

# Impact of Biosolids on Soil Stability and Plant Cover

*Yidie Feng, Qiaoyue Tang, Sonja Surjanovic, Fatema Tuz Jhohura*

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## Summary

This report examines the effectiveness of biosolids, a new kind of fertilizer that potentially enhances soil activity by providing a food source through microbial activities. In particular, we compare the magnitude of Mean Weight Diameter (MWD), a parameter measuring soil stability, with and without biosolids treatment. We also compare the magnitude of cover value, a criterion for the spread of plant species, between observations under biosolids and control. The impact of biosolids on soil stability is analyzed by a mixed effect model. The effect on the spread of plant species is addressed by linear regression, as well as a non-parametric alternative. The correlation between MWD and cover value is also examined for several species. Our results show strong evidence that biosolids significantly increase soil stability, as well as coverage of certain plant species. We also find that, for many of the plant species, there is a negative correlation between MWD and average cover value. This could be due to the growth of one species negatively affecting others. There are also several species for which there is a moderate positive correlation between MWD and average cover value.

## 1. Introduction

Soil quality is an important issue affecting agricultural activities and biodiversity. Our client, Emma Avery, is completing her thesis for Master of Soil Science degree which investigates the long term impact of biosolids on soil health and grassland plant communities. Biosolids, the main interest of the experiment, is able to provided a food source for microbial activities thus enhancing soil stability. The experiment was conducted in 2002 at OK Ranch, Jesmond, BC. Four grasslands were randomly chosen and either applied biosolids or left as control (no biosolids). Later, the data were collected in 2016 and passed on to us in January 2017 for analysis. This report's primary interest is to investigate whether Mean Weight Diameter (MWD), a parameter that quantifies soil productivity, is affected by 1) treatment type and 2) sampling date. The secondary interest is to explore the effect of biosolids on specific plant species composition. The third interest is to examine the correlation between MWD and the cover value of plants. The report assesses the significance of biosolids treatment and indicates that biosolids perform well in terms of enhancing soil stability and increasing the spread of several plant species. Starting with a description of the dataset and methods, the report addresses the above questions of interest with detailed analyses, and ends with conclusions and further discussions.

## 2. Data Description

The experiment was laid out in 4 pieces of lands with similar characteristics which are treated as blocks. The investigator randomly applied biosolids to half of each block and no biosolids to the other half. Within each half of a block, 3 equally spaced transects were arranged. MWD(mm) data were obtained from 7 fixed sample spots along each transect. This process was conducted four times: April, June, August and October 2016 respectively. The dataset given to us includes the sampling month, block index, treatment type, transect number and the MWD averaged from those 7 soil samples for each transect. So the total number of soil samples is 96 (4 sampling dates x 4 blocks x 2 treatments x 3 transects x 1 composite sample per transect).

Figure 2.1 shows the boxplots of MWD under both biosolids and control in 4 sampling dates. In April, August and October, we observe that data points under biosolids have higher MWD than those under control. This can be seen from the upward shift of data points under biosolids in the boxplots. In June, the data points under biosolids overlap with those under control, but the median under biosolids is higher. Table 2.1 shows the mean and standard deviation of MWD for 8 treatment-block combinations.

