EFFECT OF WHEAT ROTATIONS AND FERTILIZATION ON SOIL MICROORGANISMS AND ENZYMES OF A BROWN LOAM

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INTRODUCTION

On the Canadian Prairies frequent summerfallowing has led to large losses of organic matter (Campbell and Souster 1982) and has seriously impaired the productivity of the soils (Biederbeck et al. 1981; Rennie 1982). However, soil degradation caused by frequent summerfallowing can be arrested and the decline in amount and quality of organic matter can be reversed by use of appropriate agronomic practices. Thus studies with a Brown loam in the Swift Current long-term rotation experiment have shown not only a reversal in organic matter decline but also that physical properties, N-supplying power and biological activity can be improved by a combination of extending the rotation length (cropping annually), applying N and P fertilizers at rates normally recommended by the Soil Test Laboratory, and by using a stubble mulch tillage technique (Biederbeck et al. 1984).

Microbial activity is a major factor controlling fertility and in turn soil quality because it affects nutrient transformations and availability. Recent studies indicate that soil metabolic activities, as reflected by microbial biomass, respiration and enzyme levels, are affected not only by cultivation (Gupta and Germida 1986) and by tillage systems (Carter and Rennie 1982) but also by fertilization and cropping practices (Bolton et al. 1985). As our initial assessment of soil biological changes in the Swift Current rotation experiment examined only four rotation-fertilizer treatment combinations and did not include enzymic measurements, the objectives of the present study were

to determine the effects of more treatments on a wider variety of microbial populations and on some prominent soil enzymes.

MATERIALS AND METHODS

Details of the design and method of this experiment have been published elsewhere (Campbell et al. 1983); consequently, only a brief review is presented together with some additional information required for an understanding of the procedures and parameters discussed.

Table 1. Crop Rotations and Treatments

Rotation Number	Rotation[+] Sequence	Fertilizer Application
1	(Fallow)-wheat-wheat	P applied, no N applied.
2	Fallow-wheat-(wheat)	N and P applied.
3	Fallow-flax-(wheat)	N and P applied.
4	Fallow(fall rye)-wheat	N and P applied.
5	Fallow-wheat-wheat	N applied, no P applied.
6	(Oat hay)-(wheat)-wheat	N and P applied; oats cut for hay at soft dough stage.
7	Flax-wheat-wheat	N and P applied.
8	(Continuous wheat)	N and P applied.
‡9	Continuous wheat	(Fallow if less than 60 cm of moist soil exists at seeding time); N and P applied.
‡10	Continuous wheat	(Fallow if grassy weeds become a problem); N and P applied.
11	Fallow-(wheat)	N and P applied
12	(Continuous wheat)	P applied, no N applied.

[+] Special plots are indicated by brackets. These plot treatments were sampled for nutrients, soil moisture and plant growth at eight regular intervals during the growing season.

‡ Rotations 9 and 10 were cropped continuously during the first 12 yr because the criteria necessary for fallowing did not occur; these rotations were then changed to fallow-lentils.

In 1967, 12 crop rotations (Table 1) were established on 81, 0.04-ha, plots located on Wood Mountain loam, a Brown Chernozem in a 3-replicate experi-

ment. The land had previously been cropped in a fallow-wheat rotation since 1922.

Fertilizer N, as ammonium nitrate (34-0-0), was broadcast and incorporated prior to spring cultivation; fertilizer P, as mono-ammonium phosphate (11-48-0), was placed with the seed. The fertilziers were applied in accordance with treatment specifications (Table 1) and the general recommendations of the Saskatchewan Soil Testing Laboratory. Commercial farm equipment was used to perform cultural and tillage operations. Weed control was achieved by a combination of mechanical tillage and by spraying with herbicides (as required) at recommended rates.

The following seven rotation-treatment combinations were sampled for soil microbiological and biochemical analyses: the fallow and the wheat phase of the F-W rotation (i.e. #11), the stubble wheat phase from each of the three differently fertilized F-W-W rotations (i.e., #1, #2 and #5) and the two differently fertilized continuous wheat rotations (i.e., #8 and #12 in Table 1). The soils were sampled on October 25, 1983 by taking three cores, each at 10-m intervals, down the centre of each plot and then bulking these three subsamples into one representative sample per plot. All samples were immediately sieved (< 2 mm) and then stored field moist in polyethylene bags at 0°C until they were analyzed. Although several depth segments were sampled and analyzed only results from the top 7.5 cm, the biologically most active segment, are presented here.

