Effect of Soil Compaction on Some Soil Organisms

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ABSTRACT

Agricultural soil compaction has become more and more prevalent as mechanized agricultural using heavy equipment expands globally to feed a rapidly growing world population. However, there is limited knowledge and research being carried out on the biological and physical effects resulting from soil compaction. In our study, we found that in the compacted soil samples, there are increased soil fungi colony forming units and meso- and micro-fauna populations compared with un-compacted control soil. In addition, we found a significant decrease in aggregate stability in the compacted soil samples. Specifically, soil compaction destroys the physical environment including soil pore size and soil aggregates stability and these changes in turn it affect the soil organisms that inhabitat in these environments.

KEYWORDS

Soil bacteria, soil fungi, soil organisms, soil pore size, soil aggregates stability

INTRODUCTION

The magnitude of soil compaction in agricultural fields is increasing very rapidly on a global basis. Hand farming is being replaced by mechanized farming in the developing world and heavier more powerful equipment is being deployed in the developed world. As the global population increases, food security requirements only can be met if agriculture around the world pursues sustainable intensification (Abraham et al. 2014). Research shows the major cause of soil compaction on farmland is the use of heavy equipment (Alakukku 1999). Small tractors are being replaced by ever larger and heavier tractors in industrialized countries as farm and field sizes increase (Breland and Hansen 1996). Specifically, axle loads of tractors with implements and harvest wagons have increased in weight to 20 Mg or more. Penetrometer resistance of soils exceeding 4 MPa have been measured following wheel compaction, but 2 MPa is known to begin to inhibit root growth in soil (Souch et al. 2014). Soil compaction is becoming a prevalent phenomenon in agricultural farming on a global scale.

Increasing soil compaction significantly reduces the pore space in soils, particularly the larger diameter pores; this decreases soil water infiltration rate, restricts air diffusion, and increases bulk density, which can slow down crop growth by restricting root penetration (Jung et al. 2009) and nitrogen uptake (Breland and Hansen 1996). Furthermore, studies show that the major impact of soil compaction is in the compression and loss of soil pores, which are a critical component of the soil environment. Roots can penetrate pore sizes of 10^{-4} m or greater, but are impeded by smaller pore sizes (Birkas 2004). Root hairs require pore sizes of greater than about 10^{-5} m. Fungal hyphae prefer pore sizes greater than 10^{-6} m and soil bacteria prefer pore sizes of $10^{-}7$ or larger (Birkas 2004). In addition, soil animals, such as earthworms prefer pore sizes of 10^{-2} m and greater, although they can make a burrow in soil with a smaller pore size and a penetrometer reading of 3 MPa (Birkas 2004). Therefore, compaction of soil degrades these micro-environments needed by plant roots, fungi, bacteria and earthworms and could significantly reduce soil bio-activity and adversely affect soil fertility.

Microbial communities active in soil have a positive effect on the formation of the structure and aggregation of soil particles that can help retain the soil structure required for optimal fertility. Specifically, microbial communities enhance the formation of micro-aggregates and macro-aggregates in soil (Bronick and Lal 2005). Magro-aggregates are first formed around particulate organic matter (POM). However, as the POM is decomposed and microbial exudates

are released, the structure of macro-aggregates is stabilized while micro-aggregates form inside the macro-aggregates (Bronick and Lal 2005). Moreover, bacteria colonies together with their exudates form a polysaccharide capsule around clay particles that aligns the clay particles to form the micro-aggregates structure. More importantly, these micro-aggregates create a different internal environment that inhibits decomposition of Soil Organic Carbon (SOC), which preserve the soil organic matter (Bronick and Lal 2005). The stability of soil structure is measured by soil aggregation structure, which plays an important role in the functioning of soil. Thus, microbial organism activity plays an important role in the stability of soil structure.

However, it is unclear in literature how heavy soil compaction can impact these soil microbial communities. It is known that compaction can decrease porosity among soil particles (Souch et al. 2014). Thus, perhaps in very heavily compacted soil, the microbial community will behave different from un-compacted soil. Through cell culture on artificial media, we can recover about 1% of the bacteria and fungi from the original soil, which will gives a comparison on microbial number and diversity between compacted and un-compacted soil. Direct counting and microscopic counting of small insects, worms and nematodes is applied to estimate the diversity of soil macro-fauna and meso-fauna. We expect to see a decreased number or species present in soil that has a high degree of compaction.

