The Microbial Ecology of a Sustainable, Long-Term Lentil-Wheat Rotation

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Introduction

The beneficial effects of legumes (grain, forage or green manure) in crop rotations have been recognized for decades. The benefits include improved fertility and quality of soil (Wright 1990; Campbell et al. 1992; Green and Biederbeck 1995; Biederbeck et al. 1998), reduced soil nitrogen losses (Drinkwater et al. 1998) and increased soil biological activity (Lupwayi et al. 1998; Biederbeck et al. 1999). Recently, it has been proposed that some of the benefit from legume inclusion in rotations may be due to rhizobial/non-legume associations in the rhizosphere, on the rhizoplane and in the root interior (Yanni et al. 1997; Höflich 1998; Biederbeck et al. 2000a; Lupwayi et al. 2000). Since most processes that are essential to agricultural productivity are mediated by soil microorganisms (Paul and Clark 1996) an evaluation of the microbial ecology of soils influenced by legumes in rotation seems timely.

The existence of the long-term lentil-wheat rotation in the 'Old' rotations study at the Semiarid Prairie Agricultural Research Centre (SPARC) in Swift Current made it possible for us to study various aspects of the benefits related to a grain legume in frequent rotation with a cereal crop. The economic, ecological and environmental sustainability of this rotation has been discussed elsewhere (Biederbeck et al. 2000b). In this paper we are focussing on assessing the influence of a grain legume in frequent rotation with wheat on:

1. the ecology of the root associated microflora, and

2. the ecto- and endophytic colonization of wheat roots by rhizobia.

through comparison with continuous, monoculture wheat.

Materials and Methods

The 'Old' rotations study was established at the South Farm of SPARC in 1967, however, the lentil-wheat rotation within this study was started in 1979. Three replicate plots of each rotation and rotation phase are present in the study. In 1997 and 1998, plots of the wheat phase of the lentil (L)-wheat (W) rotation and the continuous, monoculture wheat (CW) were sampled at the flag-leaf stage. Both phases of the L-W rotation as well as CW were sampled in 1999 and 2000 again when wheat was at the flag-leaf stage. In each plot, five samples from the bulk (non-rhizosphere) soil were dug to 15 cm depth between crop rows and bulked into a composite sample. Five 0.5 m row lengths of crop were dug out, excess soil shaken off and then bulked

with adhering soil into plastic bags for rhizosphere soil and plant root samples. At the laboratory, rhizosphere soil was gently brushed of the roots within 24 h of field sampling. All soil samples were sieved (<2 mm) and stored field moist at 0°C. In 1999 and 2000, the plant roots were kept for analysis of the rhizoplane (root surface) and root interior (for endophytic rhizobia) by storing at 4°C.

Microbial populations were enumerated by soil dilution plating technique, using soil extract agar for bacteria and actinomycetes, rose bengal streptomycin agar for filamentous fungi and yeast and nutrient agar for spore-forming bacteria (after pasteurization of soil dilutions). Microbial diversity and community structure was assessed using the BIOLOG system for detection of specific patterns of substrate utilization by bacteria. Microbial biomass carbon was determined by substrate-induced respiration method and unamended soil microbial respiration measured during 30 days of incubation in Biometer flasks. Dehydrogenase was determined by the standard method. Ecto- and endophytic rhizobial populations were determined using the MPN plant-infection technique. Not all analyses were conducted in all years.

Results and Discussion

Overall, the L-W rotation increased most microbial parameters measured. Microbial biomass C was 37% and 46% greater in rhizosphere and bulk soil, respectively, in the wheat phase of the L-W compared to CW (Figure 1). In addition, bulk soil of L-(W) showed increased microbial diversity based on several indices generated by BIOLOG, while in the rhizosphere there were was no difference (data not shown). Dehydrogenase activity was greatly increased in L-(W) compared to CW as well, however, the other parameter measuring activity, C-mineralization (respiration), was lower in both bulk and rhizosphere soil (Figures 2 & 3). Taken together this may reflect that even though the microbial biomass in the soil is very active, more legume N (or more readily available N) may reduce respiration by converting more crop residue C into soil-building humus rather than venting it off as CO_2. This soil building is reflected in the higher levels of stable soil organic carbon found in the L-W rotation compared to CW (Figure 4).