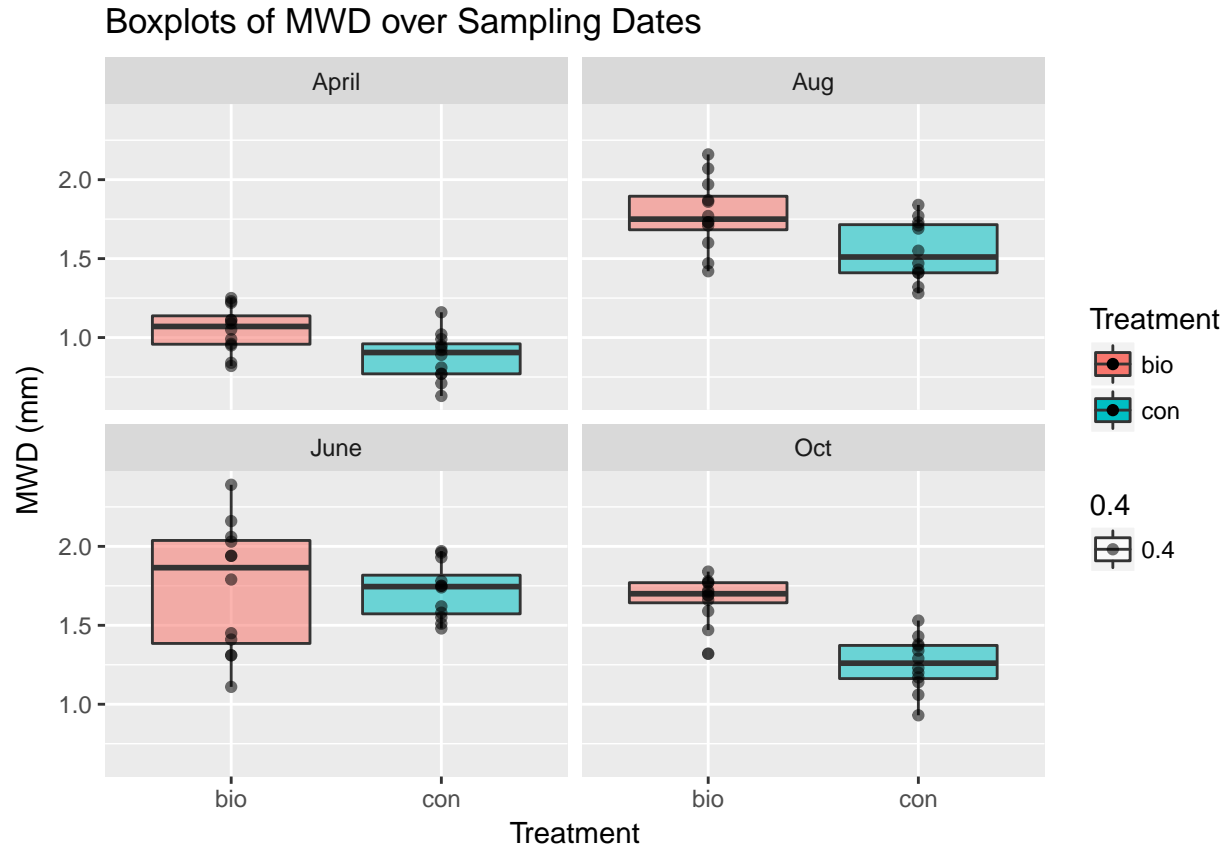


Figure 2.1 Boxplots of MWD of biosolids and control group plotted by the four sampling dates. The four plots represent the four months. MWD is plotted against treatment type (biosolids as pink, control as green) in each month.

```
## Source: local data frame [8 x 4]
## Groups: Date [?]
##
##   Date Treatment `mean(MWD)` `sd(MWD)`
##   <fctr>      <fctr>      <dbl>      <dbl>
## 1 April      bio        1.051667 0.1442115
## 2 April      con        0.880000 0.1479558
## 3 Aug        bio        1.780000 0.2230369
## 4 Aug        con        1.550833 0.1897107
## 5 June       bio        1.741667 0.4076057
## 6 June       con        1.718333 0.1732488
## 7 Oct        bio        1.667500 0.1461086
## 8 Oct        con        1.255833 0.1682238
```

Table 2.1 Mean and standard deviation of MWD by sampling dates and treatment type.

For the investigation of plants, 5 plant transects were laid out in half of a block. The researchers randomly selected 10 plots from each transect. Visual assessment was done for each plot. The species present and the corresponding abundance were recorded. The measurement of plant composition was quantified by cover class, a number from 1 to 6 that assesses the canopy cover of plant species. Cover class was then converted to cover value which is a number in percentage and is in one-to-one correspondence to cover class. For example, cover class 1 represents a cover from 0% to 5%. The midpoint 2.5% is the cover value corresponding to cover class 1. Therefore there are 6 cover values in the dataset: 2.5%, 15%, 37.5%, 62.5%, 85%, 97.5%. The dataset includes the plant species name, block index, treatment type, plot number, cover class and cover value. The

total number of plant assessments is 400 (4 blocks  $\times$  2 treatments  $\times$  5 transects  $\times$  10 samples per transect). If researchers did not observe a species for certain plots, the entry of that species was omitted instead of being recorded as 0. In the following analysis, we specifically investigate one species, *Poa pratensis* (POPR).

The histogram (Figure 2.2) describes the frequencies of cover values under biosolids and control for all species. Biosolids group has more observations with high cover values, while control group has more observations with low cover values. For the specific species POPR, Table 2.2 summarizes the frequencies of observations under 6 levels of cover values for biosolids and control respectively. The counts under two treatments are quite different, indicating that biosolids is potentially effective.