METHODS

Study site

Soil samples for my study were collected from the agricultural area in Salinas Valley. The soils samples used for compaction are Sandy Loam.

Sampling sites for intensive agriculture soil for Bulk Density measurement

Samples were taken from a field where intensive mechanized agricultural practices have been carried out with about 4 crops per year of row crop vegetables, mostly head lettuce, being grown per year for the past 45 or so years. Generally, the farmers don’t wait between crops so the soil is worked when it is wet with very heavy equipment so it goes through cycles of heavy compaction followed by mechanical breakage of large clods into smaller fragments 4 times every year. Meanwhile, in the field used for agriculture I was able to find recently made clods and also

soil which was of finer texture, but even the finer textured soil had probably gone through many cycles of mechanical compaction and mechanical fracturing. The soil collected is classified as Blanco Clay (Carpenter and Cosby 1925).

Sampling site for control and compaction treatment

I sampled soil from a site nearby the agricultural field, where there was a vegetation cover with two dominant weeds: *Atriplex patula*, (spear saltbush) and *Epilobium ciliates*, (fringed willow herb), that hasn't been affected by agricultural compaction. I took samples from the 0 to 20 cm depth and compressed them using a hydraulic press to reach a bulk density, which was the same as I measured in existing in clods made by agricultural machinery & tractors on the same soil type. The heavily compacted agricultural soil clods were found to have a bulk density of 2.1 g/cm^3. In this way I tried to find out what is the effect of a single compaction of this soil on the three biological parameters: bacteria, fungi and micro- and meso- fauna.

<table>
<tr><td>Time</td><td>Control group. Un-compacted soil samples taken from a site which has not been subject to agriculture for at least 50 years- "the control site"</td><td>Treatment 1. Soil samples, taken from the control site, which has not been subject to agriculture for at least 50 years, compressed by a hydraulic press to a known pressure to achieve 1.8 g/cm^3 bulk density & porosity which was the same as found in compacted clods formed by agricultural activity</td></tr>
<tr><td rowspan="4">7 days following compression</td><td colspan="2">1) Dilution series culture on soil extract agar to count CFU's of aerobic bacteria &actinomycetes</td></tr>
<tr><td colspan="2">2) Dilution series culture on soil Martin's acid-rose benga-streptomycin medium to count CFU's of fungi & yeasts</td></tr>
<tr><td colspan="2">3) Use a Berlese-Tullgren Funnel technique to count populations of of soil macro-fauna</td></tr>
<tr><td colspan="2">4) Carry out a visual count of earthworms and other visible macrofauna</td></tr>
<tr><td colspan="3">Note: there will be a negative control for all of the culture media. The negative-control agar culture will be added with just water to see if there any growth without the soil water.</td></tr>
</table>

Table 1. Experiment design.

Culture condition/method to get the highest total bacteria counts

To get the highest number of bacteria counts, I used a soil-extract media in an attempt to provide traces of minerals and other molecules which might be important to the bacteria in the soil. Soil extract liquid was added to plain agar and then autoclaved together so that the agar was melted and mixed up with the soil extract. To make the soil extract liquid, the soil was suspended and soaked in water overnight in the ratio of 1 kg of soil/1L of water. I used a dilution plate technique to estimate the population of Colony Forming Units (CFU) of aerobic bacteria and actinomycetes, and soil dilution of $1*10^{-4}$, $1*10^{-5}$, $1*10^{-6}$ and $1*10^{-7}$ were made.

In the Method of Soil Analysis by Soil Science Society of America from 1996, it was suggested that a higher bacteria count can be observed when using a poor-nutrient culture media. For example, the experiments performed by Dr. Andrew Watson indicated that the highest count of total bacterial CFU was achieved when cultivating with just soil extract and plain agar media. Even though, new selective media have been developed for counting certain types of soil micro-organisms. I used a non-selective, nutrient poor medium in this study to capture the highest number of bacteria and actinomycetes possible, recognizing that in any case this is only a small fraction of the total bacterial flora. I was more interested in the differences, or lack of differences between treatments, than in trying to find the absolute number. This was an observation made in few other studies including the Method of Soil Analysis by Soil Science Society of America and in Olsen and Bakken's study.