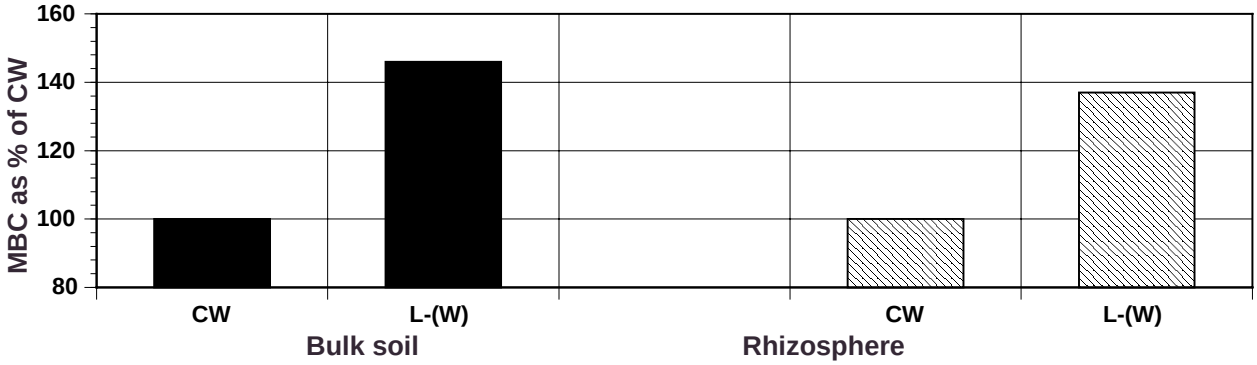

Figure 1. Effect of grain lentil in rotation with wheat (L-W) compared to continuous wheat (CW) on microbial biomass carbon (MBC) in bulk and rhizosphere soil at SPARC, 1997-1998.

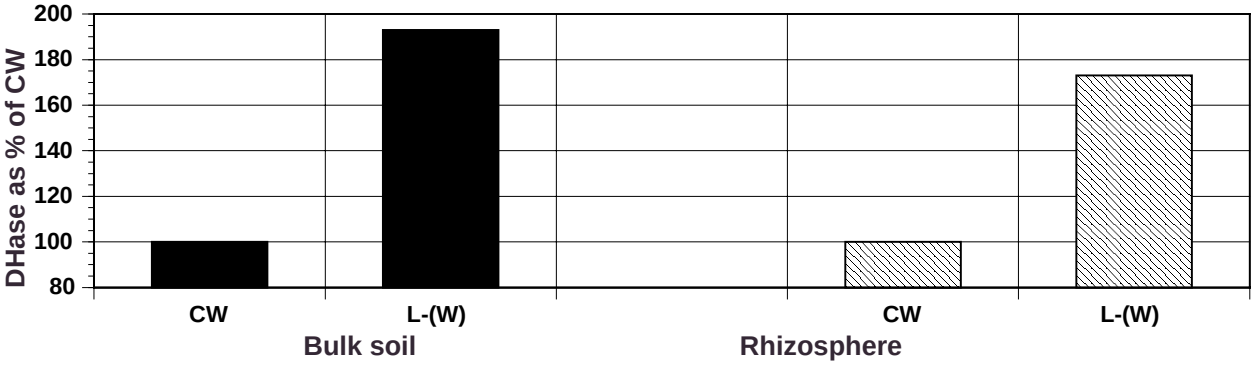

Figure 2. Effect of grain lentil in rotation with wheat (L-W) compared to continuous wheat (CW) on dehydrogenase enzyme activity (DHase) in bulk and rhizosphere soil at SPARC, 1997-1998.

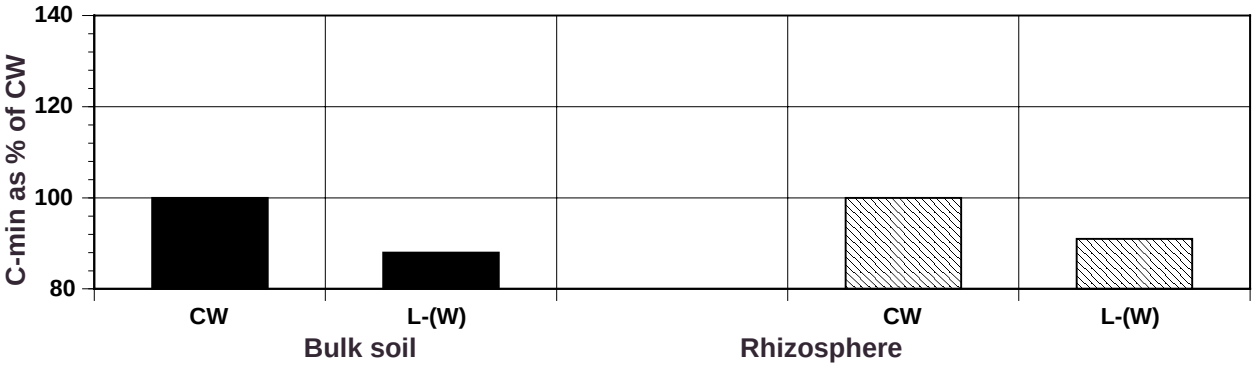

Figure 3. Effect of grain lentil in rotation with wheat (L-W) compared to continuous wheat (CW) on carbon-mineralization (C-min) in bulk and rhizosphere soil at SPARC, 1997-1998.