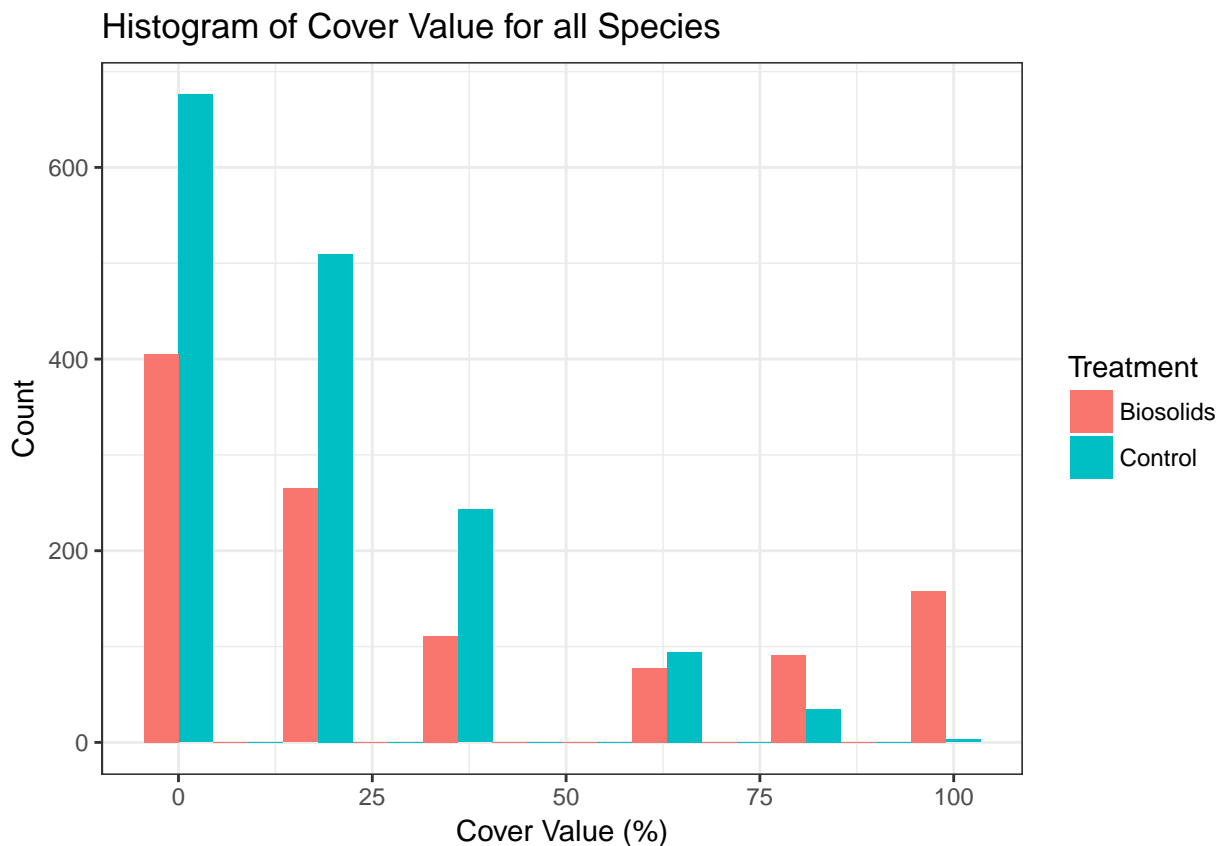


Figure 2.2 Overlapped histogram in each class of cover value for all species. The cover values are 2.5, 15, 37.5, 62.5, 85 and 97.5.

```
## $Biosolids
##
##  2.5  15 37.5 62.5  85 97.5
##  19  28  20  15  16  17
##
## $Control
##
##  2.5  15
##   4   1
```

Table 2.2 Frequencies of observations in each class of cover value presented by treatment groups for species POPR. The cover values are 2.5, 15, 37.5, 62.5, 85 and 97.5.

Figure 2.3 and 2.4 provide visualizations of change and variation in cover values under both biosolids and control for POPR. Each point represents the mean cover value under a specific treatment-block combination. The observed cover values under control group are close to 0 in all four blocks while the cover values under

biosolids are quite high. We also observe that the change in cover value for biosolids group is different in four blocks. But we do not consider any interaction effects involving block because they are not of interest. The error bars show standard errors of mean of cover values. The error bars in control group should have a lower bound of 0 since negative cover values are not intuitive. The standard error in Block 2, Control group has a value of 0 since all observations have 0 cover values.

### Comparison of POPR Cover Values between Biosolids and Control

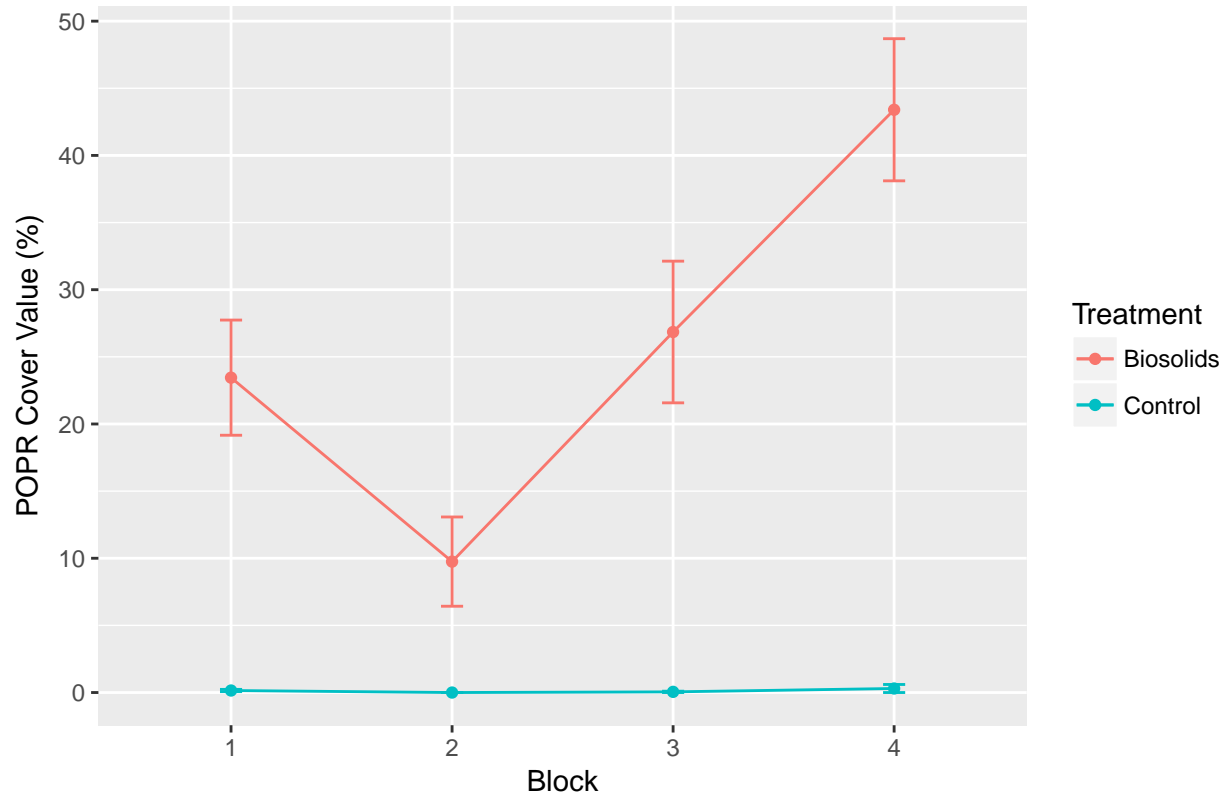


Figure 2.3 Comparison of species POPR cover values between control and biosolids group. Cover values in biosolids group are a lot higher than those in control group for all the blocks. The error bar indicates standard error of each mean cover value.

