EFFECT OF LEGUME GREEN MANURING ON MICROBIAL POPULATIONS AND ACTIVITY IN A BROWN LOAM

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Abstract

In a 7-yr plot experiment, conducted on a Brown Chemozemic loam at Swift Current, SK to evaluate four annual legumes for fallow (F) replacement green manuring (GM), the surface soil (0-10 cm) under wheat (W) stubble was sampled after 3 GM-W vs F-W cycles and 6-yr of continuous wheat (Cont W) cropping for soil biological analyses. Despite severe soil desiccation prior to sampling (in late fall 1990), the residual effects of all GM legumes on microbial populations and activities were very positive. Green manuring had improved the quantity and quality of soil organic matter as much as under N- and P-fertilized Cont W. The previous short-term legume growth and incorporation caused microbial populations to shift toward greater metabolic activity with increased respiration and enhanced enzyme levels.

Introduction

During 90 or more years of cultivation under the conventional fallow-wheat rotation most soils in southern Saskatchewan have suffered extensive degradation losing about half of the original organic matter and even more of the mineralizable N (Campbell and Souster 1982). Fallow replacement with forage, gram or green manure legumes can protect soil from erosion and degradation while adding fixed N_2 for improved fertility (Biederbeck 1990; Campbell et al. 1992b). However, on Brown or Dark Brown soils the deep-rooted perennial or biennial legumes cause excessive depletion of soil moisture reserves. Thus annual legumes are more suitable for use as short-term green manures (GM) within the drought-prone Palliser Triangle (Biederbeck 1988).

The beneficial effect of forage and green manure legumes, such as alfalfa and sweetclover, on the quality and fertility of Dark Brown, Thin Black and Gray soils in western Canada has been well documented by Campbell and co-workers (Campbell *et al.*. 1990, 1991 and 1992a). Less is known about the impact of short-term green manuring on soil quality and almost nothing about the effects of these annual legumes on soil organisms, although GMs are perceived, primarily by organic farmers, to greatly increase 'life' in the soil. Knowledge of the effects of crops and cropping practices on soil microorganisms is important because (i) changes in biomass, populations or activity can provide early indications of long-term trends in soil organic matter and fertility (Biederbeck *et al.* 1994) and (ii) almost all the nutrients in legume GM must pass through the microbial pool before being made available for plant uptake (Alexander 1977).

At Swift Current, the potential of four annual legumes for fallow replacement green manuring in spring wheat production systems has been evaluated since 1984. This field study provided an opportunity to determine, in comparison with summer-fallow (F) and continuous wheat (Cont. W), the influence of repeated short-term legume GM on:

(i) soil organic matter and microbial biomass;
(ii) populations of major types of organisms; and
(iii) respiratory and selected enzyme activities;

all within the surface layer of soil (0 to 10 cm) that was directly affected by the GM incorporation.

Materials and Methods

The plot experiment was established in 1984 on 2 ha of level land, that had previously been under conventional fallow-wheat (F-W) cropping for about 70 years, at the South Farm of the Swift Current Research Centre. The soil is classified as Swinton silt loam, an Orthic Brown Chernozem. A detailed description of this study, including experimental design and results regarding legume green manure productivity and water use, were recently published (Biederbeck ***et al.*** 1993; Biederbeck and Bouman 1994).

In this experiment we compared GM-W vs F-W and Cont. W. All rotation phases were present each year. The four annual legume species, grown as GM with tall stubble trap strips in rotation with spring wheat, cv. Leader, were black lentil cv. Indianhead; Tangier flatpea cv. Tinga; chickling vetch, also known as grasspea, NC81-3, and feedpea cv. Sirius. All treatments were arranged in a randomized complete block design with four replicates on plots measuring 6.75 m x 18 m.

Each spring the inoculated legumes were seeded into W stubble and four rows of W were planted along the west side of each plot to again provide tall stubble, after grain harvest with a head clipper, for uniform overwinter snow trapping. At full bloom, i.e., within 8-10 weeks of seeding, the legume top growth was incorporated (tandem disk) in some plots and chemically desiccated (diquat) in others and then kept as partial fallow for about 10 months until the W crop was seeded the following May.