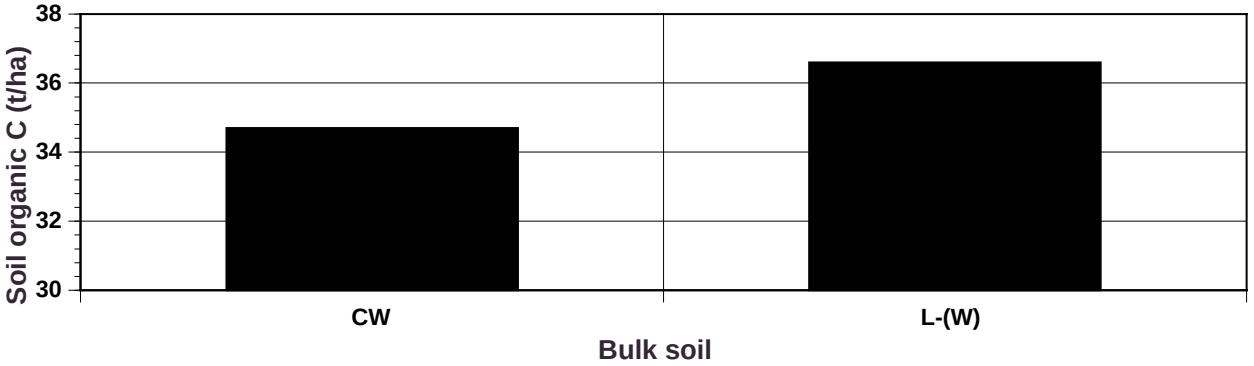

Figure 4. Effect of grain lentil in rotation with wheat (L-W) compared to continuous wheat (CW) on soil organic carbon in bulk soil at SPARC. The values presented are the mean of six samples over 20 years (1979-1999).

Bacterial populations were generally increased in both phases of the L-W rotation compared to CW in both bulk and rhizosphere soil (Figure 5). Increases in actinomycetes in the L-W rotation relative to CW were between 25 and 35% in bulk soil while in the rhizosphere actinomycete populations were considerably lower and decreased from CW to (L)-W (Figure 6). This is not surprising as actinomycetes are considered to be mainly autochthonous (slow growing) microorganisms and thus would be at a competitive disadvantage to zymogenous (rapid growing) organisms in the nutrient rich root-affected soil. Aerobic spore-forming bacteria (bacilli) were generally unaffected by rotation in bulk soil, however, there was at least a two-fold increase in both phases of the L-W compared to CW (data not shown). This indicated an enrichment in spore-forming bacteria within the rhizospheres of the L-W rotation.

Filamentous fungi were unaffected by rotation in soil except for a 12% increase found in the L-(W) rhizosphere (Figure 7). Yeast populations in the L-W rotation were consistently higher compared to CW in both bulk and rhizosphere soil with increases ranging from 20 to over 30% (Figure 8).

