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National Soil Conservation Program

SUMMARY REPORT

Response of earthworms, soil biota , and soil structure to agricultural practices in corn, soybean and cereal rotations.

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1. Introduction

Conventional cropping systems require substantial herbicide input for weed control, and reduced or zero-till methods use herbicides to prepare the seed-bed for direct drilling. The comparative effects of herbicide treatments, crop rotations and weed control practice on soil fauna, microflora, and soil microfabric features (eg. soil particle size and shape) were measured in a multifactorial experimental design. The effects of organic insecticides and fungicides on earthworms and other soil fauna have been widely reported in the literature, but effects of herbicides on soil fauna are rarely studied because destruction of vegetation by the herbicide reduces soil faunal populations inhabiting the soil beneath the vegetation, thus masking herbicide toxicity.

Generally, soil fauna responds to less intensive tillage methods by increasing in abundance, biomass and diversity, and this has been clearly demonstrated for earthworms. Increases in earthworm abundance and biomass under low or zero tillage are usually accompanied by increased soil macroporosity and increased water infiltration rates into soil. Because of the extensive availability of nutrients in earthworm casts both at the surface and within the burrows, agronomic techniques enhancing or reducing earthworm populations have significant consequences for processes involving soil microflora and soil microfauna colonizing the burrows.

We have been able to develop methods of measuring the effects of agronomic practices on microfabric soil physical features (eg. soil aggregates and voids) using image analysis techniques. We now more clearly understand how the abiotic physical environment of soil interacts with agronomic practices. We can use the same image analysis techniques at microfabric scale to trace the impact of biotic contributions to soil microfabric and structure. This is a real advance because most soil biological processes are poorly understood. These tools will allow us to construct a more complete picture of soil processes and soil ecosystem function.

2. Materials and methods

2.1. Site description and experimental design

Plots were established in 1982 at Harrow Research Station's Whelan Experimental Farm near Woodslee, Ontario (approx. 42º13'N, 82º44'W) in clay loam (Brookston series, orthic humic gleysol) to test effects of weeds, cultivation and herbicide treatments on different crop rotations. The primary treatments were corn-soybean rotation (CS), continuous corn (CC), continuous soybeans (SS), cereal-soybean rotation (WS), continuous cereal (WW), and corn-soybean-cereal rotation (CSW), all widely practised rotations in southern Ontario. Each crop rotation regimen was divided into three weed control sub-treatments (herbicide-treated, machine cultivated, and a non-weeded control), and all treatments and sub-treatments were replicated in each of three blocks. The herbicide treatments were: for corn and soybeans - pre-emergent application of metolachlor at 1.92kg ai/ha + atrazine at 1.00kg ai/ha, and for wheat (cereal) - post-emergent application of KilmorR (a 31:8:11 mixture of 2,4-D: mecoprop: dicamba) at 0.55kg ai/ha. Statistical comparisons of soil biota responses amongst treatments were performed by analysis of variance.

2.2. Sampling schedule and extraction methods for soil fauna

We began soil faunal and microfloral sampling in the spring of 1990, when crop rotations had been established for nearly nine years (Fig.1), by taking an earthworm sample, and four 5cm-diameter x 15cm-deep soil cores (two used for Tullgren funnel extractions, and remaining two cores shared for protozoan and Baermann funnel extractions, and microfloral analyses) from each block (replicate) within each sub-treatment (weed control method) within each main treatment (crop rotation). There were 3 (blocks: replicates) x 3 (sub-treatments: weed control treatments) x 6 (main treatments: rotations) = 54 earthworm samples, and 216 soil cores. Sampling was repeated for each of two dates (spring and fall) in each of 1990 and 1991. Earthworms were collected using dilute formalin solution sprinkled on 0.36m^2 quadrats, and sorted, counted and weighed (fresh weight) by species, and as juveniles or adults. Ambulatory arthropods were extracted from soil using modified

Tullgren funnels, and nematodes, tardigrades, rotifers and enchytraeids were extracted in modified Baermann funnels.

