

## ENUMERATING SOIL BACTERIAL POPULATIONS IN THE CBARC LONG-TERM PLOTS

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### Abstract

Diverse soil bacterial populations contribute to the breakdown of crop residues, nutrient cycling, and nitrogen fixation. In addition, the size and activity of bacterial population may also be indicative of overall soil quality. In this study, dilution-spread-plating methods were used to enumerate the bacterial populations from several Columbia Basin Agricultural Research Center long-term plots, in Pendleton, OR. By diluting soil samples, plating a portion of these dilutions on nutrient agar, and incubating the plates for 24 and 48 hours, the number of bacteria per unit of dry weight soil was determined. Bacterial populations, in a no-till experiment, were found to be greater in a 120 lbs/acre N treatment than in a 0 N treatment. In contrast, bacterial populations in conventionally tilled 0 N and 120 lbs/acre N treatments were similar, demonstrating that the no-till residue management, at higher N levels, will support greater bacterial populations. This study also examined seasonal changes in populations. A general decrease in bacterial numbers was observed in the fall. This general decline may have been due to colder temperatures or the depletion in available nutrients. The addition of supplementary organic material, such as manure, increased bacterial numbers, even in conventionally tilled systems.

### *Key Words*

Bacteria, Long-term experiments, Soil Management, No-till, Seasonal change

### Introduction

Bacteria compose the largest portion of soil microorganisms. They are a useful indicator of the overall diversity and health of soil communities. Even soils planted in wheat monocultures support highly diverse bacterial populations that are responsible for carbon cycling and, to some extent, the breakdown of crop residue and nitrogen fixation. Additionally, the presence of certain bacteria have been linked to increased plant growth and disease suppression.

Because of their high variability, small size, and large numbers even in the poorest of soils, recognizing the importance of bacteria in any soil community is easier than accurately enumerating them or quantifying their activity. Some techniques, like soil respiration or fluorescein diacetate hydrolysis (Smith and Albrecht 2001), attempt to determine the total microbial activity. Other methods are used to quantify the bacteria present. For instance, direct counting methods using epifluorescence microscopy allow microbiologists to count individual cells in soil sub-samples (De Fede and Sexstone 2000). Counting can also be accomplished using pour or spread plating techniques, where a dilute soil suspension is either added to warm, liquid agar and then poured into a petri dish or spread across the top of a pre-poured plate. After an appropriate incubation to allow bacteria time to multiply, colonies are counted (Alexander 1998).

In this study, spread plating was chosen for a number of reasons. Unlike direct counting that does not distinguish between living and dead cells, in spread plating viable cells grow and are counted. Pour plating, a similar technique, distributes cells throughout the agar, making counting large numbers of colonies difficult and inaccurate. One disadvantage to spread plating is that only culturable (able to grow on laboratory media) bacteria are counted.

The goals of this study were to determine the relative populations of soil bacteria residing in several Columbia Basin Agricultural Research Center (CBARC) long-term plots and evaluate any correlations between farming practices and bacterial population size. Samples were collected during summer and fall, to examine seasonal-shifts in bacterial numbers. Determining the size of bacterial populations can be an important part of assessing overall soil quality. Similarly, studying population variability may help us understand the relationship between tillage management and bacterial communities. Differences in bacterial populations may also show agriculture's long-term effects on soil quality and composition.

### **Materials and Methods**

Soil was collected from eleven treatments in the CBARC long-term plots (Rasmussen and Smiley 1994) during summer 2000, summer 2001, and fall 2001. In summer 2000, soil was collected from three different No-Till experiments: no-till wheat/fallow rotation for eighteen years, NT-A; no-till wheat/fallow rotation for three years, NT-B; and conventionally tilled wheat/fallow rotation for eighteen years, CT. The NT-A and NT-B experiments have five fertilizer treatments ranging from 0 to 180 lbs. of nitrogen/acre, but samples from NT-A and

NT-B were taken from the 0-N and 120-N only. CT 0-N and 120-N were also included. Summer and fall 2001 samples were taken from the fall burn (CRFB) and manure (CRM) treatments in the long-term Crop Residue experiment. Samples were also taken in summer and fall 2001 from the Grass Pasture (GP), Annual Wheat No-Till (AWNT), and Annual Wheat Conventional Till (AWCT). Samples came from fallow ground in NT-A, NT-B, CT, CRM, and CRFB, but for GP, AWNT, and AWCT, samples were taken from cropped or recently harvested areas. In order to reduce spatial variability, four soil cores were taken from each plot and combined to form a composite sample. Each cylindrical core was 4 in. deep with a diameter of approximately 1 in.. Sterile collection technique was used to exclude lab-based contaminants.