Culture to get highest count of the number of fungal CFU's:

In the study, I used J.P. Martins's acid, rose bengal and streptomycin medium and a dilution plates method for estimating soil fungi. Dilution of $1*10^{-4}$, $1*10^{-5}$, $1*10^{-6}$ and $1*10^{-7}$ are made.

Ingredient	1 L	3 L
Glucose	10g	30g
Peptone	5g	15g
K_2HPO_4	0.5g	1.5g
KH_2PO_4	0.5g	1.5g
$MgSO_4*7H_2O$	0.5g	1.5g
Rose bengal	30mg	90mg
Agar	12g	36g
Yeast extract	0.5g	1.5g
Streptomycin sulfate	30mg	90mg
Mix all ingredients, autoclave and cool to 50 degree celcius, streptomycin sulfate added in dissolved distilled water.		

Table 2: Ingredient to grow fungi.

Method to count/observe the diversity of meso-fauna and micro-fauna:

I used the Berlese-Tullgren Funnel Extraction method. I also did a visual examination of 100 g of each sample be adding water in a dish and make a visual count of earthworms and large soil arthropods. Also, to save the visible soil animals, I have taken out the large insects and soil animals before any treatment with a size greater than 3 cm of body length.

Detail to make serial dilution and cultivate culture

Three one-gram sub-samples of each natural soil samples taken from the field were dispersed in 100 mL of autoclaved water. The same was done on the compacted soil samples. Each one-gram sub-samples were added into three sterilized flasks and were broken down manually using a sterilized metal grinding rod for the same length of time for each sample. The soil was soaked and set in the suspension for 15 mins. This procedure was to separate the maximum number of spores from particles and from each other. After preparing this solution, a serial dilution was performed to produce a range of dilutions from $1 \times 10^{-4}$to $1 \times 10^{--7}$ dilution for bacteria and fungi counting. Then, 1 ml aliquots was taken from all the dilutions from the control soil and treatment samples and were grown on soil extract media and Martin's Rose Bengal Media. The bacteria plates were grown under 25^{o}C for two weeks (the colony forming units was counted each day for a week to see how the number of colonies is increasing). Finally, the number and types of colonies grown was recorded; the bacterial colonies were differentiated from the actinomycetes

colonies by stereomicroscope at 100X power. Different genera of fungi were identified by their morphologic appearance on the plates and microscopic identification of spores, and yeast colonies were also visually differentiated by their circular shape and slightly red color.

Data analysis

First, I used Shapiro-Wilk normality test to check the normality of each date set from control and experiment groups. I used two-sample t-test to compare if there is a difference between control (without compaction) and treatment (with compaction) groups with respect to Colony Forming Units of bacteria, fungi and number of micro- meso- fauna present.

RESULTS

For both bacteria and fungi plates, I saw the highest number of Colony Forming Units(CFUs) on the soil dilution of $1*10^{-4}$ in all the plates. Thus, I used soil dilution of $1*10^{-4}$ to compare the CFUs present in control and experimental groups for both bacteria and fungi.

Bacteria

I observed that there are more CFUs in the un-compacted soil samples than the compacted soil samples on the $1*10^{-4}$ dilution. However, this may not be quantitative because the cultures were cloudy and it was hard to distinguish the CFUs from particulate material. In addition, the variation among the plates is huge. However, there is no doubts that this could be improved if I have time to perfect my technique. I should be able to reduce the variation among plates. However, I don't think these Bacteria Colonies can be quantified because of the variation and the difficulty to make a definitive count. Nevertheless, there are more CFUs seen in un-compacted soil plates compared with the compacted soil plates. This certainly requires further investigation.

Also, on one of the plates from compacted soil sample, I observed at least 30 live Nematodes swimming on the agar. Although this is a single observation I would have expected the nematodes to have been crushed in the compaction.

