CHAPTER THREE

THE LONG TERM EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON ECOSYSTEM FUNCTIONING OF FIELD PLOTS

Abstract

The effects of annual application of 2,4 -D on the soil biosphere was studied in plots that received technical grade or the amine formulation of 2,4-D at rates of 0 kg/ha (control plot), 1X (the normal field rate of application of 1.1 kg/ha), 10X, and 100X that amount for ten consecutive years. The soils were sampled to determine the effect of herbicide on microbial C and N processes, and the structure and the metabolic status of microbial community. In the top 15 cm of soil, microbial biomass, microbial nitrogen, and total phospholipid fatty acids (PLFA) were depressed with an increase in 2,4-D concentration while rates of nitrification increased. Both nitrogen and carbon mineralization were depressed with increased 2,4-D concentration. After one month of incubation the depressive effect of 2,4-D on C mineralization was significant but this was not the case at subsequent sampling times.

The Gram negative community in the 100X plots appeared to be in the inactive phase based on PLFA signatures whilst that of the other treatments were in the active phase. The rate of microbial turnover in these plots was low and the microbial community was showing signs of adaptation. Although some herbicide -induced changes were significant ($P < 0.05$) all the depressive and stimulatory effects were relatively small (< 50 % change from the control). The effects of long term 2,4-D application were not ecologically significant to the soil microbial community nor did they significantly interfere with nutrient cycling to adversely affect soil fertility. The results suggest a trend towards disturbance of the ecosystem equilibrium if 2,4-D were to be continually and repeatedly applied to the plots at high rate.

Introduction

The role of microorganisms in agroecosystems is often understated (23) yet without microbes and their function, no other organism could be supported by the soil. Microorganisms constitute a source of and sink for nutrients in all ecosystems and play a major role in plant litter decomposition and nutrient cycling (5, 9, 39), soil structure (26), dinitrogen fixation (40), mycorrhizal associations (1), reduction in plant pathogens (10) and other alterations in soil properties that influence plant growth.

Soil microorganisms also are sensitive biological markers (31. 43) and useful for classifying disturbed or contaminated systems since diversity can be affected by minute changes in the ecosystem. Early investigators such as Waksman (42) noted that the use of microorganisms coupled with physical and chemical parameters could indicate the fertility of a soil. The microbial criteria identified then were numbers of microbes, nitrification, carbon dioxide evolution, cellulose decomposition, dinitrogen fixation, etc. Domsch et al. (13), on the otherhand, suggested other microbial parameters be measured such as those populations with greater sensitivity to perturbation including nitrifiers, Rhizobium, actinomycetes and those of moderate sensitivity, which were algae, bacteria, fungi and soil respiration, denitrification and ammonification. It is also possible that individual species can function as indicators of the status of an ecosystem. These are the keystone species in that they should reflect the response of the community they represent. There is, however, concern over the use of single populations to identify stress response of a system (6, 24). It is argued that the effect of stress on single species cannot predict what will happen

throughout the community nor identify all the interactions that may be altered (7).

Management practices influence microbial activities over the long term in agricultural lands (3, 27, 32). One such agricultural practice is the use of herbicides to control the weeds which pose a major threat to crop yields (34, 36). Therefore, there has been considerable interest in the side effect of these chemicals on non-target organisms, including soil microorganisms (16, 35). Most of the studies on this subject have focused on herbicide effects on microbially mediated processes eg. nitrification, denitrification, soil respiration and soil enzyme activity (8, 20, 46). Process level measurements, although critical to understanding the ecosystem, may be insensitive to community level changes due to the redundancy of these functions and the complexity of relationships within particular communities. This measure also does not indicate the diversity and location of the organisms responsible for such alteration. The majority of studies have used laboratory incubation techniques for investigating such side-effects (35) but there are certain difficulties in attempting to extrapolate such information to the field, especially relating it to the importance of a detected herbicide side-effect relative to the background spatial and temporal biotic variability in the field (11). The authors generally conclude that detailed field data on spatial and temporal variation in environmental variables could provide a background of natural variability against which pesticide side-effects could be assessed.

In recent times, field experiments have been conducted such as that of Wardle and Parkinson (44) who found that 2,4-D significantly influenced all microbial variables investigated but that they were transient being detectable only within the first 1-5 days of herbicide addition. Similarly, Biederbeck (2)

found that the effect of 2,4-D after 40 years of field application was not of ecological significance. Domsch et al. (13) also concluded that most detected side -effects of pesticide applications on the soil microflora were relatively unimportant because variability in natural factors may depress soil processes by over 90 %. Few have done ecosystem level studies monitoring ecosystem processes and their relation to community structure and activity.

The objective of this study was to elucidate any long term effect of 2,4-D of soil ecosystem processes carried out by the microbial community.

Materials and Methods

Soil sampling. 2,4-D was applied to the 2,4-D Gene Transfer plot at the Kellogg Biological Station, Hickory Corners, MI. during 1996 to 1998. The plot consisted of eight subplots, each 3.6 by 9.1 m (15). Each subplot was separated by a buffer zone 4.5 m wide. The first application of 2,4-D occurred in October of 1988, and was continued each year. The levels of 2,4-D application were 0X (for the control plot), 1X (which is the rate of good agricultural practice - 1.1 kg/ha), 10X the normal field rate of application and 100X the normal field rate of application. Each application rate was replicated twice. Beginning in 1996, the 2,4-D the dimethylamine ester form was applied from August each year to early October in the form of five applications every other week. One week after the last application, soil samples were collected, kept on ice and brought to the laboratory and immediately sieved through a 2 mm sieve. The same 2,4-D application scheme was followed in August 1997. However in August 1998 instead of five applications of 2,4-D, seven applications were made so that it might be possible to see differences in microbial communities not previously detectable. Soil samples were stored at -20°C °C for experimental use.

Microbial parameter measurements.