Serial dilutions of soil samples were prepared and total counts for the four principal types of heterotrophic organisms were determined by spread plate technique. Bacterial and actinomycete populations were enumerated on soil extract agar and those of filamentous fungi and yeasts on rose bengal-streptomycin agar. The number of antotrophic nitrifying bacteria was

estimated by a simplified MPN method (Sarathchandra 1979) and denitrifying bacteria were enumerated by the standard MPN method (Alexander 1965) but with the inclusion of Durham tubes to confirm gaseous evolution. Microbial biomass was determined by the chloroform fumigation-incubation technique (Jenkinson and Powlson 1976). A k value of 0.41 was used for conversion of CO_2-C to biomass carbon (Voroney and Paul 1984).

All enzyme assays were replicated three times. Dehydrogenase activity was determined by the method of Casida et al (1964). Urease activity was measured with the non-buffer method of Zantua and Bremner (1975). Acid and alkaline phosphatase and also arylsulfatase activities were determined from the release of p-nitrophenol (PNP) when soil was incubated with p-nitrophenyl phosphate or p-nitrophenyl sulfate as described by Tabatabai and Bremner (1969, 1970).

RESULTS AND DISCUSSION

The population of aerobic bacteria increased significantly (p.05) from the fallow phase of F-W with cropping and with lengthening of the rotation (Table 2). Among similar rotations bacterial numbers tended to be greater where only P fertilizer was applied. However, the actinomycete populations did not follow this trend (Table 2). In fact, numbers of these autochtonous-type organisms did not differ significantly between any rotation at 17 years after initiation of these different rotation and fertilizer treatments. The populations of filamentous fungi showed a very different trend from that observed with bacteria as numbers of fungi were greatest in the fallow phase of F-W and decreased significantly to the well-fertilized 3-yr and continuous wheat rotations and were consistently lowest in the P only fertilized 2-yr (i.e., F-W in most years), 3-yr and continuous wheat rotations (Table 2). As

Table 2. Effect of crop rotation and fertilization on soil microbial populations, biomass and respiration after 17 years

	Rotation and fertilizer treatment						
	F-W $\underline{N}$,P	F-$\underline{W}$ N,$\underline{P}$	F-W-$\underline{W}$ N,P	F-W-$\underline{W}$ P	F-W-$\underline{W}$ N	Cont. W N,P	Cont. W P
Microbial counts (organisms/g soil)							
Bacteria (x 10^6)	65	80	101	116	112	113	123
Actinomycetes (x 10^6)	25	23	28	28	24	29	26
Filamentous fungi (x 10^4)	69	26	55	35	44	51	33
Yeasts (x 10^4)	31	10	20	12	14	20	6
Denitrifiers (x 10^3)	6.5	2.3	13.3	1.0	13.3	13.7	2.0
Nitrifiers (x 10^3)	17.1	5.0	25.2	11.5	9.6	5.6	2.3
Microbial biomass (µg C/g soil)	203	266	285	334	309	319	394
Respiration, cumulat. 14 days (µg CO_2-C/g soil)	62	183	244	220	244	243	291

the dilution plate count technique is known to enumerate primarily those soil fungi that were originally present as spores the very high population found under fallow could suggest that the fungus flora in this soil is metabolically rather inactive. However, the yeast counts in Table 2 show that these 'unicellular' fungi, which are a small but metabolically rather active component of the large mycoflora (i.e., eucaryotes) of surface soils, followed the same trend as observed with filamentous fungi except that continuous wheat receiving only P had by far the lowest number of yeasts, thus indicating a low level of activity in this highly N-starved soil. Thus it appears that fertilization with only P generally depressed the population of both types of eucaryotic soil organisms while it tended to increase the population of bacteria. These population shifts seem to corroborate our earlier suggestion (Biederbeck et al. 1984) that qualitative changes within the soil microflora were brought about not only by extended cropping but also by differences in fertilization.

Numbers of potential denitrifiers were not influenced by rotation length but they were greatly affected ($p < .01$) by nitrification and N-fertilization as numbers in the fallow phase (with high NO_3-N level) of F-W and in all N-fertilized rotations were 3- to 10-fold greater than in those rotations that received no N (Table 2). A similar pattern of denitrifier populations was found when the same rotations were sampled again on July 19, 1985 (data not shown). Thus the enhancement of denitrifiers by soil nitrate accumulation and N-fertilization seems to be very consistent and was evident despite the severe drought during the summer of 1985. The nitrifier population too was significantly enhanced by N-mineralization and by N-fertilization, not only in late fall of 1983 (Table 2), but also in mid-summer of 1985 (data not shown).

It is interesting that changes in microbial biomass across the seven rotation-treatment combinations followed the same pattern as that found for

aerobic heterotrophic bacteria and these resutls confirm our earlier report (Biederbeck et al. 1984) of soil microbial responses found with four of the seven rotation treatments. Biomass-C increased significantly from the fallow phase of F-W with cropping and with lengthening of the rotation and also with P-fertilization and, as with the bacteria, the amount present in continuous wheat receiving only P was roughly double that found in the fallow soil (Table 2).