On October 19, 1990, i.e., two months after harvest of the wheat crop, that had been solid-seeded across all plots, and about 15 months after incorporation or desiccation (on July 25, 1989) of the preceding legume GM; soil samples from the O-10 cm depth were taken at four equidistant locations per plot and bulked into one representative sample for physical, chemical and microbiological analyses relevant to soil quality. All samples were sieved (< 2 mm) and then part of each sample was stored field-moist in polyethylene bags at 0°C for subsequent biological analyses.

The samples were analyzed for organic N concentration by a Kjeldahl method and for organic C concentration by dry combustion. Microbial biomass N and C were determined by the chloroform fumigation-extraction technique and measurement of ninhydrin-reactive N (Amato and Ladd 1988). For population estimates, serial dilutions of soil samples were prepared and colony 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 autotrophic 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. In vitro soil respiration was measured at 21°C for 30 days in biometer flasks (Biederbeck ***et al.*** 1994). All enzyme assays were replicated three times for colorimetric analysis. Dehydrogenase activity was determined by the method of Casida ***et al.*** (1964) with minor modifications and arylsulfatase activity according to the procedure by Tabatabai and Bremner (1970). Standard methods of analysis of variance were used to analyze all results from our study and LSD values were calculated to compare treatment means.

Results and Discussion

Over the 7 yr period of the field experiment, the growing season weather was characterized by wet conditions in 1986 and 1989, normal in 1990, dry in 1984,1985,1987, and extremely hot and dry in 1988 aggravated by low spring soil water (Biederbeck and Bouman 1994).

During the three GM-W cycles, prior to our soil sampling for biological analyses, the cumulative aboveground GM dry matter (legumes plus weeds) produced and soil incorporated was 7480 kg/ha for black lentil, 7500 for Tangier flatpea, 8950 for chickling vetch, and 10340 for feedpea. The aboveground total GM production varied from year to year and, when averaged across all four legumes, it was 2110 and 2400 kg/ha in 1985 and 1987, both dry years, but 4070 kg/ha in the wet growing season of 1989 (Biederbeck et al. 1993). After three cycles of GM-W, incorporation of all aboveground plant material in the GM phase had resulted not only in significant enrichment of soil organic N in all GM treatments relative to F-W, but it had also increased soil organic C contents in three of the four GM treatments by as much or more than in Cont. W (Table 1). The highest enrichment of soil organic C and N in the surface soil was found when feedpea was the GM and reflected the consistently greater biomass production by this annual legume species (Biederbeck *et al.* 1993). The increases

Table 1. Effect of fallow replacement green manuring (GM) with annual legumes on selected soil biochemical characteristics in top 10 cm of Swinton silt loam sampled in October 1990 under wheat (W) stubble after 3 cycles of GM-W cropping

{ **---Table 1 at end of paper---** }

in soil organic N with green manuring corroborate our finding from an earlier micro-plot study with 15N-labelled GM that showed most of the legume-N remaining after incorporation had become part of the soils organic N reserves (Janzen et al. 1990) and had greatly enhanced the more active or mineralizable fraction of soil N (Biederbeck *et al.* 1995). However, the magnitude of soil organic C increases due to short-term GMs in the present study was unexpected. After 30 years of crop rotations on a thin Black Chemozem at Indian Head, Campbell et al. (1991) found soil organic C increases with sweetclover GM-W-W and with grass-legume hay in wheat rotations that were barely significant when compared to F-W-W.

After 6 yr of N- and P-fertilized Cont. W there was no significant increase in soil microbial biomass-C and -N relative to the F-W system (Table 1). However, after only three GM-W cycles, biomass-C and -N in practically all GM treatments were definitely greater than in F-W. A maximum biomass increase of 65% occurred when the black lentil was used as GM (Table 1). This was rather surprising because in our plot experiment this species did neither produce much dry matter nor did it fix large amounts of N_2 relative to some other annual legumes (Biederbeck et al. 1993, 1995). In the long-term rotation study at Indian Head, the unfertilized sweetclover GM-W-W had resulted in greater microbial biomass-C and -N than under F-W (Campbell *et al.* 1991).

