EFFECTS OF TILLAGE AND CROP ROTATION ON MICROBIAL DIVERSITY IN WHEAT RHIZOSPHERE

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ABSTRACT

The effects of tillage and preceding crops in a legume-based rotation on the diversity of microbial communities in the rhizosphere of wheat were assessed in a field experiment. Zero tillage and conventional tillage systems were compared and the crops that preceded wheat in the rotation were field peas, red clover green manure, summer fallow and wheat. Biolog™ media were used to evaluate microbial functional diversity by assessing the numbers and types of substrates that could be utilized by the soil bacterial community. There were no significant differences in microbial diversity between treatments prior to seeding. In the rhizosphere, microbial diversity was significantly greater under zero tillage than under conventional tillage at four-leaf stage, but the difference was not significant at flag-leaf stage. In the bulk soil, conventional tillage resulted in significantly greater microbial diversity at four-leaf stage, but zero tillage had significantly greater diversity at flag-leaf stage. Preceding crops did not significantly affect microbial diversity at any sampling time. Principal component analysis revealed that two components accounted for 7 1-97% of the variation in microbial diversity, with one component attributable to differences in the ability of microbes to utilize amines/amides, amino acids and carboxyllic acids. and the other component due to differential utilization of carbohydrates, polymers and other substrates.

INTRODUCTION

Conservation tillage systems are becoming increasingly popular in the Peace River region. Because they conserve residue cover and reduce soil erosion and compaction, such tillage systems affect soil structure, soil water, soil organic matter and soil organisms and may improve soil fertility and crop response. Cereals dominate crop production in the Peace River region, with canola as the only significant broad-leaf crop in the rotation. Economic pressure on cereal production will cause farmers to consider well-planned crop rotations. especially those that utilize legumes as break crops. As farmers adopt conservation tillage practices and alternate cropping sequences, changes in soil

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quality and crop responses will occur.

The objective of this work was to investigate the effects of zero tillage and preceding crops in a rotation on soil microbial diversity. We used the method proposed by Zak et al. (1994) to quantitavely evaluate functional microbial diversity based on patterns of substrate usage. In this context, microbial functional diversity is defined as the numbers. types. activities and rates at which a suite of substrates (carbon sources) are utilized.

MATERIALS AND METHODS

The study was conducted on an existing crop management trial. which was established in 1992 on a sandy-loam dark-gray Luvisol at Fort Vermillion in northern Alberta. The trial was arranged in a split-plot randomized complete block design. The main plots were tillage treatments: zero tillage (ZT) and conventional tillage (CT). The sub plots consisted **of** different crop sequences in rotation. Data for this study was collected in 1995 in wheat plots that had been preceded by field peas (FP), red clover green manure (GM), summer fallow (SF) or continuous wheat (CW). Soil samples were collected prior to seeding and at four-leaf and flag-leaf stages of wheat growth from 0-7.5cm depth. Prior to seeding, ten samples were collected at random from each plot and bulked. At four-leaf and flag-leaf growth stages, 120 wheat plants were carefully excavated from each plot. Loose soil was shaken off the roots. The soil that adhered strongly to the roots was carefully scraped and kept separately as rhizosphere soil. Non-rhizosphere (bulk) soil was sampled between wheat rows and kept separately.

A ten-fold dilution series was made from 1 g sub-samples of the bulked soil samples. To standardize inoculum density, the optical density (at **36011111)** of the 10^{-3} dilution was measured with a spectrophotometer and the samples were adjusted to have uniform optical density at $1\ 0^{-3}$ dilution according to a pre-established calibration curve. Inoculum aliquots of 150µl were added to each of 96 wells of Gram-negative (GN) and Gram-positive (GP) Biolog™ microplates (Biolog Inc., 3938 Trust Way, Hayward, CA 94545, USA). The plates were incubated at 25°C. Optical densities in the wells, which reflected the density and activity of the bacterial species capable of utilizing the substrate contained in each well, were read with an ELISA plate reader (at 490nm) after 48h of incubation for rhizosphere samples and 72h for non-rhizosphere samples. These times were selected because they produced the best resolution of results.