The potential nitrification rate is the enzymatic oxidation of ammonia to nitrates by the nitrifiers (viz Nitrosomonas and Nitrobacter) and this rate was determined by the shaken soil- slurry method for assessing the maximum rate (Vmax) of nitrification for a soil sample (18). Samples were incubated under laboratory conditions that had been optimized with respect to water content, ammonium, aeration and P availability. Fifteen gram of sieved, field-most soil was put into a 250-ml Erlenmeyer flask. with 100 ml of combined solution (0.3 mM KH_2PO_4, 0.7 mM K_2HPO_4 and 0.75 mM NH_4SO_4) and the flask covered with a vented cap. All flasks were placed on an orbital shaker and shaked at approximately 180 rpm for 24 h. At 2 h intervals aliquot samples (5 ml) were collected, 1 ml of 2N HCL was added and centrifuged. The supernatant was then stored at -20°C and later analyzed for NH_4^+ and NO_3^- contents.

The N mineralization rate is the microbial release of organically bound nitrogen to inorganic mineral forms and it was determined using the sieved field-moist soil that was placed in plastic caps and put in a Mason jar sealed with a lid, Hart et al., (18). The soil was then incubated for at least 100 days at room temperature (25° C) in the dark. Ammonium and nitrate concentrations were determined by extracting soil subsamples with 1N KCl before the experiment began and every tenth day of incubation.

Carbon mineralization was measured by incubation of soil for > 100 days at room temperature in the dark in Mason jars. Accumulated CO_2 was collected in a plastic vial containing 1N KOH that was placed near the soil sample in the jar. Every ten days the CO_2 released was determined and fresh vial containing 1N KOH was placed in the Mason jar.

Microbial biomass was determined by the chloroform - fumigation incubation method (CFI) as described by Howarth and Paul (21). Chloroform was used to lyse living microbial cells in a soil sample whilst an identical sample remained unfumigated. The soil samples were then incubated for a period of 10 days in sealed Mason jars. The temporary flush of carbon dioxide was primarily due to decomposition of microorganisms (22). In addition, an increase in the NH_4^+ pool occurred as a result of mineralization of nitrogenous constituents from lysed microorganisms. The increase in CO_2 evolution and extractable NH_4^+ from fumigated samples were used to estimate the size of soil microbial biomass carbon and microbial biomass nitrogen, respectively. The microbial respiration rate was determined from the CO_2 evolved from the unfumigated soil after the ten day period of incubation. Thus differences in incubation period distinguished microbial respiration from carbon mineralization.

Statistical analyses. Analyses of variance (ANOVA) were performed with the general STATA program for the microbial biomass, microbial respiration, microbial nitrogen and carbon mineralization. The number of replicates was three. To determine the standard error of the metabolic status of microorganisms n was two.

PLFA analyses. Samples of soil for phospholipid fatty acid analysis (PFLA) were removed as soon as soils were sieved and kept frozen at -20 °C. The samples were then sent on dry ice to the Microbial Insight Incorporated at Rockford, Tennesee. The **metabolic status** of the Gram negative population was determined as follows;

$$\frac{\text{cy 17:0}}{\text{16:1w7c}} + \frac{\text{cy19:0}}{\text{18:1w7c}}$$

which is based on the fact that in Gram negative bacteria 16:1w7c and 18:1w7c are converted to cylopropyl fatty acids (cy 17:0 and cy 19:0) as microbes move from log to a stationery phase (17).

Turn over rate= $\frac{1}{\text{cy 17:0/16:1w7c + cy19:/18:1w7c}}$

Stress indicator of microbes: Gram negative bacteria generate trans fatty acids to minimize the permeability of their cell membranes as protection against changes in the environment such as toxicity or starvation. Hence

$$\frac{\text{16:1w7t}}{\text{16:1w7c}} + \frac{\text{18:1w7t}}{\text{18:1w7c}}.$$

if the sum of the two is > 0.1 then the starvation state is indicated (19).

Results

Effect of 2,4-D on non-target organisms: The total PFLA for the control was the highest (29,182 pm/g soil, Table 1) suggesting that this plot had the highest viable cells. This value decreased by 42 % in the plots with the highest 2,4-D. Generally as the 2,4-D concentration increased the total PLFA also decreased. 2,4-D depressed the Gram negative population (represented as the monoenoics) more than the other populations (Fig. 1 a and Fig. 1d). In this comparison the 4 % decrease in the Gram negatives was compensated by a slight increase in the Gram positive (mostly represented by terminally branched saturated fatty acid) and the actinomycete populations (also represented by the mid chain branched saturated fatty acid). The % Gram positive bacteria on the otherhand increased slightly in the 10X 2,4-D treatment and then decreased again. The proportions of the

actinomycete and the eukaryote populations remained relatively unchanged in the rest of the treatments.

Effect of 2,4-D concentrations on microbial parameters: High 2,4-D concentrations reduced the microbial biomass as measured by the chloroform fumigation method by 22 % in the 100X plots as compared to the control plots and the other treatments (Table 1), Similarly microbial respiration declined indicating that there was a reduction in microbially related activities with an increase in 2,4-D concentration although no difference was statistically significant. Microbial biomass nitrogen on the otherhand increased with 2,4-D concentration and the differences in treatment were significant. The low C:N ratio especially in the 100X treated plot suggest that nitrogen was being immobilized into the microbial cells rather than being released into the envinronment for the plant community. The C:N ratio for the other treatments remained relatively high (Table 1).

The Gram negative population contributed more to microbial respiration with increasing 2,4-D (r 58, Table 2) whilst the converse was observed for the Gram positive population and eukaryote populations (Table 2). A negative correlation coefficient was observed between microbial nitrogen and the % of Gram negative population in the microbial community implying that an increase in microbial nitrogen was not favorable to the Gram negative population. Generally microbially biomass carbon was negatively correlated with microbial biomass nitrogen (Table 2).