2.3 Soil microbiology analyses

Fungal populations were counted by subjecting soil samples to the soil dilution plate method using rose bengal streptomycin agar. Soil nitrite (nitrification) was analyzed by a diazotization method with sulphanilic acid, á-naphthylamine hydrochloride and sodium acetate buffer. Soil phosphatase was evaluated by hydrolysis of p-nitrophenol disodium orthophosphate. Dehydrogenase activity was measured by the formation of formazan following incubation of soil samples with 2,3,5-triphenyl tetrazolium chloride.

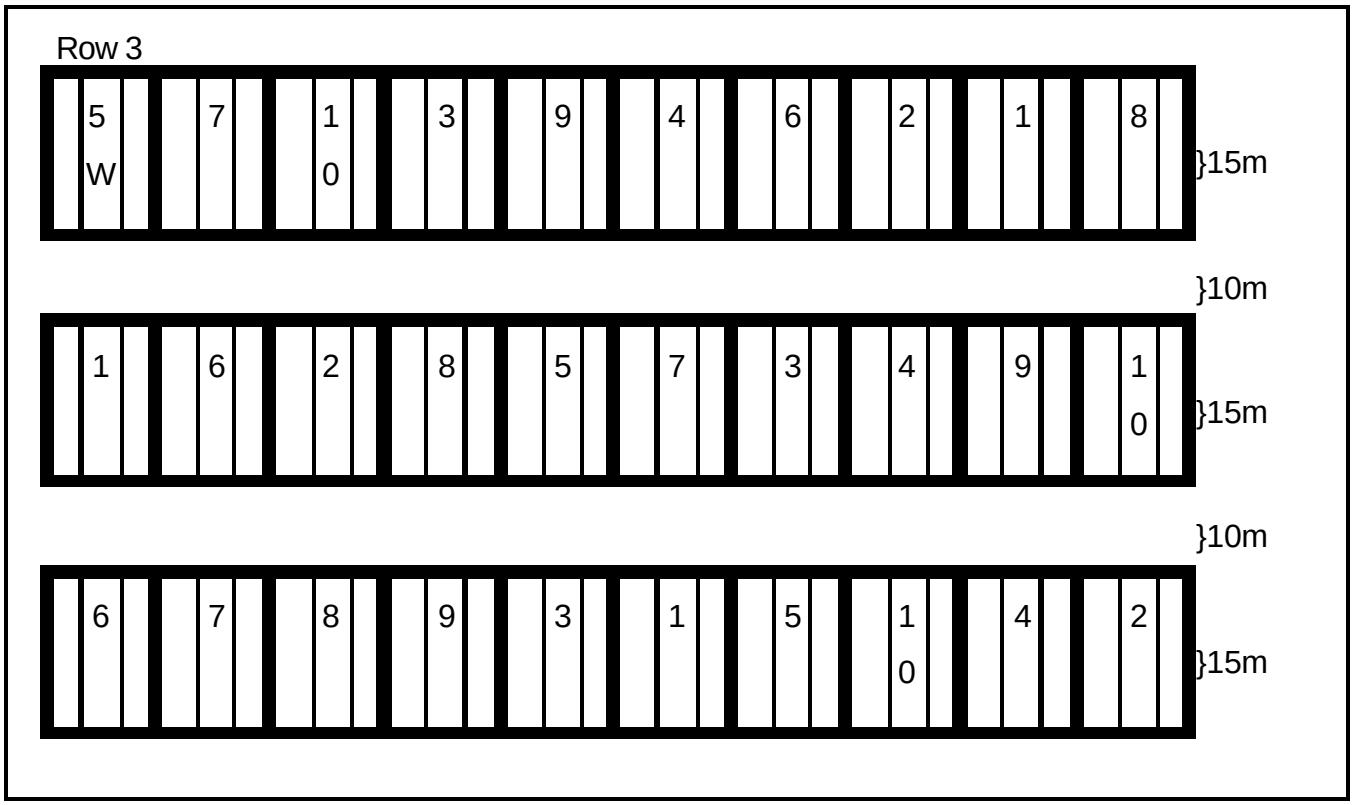

Figure 1. Long-term crop rotation plots at E. Whelan Research Station, North Woodslee, Ontario. H=Herbicide, M= Machine Cultivation, W=Non-weeded control.