In evaluating functional diversity, we combined the results from GN and GP microplates in order to use a large number (128) of different substrates, consisting of 45 carbohydrates, 29

carboxyllic acids, 7 polymers, 7 amines/amides. 20 amino acids and 19 miscellaneous substrates (Zak et al., 1994). The results that we report here are based on mean optical densities of the six categories of substrates. Four indices of diversity were calculated for each sample (mugurran. 1988 and Zak et al, 1994): Shannon's diversity index (H), which is a measure of overall substrate diversity encompassing substrate richness and substrate evenness, substrate richness (S). which is the number of different substrate types that were utilized. substrate evenness (E). which is a measure of how equally abundant the different types of bacteria were. and sum of activities (N), i.e.. the sum of optical density values of the six substrate types.

For each sampling time, analysis of variance was conducted on the H. E and N data separately for rhizosphere and bulk soils according to the split-plot design of the experiment. Since all the six substrate types were used in all cases, no statistical analysis was conducted on S. There was insignificant variation in E between treatments at all sampling times. For that reason. H and N varied similarly between treatments; but N was more sensitive than H. Therefore, we report sum of activities (N) data as indicative of microbial diversity.

In order to explain the diversity differences in terms of the types of substrates that were utilized, we used principal component analysis (PCA) to analyze the activity levels (optical density values) on each substrate type for each treatment (SYSTAT, 1992). This procedure, in which we factored a correlation matrix, summarized the six-dimensional data (for the six substrate types) using two components and showed which substrate types explained the differences within the components Pielou, 1984).

RESULTS AND DISCUSSION

There were no significant differences in sum of activities (N) between tillage systems or preceding crops prior to seeding, when the soil was all non-rhizosphere (Fig. 1). At four-leaf growth stage of wheat, microbial diversity was significantly greater under ZT than under CT in wheat rhizosphere, but the reverse was true in bulk soil (Fig. 1). Preceding crops in rotation had no significant effect on microbial diversity in the rhizosphere or bulk soil, and interaction between tillage and preceding crop was also not significant. At flag-leaf stage, neither tillage nor preceding crops had significant effects on microbial diversity in the rhizosphere. but diversity was significantly greater under ZT than CT in the bulk soil (Fig. 1).

Fig. 2 shows the separation of treatments into two principal components for pre-seeding soil. PC1 separated CTGM, ZTFP, ZTCW and CTCW from CTSF, ZTGM. CTFP and ZTSF while PC2

