ggplot(regen,aes(x=Regen,y=Number,col=Taxa)) + geom_jitter() +
geom_boxplot(alpha=0.2) +
  facet_wrap(~Taxa) +xlab("Regenerative score") + ylab("Number of taxa")
```

```
#graph data for individuals
number<- ggplot(regen,aes(x=Regen,y=Individuals, col=Taxa)) + geom_jitter() +
geom_boxplot(alpha=0.2) +
  facet_wrap(~Taxa) +xlab("Regenerative score") + ylab("Number of taxa")


#putting graphs together
ggarrange(taxa, number,
          labels = c("A", "B"),
          ncol = 2)


##========== Linear Models ==========##
?lm


#input file
mites <- read.csv(file="Dane LM.csv", sep =",", header=T, row.names=1)
#running linear model for number of mites
fitm <- lm(Acari ~ Regen.Score, data = mites)
lm1 <- ggplot(fitm$model, aes_string(x = names(fitm$model)[2], y =
names(fitm$model)[1])) +
  geom_point() +
  stat_smooth(method = "lm", col = "red") + xlab ("Regenerative Score") + ylab ("Number
of Acari") +
  geom_label(aes(x = 3, y = 300), hjust = 0,
             label = paste("Adj R2 = ",signif(summary(fitm)$adj.r.squared, 4),
                           " \nSlope =",signif(fitm$coef[[2]], 4),
                           " \nP =",signif(summary(fitm)$coef[2,4], 4)))
lm1


#running linear model for collembola abundance
fitc <- lm(Total.Collem ~ Regen.Score, data = mites)
```

```
lm2 <- ggplot(fitc$model, aes_string(x = names(fitc$model)[2], y = names(fitc$model)[1])) +
  geom_point() +
  stat_smooth(method = "lm", col = "red") + xlab ("Regenerative Score") + ylab ("Number of Collembola") +
  geom_label(aes(x = 6, y = 30), hjust = 0,
          label = paste("Adj R2 = ",signif(summary(fitc)$adj.r.squared, 4),
                    " \nSlope =",signif(fitc$coef[[2]], 4),
                    " \nP =",signif(summary(fitc)$coef[2,4], 4)))
lm2


#running linear model for collembolan diversity
fitd <- lm(Collembola ~ Regen.Score, data = mites)
lm3 <- ggplot(fitd$model, aes_string(x = names(fitd$model)[2], y = names(fitd$model)[1])) +
  geom_point() +
  stat_smooth(method = "lm", col = "red") + xlab ("Regenerative Score") + ylab ("Collembolan Families") +
  geom_label(aes(x = 6, y = 3.5), hjust = 0,
          label = paste("Adj R2 = ",signif(summary(fitd)$adj.r.squared, 4),
                    " \nSlope =",signif(fitd$coef[[2]], 4),
                    " \nP =",signif(summary(fitd)$coef[2,4], 4)))
lm3


#putting all three graphs together
ggarrange(lm1, lm2, lm3,
        labels = c("A", "B", "C"),
        ncol = 2, nrow = 2)


##========== Running NMDS ==========##
#input file
dcf <- read.csv(file="Book2.csv", sep=",", header=T, row.names=1)
```

```
#look at variables
str(dcf)
#change regen score and environmental variables to factors
dcf$Score <- as.factor(dcf$Score)
dcf$Crop.Div <- as.factor(dcf$Crop.Div)
dcf$CC <- as.factor(dcf$CC)
dcf$Till <- as.factor(dcf$Till)
dcf$Fert <- as.factor(dcf$Fert)
dcf$Pest <- as.factor(dcf$Pest)
dcf$Crop <- as.factor(dcf$Crop)
str(dcf)

#run NMDS
nmds1 <-metaMDS(dcf[,1:12], "bray", 5, autotransform=T)
nmds1
plot(nmds1)
#extract NMDS scores (x and y coordinates)
data.scores = as.data.frame(scores(nmds1)$sites)
#adding back in environmental factors
data.scores1 <- cbind(data.scores, dcf[,13:19])