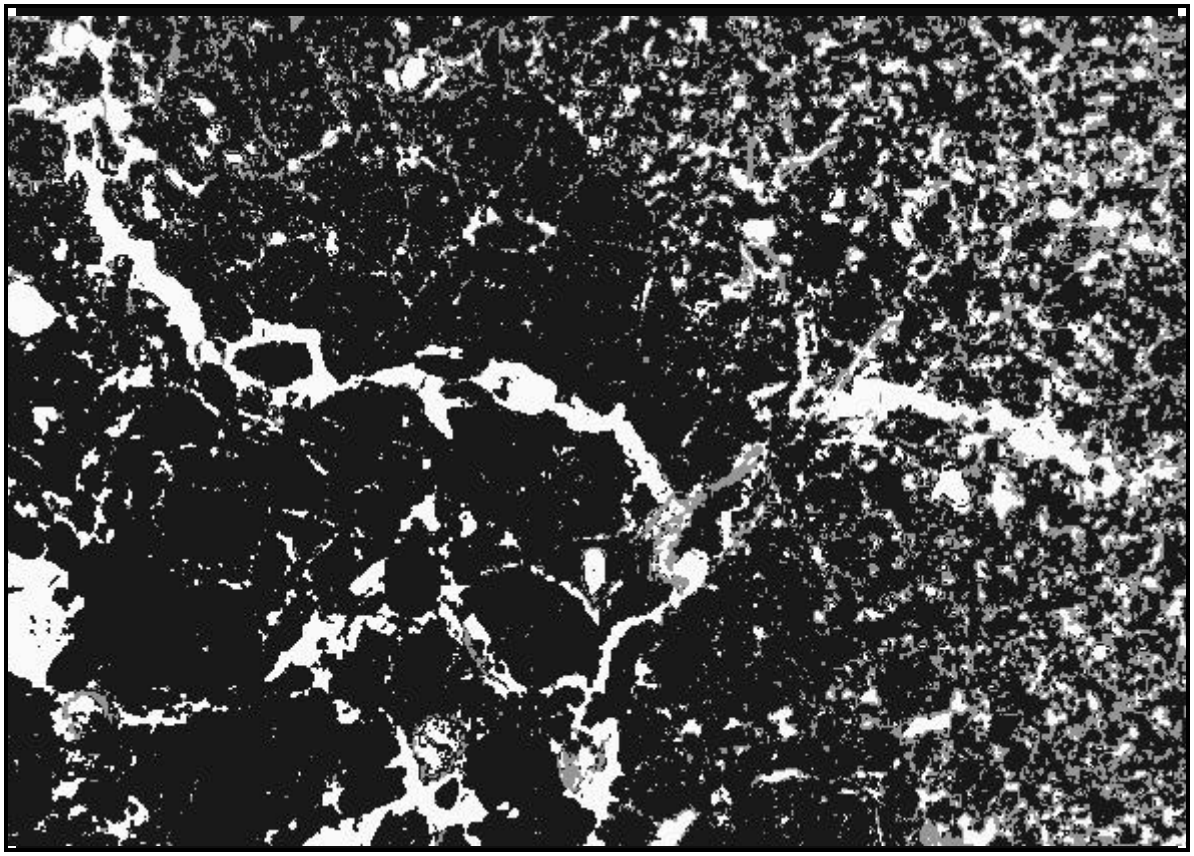

Figure 2. Image of soil thin section taken from hand-hoed (weedy check) wheat plot.