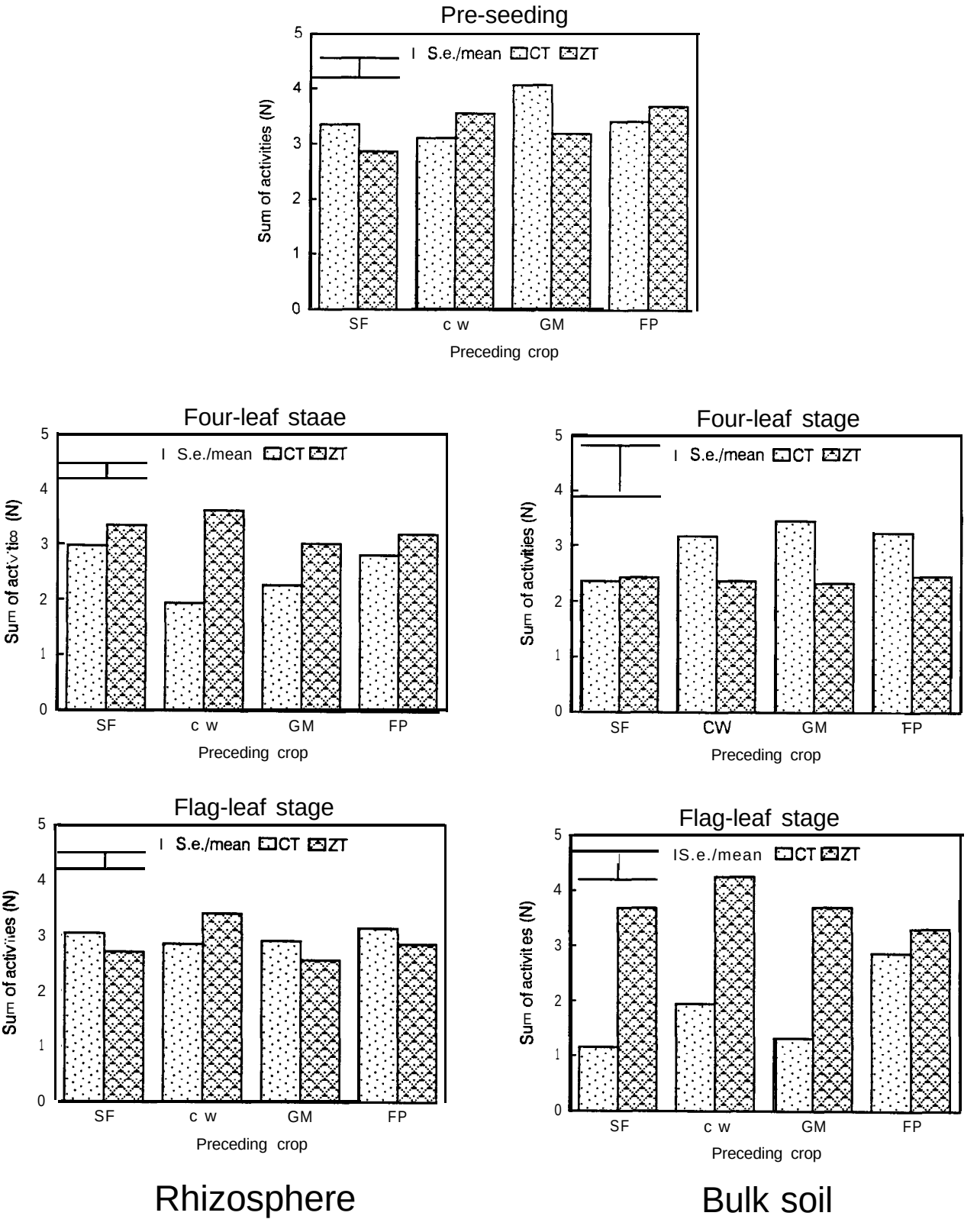

Figure 1. Effects of tillage and preceding crops on microbial diversity in the rhizosphere and bulk soil at three sampling times.