In the present study, numbers of viable aerobic bacteria under wheat stubble after F and in Cont W (Table 2) were drastically reduced from the earlier reported 60 to 80 million/g for F-W and 120 to 200 million/g range for Cont W in fall samples of this silt loam taken from the nearby long-term rotation study (Biederbeck *et al.* 1984; Biederbeck and Campbell 1987). Bacterial populations near the surface were greatly depressed because of severe soil desiccation following the wheat harvest in 1990. Throughout September the weather was abnormally hot, dry and windy and although October was cool it was again very dry and windy, consequently, when the plots were surface-sampled on October 19, 1990 all soils were 'bone'-dry (with an avg. water content of 4% in a soil that has a PWP of 10%) and very hard.

Despite the extreme desiccation, residual effects of legume incorporation on aerobic bacteria, after three GM-W cycles, were highly significant and very positive. Bacterial population levels at 15 months after the four GM legumes were grown were still from 3- to 4-fold greater than the population in F-W and more than double that of Cont W (Table 2). However, the actinomycete populations did not follow this trend. Numbers of these autochthonous-type organisms varied little between cropping systems, as had also been found earlier with population estimates from the Swift Current long-term crop rotation study (Biederbeck and Campbell 1987). Only black lentil and feedpea (GM) caused a significant increase in actinomycetes relative to population levels in F-W and Cont W (Table 2). As a result of rather static behaviour by the actinomycetes vs the dynamic and extensive bacterial population response to legume GM, the bacteria/actinomycete

Table 2. Effect of fallow replacement green manuring (GM) with annual legumes on microbial populations in top 10 cm of Swinton silt loam sampled in October 1990 under wheat (W) stubble after 3 cycles of GM-W cropping

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All microbial population estimates for GM-W are from plots where the previous legume growth was incorporated at full bloom.

ratio increased from an unusually narrow 1.1 for F-W to 1.7 under Cont W and up to ratios ranging from 3.2 to 4.1 for the GM-W treatments (Table 2). The widening of this ratio indicates a qualitative shift within the prokaryotic flora towards greater dominance by zymogenous-type organisms in green manured soils. In the nearby long-term rotation study, the bacteria/actinomycete ratio was found to widen as the cropping frequency was increased (Biederbeck et al., 1984).

Numbers of filamentous fungi in the dried-out soils were high enough to suggest that the colonies counted on RBSA plates originated mainly from fungi present as dormant spores rather than vegetative structures. Although the population size did not differ between F-W and Cont W, fungal populations in the four GM-W treatments were generally twofold greater indicating a definite population enhancement from legume incorporation (Table 2). In contrast to filamentous fungi, the yeast population is considered to represent a small, but metabolically active, component within the large mycoflora of surface soils. The number of viable yeasts was significantly increased with chickling vetch and feedpea as GM (Table 2); however, populations of yeasts in all plots had been severely decimated by the extreme soil desiccation and were about two orders of magnitude lower than population levels reported earlier for various treatments of the nearby long-term rotation study (Biederbeck *et al.* 1984; Biederbeck and Campbell 1987). Populations of chemo-autotrophic nitrifiers were increased, relative to F-W and N-fertilized Cont W, by two of the four GM legumes (viz. Tangier flatpea and chickling vetch) reflecting the enhanced N-mineralization in the green manured soils; while denitrifier populations had been so severely depressed by the extensive prior soil desiccation that numbers did not differ between F-W and GM-W systems (Table 2).

Rates of in vitro soil respiration were rather low and did not differ between F-W and Cont W as would have been expected (Biederbeck and Campbell 1987). It is possible that these treatment effects were masked by microbial repression due to the severe soil desiccation prior to field sampling. However, the respiratory activity in soils from the GM-W plots was 40 to 55% higher than in F-W (Table 1), even 15 months after the legumes had been incorporated. The increases in respiration followed a trend similar to that of changes in microbial biomass. The dehydrogenase activity was also lowest in F-W and increased significantly with all four legume GMs (Table 1).