2.4 Image Analysis of soil particles

On July 5th, 1990 one soil block was taken from each treatment (cultivated, hand-hoed, and non-weeded control) for each crop (continuous wheat, corn, and beans) making nine blocks in total. These blocks were taken 2m in from the south boundary of each plot. The blocks were impregnated with 3-hydroxyl butyl methyl methacrylate and thin section (50 µm thick) slides prepared (Fig.2). Preliminary analysis was done by digitizing micrographic views of each impregnated soil section; the digitized images were segregated into three component categories: voids, particles, and features that were a combination of small particles (roughly soil aggregates). An image analysis software program was used to determine particle size and

shape distribution. Statistical comparisons of soil particle features amongst treatments were performed by Kruskal-Wallis one-way analysis of variance.

5. Conclusions

5.1 Soil Faunal Populations

Herbicide treatments reduced earthworm (Table 1) and some mite populations (Table 3: Cryptostigmata, Prostigmata and Astigmata) as much as machine cultivation for weed control; however, herbicide treatments did reduce abundance of Entomobryidae and Mesostigmata compared to machine cultivation and a non-weeded control. Continuous soybean rotations reduced abundance of earthworms (Table 2), mites (Table 3: Mesostigmata, Cryptostigmata, Prostigmata and Astigmata), and springtails (Onychiuridae, Isotomidae, Entomobryidae, and Sminthuridae)), compared to rotations containing cereals and continuous corn rotations. Crop rotation and machine cultivation were important treatment effects for several soil faunal taxa, and crop rotation (but not herbicide treatment) was a significant treatment effect for fungal numbers and several microfloral process in soil. Most of the faunal and microfloral increase effects can be ascribed to increases in available soil organic matter in the case of corn and wheat plots; reductions in faunal or microfloral activity, particularly notable in the case of soybeans can probably ascribed to the removal of available organic matter due to the way that soybeans grow and are harvested.

5.2 Image Analysis of Soil Fine Structure

Image analysis is a powerful tool which allows the physical associations of minerals, aggregates, organic matter, and biotic components of soil to be measured *in situ*. Statistical analysis of image analysed microfabric scenes taken from the resin-impregnated soil blocks revealed differences in particle size (area) for both the herbicide and hand-hoed plots but not for the non-weeded control. The particle shape parameter showed a similar result, except that there was no significant difference for herbicide treatment. This could be due the 'homogenizing' effect of the weeds (at least their roots) on soil physical structure.

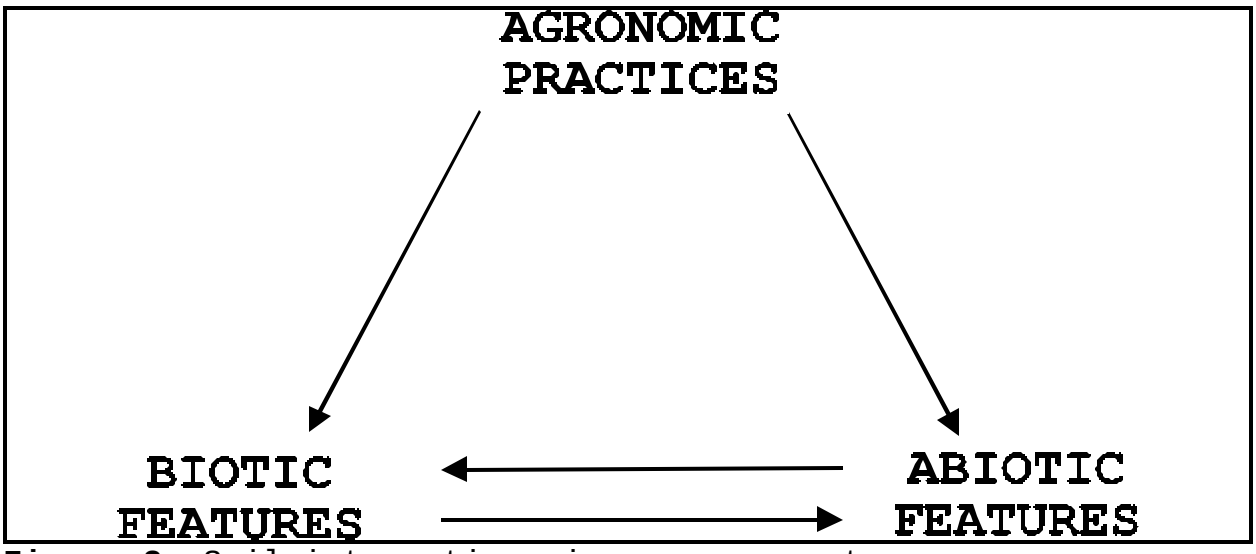

Figure 3. Soil interactions in agro-ecosystems.