Transformation of carbon was depressed in the 100X plots but not in the other treatments (Fig. 2). Significant differences in the treatments were observed for carbon mineralization on the 30th day of incubation but not on the fiftieth

and seventy days of incubation (Table 1). Likewise, the rate of carbon transformation declined with an increase in 2,4-D. The Gram negative population played a significant role in C mineralization as 2,4-D concentration increased (r being 98%) as compared to the Gram positive and the eukaryote populations (Table 2). Net nitrogen mineralization also declined in the other plots. This is reflected in the decrease in nitrogen mineralization rate as 2,4-D concentration increased (Fig.3). However, there were no significant differences in the treatment after 30, 60 and 70 days of incubation . At all levels of 2,4-D treatment, correlation between the rate of carbon and nitrogen mineralization was not high and was even negative in the 10X 2,4-D treated plots (Table 2) implying that the two processes were not closely related. Beyond 70 days of incubation, N immobilization was observed in some of the higher 2,4-D treated plots, for instance in the 10X and the 100X plots.

The nitrification rate, on the otherhand, was slightly higher (4.57 ug/g soil) with the highest 2,4-D concentration. A graphic plot of the nitrification vs time revealed a biphasic activity (Fig. 4). The control, 1X 10X plots had 4.46, 3.27, 3.71 ug/g soil as their nitrification rates, respectively. The rate of N mineralization for all 2,4-D treatments was similar, however, the rate of C mineralization for the control plot was higher than the rest of the treatments (Table 3). This indicates that long term 2,4-D application has the tendency to affect the carbon cycle.

Metabolic status of the microbial community: According to the PLFA analysis, the gram negative community was in the inactive phase whilst for the other treatments they were in the active phase (Table 1). Moreover the microbial turnover rate declined from the control plots to the higher 2,4-D treated plots. The turnover rate in such higher treated plots was 0.43 as compared to 0.74 for the

control plots. Also the microbial community showed signs of adaptation to the environmentally induced stress from either toxicity or starvation. Ammonification on the otherhand was not affected by the increase in the 2,4-D concentrations, hence the rate of ammonification remained the same in all the plots.

Discussion

With increase in 2,4-D concentration, microbial respiration declined which might not be beneficial to soil fertility since it can reduce the rate of herbicide dissipation leaving a herbicide concentration which might be injurious to more sensitive 2,4-D degraders. The effect of high 2,4-D concentrations on the microbial community might be due to the accumulation of 2,4-dichlorophenol especially when 2,4-D is applied at high rates (28). Chlorophenols, are classic cytochrome uncouplers and are not always readily used as sole carbon source because of toxicity at low substrate concentration e.g. 100 ppm, (33). Recently 2,4-dichlorophenol was isolated by Smith (38) from laboratory-incubated soils and identified as a soil degradation product of 2,4-D.

Another reason why high 2,4-D concentration appear toxic to the microbial community might instead be due to the decrease in available soil organic matter or altered soil conditions due to the lack of plant growth. The higher 2,4-D treated plots were virtually weed free even after the planting season and this was observed at least for 2 years. This could possibly lead to decrease in organic matter, causing significant changes in the microbial populations hence affecting nutrient transformation. Significant changes in the microbial populations were observed in a dwarf apple tree orchard treated annually with atrazine at 4 kg/ha for fifteen years (44). In particular anaerobic sporeforeming bacteria and cellulolytic microorganisms were permanently reduced. The authors ascribed

these changes to the long-term elimination of direct vegetative cover and concomitant loss of organic matter input to the atrazine treated soil. Also lack of substrates in the 100X plots for the microorganisms could have contributed to some of the observed side effects. If carbon in the form of crop residue from surrounding plots had been addded to that plot, the C:N ratio would likely not have been as low as in the 100X plots. The microbes would have used the carbon for growth and microbial biomass might not have been depressed to that extent.

Any interpretations of antimicrobial herbicide effects on soil fertility is difficult, because herbicide induced stress in the surface soil can cause changes which can be both inhibitory and stimulatory to microbial populations and their activities which although statistically significant, are neither of ecological consequence nor of practical agricultural significance. The microbial community in these Gene transfer plot mnay have been sustainable by adding crop residue to the plot from time to time so that the effects of 2,4-D could be seperated from the effect of carbon loss.

The absence of any significant effect of 2,4-D concentration on nitrogen mineralization suggests that over the many years of 2,4-D application the major groups of organisms within the soil microflora have become adapted to 2,4-D . Similar soil microbial adaptations in response to repeated applications have also been reported from studies of long-term effects of other herbicides (14, 15, 44). The stimulatory effect of 2,4-D on nitrification was unexpected because it had been reported that phenoxy herbicides significantly, albeit temporarily, reduce nitrification, (16, 25, 28, 37). The increase in nitrification I observed was not relevant to the maintenance of soil fertility because the nitrate could easily be leached from the ecosystem and burden the receiving waters. In the current

study, the nitrifier population might have adopted to the 2,4-D application an explanation noted by other authors who saw adaptation of nitrifiers to phenoxy herbicides after repeated application (41). The slightly higher nitrification rate observed might also be due to the effect of higher organic N added through the usage of the dimethylamine form of 2,4-D. This form of 2,4-D is 20 % N by weight and organic N released could stimulate growth of the nitrifier population. The biphasic form of NO_3^- production suggested that nitrifiers initially used soil N and later used the N from the dimethylamine salt of the 2,4-D for growth. The low C:N ratio of the microorganisms might have a negative impact on the ecosystem because the nitrogen is not made available for plant growth but immobilized by microorganisms. The low microbial turnover rate also suggests that it takes a long time for this N to be released to the environment. It is not surprising that after 70 days of incubation that N immobilization was observed especially in the 100X and the 10X soils.