The respiratory activity was very low in the bare fallow soil and increased sharply with cropping (Table 2), but it was not significantly affected by fertilization within the 3-yr rotations or in continuous wheat.

The dehydrogenase activity was also lowest in bare fallow and increased sharply with cropping but also with increasing rotation length (Table 3). Under continuous wheat the activity increased significantly when fertilized only with P. The levels of activity found in all but the bare fallow soil appear to be rather high when compared to literature values (Bolton et al. 1985; Gupta and Germida 1986). However, as the dehydrogenase enzymes are thought to be linked with microbial activity associated with initial breakdown of organic matter (Ross 1971) it is reasonable that their activity should be very high at the surface of cropped soils in fall when large amounts of straw and roots become available for microbial decomposition. Dehydrogenase is also considered to be an index of endogenous soil microbial activity (Moore and Russell 1972) because its assay involves no addition of substrate that would preferentially stimulate any particular group of soil organisms. Thus the pattern of dehydrogenase activities found in the present study (Table 3) would indicate that soil microbial metabolism increased significantly with increasing rotation length.

Table 3. Effect of crop rotation and fertilization on selected soil enzyme activities after 17 years

Type of enzyme	Rotation and fertilizer treatment						
	F-W N,P	F-W N,P	F-W-W N,P	F-W-W P	F-W-W N	Cont. W N,P	Cont. W P
Dehydrogenase (µg TPF/g soil/hr)	154	288	359	381	442	518	667
Urease (µg urea hydrol./g soil/hr)	33	55	60	78	77	75	107
Acid phosphatase, pH 6.5 (µg PNP/g soil/hr)	1223	827	1161	862	1232	1338	959
Alkaline phosphatase, pH 8.5 (µg PNP/g soil/hr)	712	474	620	522	629	710	536
Arylsulfatase (µg PNP/g soil/hr)	45	50	56	70	44	57	83

Urease activity followed a similar pattern as that observed for dehydrogenase with rotation and fertilization effects again being quite singificant (Table 3). The activity of acid phosphatase was affected neither by cropping nor by rotation length but it was very signfiicantly ($p < .01$) reduced wherever the soil had been fertilized only with P (Table 3). Although the activity of alkaline phosphatase was considerably lower than that of acid phosphatase in all soils (Table 3) it followed the same pattern of activity changes being always reduced in response to P-fertilization.

Arylsulfatase activity increased very little with cropping and with rotation length but was very signfiicantly increased in those 3-yr and continuous wheat rotations that received only P (Table 3). The arylsulfatases are enzymes that hydrolyze organic sulfate esters in the large fraction of HI-reducible, rather labile soil-S and they are thought to play an important role in the processes of organic-S mineralization in surface soils (Tabatabai and Bremner 1970; Cooper 1972). Thus our results would suggest that long-term fertilization with only phosphates could result in an increase in the rate of sulfur mineralization.

An examination of all enzymes measured in this study indicates that their activities generally increased with cropping and rotation length (Table 3). This trend was not unexpected considering the corresponding increases in microbial biomass and substrate inputs and it is in agreement with the recent finding by Gupta and Germida (1986) that the activities of all soil enzymes they had studied were very high in native soils, dropped sharply upon cultivation and decreased further with cultivation systems that included summerfallow. However, what is surprising is the fact that we found the activities of some enzymes (e.g., arylsulfatase, urease and dehydrogenase) to be significantly increased by fertilization, particularly where only P was applied. Our

results seem to disagree with the fertilizer effect study by Bolton et al. (1985) that found levels of urease, phosphatase and dehydrogenase were always significantly higher under an organically fertilized (legume green manure) winter wheat system than under the corresponding chemically fertilized system in eastern Washington. It may be that these reductions in soil enzyme activity were due to the three- to five-fold higher rates of fertilizer used in the farm management system at Washington as compared to the low rates applied at Swift Current.

CONCLUSIONS

Our results confirmed earlier indications that soil microbiological and biochemical responses to different agronomic practices under monoculture wheat are extensive and persistent near the soil surface. Thus summerfallowing and N-fertilization, even at the low rates (avg. 32 kg N/ha/yr) used in this experiment, significantly increased the populations of denitrifiers and nitrifiers. P-fertilization consistently reduced the activity of both phosphatases, but increased the activity of arylsulfatase, urease and dehydrogenase. The extensive shifts among the populations of heterotrophic organisms indicate that differences in rotation length and fertilization have effected not only quantitative but also qualitative changes in the soil microflora.

The increases in microbial numbers, biomass and enzyme activities from the typically low level under bare fallow with cropping, increasing rotation length and adequate fertilization prove that marked improvements in the metabolic state and nutrient cycling -- hence the quality -- of a Brown soil can be achieved with progressive agronomic practices.

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