As a result of concurrent research that we have conducted on another site at Elora, Ontario using these same techniques plus spatial mapping (impregnated blocks, image analysis, and spatial mapping of elements in earthworm fecal pellets in earthworm burrows), we have been able to trace the micro-scale interactions occurring in earthworm burrows. I enclose a copy of the paper we published in Geoderma (1993) that outlines the advance we have been able to make with these techniques. We now know that it is technically feasible (we have demonstrated it) to measure micro-scale interactions of soil biota using impregnated soil blocks and image analysis techniques.

Applications and Future Research

As a consequence of this research we can predict the effect of tillage practices and weed control methods on populations of earthworms, mites with some confidence; we have also been able to develop image analysis techniques of soil microfabric and fine soil structure and measure microfabric response to cropping, tillage and weed control methods, and segregate these differences with high statistical confidence. We now need to separate (tease apart) the contribution of agronomic practices from faunal/biotic contributions to soil microfabric (Fig.3). As a result of this research, we now see a method

of accomplishing this goal by tracking identifiable microfabric-scale pedofeatures (eg. earthworm or oribatid mite fecal pellets) in the various treatments, and subjecting those features to fluorescence imaging and statistical analysis to establish their spatial distributions (which must differ for different agronomic treatments, and should be statistically separable). The imaging method is now possible using fluorescence microscopy that incorporates an ultra-sensitive colour video camera and image analysis software. Further work could emphasise the relationship between fauna, soil structure, and plant roots.

Table 1. Earthworm response to weed control treatments in long-term rotation plots at North Woodslee, Ontario.

		Numbers/m^2 for indicated weed control method		
		Machine cultivated	**Weedy check**	**Herbicide treated**
Fall, 1990	Imm. L.terrestris	6.2	5.4	5.1
	Adult L.terrestris	2.6	3.7	2.3
	Imm. A.turgida	25.0	47.2a	31.8
	Adult A.turgida	4.6	7.7	4.9
	Totals	38.4	64.0	37.3
Fall, 1991	Imm. L.terrestris	9.1	13.0	6.5
	Adult L.terrestris	3.1	2.6	1.7
	Imm. A.turgida	14.8	25.9	13.7
	Adult A.turgida	16.1	19.9	14.6
	Totals	43.1	61.4	36.4

a - Significantly different ($P<0.02$)

Table 2. Earthworm response to crop rotation treatments at North Woodslee, Ontario in 1990 and 1991 (C=corn, S=soybean, W=cereal)

Sampling Period		Numbers/m² for indicated crop rotations					
		CSC	CC	SS	SW	WW	CSW
Fall, 1990	Imm. L. terrestris	0.94	2.02	0.066	0.58	1.00	3.87
	Adult L. terrestris	0.44	1.56	0.78	0.33	1.00	2.11
	Imm. A. turgida	12.44	18.56	6.67	9.33	10.89	12.11
	Adult A. turgida	1.44	3.67	1.78	2.44	1.11	2.00
Fall, 1991	Imm. L. terrestris	0.65	1.22	0.09c	0.09c	1.14	2.32
	Adult L. terrestris	0.17a	1.12	0.70a	1.01	5.65	7.18
	Imm. A. turgida	5.56	7.33	4.89	1.67	6.89	12.89
	Adult A. turgida	4.11	5.11	5.00	5.78	7.89	8.49

a = $P < 0.05$; b = $P < 0.01$; c = 0.001

Table 3. Effect of crop rotations (A) and weed control method (B) on abundance of soil mites at the experimental farm, Woodslee, Ontario in the fall of 1991; rotations: CS=corn-soybean, CC=continuous corn, SS=continuous soybeans, WS=cereal-soybean, WW=continuous cereal, and CSW=corn-soybean-cereal rotation; weed control methods were: M=machine cultivated, W=weedy check, and H=herbicide.