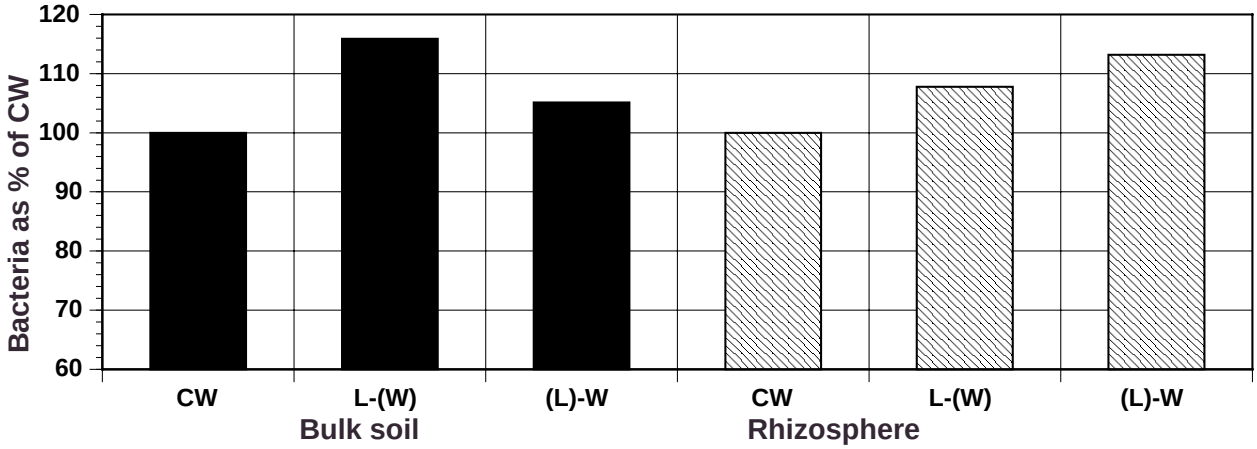

Figure 5. Effect of rotation on bacteria in bulk soil and rhizosphere soil at SPARC (1999-2000).

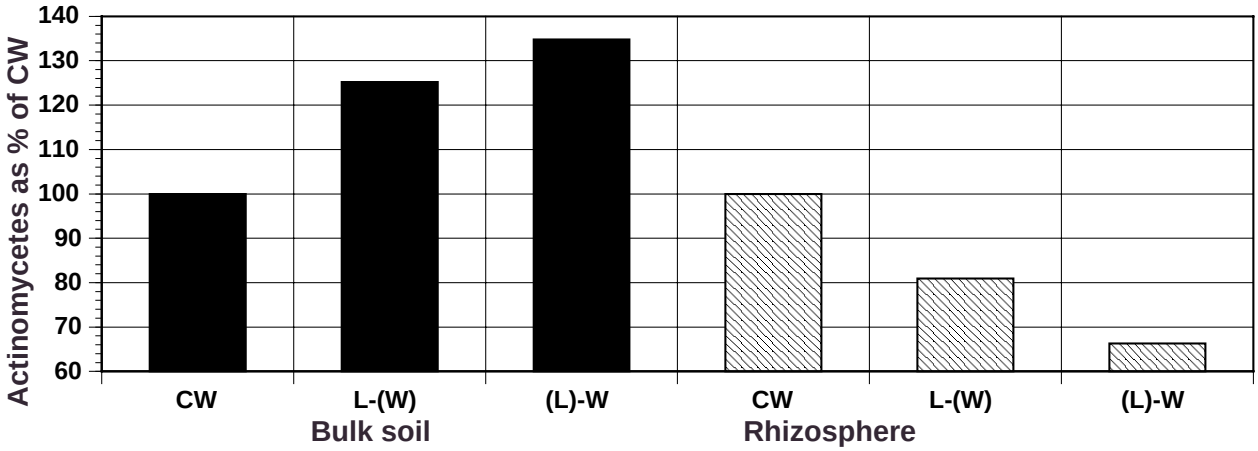

Figure 6. Effect of rotation on actinomycetes in bulk soil and rhizosphere soil at SPARC (1999-2000).