Soil from each sample was used to determine soil moisture and to perform a dilution series (Alexander 1998). A sub sample was diluted according to procedures outlined by Alexander (1998). Three nutrient agar plates were spread at each of three dilution levels--1:10,000; 1: 100,000; and 1:1,000,000.

During incubation, humidity and temperature approximated soil conditions (Germida 1993). After incubation at 30°C for both 24 and 48 hours, each colony on the plate was counted. When plates were counted at 24 hours, they were kept closed to prevent any contamination that would influence the 48 hour count. The 48 hour incubation allowed slow growing bacteria time to form colonies, but prevented the growth of fungi that begin to overgrow plates after 2 days.

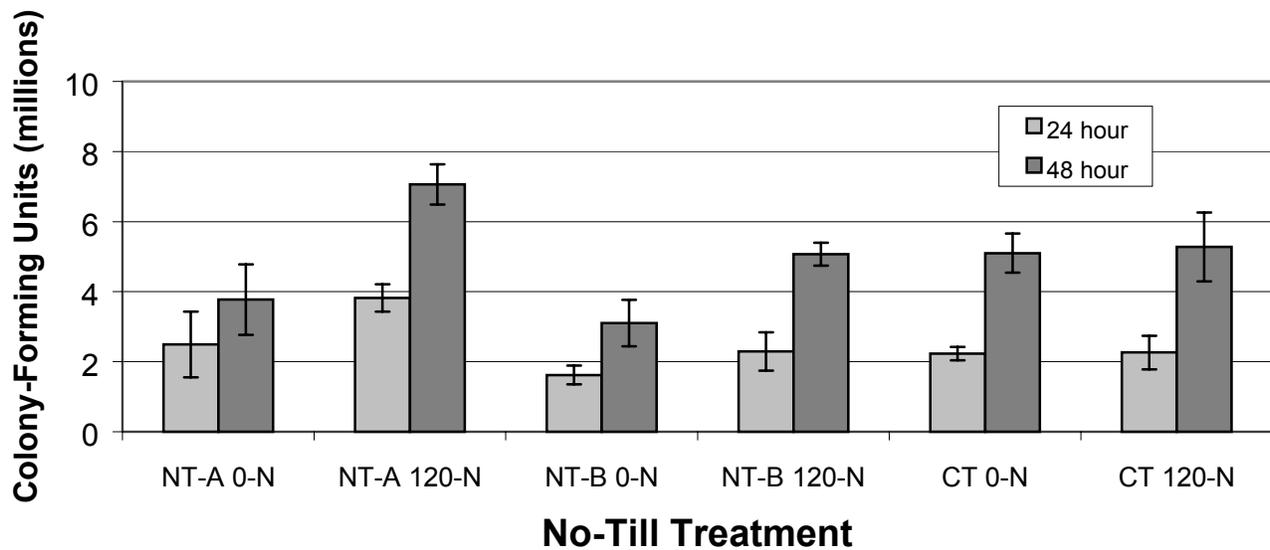
Plates with 30 and 300 colony-forming units (CFUs) were selected for counting. Colony

forming units ranged in size from pinpoints (diameter less than .04 in ) to dime size (diameter of approximately half an in) colonies that were visible to the naked eye. Plates containing more than 300 colonies were not counted to avoid inaccuracies (Bailey and Scott 1966). CFUs were corrected for dilution level and moisture content before the final counts were expressed as the number of 100 culturable bacterial cells per .035 oz. (1g) of dry soil.

## Results

The plate counts for six different tillage/fertilizer treatments in the No-Till experiment are summarized in Figure 1. The numbers of CFUs are the mean of four field replications and two lab replications. Error bars were calculated using the standard error of the mean (SEM).

Figure 1. Mean colony-forming units (CFUs) for three No-Till tillage treatments and two fertilizer treatments in the long-term plots at CBARC, Pendleton, OR, summer 2000.