Mites		Most	Mean Nos./m^2	Least	Mean Nos./m^2
Mesostigmata	A	WW,CCc	1718,1251	SS,WS	297,573
	B	M,Wc	1262,1124	H	477
Cryptostigmata	A	CCc	21590	SS,CSW	4263,6532
	B	Wc	14464	H,M	7773,9650
Prostigmata	A	WS,WW,CCc	35460,34230,31473	SS,CS,CSW	19236,19533,23499
	B	W,Ha	30773,28758.5	M	22184
Astigmata	A	CSW,CCc	551,509	SS,WS,WW,CS	21,21,21,64
	B	Wa	361	H,M	85,148

a b c - Significant differences amongst treatments at P<0.05, 0.01, and 0.001 respectively
(ns) - no significant differences (P>0.05)

Table 4. Effect of crop rotations on soil microfloral parameters at the experimental farm, Woodslee, Ontario in 1991; rotations: CS=corn-soybean, CC=continuous corn, SS=continuous soybeans, WS=cereal-soybean, WW=continuous cereal, and CSW=corn-soybean-cereal rotation.

	Spring		Fall	
	Most	Least	Most	Least
Fungus	CS,CC,CS Wa	WS	CC,CSc	SS,WS
NO_2-nitrification	CC,WW,CS Wb	SS,WS	CCb	CSW,WW,SS ,WS,CS
Phosphatase	WWb	CS	ns	ns
Dehydrogenase	WWb	SS,WS	WWa	CC,CS,SS

a b c - Significant differences amongst treatments at P<0.05, 0.01, and 0.001 respectively
ns - no significant differences amongst treatments

Table 5. Statistical summary of soil particle analysis from image analysed soil thin sections prepared from long-term rotation plots July 5, 1990.

PARTICLE AREA

	MACHINE CULTIVATED			HERBICIDE TREATED			WEEDY CHECK		
CROP	WHEAT	CORN	BEANS	WHEAT	CORN	BEANS	WHEAT	CORN	BEANS
COUNT	142	1949	1037	154	125	1312	194	244	250
MEAN	7.52e+06	3.77e+05	7.72e+08	6.52e+06	7.64e+06	6.69e+05	5.36e+06	4.10e+06	4.10e+06
STD.DEV.	8.88e+07	6.35e+06	8.50e+06	7.85e+07	7.54e+07	8.73e+06	7.26e+07	6.12e+07	5.40e+07
VARIANCE	7.89e+15	4.04e+13	6.46e+14	6.16e+15	5.69e+15	7.62e+13	5.28e+15	3.75e+15	2.92e+15
ANOVA*	P=0.0000			P=0.0000			P=0.2775		

PARTICLE SHAPE**

(area=529 µm²/shape=1.57 dropped)

	MACHINE CULTIVATED			HERBICIDE TREATED			WEEDY CHECK		
CROP	WHEAT	CORN	BEANS	WHEAT	CORN	BEANS	WHEAT	CORN	BEANS
COUNT	120	1716	951	126	110	1180	169	203	218
MEAN	0.55	0.46	0.44	0.49	0.45	0.45	0.47	0.46	0.46
STD.DEV.	0.16	0.16	0.17	0.15	0.19	0.17	0.15	0.17	0.16
VARIANCE	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.03	0.02
ANOVA*	P=0.0000			P=0.0590			P=0.9977		

*Non-parametric Kruskal-Wallis one-way ANOVA

**'Closeness to a circle' - where circle = 1.000 and line = 0.000 as defined by shape factor = (4pi x area) / perimeter²