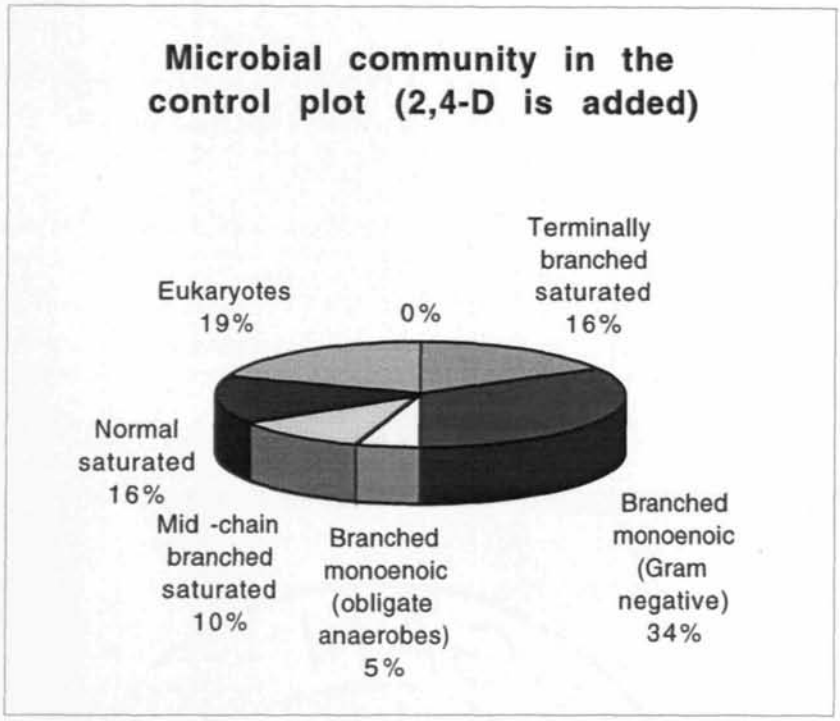
Through C mineralization, energy is released for other ecosystem functions. Hence any decrease in energy released in an ecosystem causes the system to degrade. The reduction in the microbial biomass with increasing 2,4-D concentration could mean that the net primary productivity and soil organic matter levels are affected because of the high correlation of microbial biomass and net primary productivity (30). But, in the present study such a reduction was obvious only in the Gram negative populations possibly because of the differences in cell wall composition. The Gram positive bacteria have a thicker cell wall peptidoglycan with no outer membrane and have techoic acids whilst the Gram negative bacteria have a thin layered peptidoglycan layer. Zelles et al (47)

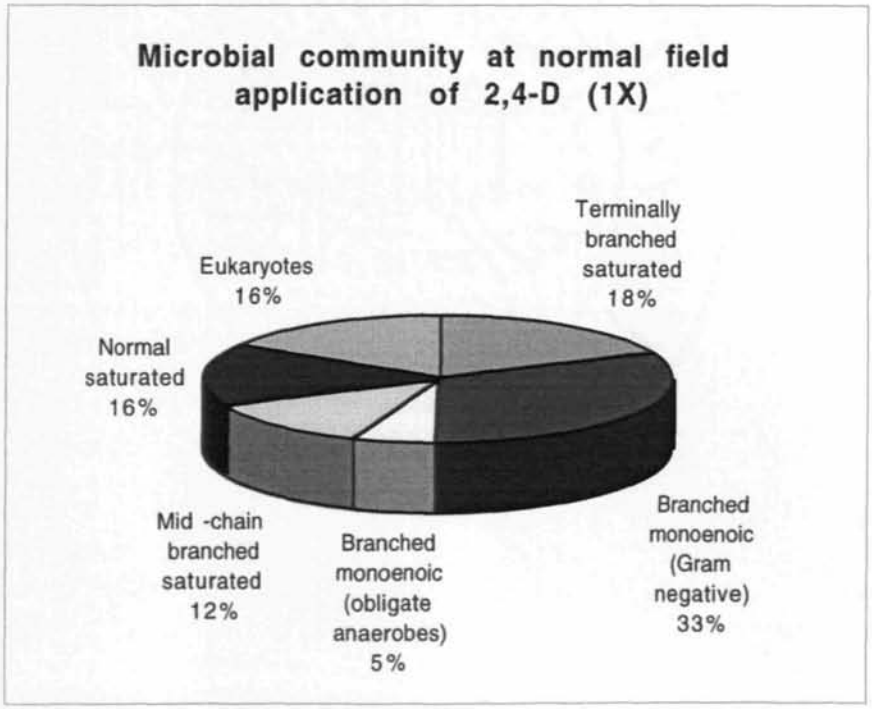
also observed that Gram negative populations were heavily affected during chloroform fumigation whilst the Gram positive only was slightly affected.

Because the effects of a toxic chemical are a function of both dose and time, the possibility of harm to the soil microflora is greater in situations where repeated applications are used over a long period. The use of PLFA in the current studies instead of soil enzymes as a measure of the microbial status is advantageous because it also gives more information on "health" of the microbial community. Domsch (12) stated that the duration and magnitude of the response to herbicide induced stress should be compared to that of the naturally occurring stress situations or "catastrophes" in soil microhabitats such as drought, flooding, freezing etc. From the result of many soil biological studies it was observed that a depression of 50 % or more in biomass or biochemical process that persist for 30 days or longer frequency occurs in surface soils under natural conditions (16). Hence Domsch (12) states that ecologically critical situations occur when the herbicide-induced depressions of soil microbial biomass or function last for more than 60 days and the reduction is > 50 % of the control. Assessment of our data according to this European system for chemical stress evaluation shows that that none of the herbicide-induced depressions of soil microbial biomass or functions met the criteria. Further, these 2,4-D effects were generally confined to the surface 10-15 cm and, as stated by Greaves and Malkomes (16), side- effects that are restricted to the surface layer may be ignored since a large volume of underlying rooted soil will maintain normal biological processes.