##========== Plotting NMDS ==========##
dcfNMDSplot<-ggplot(data.scores1,aes(NMDS1, NMDS2, color=Score))+
  geom_point(position=position_jitter(.05), size = 2)+
  annotate(geom = "label", x = 1.25, y = -1.6, size = 5,
           label = paste("Stress: ", round(nmds1$stress, digits = 3))) +
  stat_ellipse(type='norm',size =1)+
  scale_color_manual(values=c("royalblue1", "#009E73", "yellow2", "darkorange",
                              "magenta", "red1", "purple2", "turquoise", "darkblue" ))+
  theme_bw()
```

dcfNMDSplot

```
#sites don't cluster well by site regenerative score, too much overlap except for at extreme ends
#running stressplot
stressplot(nmds1)
#but the stress plot is ok and stress level is acceptable

#let's try with just Collembola, but will need to remove 0 value samples
#creating object with only collembola data
collem <-(dcf[,2:12])
collem
#checking for 0 values
sp.abund <- rowSums(collem)
names(sp.abund)[which(sp.abund<1)]
#indicates Hunt 5, Seif 1, and Park 5 have 0 collembola

#input trimmed file
dcf_tr <- read.csv(file="collem2.csv", sep=",", header=T, row.names=1)
dcf_tr
str(dcf_tr)
#change regen score and environmental variables to factors
dcf_tr$Score <- as.factor(dcf_tr$Score)
dcf_tr$Crop.Div <- as.factor(dcf_tr$Crop.Div)
dcf_tr$CC <- as.factor(dcf_tr$CC)
dcf_tr$Till <- as.factor(dcf_tr$Till)
dcf_tr$Fert <- as.factor(dcf_tr$Fert)
dcf_tr$Pest <- as.factor(dcf_tr$Pest)
dcf_tr$Crop <- as.factor(dcf_tr$Crop)
str(dcf_tr)
```

```
#run NMDS
nmds2 <-metaMDS(dcf_tr[,1:11], "bray", 5, autotransform=T)
nmds2
plot(nmds2)
#extract NMDS scores (x and y coordinates)
data.scores2 = as.data.frame(scores(nmds2)$sites)
#adding back in environmental factors
data.scores3 <- cbind(data.scores2, dcf_tr[,12:18])

#Plot the NMDS
dcf_trNMDSplot<-ggplot(data.scores3,aes(NMDS1, NMDS2, color=Score))+
  geom_point(position=position_jitter(.05), size = 2)+
  annotate(geom = "label", x = -1.15, y = -2.4, size = 5,
           label = paste("Stress: ", round(nmds2$stress, digits = 3))) +
  stat_ellipse(type='norm',size =1)+
  scale_color_manual(values=c("royalblue1", "#009E73", "yellow2", "darkorange",
                              "magenta", "red1", "purple2", "turquoise", "darkblue" ))+
  theme_bw()
dcf_trNMDSplot
#running stress plot
stressplot(nmds2)

#plotting both NMDS figures together

ggarrange(dcfNMDSplot, dcf_trNMDSplot,
          labels = c("A", "B"),
          ncol = 2, nrow = 1)
##========== Adonis =========##
```

```
#create distance matrix
dcf_tr.bc <- vegdist(dcf_tr[,1:11], method="bray")
dcf_tr.bc

#create Regen object with score
Regen <- dcf_tr[c(12:18)]
Regen

#run adonis for score
adonis2(dcf_tr.bc~Score,data=Regen, permutations = 999)
#Score is highly significant (0.001)but low R^2 value (0.223), with 6 df
#Can re-run on additional environmental variables added in
adonis2(dcf_tr.bc~Crop.Div, data=Regen, permutations = 999)
#Non-significant
adonis2(dcf_tr.bc~CC, data=Regen, permutations = 999)
# Significant but less than Score, F-value = 0.003, R^2 = 0.09633
adonis2(dcf_tr.bc~Till, data=Regen, permutations = 999)
#non-significant
adonis2(dcf_tr.bc~Fert, data=Regen, permutations = 999)
#Significant, but less than score, F=0.004, R^2 0.0896, df2
adonis2(dcf_tr.bc~Pest, data=Regen, permutations = 999)
#Significant but less than Score, F=0.003, R^2=0.09565, df 2
adonis2(dcf_tr.bc~Crop, data=Regen, permutations = 999)
#Non-significant
```