Microbial Population as A Function of Woody Biomass Removal Treatments

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Introduction

Collecting small-diameter woody biomass will alter post-timber harvesting landscapes (Figure 1). The study objective of this portion of the NARA project is to determine potential changes in long-term nutrient ecology by measuring changes in microbial soil populations as a function of woody biomass removal treatments. This will help to understand the environmental impacts of residual ground cover (biomass) removal in the production of jet fuel in the Pacific Northwest.

FIGURE 1. NARA LTSP Plots in Springfield , OR

Hypothesis

H_o: Changes in soil moisture as a result of biomass removal will impact microbial community indicating potential long-term implications to nutrient dynamics.

H_a: There will be no changes in microbial community.

Methodology

Sample Collection

Soil samples were collected in May 2014 from all 28-1 acre LTSP (Long Term Site Productivity) plots of Weyerhaeuser Company located in Springfield , OR to perform DNA extraction test in the laboratory. Nine soil samples were collected from each plot in the following pattern: South-West , South, South-East, North-West, North, North-East, Center, Mid-West, and Mid-East. The samples were taken at a depth of 0-20cm using a hand shovel. Rubber gloves were used at the time of collecting soil samples and the shovel was always cleaned properly after taking samples from every location.

The soil samples were kept in 8-ounce, air tight jars and were preserved in coolers at a temperature of less than 4°C to keep the microbial community safe. Dry ice was used to maintain the temperature of the coolers. A total of 252 samples were collected from plots for subsequent DNA Extraction testing in the laboratory (see Figure 2). The samples were kept in a -20°C temperature freezer in the laboratory to preserve them for a long time.

Lab Procedure

MO Bio's Power Soil DNA isolation kit was used in the laboratory to extract DNA from the collected soil samples. MO BIO has developed a standard protocol to extract DNA from any soil using this kit which has been followed in our analysis. According to the protocol 0.25g soil was taken from each 8-ounces jar of soil and then six different solutions were used in different stages of the experiment. After isolating the DNA following the above protocol, a Nano Drop 2000c Spectrophotometer was used to measure the concentration of DNA. Between each use of the Nano Drop meter it was cleaned using sterile DNA-free PCR Grade water. A software program developed for the kit was installed on the connecting computer to calculate the concentration of DNA in ng/µl. Results are summarized below.

FIGURE 2. Power Soil DNA Isolation kit & Other Instruments used in the lab to extract DNA

Treatment processes

A - **No Compaction Bole Only** – Bole only harvest to a saw log top (5" top) all limbs and tops remain on the site. No ground trafficking.

B - **No Compaction Total Tree -** Whole-tree type harvest where ~75+% of limb/top material is removed along with the bole. Remaining material will be dispersed. No ground trafficking.

C - **Compaction Bole Only -** Bole only harvest to a saw log (5") top – all limbs and tops remain across the whole site. Fixed Traffic lanes.

D/F – **Compaction Total Tree -** Whole-tree type harvest where ~75+% of limb/top material is removed along with the bole. Remaining material will be dispersed and equal across like plots. *Fixed traffic lanes* .

E/G - **Compaction Total Tree + FF -** Whole-tree type harvest where ~90-95% of limb/top material is removed along with the bole. Forest floor and legacy woody debris also removed. Compaction on this treatment will be the baseline for all compaction treatments.

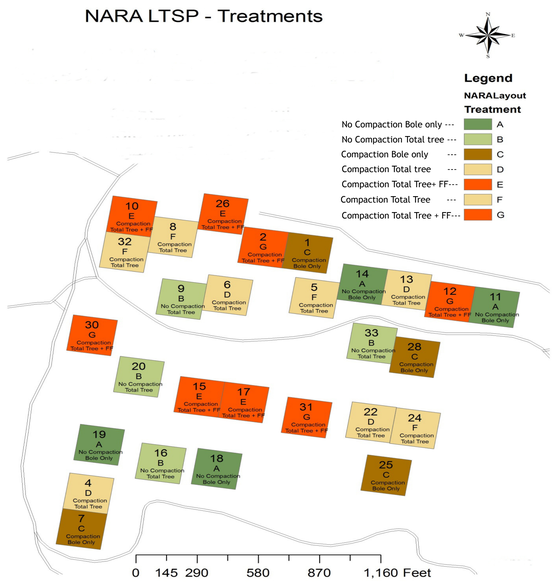

FIGURE 3. Location of the LTSP Sites and Treatment Processes

Results

Treatments		Plots	Average DNA Concentration (ng/µl)
A	- No Compaction Bole only	P#14	**51.84**
		P#19	**37.14**
		P#11	**29.06**
		P#18	**12.08**
B	- No Compaction Total tree	P#9	**37.85**
		P#20	**63.77**
		P#33	**20.69**
		P#16	**31.75**
C	- Compaction Bole Only	P#1	**56.40**
		P#7	**38.48**
		P#28	**45.84**
		P#25	**20.27**
D	- Compaction Total tree	P#13	**35.14**
		P#4	**33.08**
		P#6	**24.70**
		P#22	**21.49**
E	- Compaction Total tree + FF	P#10	**14.64**
		P#15	**20.35**
		P#26	**28.92**
		P#17	**23.19**
F	- Compaction Total tree	P#5	**49.06**
		P#32	**30.07**
		P#8	**19.31**
		P#24	**27.51**
G	- Compaction Total tree + FF	P#2	**27.60**
		P#30	**28.32**
		P#12	**16.13**
		P#31	**15.01**
	No treatment	Unharvested Site	**15.44**

Table 1. Results of the DNA Extraction tests for the LTSP sites

Discussion

The average DNA concentrations (ng/µl) are different for different plots; even for those undergoing the same treatment process. For example, plot #14 and plot #18 both have the same treatment of "No Compaction - Bole Only" but the average DNA concentration is 51.84 ng/µl and 12.08 ng/µl, respectively whereas considering plot #19 and plot #9 it has been found that the average DNA concentrations are 37.14 ng/µl and 37.85 ng/µl (although the treatment processes are different). We only recently completed the laboratory analysis so while the reason(s) behind this is not clear, we are still trying to find explanations by looking at soil types and variability. Four samples were taken from an unharvested control site where the average DNA concentrations is 15.44 ng/µl.

Conclusion

Nine soil samples were collected from each 1-acre site and four DNA extraction test were performed for each sample. The average DNA concentration (ng/µl) per plot showed in the table is the average of the result of 36 DNA extraction tests for that plot. PRELIMINARY analysis of average DNA Concentration does not show any clear trend which would indicate that microbial variation due to different treatment processes is nonexistent and apparently nullify the hypothesis. For a final result, more analysis of the DNA extraction test data are required and we are working on it.

References

Power Soil DNA Isolation Kit Protocol, MO BIO, http://www.mobio.com/images/custom/file/protocol/12888.pdf.

Holub S., Meehan N., Meade R., Carrier B., Johnson G., Harrison R., Betts M., Barber M. et al. January 2013. Sustainable Feedstock Production System – Long Term Site Productivity (LTSP).

NARA

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