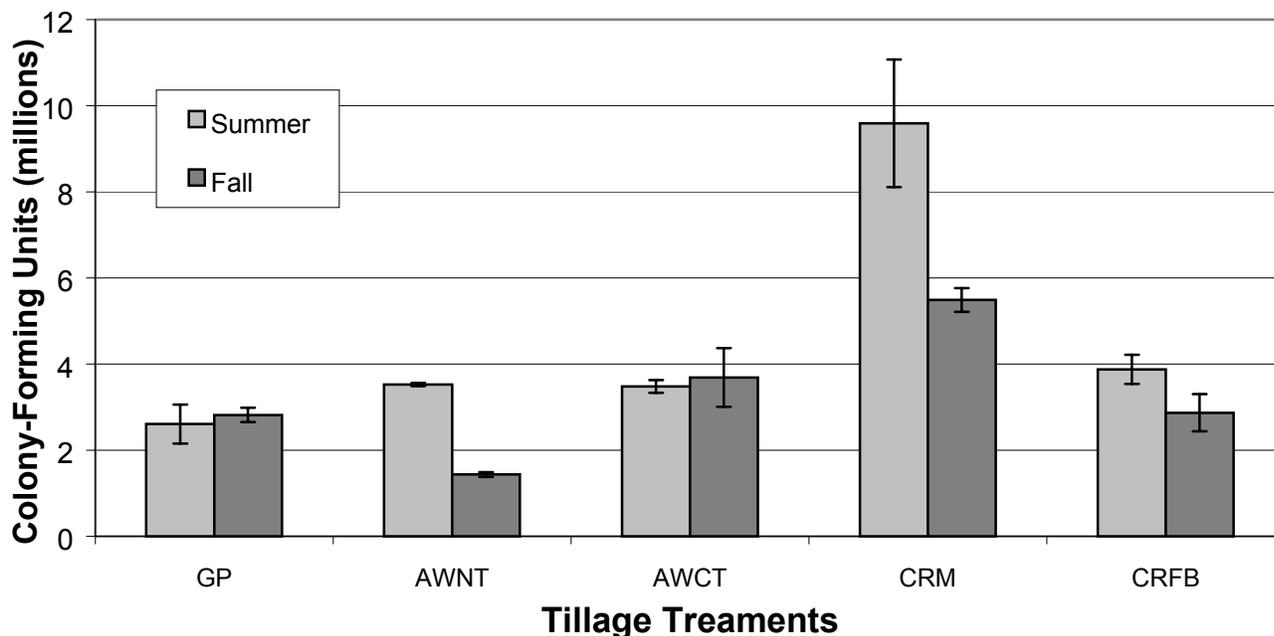
Plots include NT-A no-tilled for eighteen years, NT-B no-tilled for three years, and CT conventionally tilled. Fertilizer treatments include O-N (no nitrogen added) and 120-N (lbs/acre). Errors were calculated from the standard error of the mean between four field replications and two lab replications.

Twenty-four hour CFUs for the No-Till plots ranged from a low of 1.6 million in the NT-B 0-N to a high of 3.8 million in the NT-A 120-N. All six No-Till treatments showed increasing bacterial numbers between 24 and 48 hours, although the rate of increase varied among treatments (rate calculations are not shown). At the end of 48 hours, CFUs ranged from 3.1 million in NT-B 0-N to 7.1 million in the NT-A 120-N. The NT-A treatments had the greatest variability with an increase of approximately 47 percent from no nitrogen to 120 lbs/acre. In contrast, the CT were similar with 5.1 million for 0-N and 5.3 million for 120-N.

Five CBARC long-term experiments were sampled during the summer and fall of 2001, to study possible seasonal changes and differences among farming systems (Figure 2).

Summer samples ranged from 2.6 million in the GP to 9.6 million in the CRM. The fall range was lower, between 1.43 million for AWNT to 5.5 million in CRM. A decline in the number of bacteria from summer to fall was observed in the CRM, CRFB, and AWNT which decreased by almost 60 percent. The CRM also showed a substantial decline of approximately 43 percent. The results from the GP and AWCT registered a slight increase from the summer to fall sample.

Figure 2. Mean colony-forming units (CFUs), during summer and fall 2001, inside five long experiments at the Pendleton, OR experiment station.



Soil was collected from the Grass pasture (GP), Annual wheat no-till (AWNT), Annual wheat conventional till (AWCT), Crop residue manure (CRM), and Crop residue fall burn (CRFB). Error bars were calculated using the standard error of the mean between three lab replications.