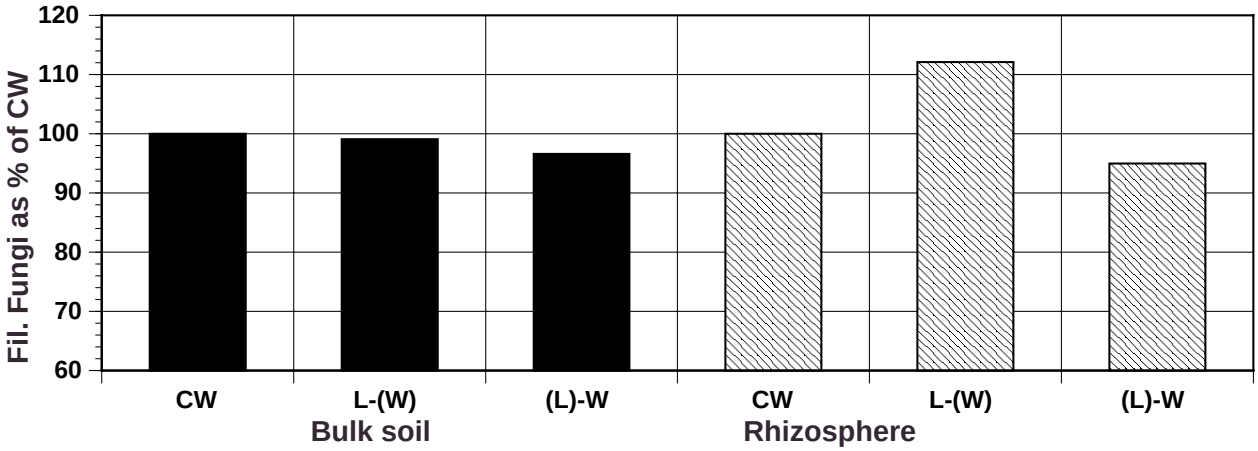

Figure 7. Effect of rotation on filamentous fungi in bulk soil and rhizosphere soil at SPARC (1999-2000).

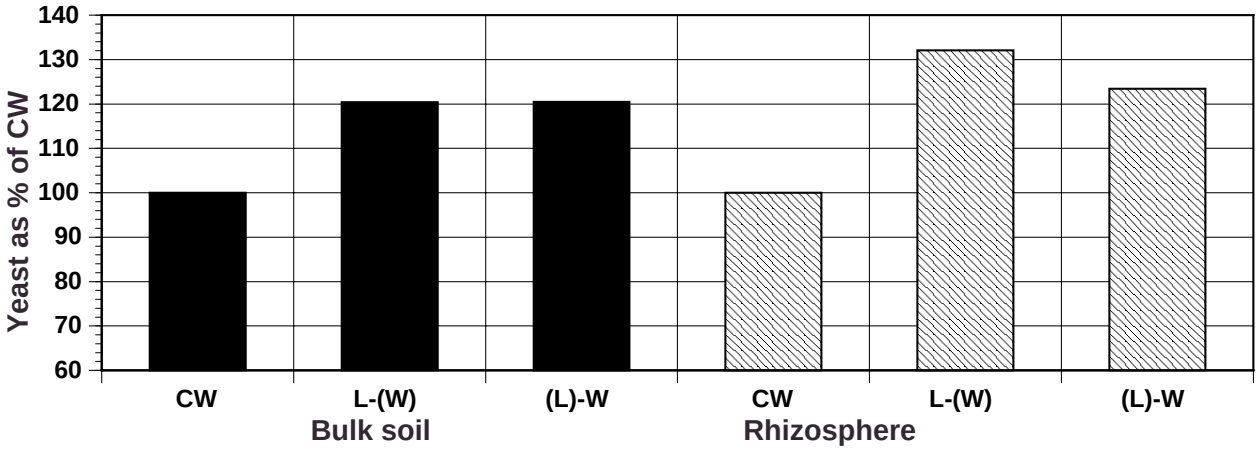

Figure 8. Effect of rotation on yeast in bulk soil and rhizosphere soil at SPARC (1999-2000).

On the rhizoplane (root surface), there were very large increases, ranging from at least two-fold to eight-fold, in the populations of all microorganisms in the (L)-W phase compared to the wheat rhizoplanes (data not shown). In addition, bacteria/actinomycetes ratios increased while filamentous fungi/yeasts ratios decreased (data not shown) indicating higher metabolic activity with increased proximity to live roots for all rotation phases (Biederbeck et al. 2000a).

An examination of the rhizobial populations revealed that neither bulk nor rhizosphere soil of CW contained any rhizobia while soils from both phases of L-W had populations ranging from 240 x 10^3 to 1.2 x 10^6 cells per gram (Table 1). On the root surfaces of L-(W) there were over a million times more rhizobia than in the rhizoplane of CW and even more importantly, there were large numbers of rhizobia inside the roots of wheat after lentil compared to the essentially rhizobia-free root interior of CW (Table 1). The numbers of endophytic rhizobia found in the roots of wheat from our 22 yr L-W rotation are over ten-fold larger than those Lupwayi et al, (2000) found in roots of barley and canola following peas in short-term rotations. This

comparison suggests that colonization of root interiors of cereals is likely to intensify with increasing frequency of the legume in the crop rotation. The presence of endophytic rhizobia in cereal crops has been shown to increase many characteristics of plant growth including grain yield significantly under field conditions (Yanni et al. 1997). Whether the same increases in wheat yield found in the L-W compared to the CW at SPARC are due to the presence of endophytic rhizobia has yet to be determined.

Table 1. Effect of Rotation on Populations of Rhizobia in Bulk Soil, Rhizosphere, Rhizoplane and Root Interior of Wheat and Lentil.