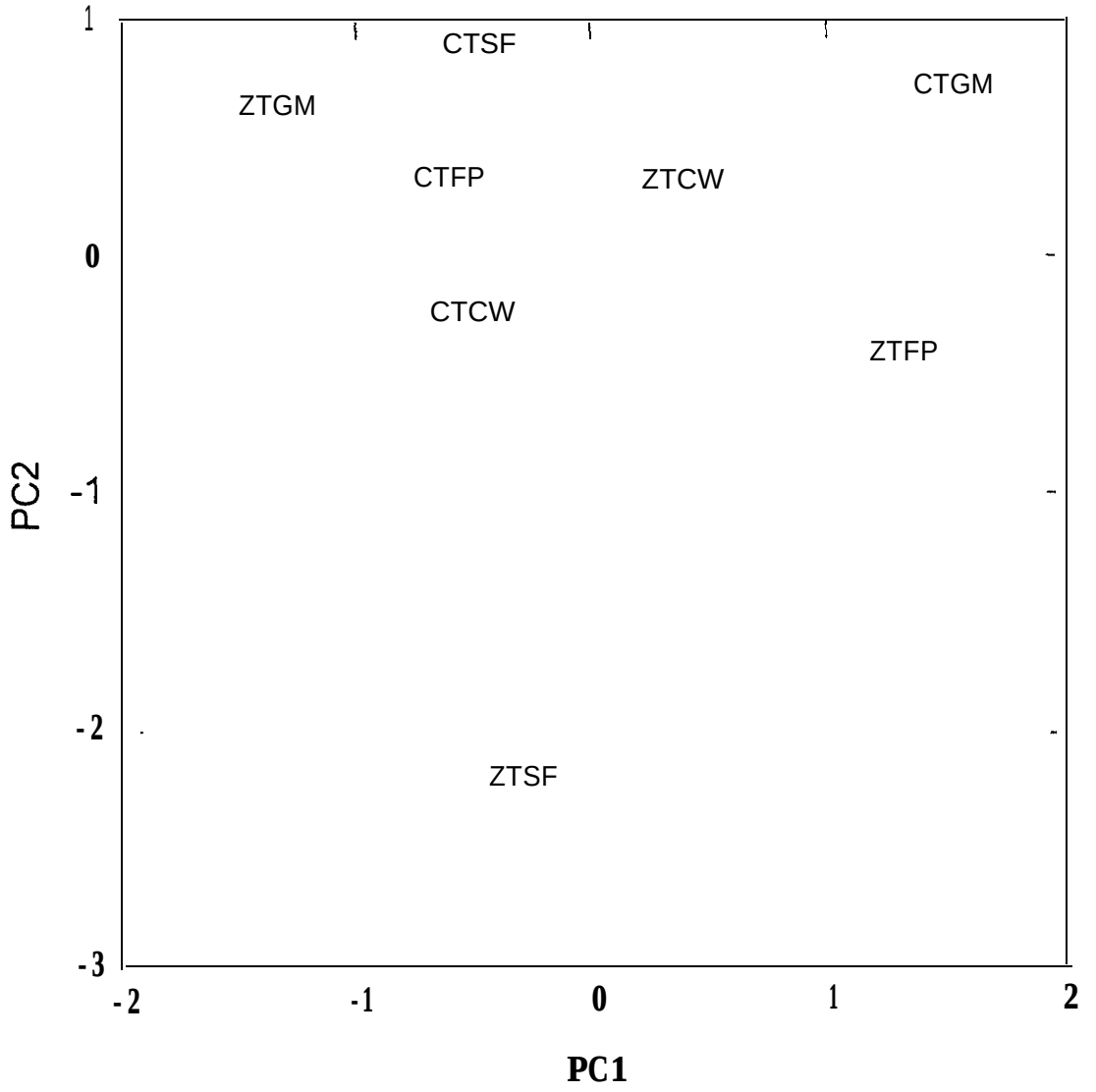

Figure 2. PCA ordination of tillage and preceding crop treatments prior to seeding.

separated ZTSF from all other treatments. In these groups of treatments. tillage or preceding crop treatments are not clustered together, and this is consistent with microbial diversity results which showed no significant differences between these treatments prior to seeding (Fig. 1). Table 1 shows that PC1 separated treatments in which the bacteria utilized polymers. carboxyllic acids. amines/amides and amino acids differently. (This is indicated in principal component analysis results by high PC 1 loadings for these substrate types.) Thus, the bacteria in CTGM, ZTFP. ZTCW and CTCW utilized polymers less, but utilized carboxyllic acids aminesiamides and amino acids more than the bacteria in CTSF, ZTGM. CTFP and ZTSF. Similarly. PC2 separated treatments in which the bacteria utilized miscellaneous substrates and carbohydrates differently. i.e.. bacteria in ZTSF utilized these substrates less than bacteria in all the other treatments. With this kind of analysis, the treatments were separated into four groups as shown in Table 1. although in this case there were no treatments in one of the four groups.

Table 1. Separation of treatments according to substrate utilization: pre-seeding bulk soil

1. Utilized polymers **more,** but carboxyllic acids, amines and amino acids **less** **2.** Utilized miscellaneous substrates and carbohydrates **more** CTSF, ZTGM & CTFP	1. Utilized polymers less. but carboxyllic acids. amines and amino acids **more** **2.** Utilized miscellaneous substrates and carbohydrates **more** CTGM, ZTFP. ZTCW & CTCW
ZTSF 1. Utilized polymers **more,** but carboxyllic acids, amines and amino acids less 2. Utilized miscellaneous substrates and carbohydrates less	 1. Utilized polymers less, but carboxyllic acids. amines and amino acids **more** **2.** Utilized miscellaneous substrates and carbohydrates less

Results of similar analyses at four-leaf stage are presented in Table 2 for rhizosphere soil and Table 3 for bulk soil. Tables 4 and 5 show corresponding results for flag-leaf stage data. The two principal components accounted for 7 1% (pre-seeding) to 97% (flag-leaf stage bulk soil) of the variation in the data. The results show that in pre-seeding bulk soil (Table 1) and in four-leaf stage rhizosphere (Table 2), amines/amides, amino acids and carboxyllic acids separated treatments along

PC 1 while carbohydrates. polymers and miscellaneous substrates separated treatments along PC2. The reverse occurred in four-leaf stage bulk soil and flag-leaf stage rhizosphere and bulk soils (Tables 3-5), where carbohydrates, polymers and miscellaneous substrates separated treatments along PC 1 and amines/amides. amino acids and carboxyllic acids separated treatments along PC2. Because PC 1 always explained more variation in data than PC2, then differential utilization of amines/amides, amino acids and carboxyllic acids was more important for explaining treatment differences in pre-seeding bulk soil and four-leaf stage rhizosphere than utilization of carbohydrates. polymers and miscellaneous substrates, and vice-versa for four-leaf stage bull; soil. flag-leaf stage rhizosphere and flag-leaf stage bulk soil.