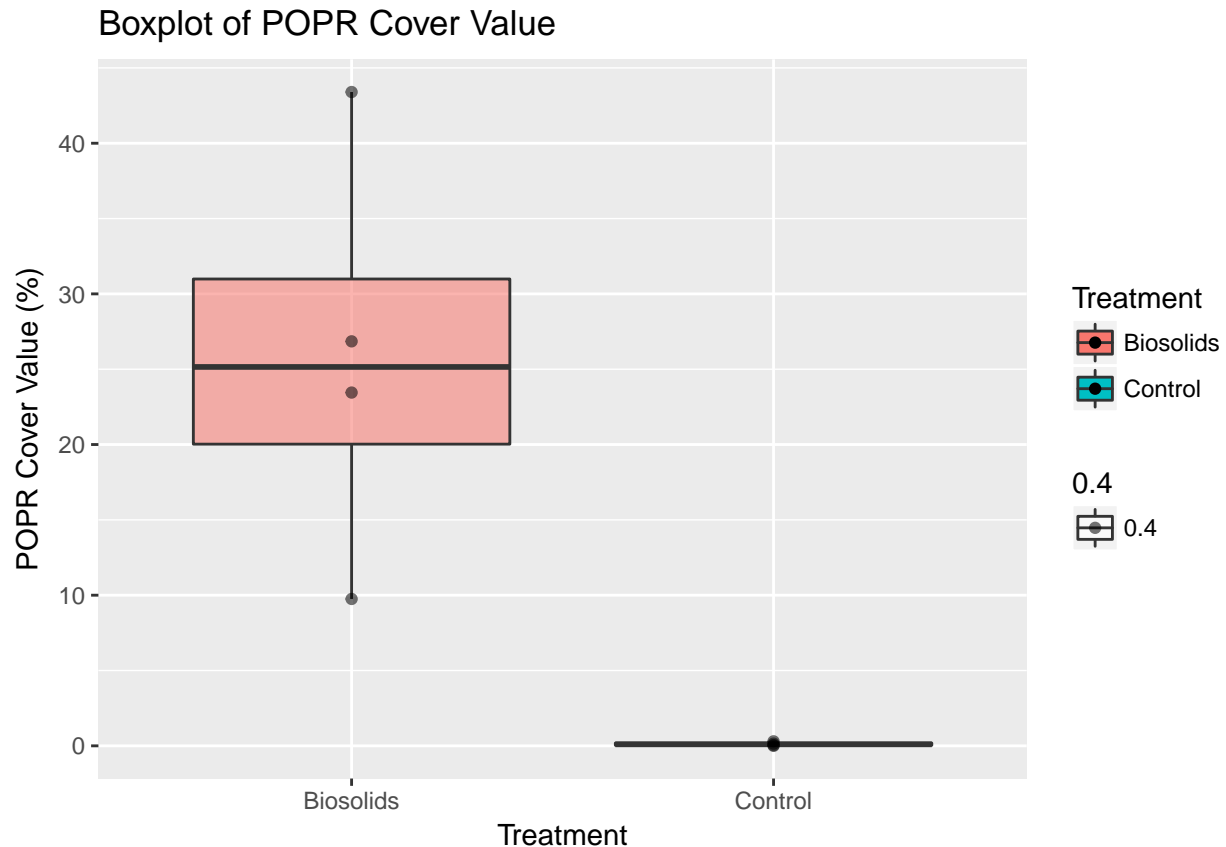


Figure 2.4 Boxplot of cover value of species POPR in biosolids and control group.

### 3. Methods

#### Long term impact of biosolids on soil (MWD)

We use a mixed-effect linear model which has both fixed and random effects. In our context, MWD is the response variable. Treatment is a fixed effect, because the experimenters directly manipulate the application of treatment to the soil. Date is also a fixed effect, because the four sampling dates are predetermined by the researchers. Block is treated as a random effect, because we use random effect to handle the issue of dependencies that arise from the experimental design.

There are two features in the design:

“Repeated measurements” is the case where observations are taken from the same subject several times. In our case, MWD is measured in roughly the same place four times during the growing season. These four observations are dependent, because they are affected by common characteristics belonging to the same sampling location.

Another feature is the transects setup. It results in the problem of “pseudoreplication” where replicated observations are not independent. There are two sources of pseudoreplication in our case:

- The seven soil samples along one transect are pseudo replicates. Because the soil composition measurement in one place is highly correlated with that five feet away.
- In every half of the block, the three transects are pseudo replicates. Because they belong to the same experimental unit. The conditions affecting one transect also affect another transect in that half of the block.

Therefore, both repeated measurements and pseudoreplication violate the assumption of independence that is assumed in linear models. The way we tackle this problem is to introduce a random block effect into the model. This mixed-effect linear model then analyzes the data in a way that is similar to randomized complete block design but with a random block effect. Although transect is not included in the model, it is not of particular interest to the researchers.

### **Long term impact of biosolids on plant cover**

The same problem of pseudoreplication also appears in the plant cover dataset. The observations within and between 5 plant transects are dependent for the same reasons described in soil transects. The method we use is to take the average of cover value over each treatment-block combination. Then we have four independent observations from the four blocks for both biosolids and control treatment groups. For a block-treatment combination in which experimenters did not observe any presence of POPR, we add a cover value of 0 for that specific combination. Then we fit a usual linear model to this averaged dataset with the averaged cover value as the response variable and treatment as the explanatory variable.

In addition, averaging observations from each block-treatment combination relieves the problem that the response variable is discrete. Since we take an average over 50 discrete observations for each combination, the averaged cover value resembles the underlying continuous values. Therefore the usual linear model introduced above applies.

## **4. Results**

### **Long term impact of biosolids on soil**

Long term impact of biosolids on soil is analyzed by mixed effect model. First, we want to check the most important assumption of mixed effect model which is normality of the underlying population. The normality assumption is validated by the histogram of MWD (Figure 4.1) and normal quantile-quantile plot (Figure 4.2). The histogram shows that the sample distribution of MWD is approximately normal, indicating a normal underlying distribution of MWD. The normal QQ plot shows the sample quantiles from MWD dataset against the theoretical quantiles from the standard normal distribution. The fact that most of the points align well with the straight line suggests normality.