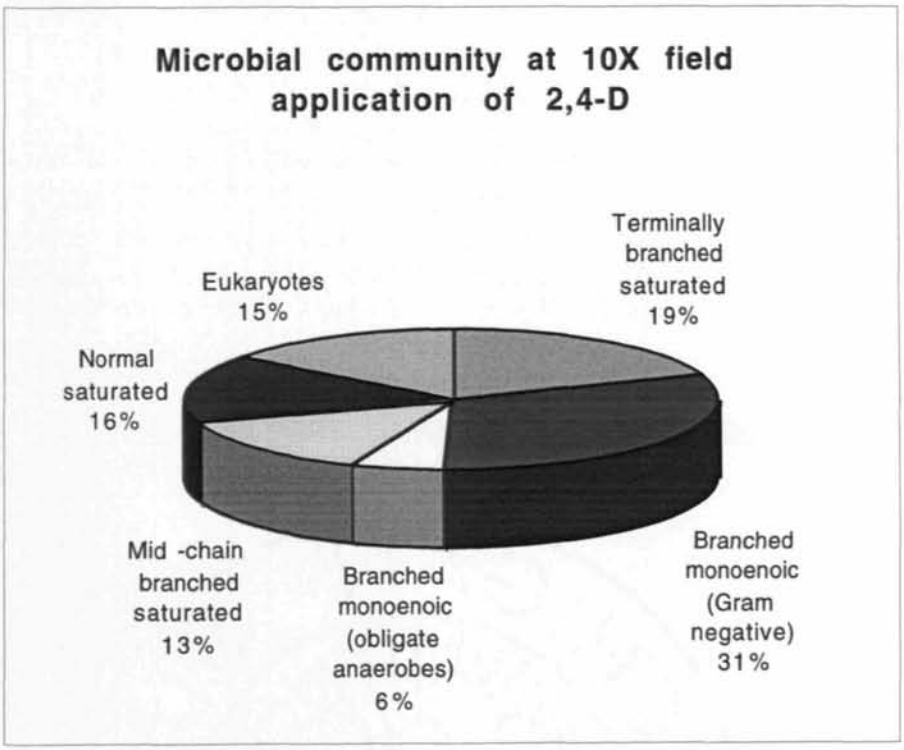
Since 10 years of 2,4-D at four different concentrations viz 0X, 1X, 10X, and 100X, produced temporary and minor soil side effects that did not significantly interfere with the normal cycling of C and N in surface soil under

field conditions, we conclude that there was no agronomically significant effect of long-term 2,4-D applications on soil fertility. However, it is possible that with time the effect of 2,4-D especially on the 100X plot might be of ecological significance since the microbial populations were not "healthy". These findings show that the repeated use of 2,4-D at high concentration (100X) might result in a disturbance of the biological equilibrium in the soil and possibly lead to eventual loss of fertility. While the microbial ecosystem was not seriously perturbed, the plant - soil ecosystem was since primary productivity (plant growth) was seriously reduced. Hence the plant community was much more sensitive to herbicide-induced effects.





Figs. 1a-d. Distribution of microbial community components as determined by PLFA . 2,4-D additions are as follows a=0X, b= 1X, c=10X, d=100X. Data are mean values of 2 replicates.


Microbial community at 10X field application of 2,4-D
Terminally branched saturated 19%
Eukaryotes 15%
Normal saturated 16%
Mid -chain branched saturated 13%
Branched monoenoic (obligate anaerobes) 6%
Branched monoenoic (Gram negative) 31%

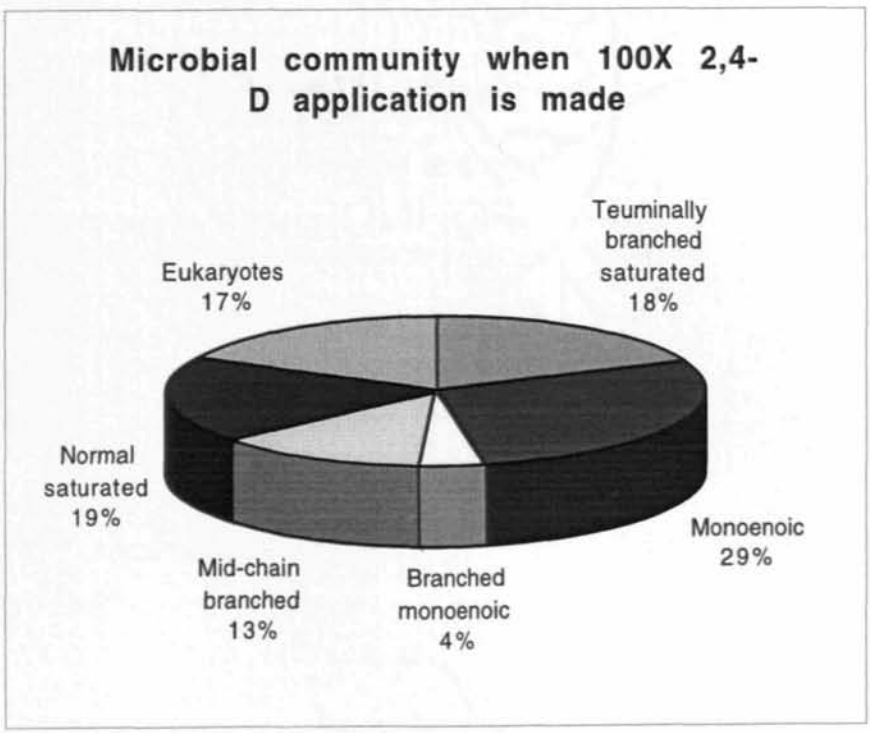

Microbial community when 100X 2,4-D application is made
Teuminally branched saturated 18%
Eukaryotes 17%
Normal saturated 19%
Mid-chain branched 13%
Branched monoenoic 4%
Monoenoic 29%

Fig. 1c and 1d.

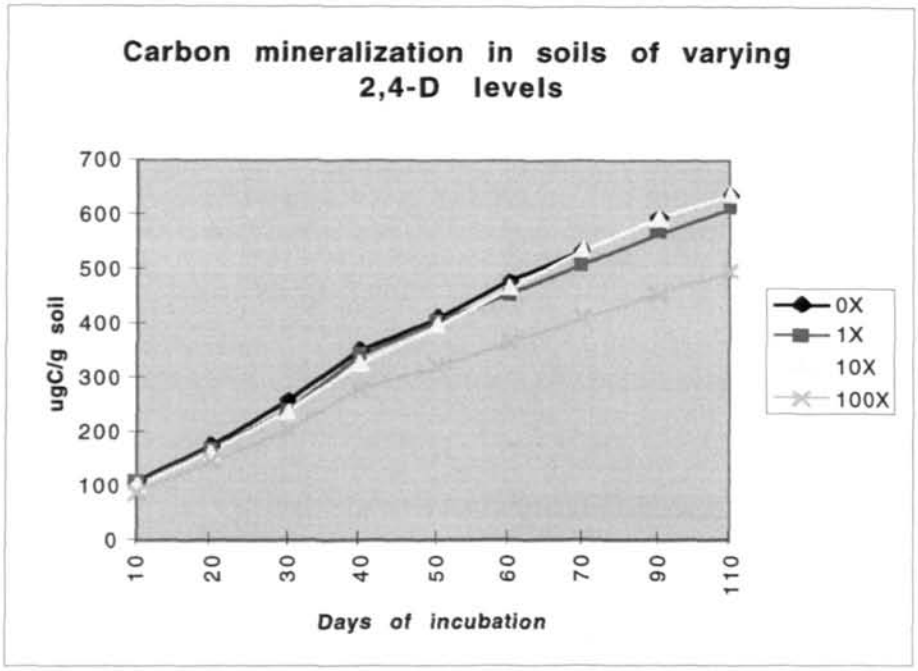


Fig. 2. Cumulative C mineralization with time. The plots were amended with the indicated 2,4 -D concentrations.