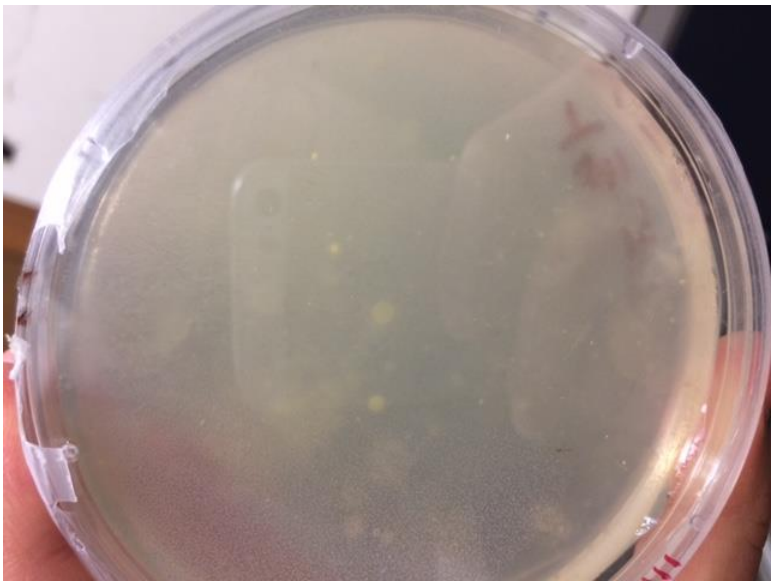
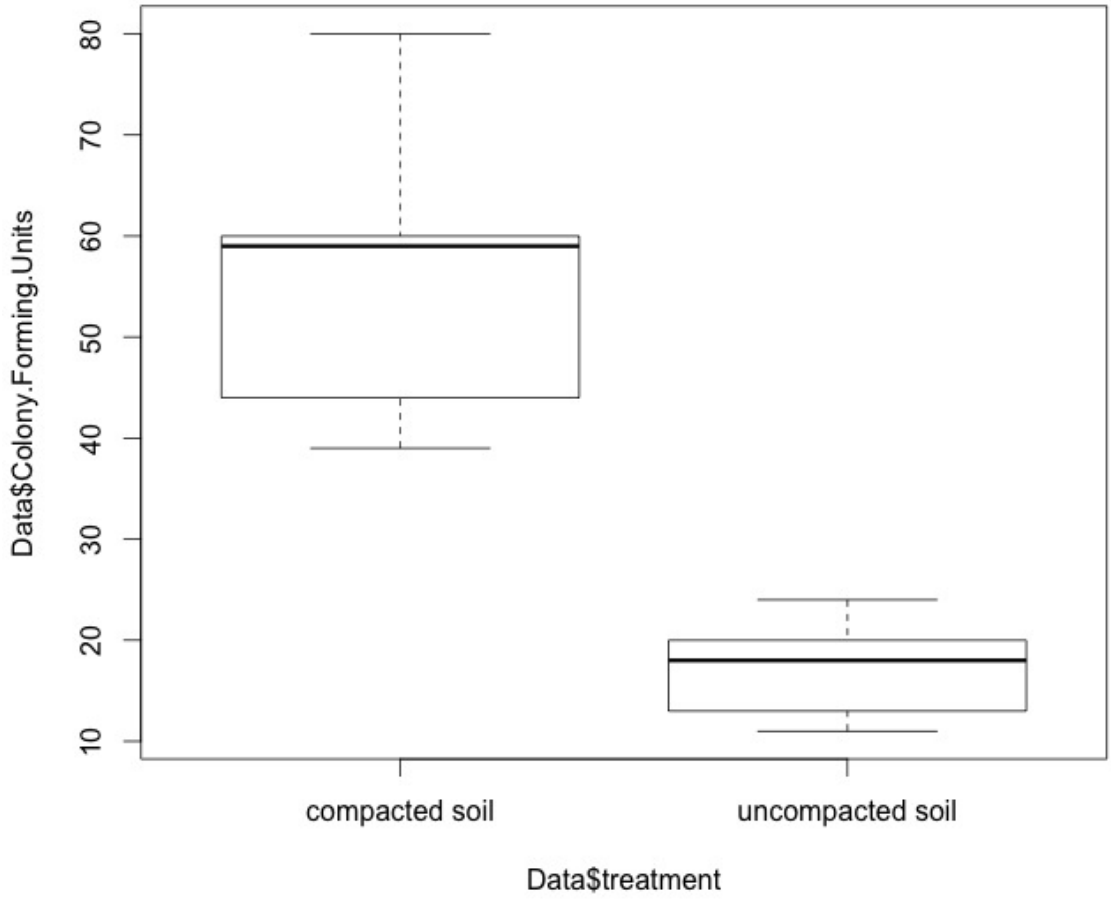