This enzyme can provide an index of endogenous soil microbial activity because its assay involves no addition of a substrate that would preferentially stimulate any particular group of soil organisms. Thus the pattern of dehydrogenase in the present study indicates that soil microbial metabolism was greatly enhanced by legume green manuring. Our results corroborate the finding by Bolton et al. (1985) that dehydrogenase levels in eastern Washington were always higher in legume green manured than in chemically fertilized soils of winter wheat production systems. Arylsulfatase activities were also increased significantly by all four legume GM treatments relative to the level of activity in F-W (Table 1) suggesting that green manuring may enhance the rate of organic-S mineralization near the soil surface.

Conclusions

Soil biochemical and microbiological analyses of surface samples taken in wheat stubble after three cycles of GM-W in comparison with F-W and six years of Cont W showed that most biological soil characteristics assessed were increased or improved by legume green manuring. Despite extensive desiccation of the Brown silt loam prior to sampling of the plots in late fall 1990, partial replacement of fallow with annual legumes, grown as GM, was found to have resulted in soil organic matter and microbial biomass C and N greater than in F-W and generally as high as under Cont W. Green manuring also produced large quantitative (bacteria and filamentous fungi) and some qualitative (widening of bacterial/actinomycetes ratio) population shifts toward a more 'zymogenous' or metabolically active microflora which was also reflected by enhanced ***in vitro*** soil respiration and markedly increased dehydrogenase and arylsulfatase activities.

Acknowledgements

This study was partly funded under the federal ERDAF and Energy R&D programs and also through support from the Alberta Agricultural Research Institute (AARI). The authors wish to acknowledge the technical assistance of Gary Winkleman, Richard St. Jacques, Jon Geissler and Lance Sawatsky.

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Table 1. Effect of fallow replacement green manuring (GM) with annual legumes on selected soil biochemical characteristics in top 10 cm of Swinton silt loam sampled in October 1990 under wheat (W) stubble after 3 cycles of GM-W cropping

Soil biochemical characteristics'	Previous crop or treatment						LSD
			Legume green manures				
	Summer-fallow	Continuous wheat	Black lentil	Tangier flatpea	Chickling vetch	Feedpea	(P<0.05)
Organic carbon (g/kg)	15.6	17.1	16.6	17.4	17.0	17.8	1.6
Organic nitrogen (g/kg)	1.70	1.85	1.84	1.83	1.80	1.86	0.09
Microbial biomass-C (pg C/g soil	242	307	398	373	374	336	97
Microbial biomass-N (µg N/g soil	35.7	45.0	58.8	55.0	55.0	49.5	14.1
Respiration, 30 day cumulative (µg CO,-C/g soil)	152	196	234	236	219	213	51
Dehydrogenase (µg TPF formed/g soil/day)	57.6	63.7	81.9	75.2	74.4	71.6	13.7
Arylsulfatase (µg PNP released/g soil/hour)	42.0	49.2	61.8	53.7	51.4	55.5	11.3

• Values for organic matter and respiration in GM-W are from plots where previous legume growth was incorporated while biomass and enzyme values are from plots where legume growth was desiccated.

Table 2. Effect of fallow replacement green manuring (GM) with annual legumes on microbial populations in top 10 cm of Swinton silt loam sampled in October 1990 under wheat (W) stubble after 3 cycles of GM-W cropping

	Previous crop or treatment						
			Legume green manures				
Microbial counts' (organisms/g O.D. soil)	Summer-fallow	Continuous wheat	Black lentil	Tangier flatpea	Chickling vetch	Feedpea	LSD (P<0.05)
Bacteria (x 10')	16.8	24.5	60.0	54.1	67.7	60.4	9.5
Actinomycetes (x 10^6)	14.6	14.2	17.3	16.6	16.4	17.7	2.5
Bacteria/actinom. ratio	1.1	1.7	3.5	3.2	4.1	3.4	0.3
Filamentous fungi (x 10^3)	58	66	130	103	112	102	23
Yeasts (x 10^3)	0.7	1.0	0.7	1.4	1.8	1.6	0.9
Nitrifiers (x 10^3)	17.1	7.1	34.4	54.9	52.0	23.5	31.7
Denitrifiers (x 10^3)	1.6	0.5	8.1	6.9	7.0	7.6	7.0

• All microbial population estimates for GM-W are from plots where the previous legume growth was incorporated at full bloom.