## Discussion

An increase in the number of CFUs were observed in NT-A, NT-B, and CT from 24 to 48 hours. CT 0-N and 120-N both had increases of approximately 56 percent between 24 and 48 hours, but the NT-A 0-N only increased 25 percent. The different rates of increase may be due to differences in the bacterial compositions between soil samples. For example, the NT-A 0-N soil may have included bacteria that were dormant at the time of sampling and required a longer incubation before sufficient growth could occur.

A possible relationship between management and the number of culturable bacteria was observed in summer 2000. The largest differences in 48 hour 0-N and 120-N were in the NT-A, showing that bacteria were present in greater numbers when fertilizer was added. The length of time that plots were no-tilled rather than conventional-tilled could have also contributed to NT-A numbers, because it had been no-tilled fifteen years longer than NT-B. The number of organisms may have increased in relation to nitrogen fertilization levels because the fertilization may provide more available nitrogen for bacterial populations or because higher nitrogen levels promote crop growth, which in turn generates more residue, hence, providing more material to support bacterial growth and development.

The application of other fertilizers, such as manure, may similarly increase bacterial populations, as in CRM. A difference of almost 60 percent was observed in 2001 between the CRM and the CRFB. This increase may be a result of an added nutrient base, which encouraged the growth of bacteria already in the soil. Another possibility is that application of manure

introduced more bacteria. The lower numbers in CRFB may reflect a lack of usable carbon.

A combination of factors may have contributed to the population decreases from summer to fall in both the CRM and CRFB. One possible reason for the large decline in the CRM could be the time between addition of the manure and collection of the sample. Manure was added 47 days before the summer sample; in contrast, the fall sample was taken over 150 days after manure addition. Regardless of the reason for this initial increase in bacteria immediately following manure addition, at least some of these bacteria may not survive to reproduce (Albrecht et al. 1983). At least two other environmental changes may have contributed to the CRM and CRFB decreases. The bacteria may have had a limited carbon source in November or may have been killed by low temperatures.

With the exception of CRM, the remaining results were similar. Although the CRFB showed a slight elevation over AWCT, there was no substantial difference. A comparison of AWCT and CRFB showed that the size of bacterial populations was the same for fallow and continuous wheat. Numbers from treatments where crops were present may be low for the same reason that the CRFB numbers were low. Growing plants may tie up nutrients, thereby decreasing the number of bacteria.

The decrease in bacterial populations between summer and fall from AWNT may have been due to an unidentified environmental stress. One of the largest obstacles to accurately enumerating bacterial populations is spatial variability, even in a small geographic area. Although a composite sample was taken from each treatment, pockets containing unusually

composite sample was taken from each treatment, pockets containing unusually large or small populations of bacteria could have biased results. Also, interactions of bacteria with other microorganism have not been considered. For example, the AWNT decline may have been the result of competition from more successful fungi, rather than a lack of carbon source.

Plate count results illustrated possible correlations between farming practices and bacterial numbers. For example, long-term no-till farming may have contributed to higher bacterial populations. Established populations of bacteria may be able to use increased nitrogen levels for growth, or nitrogen fertilizer, resulting in increased plant productivity, provides an energy source for larger soil bacterial populations. Similarly, the plate count data showed a distinct increase in the bacterial populations, when manure is added. Given the decline in the CFUs as the time between manure addition and sampling increased, further tests could determine if populations eventually stabilize and if this level is above or below other treatments. Collecting data in winter and spring could further our understanding of bacterial responses to environmental stresses. Given the variability among plots at any one time, future studies should rely on a greater number of samples per area. Increased sampling cannot guarantee, however, that the great diversity and uneven distribution of bacterial populations will be adequately represented.

### Summary

Bacterial populations in the soil of various CBARC long-term plots were determined using traditional dilution, spread plating methods. By diluting soil samples, plating on nutrient agar, and growing for 24 to 48 hours the number of bacteria per unit of soil

could be determined. Comparison of the 0-N and 120-N data collected from the No-Till experiments in 2000 showed a possible link between no-till farming and an increase in bacterial populations. Comparison of bacterial numbers between the summer and fall of 2001 for five different long-term experiments showed a general decrease in populations in the fall. A bacterial population increase was observed in the Crop Residue treatment with periodic manure additions, when compared to the Crop Residue treatment with a fall burn and no addition of fertilizer.

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