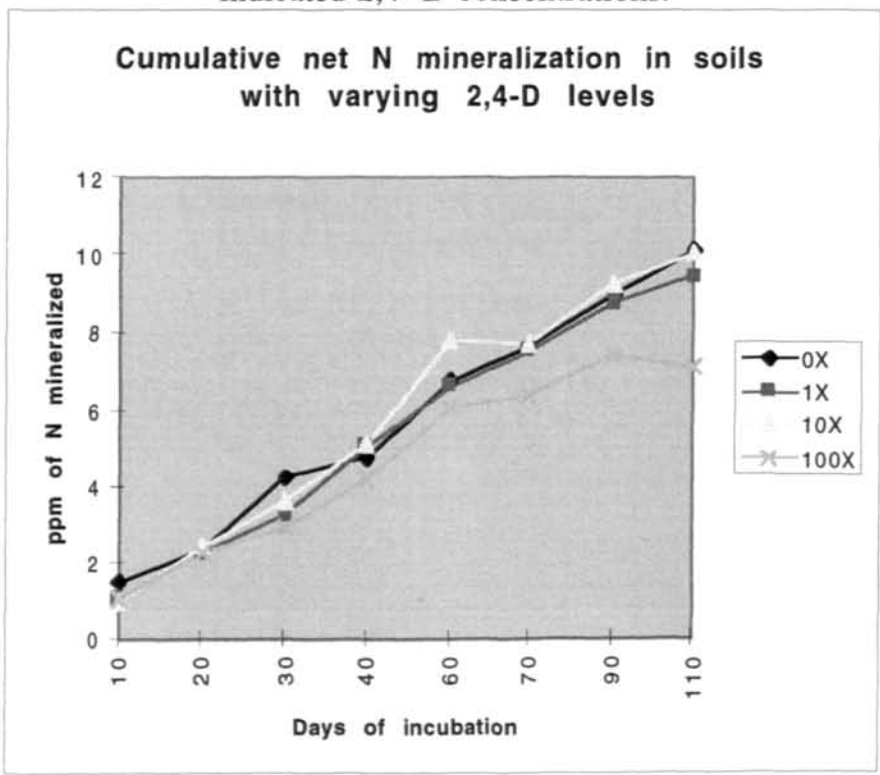


Fig. 3. Cumulative net N mineralization with time amended with different 2,4-D concentrations. The plots were amended with the indicated 2,4 -D concentrations.

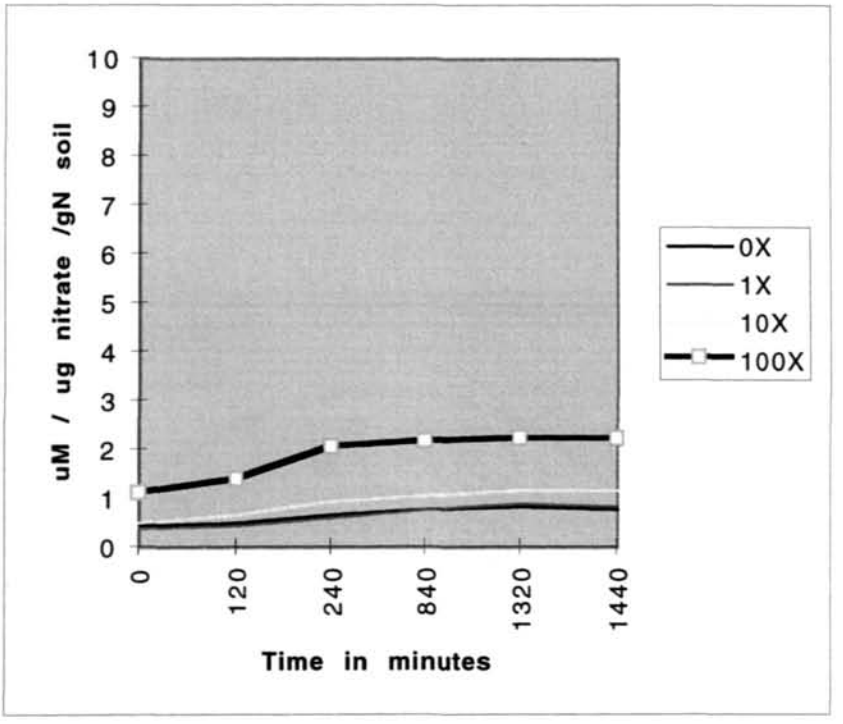


Fig. 3. Release of nitrate during incubation studies of soils amended with the indicated 2,4-D concentration

Table 1. Effect of long-term use of 2,4-D on microbial parameters and ecosystem functioning