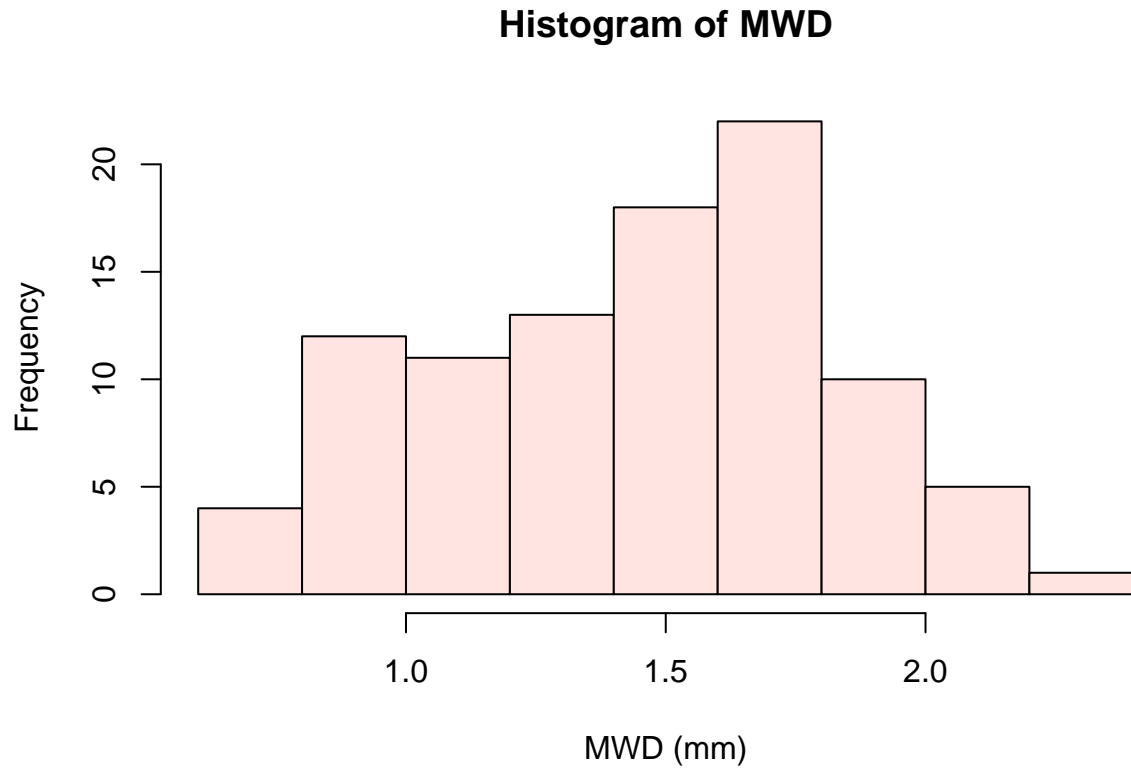


Figure 4.1 Histogram of MWD.

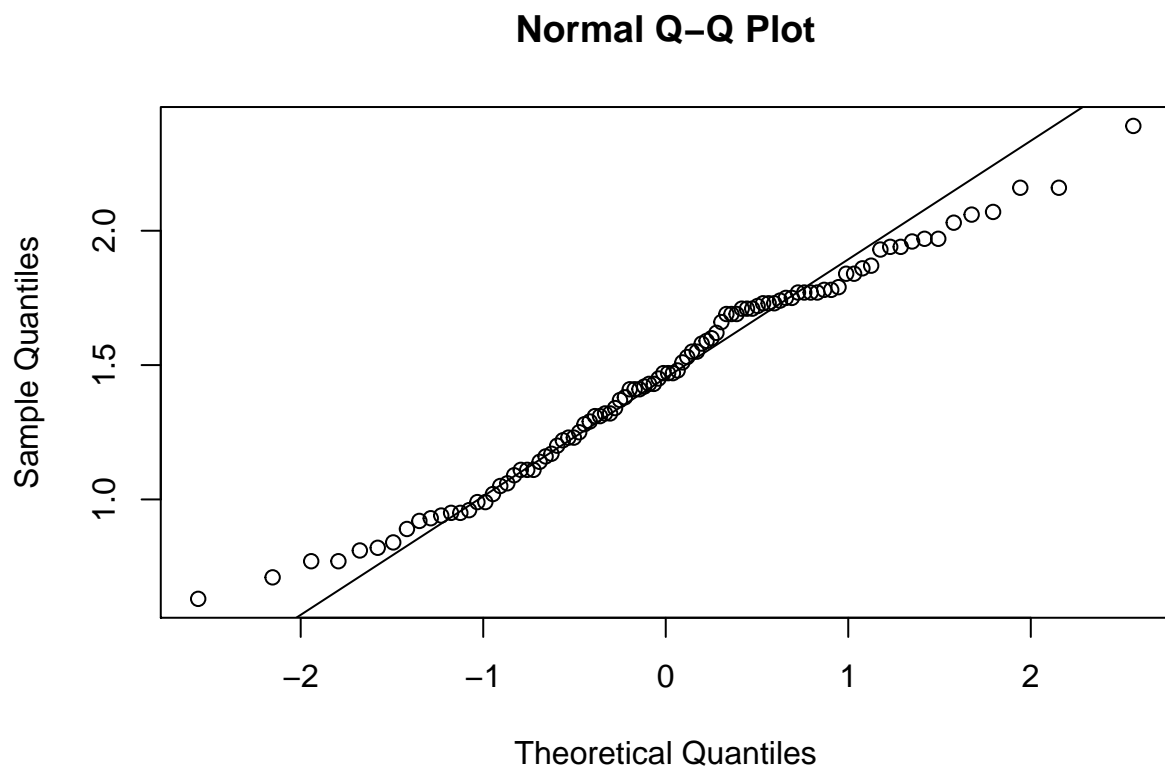


Figure 4.2 Normal quantile-quantile plot of MWD.