Image 1. Colony forming units (the very small white circles and some cannot be seen on the photo) for bacteria appeared on my culture plates.

Fungi

Effect of compaction on Fungi population

I found that there are more fungal CFUs in the compacted soil samples than the un-compacted soil samples across all the dilution series. There was a significant increase in colony forming units (CFUs) of fungi for un-compacted soils in the dilution of $1*10^{-4}$. The average CFUs of fungi found in un-compacted soil is 17.4, and the average colony forming units of fungi in compacted soil is 56.0. According to the t-test, p-value is equal to $1.629*10^{-5}$ and is less than 0.05, and compacted soil has a significantly higher CFUs.



Graph 1. Results of CFUs for fungi present in compacted and un-compacted soil.

Image 2. Colony forming units for fungi seen on my media plates. 3 days after inoculation on the left and 25 days after inoculation on the right (5 day in refrigerator).

Effect of compaction on different fungi genera

25 days after the inoculation of the fungi plates, the plates were held in ambient conditions (18-22 ^{o}C) in daylight with no direct sun to stimulate the formation of spores. In order to identify the genera, the colonies were examined with a stereo microscope while the sporulating colonies were examined with a compound microscope. I attempt to identify the genera of the fungus in discrete colonies using the illustrations in the "Illustrated Genera if Imperfect Fungi" by Barnett and Hunter. I was able to identify 7 genera, which represented 86-90% of the colonies.

The number of CFUs of Penicillium is 31.5 in compacted soil samples, which is significantly higher than 8.5 found in un-compacted soil (P-value = 0.00012 <0.05). The number of CFUs of Yeast is significantly higher in compacted soil (P-value = 0.01<0.05). There is no significant difference between other genera between compacted and un-compacted soils. However, the number of CFUs in all the genera appeared to be higher in compacted soil samples. And the total of CFUs on the plates from compacted soil is significantly higher than the un-compacted soil samples.

	Fungal Genus							
	Penicillium	Mortierella	Gliocladium	Yeasts	Stemphylium	Mucor	Dinemasporium	Unidentified
Compacted (Avg. CFUs)	31.5 (56.5%)	5.5 (9.9%)	7.8 (13.9%)	4.8 (8.5%)	0 (0%)	0.5 (0.9%)	0 (0%)	5.8 (10.3%)
Un-compacted (Avg. CFUs)	8.5 (39.1%)	2.8 (12.6%)	3.5 (16.1%)	2 (9.2%)	1.8 (8.0%)	0 (0%)	0.3 (1.1%)	3 (13.8%)

Table 3. Identification of Fungal Genera of Dilution Plates of Martin's Rose Bengal, Peptone Fungal Medium from Compacted and Un-Compacted Soil. The average Colony Forming Units (CFUs) from different Genera for fungi are recorded above.