Most probable number of rhizobia				
Rotation phase	Bulk soil	Rhizosphere	Rhizoplane	Endophytic
	Cells/g soil		Cells/g root	
CW	0	0	22	2
L-(W)	646 000	240 000	31 600 000	67 600
(L)-W	457 000	1 175 000	n.d.	n.d.

n.d. = not determined

Conclusions

- Grain lentil in rotation with wheat led to greater microbial biomass C and dehydrogenase activity in soil while lowering C-mineralization resulting in higher levels of stable soil organic C compared to continuous monoculture wheat.

- Bacteria and yeast tended to have relatively higher populations, especially in rhizosphere soil, in both phases of the L-W rotation compared to CW indicating enhanced metabolic activity.

- Populations of all microorganisms enumerated were always several-fold higher on the rhizoplane of lentil than in the wheat phase or in continuous wheat.

- The million- to many thousand-fold greater populations of rhizobia near and inside the roots of wheat after lentil, as compared to monoculture wheat, attest to the ecological and agronomic benefits of legumes and suggest that colonization of cereal roots by rhizobia intensifies with frequency of legumes in the rotation.

References

Biederbeck, V.O., Campbell, C.A., Rasiah, V., Zentner, R.P. and Wen, G. 1998. Soil quality attributes as influenced by annual legumes used as green manure. Soil Biol. Biochem. 30: 1177-1185.

Biederbeck, V.O., Lupwayi, N.Z., Rice, W.A., Hanson, K.G. and Zentner, R.P. 1999. Crop rotation effects on soil microbial populations, biomass and diversity under wheat in a Brown

loam. p. 594-602. *In* Soils & Crops Workshop, Univ. of Saskatchewan, Saskatoon, SK, Feb. 25-26, 1999.

Biederbeck, V.O., Lupwayi, N.Z., Hanson, K.G., Rice, W.A. and Zentner, R.P. 2000a. Effect of long-term rotation with lentils on rhizosphere ecology and on endophytic rhizobia in wheat. Poster paper PF18 p. 80 *In* Program and Abstracts, 17th North American Conference on Symbiotic Nitrogen Fixation. Laval University, Sainte-Foy, Quebec, Canada, July 23-28, 2000.

Biederbeck, V.O., Zentner, R.P., Selles, F., Lupwayi, N.Z. and Rice, W.A. 2000b. What makes the lentil-wheat rotation at Swift Current so sustainable? Paper presented at Third Pulse Crop Research Workshop. Greenwood Inn, Winnipeg, Manitoba. November 19-21, 2000.

Campbell, C.A., Zentner, R.P., Selles, F., Biederbeck, V.O. and Leyshon, A.J. 1992. Comparative effects of grain lentil-wheat and monoculture wheat on crop production, N economy and N fertility in a Brown Chernozem. Can. J. Plant Sci. 72: 1091-1107.

Drinkwater, L.E., Wagoner, P. and Sarrantonio, M. 1998. Legume-based cropping systems have reduced carbon and nitrogen losses. Nature 396: 262-265.

Green, B.J. and Biederbeck, V.O. (eds.) 1995. Farm facts: Soil Improvement with Legumes, including legumes in crop rotations. Canada-Saskatchewan Agreement on Soil Conservation Bulletin, ISSN0840-9447, 20 pp.

Höflich, G. 1998. Colonization and growth promotion of non-legumes by *Rhizobium* bacteria. Paper presented at 8th International Symposium on Microbial Ecology, Halifax, Canada, August 9-14, 1998.

Lupwayi, N.Z., Rice, W.A. and Clayton, G.W. 1998. Soil microbial diversity and community structure under wheat as influenced by tillage and crop rotations. Soil Biol. Biochem. 30: 1733-1741.

Lupwayi, N.Z., Rice, W.A., and Clayton, G.W. 2000. Endophytic rhizobia in barley and canola in rotation with field peas. Poster paper PF19, p. 80 *In* Program and Abstracts, 17th North American Conference on Symbiotic Nitrogen Fixation. Laval University, Sainte-Foy, Quebec, Canada, July 23-28, 2000.

Paul, E.A. and Clark, F.E. 1996. Soil Microbiology and Biochemistry, 2nd Edition, Academic Press Inc. San Diego, CA.

Wright, A.T. 1990. Yield effect of pulses on subsequent cereal crops in the northern prairies. Can. J. Plant Sci. 70: 1023-1032.

Yanni et al. 1997. Natural endophytic association between *Rhizobium leguminosarum* bv. *trifolii* and rice roots and assessment of its potential to promote rice growth. Plant Soil 194:99-114.