We use Chi-square test to investigate the significance of treatment effect, date effect and treatment-date interaction effect respectively:

P-values of 0.006, less than 0.001 and 0.008 respectively show strong evidence that the treatment and date main effects and the treatment-date interaction effect are all significant at 1% significance level. These results show that biosolids and application date affect the MWD both individually and interactively.

Two treatment types and four sampling dates give us eight combinations. The estimated intercept 0.88 is the mean of MWD of control group in April (baseline group). From April to June, the mean of MWD of control group increases by 0.838mm [0.719mm, 0.956mm] with 95% confidence. This is the most dramatic increase in MWD among the four sampling dates as Figure 4.3 shows. Moreover, all the estimates for the main effects of dates are positive. It means that MWD always increases as sampling dates change compared to April. Keeping date fixed in April, June, August, and October, MWD increases by 0.172mm [0.054mm, 0.261mm], 0.023mm [-0.235mm, 0.248mm], 0.229mm [0.058mm, 0.422mm], and 0.412mm [0.265mm, 0.522mm] respectively with 95% confidence. These increases are due to the treatment effect only.

The treatment-date interaction effect accounts for the extra change in MWD that is not explained by adding up the main effect of treatment and date when compared with the baseline group. For example, the mean MWD for biosolids group in October is 1.668mm, which is 0.788mm larger than that for baseline group. There is an increase of 0.376mm in the mean of MWD from April to October. There is another increase of 0.172mm in the mean of MWD from control group to biosolids group. But they do not add up to 0.788mm. The rest of the difference between the two groups is explained by the interaction effect between biosolids and October (0.240mm with 95% confidence interval [0.085mm, 0.409mm]). Visually from the interaction plot (Figure 4.3), we can see that the change in the mean of MWD over the four sampling dates is quite different for the two treatment groups. Especially from June to August, the mean of MWD increases for biosolids group, while the mean of MWD decreases for control group. This corroborates the significance of the interaction effect between treatment and date.

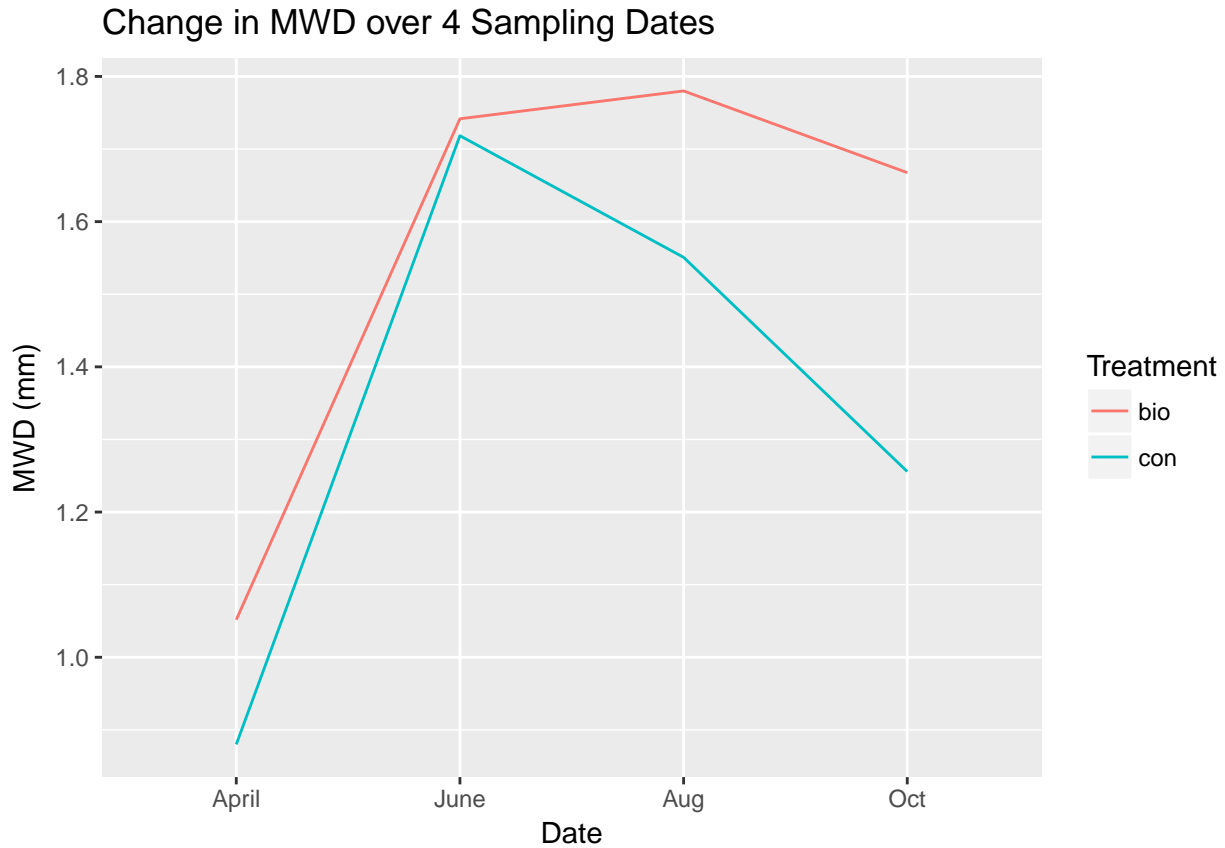


Figure 4.3 Change in mean of MWD over the four sampling dates.