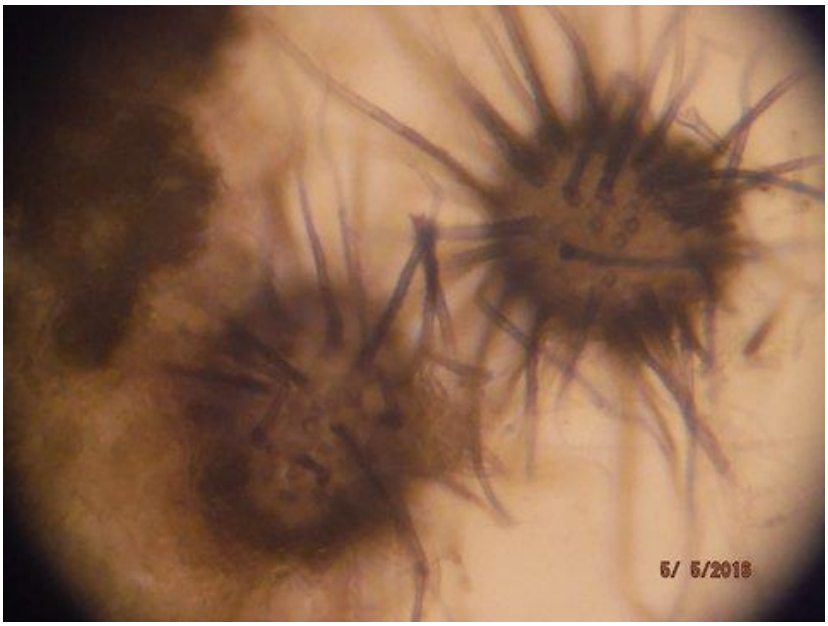


Image 3. Spores from Dinemasporium.

Arthropods

Because springtails were very frequent in the Berlese funnel extraction, I used these as an indicator organism for the meso-fauna. I found that there was an average of 13 individuals of springtails in 100g of un-compacted soil samples, and there were no springtails observed in compacted soil sample extracts. We conclude that pressure from compaction has wiped out all the springtails in the compacted soil samples, or at least immobilized them.

Image 4. Springtails observed under microscope (magnification: 400x).

Aggregates stability

Through a wet sieving method, I found that the compacted soil has a significant decreased aggregates stability. This difference is quantified by the calculating the dry weight of stable aggregates/(stable aggregates + unstable aggregates). The result for compacted soil is 0.51 and 0.67 for compacted soil.

DISCUSSION

Introduction

Gaining knowledge on the effect of soil compaction on microorganisms is an important aspect of understanding the effect of soil compaction resulting from modern agricultural practice. In my study, I found that agricultural soil compaction lead to a disappearance of meso-fauna in compacted soil. The difference in bacteria populations between compacted and un-compacted soil showed that CFUs of bacteria in compacted soil has been decreased significantly. Surprisingly, results of comparison of fungi population between compacted and un-compacted soil show that fungi population was actually increased one week following the compaction treatment. Decreased meso-fauna and bacteria population after compaction treatment support my hypothesis that if the soil pore size decreases as the bulk density increases, it affects the environment's soil microorganisms and soil fauna. However, the increased CFUs in fungi, although it was unexpected it can perhaps be explained. Generally, these results, on the one hand indicate how important it is for the soil organisms including small microorganisms to have the soil pore size and the structure they need for their survival, and on the other hand, the measure of stability between compacted and un-compacted soil shows how soil compaction has broken soil aggregates into smaller pieces.

Effect of soil compaction on bacteria

We found that the bacteria population decreased in compacted soil. One study showed the survival rate of bacteria between igneous rock layers after shock compression, and found that bacteria are pretty resilient to shocks (Horneck et. al, 2008). Specifically, they discovered that with a shock compression ranged from 10-50 GPa, there is till one type of Bacteria

(Chroococcidiopsis) survived with a survival fraction ranged from $1/10^{-1}$ to $1/10^{-8}$ (Horneck et. al, 2008). Chroococcidiopsis is known to be one of the most extreme-tolerant cyanobacteria, which is desiccation resistant and ionizing-radiation resistant (Horneck et. al, 2008). Thus, we would expect to see similar number of CFUs on both compacted and un-compacted soils since we applied relatively minute pressure. However, it seems we got an opposite result, but our compaction process was a much slower process and exerted a much smaller pressure. Our compaction takes several minutes for each compression cycle, whereas Horneck et. al, 2008 made a instant shock. We may do different types of cultures in the future to grow different types of bacteria since different bacteria have different resistance to environmental pressures. We are only seeing the effect of compaction on the few specific types of bacteria recovered by our soil-extract media. We may also leave the culture plates for observation for a longer period of time so we can actually quantify the effect.