Table 2. Separation of treatments according to substrate utilization: four-leaf stage rhizosphere

1. Utilized amines, amino acids and carboxyllic acids less **2.** Utilized polymers, carbohydrates and miscellaneous substrates **more** ZTFP	1. Utilized amines, amino acids and carboxyllic acids more 2. Utilized polymers. carbohydrates and miscellaneous substrates **more** ZTCW
CTCW, CTGM & CTFP 1. Utilized amines, amino acids and carboxyllic acids **less** **2.** Utilized polymers, carbohydrates and miscellaneous substrates less	CTSF, ZTSF & ZTGM 1. Utilized amines, amino acids and carboxyllic acids **more** 2. Utilized polymers, carbohydrates and miscellaneous substrates less

These results also show that where microbial diversity was significantly different between tillage systems, i.e.. four-leaf stage rhizosphere, four-leaf stage bulk soil and flag-leaf stage bulk soil (Fig. 1), clustering of tillage treatments was evident in corresponding PCA results and that the tillage treatments which had low microbial diversity clustered in the lower left-hand sections of Tables 2. 3, and 5, i.e., the microbes utiiized less of all substrate types. But even when two treatments had the same microbial diversity, PCA showed that the microbial communities in those treatments did not necessarily have the same substrate utilization patterns.

Preceding crops did not have significant effects on microbial functional diversity The effects of crop rotation on microbial function are likely to be most pronounced immediately following crop residue incorporation after harvest. but our sampling times were all later than that.

Table 3. Separation of treatments according to substrate utilization: four-leaf stage bulk soil

1. Utilized carbohydrates, polymers and miscellaneous substrates less 2. Utilized amines/amides, amino acids and carboxyllic acids **more** CTFP	1. Utilized carbohydrates. polymers and miscellaneous substrates **more** **2.** Utilized amines/amides, amino acids and carboxyllic acids **more**
ZTSF, CTSF, ZTCW, ZTGM & ZTFP 1. Utilized carbohydrates polymers and miscellaneous substrates less 2. Utilized amines/amides, amino acids and carboxyllic acids less	CTCW & CTGM 1. Utilized carbohydrates. polymers and miscellaneous substrates **more** **2.** Utilized amines/amides, amino acids and carboxyllic acids less

Table 4. Separation of treatments according to substrate utilization: flag-leaf stage rhizosphere

1. Utilized carbohydrates. miscellaneous substrates and polymers less **2.** Utilized carboxyllic acids and amino acids **more** CTGM. ZTSF, ZTGM & CTCW	1. Utilized carbohydrates. miscellaneous substrates and polymers **more** 2. Utilized carboxyllic acids and amino acids **more** CTSF. CTFP & ZTCW
  1. Utilized carbohydrates, miscellaneous substrates and polymers less 2. Utilized carboxyllic acids and amino acids **less**	ZTSF 1. Utilized carbohydrates. miscellaneous substrates and polymers **more** 2. Utilized carboxyllic acids and amino acids **less**

Table 5. Separation of treatments according to substrate utilization: flag-leaf stage bulk soil

1. Utilized miscellaneous substrates carbohydrates and polymers less **2.** Utilized amines/amides, amino acids and carboxyllic acids **more** ZTGM	1. Utilized miscellaneous substrates carbohydrates and polymers **more** 2. Utilized amines/amides, amino acids and carboxyllic acids **more** CTCW
CTCW, CTGM & CTSF 1. Utilized miscellaneous substrates carbohydrates and polymers less **2.** Utilized amines/amides, amino acids and carboxyllic acids less	ZTFP, CTFP & ZTSF 1. Utilized miscellaneous substrates carbohydrates and polymers **more** 2. Utilized amines/amides, amino acids and carboxyllic acids less

It is apparent in this work that microbial functional diversity in wheat rhizosphere is greater in zero tillage than in conventional tillage implying that zero tillage would be more sustainable.

Since these are physiological differences in soil microorganisms. they are likely to drive microbial processes like decomposition of organic residues and nutrient cycling. In a critical analysis of this method of evaluating microbial diversity. Haak et al. (1995) confirmed that whole-community substrate utilization profiles were reproducible signatures of a given bacterial community. but they questioned whether the differences indicated real differences in community function or metabolic potential. It is worth noting that the Biolog system was developed for bacterial identification purposes (Biolog, 1993). A protocol aimed at evaluating functional diversity in soils could be developed in which the substrates included represent those found in soils. Such a protocol should include other microorganisms, particularly fungi.

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