## Long term impact of biosolids on plant cover

Long term impact of biosolids on plant cover is analyzed by a linear model. We check the following assumptions before applying a linear model:

- Normality assumption is checked by histogram of cover value (Figure 4.4). The distribution of cover values of POPR does not look normal. This is potentially due to the fact that cover value is not normally distributed in nature. In addition, the sample size of the averaged dataset is too small to appear normal. Since averaging and using linear regression is our second best solution to deal with discrete cover value, we assume that this violation of normality would not create significant bias on results.
- Common variance assumption is achieved by a weaker condition that we have equal number of observations ( $n=4$ ) under biosolids and control.
- Independence is automatically achieved by averaging dependent observations. Since we only have one observation from each experimental unit (half of a block), the observations satisfy the independence assumption.

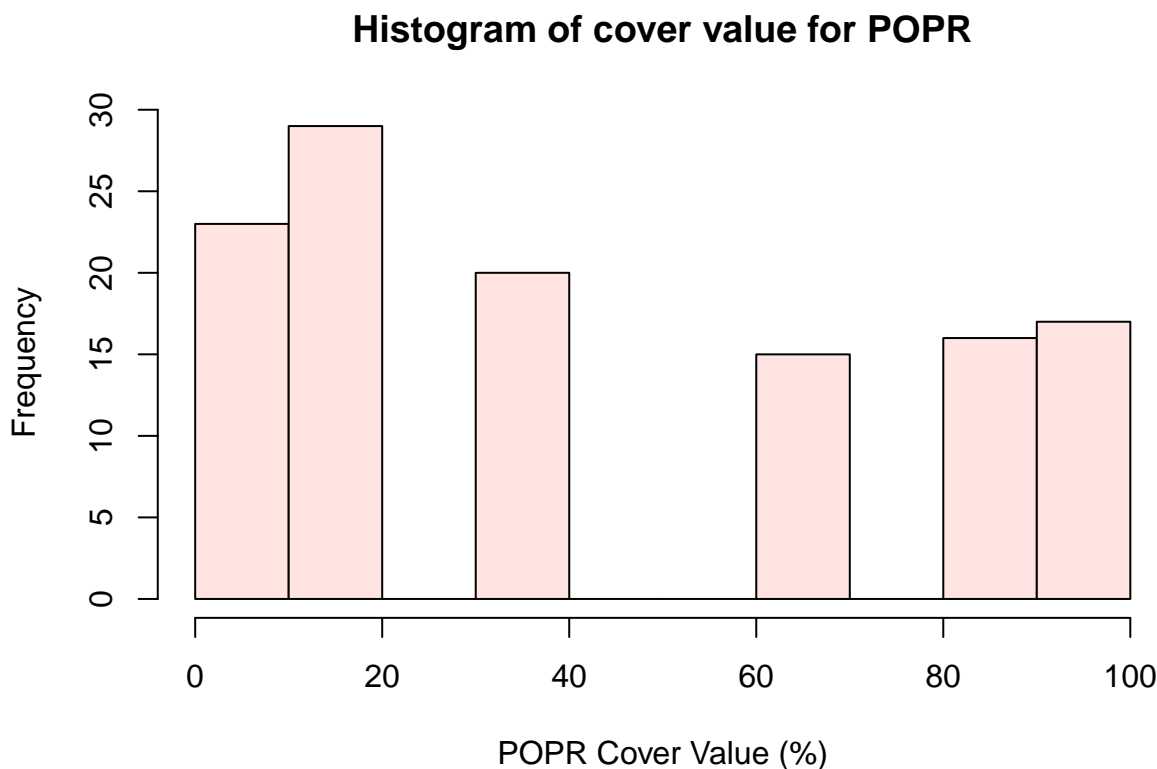


Figure 4.4 Histogram of cover value for species POPR.

After fitting a regression model with averaged cover value per block as a response variable and treatment factor as an explanatory variable, we obtain a p-value of 0.00984 for biosolids treatment. With a p-value smaller than 0.01, we find strong evidence to reject the null hypothesis that biosolids and control produce indifferent cover values. When changing from control to biosolids, we expect an estimated increase of 25.738% [8.815%, 42.661%] in cover value of POPR with 95% confidence.

## Correlation between MWD and POPR cover value

Since there are an unequal number of observations of MWD and cover value, we test correlation using the eight means from the eight treatment-block combinations for both MWD and cover value. The estimated

correlation is 0.645 which indicates a moderately strong positive relationship between MWD and POPR cover value. It means that when soil becomes more stable (larger MWD), the canopy cover of POPR increases, which is intuitive.

## 5. Broader Scope of the Study

The plant species composition dataset contains a total of 50 plant species. The above analyses have investigated the relationship between the average cover value and the treatment (biosolids or control) for a single plant species: Kentucky bluegrass (POPR). Our objective is to expand these analyses to a larger set of species.

Our client has specified that they are interested in the following species: Kentucky bluegrass (POPR), bluebunch wheatgrass (PSSP), Nevada bluegrass (POJU), needle-and-thread grass (HECO), nodding onion (ALCE), low pussytoes (ANDI), common dandelion (TAOF), soil (SOIL) and microbotic crust (BRYO). We thus expand the analysis to include these 9 species. The client has also pointed out that they are interested in any species with a cover greater than 5% across all the sites. However, we find that none of the species occur in all of the 400 sites ( $4 \text{ blocks} \times 2 \text{ treatments} \times 5 \text{ transects} \times 10 \text{ samples per transect}$ ). We thus change the criterion to include species whose averages over the (occurring) transects and plots, for every block-treatment combination, are all greater than 5%. This criterion adds 2 further species to the study, junegrass (KOMA) and litter (LITT), resulting in a total of 11 species of interest.

### Long term impact of biosolids on plant cover: Linear regression

For every plant species, we follow the same analysis methods as before. Specifically, we take the average cover value over each treatment-block combination, adding a cover value of 0 for any combination in which there is no presence recorded. We then fit a simple linear model with the average cover value as the response variable and treatment as the explanatory variable.