Effect of soil compaction on fungi population and Genera

Effect of soil compaction on fungi population

We found that fungi population was increased in soil samples with compaction treatment that increased the soil dry bulk density to about 1.8 g/cm^3. One study has discussed that with a dry bulk density of 1.7 g/cm^3 or higher, population of soil microorganisms can be affected (Nawaz et. al, 2013), but no research has done on measuring the effect. The reason I think we got higher population of fungi is that the compacting process might be breaking up soil macro & micro aggregates and or separating spores from compound conidiphores. These aggregates can

have multiple fungal components but when they are not broken they would tend to produce only one colony on the plate. On contrast, when they are broken, they produce multiple colonies. In the future, we may use genomic analysis on diversity of microorganisms present in our soil sample. I could also grow fungi for a shorter than 2 days since the fungi grow very rapidly, and it can become hard to count the total fungi colony forming units on the plates if we wait too long. I was quite surprised not to observe fungal colonies at 10^5 dilution, since the literature indicates that I should observe colonies (Watson, 1971).

Effect of soil compaction on different genera of fungi

Even though, compacted soil does have a greater number of CFUs across all genera especially Penicillium and Yeast, the number of plates examined was very small so the results are not statistically significant. Nevertheless, they do seem to indicate that compaction of soil to 1.8 g/cm^3 bulk density do result in a higher number CFUs of certain genera of fungi such as Penicillium and Yeast. A great deal more work will need to be carried out to confirm these initial results and begin to determine what effects, if any, this will have on soil health and fertility and the ability of soil to support optimal plant growth.

Effect of soil compaction on meso- and micro-fauna

Using the Berlese funnel analysis, we saw only springtails in our samples extracted from soil. And we found that compaction has lead to a disappearance of springtails. Studies have found that there are major effects from soil compaction on larger soil organisms such as earthworm and other small arthropods (Schneider. Et al 2011). The results here are consistent with our hypothesis. We hypothesized that if we decrease the pore space between soil particles,

the small pore space will have significant impact on meso- and micro-fauna in soil. Next time, we may try different method to capture more soil organisms. For example, researches have shown that Pitfall traps can capture the maximal taxa compared with Winkler and Berlese funnel (Sabu et. al, 2011). Since we are just looking for a maximum number and diversity of microorganisms in soil, it will be better if we can capture more soil organisms in future experiment.

Limitations

Because the culture method can only recover a small fraction (about 3%) of microorganisms, we cannot conclude if the compaction can also impact other species of bacteria and fungi other than the ones we recovered using culturing method. However, the method we applied (Schneider. Et al 2011) is adequate to draw a conclusion to compare microorganism and meso-fauna population on particular types in compacted and un-compacted soil, which shows that there is a different number of bacteria and fungi growing on compacted and un-compacted soil. Long-term effect of soil compaction hasn't been studied because of the time restriction.

Future research

Since we found that the number of bacteria and fungi is different between compacted and un-compacted soil, it will be interesting if further study can be carried to see how different types of microorganisms behave as soil compaction increases. However, it can be a challenge since this method will need to detect only live bacteria and fungi so that we won't be counting the

already/previously dead bacteria and fungi. It will also be interesting to look how crop production has been affected by soil compaction globally.

Conclusion

The study of how soil compaction can affect soil organism helps us understand more on the mechanism of soil compaction. Modern agricultural compaction has become prevalent with more intensive machinery use in the field as more food is needed to feed the growing global population. This study is intended to begin to reveal what happen in soil in a compaction event. Specifically, the compaction carried out by a hydraulic press surprised us how soil with certain water content can flow out of the container used to hold the soil with high pressure. Evidently all as the compaction proceeds the total pore space is reduced, air is compressed and pushed out of the soil and all the pores become water filled, at which time the soil cannot be compressed further. More over, this research gives us more understanding on the significant impact of soil compaction on soil organisms. Therefore, with more understanding of negative and long term effect of soil compaction on soil organisms and the physical structure of soil, we can really identify and quantify the mechanisms and biological reactions in compacted soil and begin to devise agricultural practices which will minimize or eliminate the use of compaction practices.

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