Variables measured	2,4-D Treatment levels				Significance of F ratio (probability)
	0X	1X	10X	100X)	
			Microbial biomass		
Carbon ugC/g soil	113.39±18.31	121.00±6.9	121.58±9.94	84.04±2.31	Prob>F (0.00001)
Nitrogen ug/g soil	47.92±2.7	48.73±3.9	57.07±2.76	61.11±1.65	Prob >F (0001)
C/N Ratio	4.38	5.42	5.47	2.49	
			Microbial respiration in CO_2-C(ug/g soil)		
10 day incubation	94.7±2.25	98.00±13.3	85.7±18.7	66.00±15.87	Prob >F (0.1227)
			Microbial metabolic status		
Total PLFAs pm/g soil	29,182	26,802	23,637	18,166	
[a]Growth phase	1.34	1.02	1.47	2.35	
[b]Turn over rate	0.74	0.98	0.68	0.43	
[c]Signs of adaptation	0.10	0.10	0.11	0.17	
			Carbon mineralization ($ugCO_2$/g soil)		
30 day incubation	83.36±2.1	73.01±3.46	72.27±8.19	59.42±10.12	Prob>F (0.0001)
50 day incubation	82.43±21.91	64.16±7.27	75.18±9.91	50.13±13.7	Prob.F (0.1358)
70 day incubation	64.12±7.84	59.03±14.8	71.34±4.195	46.24±4.95	Prob>F (0.3676)
			Nitrogen mineralization (ppm)		
30 day incubation	6.26±1.99	5.56±1.64	4.54±0.05	4.62±0.081	Prob>F (0.3778)
60 day incubation	7.53±0.42	7.49±0.73	8.75±1.44	8.313±1.26	Prob>F (0.427)
70 day incubation	9.81±0.35	10.53±0.89	9.64±0.13	11.29±0.57	Prob>F (0.025)

a. Phase of growth=cy17:/16:1w7c+cy19:0/18:1w7c

b. Turn over rate= 1/phase of growth

c. Stress indicator of microbes=16:1w7t/16:1w7c+18:1w7t/18:1w7

All values are means of three replicates ± standard deviation

Table 2. Effect of increasing 2,4-D on relationships of the microbial parameters

Variable	r (% Correlation coefficient)	Regression equation
	Microbial Respiration	
Gram negative vs microbial respiration	58	y=-80+5.36 % Gram negative in population
	Microbial Nitrogen	
Microbial carbon vs microbial nitrogen	-65	y=79.87-0.24x
Gram negative vs microbial nitrogen	-81.5	y=134.2-2.54x
	Carbon mineralization (C min)	
Gram negative vs C min	98	y=-81.7+4.86x
Gram positive vs C min	-66	y=170.3+5.48x
	n=8	

Table 3. Effect of 2,4-D concentration on nutrient transformation

2,4-D treatments	nitrification rate	N mineralization rate within the first 30 days of incubation in ug/g soil	C mineralization rate within the first 30 days of incubation in ug/g/day
0X	4.46 ±1.5	0.14	7.5
1X	3.27 ±63	0.1	6.8
10X	3.71 ±.68	0.12	6.7
100X	4.75 ±1.3	0.97	6.0

All values are means of three replicates.

rate of N min=$\frac{(NH_4^+\text{-N} + NO_3^-\text{-N in ppm})_{t+1} - (NH_4^+\text{- N} + N0_3^-\text{-N})_{to}}{30 \text{ days (incubation period)}}$

References

1. **Barea, J.M.** 1991.Vesicular-arbuscular mycorrhizae as modifiers of soil fertility. Adv. Soil Sci **15**: 2-40.

2. **Biederbeck, V.O; C.A. Campbell and A.E. Smith.** 1987. Effects of long term 2,4-D field application on soil biochemical processes. J. Environ. Qual. **16**: 257-262.

3.**Bolton, H., L.F. Elliott, R.I. Papendick and D. F. Bezdicek.** 1985. Soil microbial biomass and selected soil enzyme activities: effects of fertilization and cropping practices. Soil Biol. Biochem. **17**: 297-302.

4. **Breazeale F.W.and N.D.Camper.** 1970. Herbicidal effects on certain soil microbial activities in some Brown soils of Saskatchewan. Weed Res. **4**: 54-63.

5. **Cambardella C.A. and E.T. Elliott.** 1992. Particulate soil organic matter changes across a grassland cultivation sequence. Soil Sci. Soc. Am. J. **56**:777-783.

6. **Cairns J. Jr.** 1983. Are single species test alone adequate for estimating hazard. Hydrobiologia. **137:**271-278.

7. **Cairns J. Jr. Pratt J.R. and Niederlehner B.R. 1985**. A provisional mutispecies toxicity test using indigenous organisms. J. Test. Eval. **13**:316-319.

8. **Carlisie S. M. and J.T. Trevors** 1986. Effect of the herbicide glyphosphate on respiration and hydrogen consumption in soil. Water Air Soil Poll. **27**: 391-401.

9. **Collins H.P., P.E. Rasmussen and C.L. Douglas Jr.** 1992. Crop rotation and residue management effects on soil carbon and microbial dynamics. Soil Sci. Soc Am. J. **56**:783-788.

10. **Cook, R.J. aand K.F. Baker.** 1983. The nature and practice of biological control of plant pathogens. Amer. Phytopath. Soc., St Paul, Minnesota. 539p.

11.**Cook R and M.P.Greaves**. 1987. Herbicides and the soil environment in Canada. Can. J. Soil Sci. **62**: 433-460.

12. **Domsch, K.H.** 1984. Effects of pesticides and heavy metals on biological processes in soil . Plant Soil. **76**:367-378.

13. **Domsch K. H. , G. Jagnow and T.H. Anderson.** 1983. An ecological concept for the assessment of side-effects of agrochemicals on soil microorganisms. Residue Rev. **86**: 65-105.

14. **Fryer, J.D. 1981**. Herbicides: Do they affect soil fertility? Span. **24**: 5-10.

15. **Greaves , M.P.** 1979. Long-term, effects of herbicides on soil microorganisms, Ann. Appl. Biol. **91**:129-132.

16. **Greaves, M.P. and H.P. Malkomes**. 1980. Effects on soil microflora. p. 223-253. *In* R.J. Hance (ed.) Interactions between herbicides and the soil. Academic Press, London, U.K.

17. **Guckert J.B., M.A. Hood, and D.C. White.** 1986. Phospholipid ester-linked fatty acid profile changes during nutrient deprivation of *Vibrio cholerae*: increase in th *trans /cis* ratio and proportions of cyclopropyl fatty acids. Appl. Environ. Microbiol. **52**:794-801.