Table 5.1 shows the results of the linear regression models, with p-values and estimates corresponding to the slope coefficient, i.e. the difference in average cover value between the biosolids and control treatment levels. We find that there is a significant treatment effect (p-value  $< 0.05$ ) for more than half of the species. This indicates that, for these species, there is strong evidence to reject the null hypothesis that biosolids and control produce indifferent average cover values. For five species (ALCE, HECO, POJU, PSSP, TAOF), the p-value is somewhat large, indicating that biosolids do not have a significant impact on the average cover values of these plant species.

Species	p.value	Regression.Coefficient
ALCE	0.81	-1.41
ANDI	0.03	-1.64
BRYO	0.00	-30.70
HECO	0.74	2.38
KOMA	0.03	-4.99
LITT	0.00	54.35
POJU	0.28	7.05
POPR	0.01	25.74
PSSP	0.13	5.69
SOIL	0.02	-4.91
TAOF	0.12	4.36

Table 5.1 Results of the simple linear regression of average cover value as a function of treatment, for each plant species of interest.

### Long term impact of biosolids on plant cover: Non-parametric approach

We note that the simple linear regression approach above has several disadvantages. The p-values are obtained from a t-test on the slope coefficients, which has several underlying assumptions. Similar to Figure 4.4, histograms of average cover value for each plant species show that the distributions are highly non-Normal, which violates the Normality assumption required by the t-test. In addition, the sample size is far too small to justify the assumption of Normality of the coefficient estimates that can be made for large sample sizes.

Furthermore, the equal-variance assumption underlying the t-test is severely violated. Boxplots of average cover value for each plant species, similar to Figure 2.4, show extreme differences in variability between the two treatment levels for many of the plant species. There is a possibility that these violations of the t-test assumptions are invalidating the conclusions resulting from the simple linear regression analysis.

Thus, we implement a non-parametric alternative to test for the difference in the average cover values between the biosolids and control groups: the Wilcoxon signed rank test. This is the non-parametric equivalent of the paired t-test, and it is appropriate because the observations belonging to each treatment level are paired according to the block from which they come. The Wilcoxon signed rank test analyzes whether the distribution of the within-pair differences in average cover value is symmetric about zero. It is more appropriate for this setting than its parametric alternative, because it requires no distributional assumptions.

Table 5.2 shows the results of the signed rank tests, with p-values and estimates corresponding to the median difference in average cover value between the biosolids and control treatment levels. With the non-parametric analysis, we find that there are no significant treatment effects for any of the species, which is different from our previous results in Table 5.1. This is due to the fact that, as a non-parametric test, the Wilcoxon signed rank test tends to have less power than its parametric counterpart, where power is defined as the ability to detect an existing treatment effect. This is particularly true when the sample size is small, as is the case for this dataset. Therefore, the non-parametric test has some difficulty identifying these underlying differences.

Species	p.value	Estimate
ALCE	0.79	-2.29
ANDI	0.12	-1.19
BRYO	0.12	-30.36
HECO	0.62	2.38
KOMA	0.12	-4.66
LITT	0.12	54.24
POJU	0.25	2.49
POPR	0.12	25.74
PSSP	0.12	5.15
SOIL	0.12	-4.91
TAOF	0.12	3.06

Table 5.2 Results of the Wilcoxon signed rank test of average cover value as a function of treatment, for each plant species of interest.

### Correlation between MWD and cover value

Finally, we analyze the correlations between aggregate stability (in terms of MWD) and plant cover for each of the species of interest. Earlier, we have found that there is a moderately strong positive correlation between MWD and cover value for the species POPR. In this section, we repeat the analysis for all 11 species of interest.

Table 5.3 shows the correlation between MWD and average cover value, for each of the 11 species. From these results we can say that, for many of the plant species, there is a negative correlation between MWD and average cover value. This could be due to the fact that the growth of one type of species might negatively affect several other types. As with POPR, three other species (LITT, POJU and PSSP) have moderate positive correlations. One species (TAOF) has a very low positive correlation between MWD and average cover value.

Species	Correlation
ALCE	-0.10
ANDI	-0.41
BRYO	-0.41
HECO	-0.44
KOMA	-0.38
LITT	0.62
POJU	0.62
POPR	0.64
PSSP	0.42
SOIL	-0.41
TAOF	0.03

Table 5.3 Correlations between MWD and average cover value, for each plant species of interest.

## 6. Conclusions & Further Discussion

There is strong evidence that biosolids treatment and sampling dates have significant impact on improving soil stability. They affect MWD interactively instead of individually. There is also strong evidence that biosolids application increases the spread of more than half of the selected species, but not for all species.

In terms of correlation between MWD and average cover value, the results shows that for many of the plant species of interest there is a negative correlation. This means that as the MWD increases, the average cover value decreases. This issue might be due to the fact that the growth of some species negatively affects the growth of some other species. This would explain why the biosolids treatment does not always significantly affect the plant species composition, and why the correlation between MWD and average cover value is negative for many of the species. We also note that for some other species there is a moderate to low positive correlation between MWD and average cover value.

In conclusion, biosolids are an effective treatment that enhances soil stability and sometimes the plant canopy cover. For further studies, ordinal regression is more appropriate for plant investigation because cover value is a discrete variable. In terms of the experimental design, we recommend that researchers increase the number of blocks instead of transects. With an increase in the number of independent observations, the response variable would be closer to normality and the standard errors of the estimated coefficients would be smaller. Moreover, soil and plant samples could be taken randomly in blocks instead of in fixed transects since proper randomization may eliminate potential bias at the design stage.

## 6. Appendix

link to our Github repository for R code and output