18. **Hart, S.C., J.M. Stark, E.A. Davidson and M.K. Firestone.** 1994. Nitrogen mineralization, immobilization and nitrification. p. 985-1018. *In* R.W. Weaver, J.S. Angle and P.S. Bottomley(ed.), Methods of soil analysis. Part 2 - microbiological and biochemical properties. SSSA Book Series: 5, Soil Scil Soc. Amer. Madison, Wisc.

19. **Heipieper, H-J, R. Diefenbach and h. Keweloh.** 1992. Conversion of cis unsaturated fatty acids to trans, a possible mechanism for the protection of phenol-degrading *Pseudomonas putida* p8 from substrate toxicity. Appl. Environ. Microbiol. **58**:1847-1852.

20. **Helweg** A. 1986. Side effects caused by pesticide combinations. p 385-393. *In* V. Jenson, A. Kjoller and L.H. Sorensen (ed.), Microbial communities in soils. Elsevier Applied Science Publishers, London and New York.

21. **Howarth, W.R. and E.A. Paul.** 1994. Microbial biomass. p. 753-763. *In* R.W. Weaver, J.S. Angle and P.S. Bottomley (ed.), Methods of Soil Analysis. Part 2- Microbiological and Biochemical Properties . SSSA Book Series : 5. Soil Scil Soc. Amer. Madison, Wisc.

22. **Jenkinson, D.S. 1966.** Studies on the decomposition of plant material in soil. II. Partial sterilization of soil and the soil biomass. J. Soil Sci. **28**: 280-208.

23. **Kennedy and Smith,** 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil. **170:** 75-86.

24. **Kimbnall, K.D. and S.A. Levin**. 1985. Limitations of laboratory bioassays; the need for ecosystem level testing. BioScience **35**: 165-171..

25. **Koike, H., and P.L. Gainey**. 1952. Effects of 2,4-D and CADE, singly and in combination, upon nitrate and bacterial content of soils. Soil Sci. **74**:165-172.

26. **Lynch, J.M. and E. Bragg.** 1985. Microorganism and soil aggregate stability. Adv. Soil Sci. **2**:133-171.

27. **Martynuik, S. and G. H. Wagner**.1978. Quantitative and qualitative examination of soil microflora associated with different management systems. Soil Sci : **125:** 343-350.

28. **Newman, A.S.** 1947. The effect of certain plant growth-regulators on soil microrganisms and microbial. processes. Soil Sci. Soc. Am. Proc. **12**: 217-221.

29. **Ou Li-Tse, D.F. Rothwell, W.B. Wheeler, and J.M. Davidson**. 1978. The effect of high 2,4-D concentrations on degradation and carbon dioxide evolution in soils. J. Environ. Qual. **7**:241-246.

30. **Paul E.A. and F.E. Clark.** 1989. Soil microbiology and biochemistry,. Academic Press, San Diego. 273p.

31. **Powlson, D.S. P.C. Brookes and B.T.Christensen.** 1987. Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. Soil. Biochem. **19:**159-164.

32. **Ramsay, A.J., R.E. Stannard, G.J. Churchman** . 1986. Effect of conversion from ryegrass pasture to wheat cropping on aggregation and bacterial populations in a silt loam soiil in New Zealand. Aust. J. Soil Res. **24**: 253-264.

33. **Radehaus, P.M. and S.K. Schmidt.** 1992. Characterization of a novel *Pseudomonas* sp; that mineralizes high concentration of pentachlorophenol. Appl. Environ. Microbiol. **58**: 2879-2885.

34. **Shea, P.J**. 1985. Detoxification of herbicide residues in soil. Weed Sci.**33:** 33-41.

35. **Simon-Sylvestre, G., and J-C. Fournier**. 1979. Effects of pesticides on the soil microflora. Adv. Agron. **31**: 1-92.

36. **Somerville, L.** 1987. Perspectives on side-effect testing. p. 5-13. In Pesticide effects on soil microflora. (Eds.) L. Somerville and M.P Greaves. Taylor and Francis, London.

37. **Smith, N.R., V.T. Dawson, and M.E. Wenzel**. 1945. The effect of certain herbicides on soil microorganisms . Soil Sci. Soc. Am. Proc. **10**: 197-201.

38. **Smith A.E.** 1989. Degradation, fate, and persistance of phenoxyalkanoic acid herbicides in soil. Rev. Weed Sci. **4**: 1-24.

39. **Smith J.L. and E.A.** Paul 1990. The significance of soil microbial biomass estimations in soil. p. 357-396. *In* J. M Bollag and G Stotzky (ed.). Soil Biochemistry. Vol. 6. Marcel Dekker, New York, NY.

40. **Sprent, J.L.** 1979. The biology of nitrogen-fixing organisms. McGraw Hill, London. 196 p.

41. **Teater, R.W., J.L. Mortensen, and P.F. Pratt.** 1958. Effect of certain herbicides on rates of nitrification and carbon dioxide in soil. J. Agric. Food Chem. **16**: 214-216.

42. **Waksman S. A.** 1927. Principles of soil microbiology. The Williams and Wilkins Co., Baltimore. MD, 897 p.

43. Wardle D. A. 1992. A comparatove assessment of factors which influence microbial biomass carbon and nitrogen levels in soils. Biolog. Rev. **67**: 321-358.

44. **Wardle, D.A. and D. Parkinson**. 1990. Influence of the herbicide glyphosate on soil microbial community structure. Plant and Soil. **122**: 29-37.

45. **Voets, J. P. , P. Meerschman, and W. Verstraete**. 1974. Soil microbiological and biochemical effects of long-term atrazine applications. Soil Biol. Biochem. **16**: 9-14.

46.**Yeomans J.C. and J.M. Bremner.** 1987. Effects of dalapon, atrazine, and simazine on denitrification in soil. Soil Biol. Biochem. **19**, 31-34.

47. **Zelles, L., A. Palojarvi, A. Kandeler, E. Lutzow, M. von. Winter, and K. Bai**. 1997. Changes in soil microbial proerties and phospholipid fatty acid fractions after chloroform fumigation . Soil Biol. Biochem. **29**: 1325-1336.