ASSESSING SOIL MICROBIAL COMMUNITY AFTER LONG TERM 2,4-D APPLICATION IN THE FIELD

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ABSTRACT
2,4-dichlorophenoxyacetate was used as a model carbon substrate and applied to field plots at four different rates, 0X- control, 1X (the normal field rate of application of 1.1 kg/ha), 10X and 100X for ten consecutive years to assess the long-term effect on soil microbial community structure and the impact on carbon and nitrogen cycling. With 2,4-D application, decrease in the PLFA signatures such as the monoenoics, branched monoenoics, and the eukaryote were observed especially in the 100X treatment whilst increases in the PLFA signatures such as normal saturates, mid chain branch saturates also occurred. The Shannon Weiner Diversity index was 1.6 for almost all the treatments and the Jaccard evenness ranged from 0.856-0.998 for all the treatments reflecting the evenness of all the treatment plots. No major shift in microbial community was observed in all the treatment plots according to PLFA analysis. Biomass PLFA and microbial biomass carbon by chloroform fumigation declined with 2,4-D application, p<0.031 and p<0.14 respectively. Generally, carbon mineralization was significantly depressed whilst nitrogen mineralization was not significantly depressed with increase in 2,4-D application. Increase in the cis fatty acid to the trans fatty acid and cis fatty acid to the monoenoic precursor was observed for the 100X treatment plots indicating the stressed state of the Gram negative bacteria.

INTRODUCTION
2,4-D is a broad leafed pesticide that has been used for the past decades on lawns, gardens, golf courses, cereal crops and pastures. Because of the millions of hectares of land involved when 2,4-D is applied, interest in the environmental distribution and effect has intensified in recent years. After 40 successive years of application of 2,4-D, soil sampled from that field, had less than 0.02mg/kg of residual 2,4-D (Smith et al., 1989). Similarly, after applying 2,4-D amine or the ester form at rates of 1.12kg/ha for 35 consecutive years, Biederbeck et al. (1987) concluded that, the effects of long-term 2,4-D application were neither ecologically significant nor did it interfere with nutrient cycling to adversely affect soil fertility. Most studies involving the use of high 2,4-D rates have been short term conducted in microcosms whilst less studies have been done on
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field. In one of such microcosm studies, at least 100mg/kg of 2,4-D was required to obtain an apparent change in the community structure visualized by Denaturing gradient gel electrophoresis (DGGE). Also, the greatest diversity of 2,4-D degrading isolates was obtained from the 10mg/kg treatment (Macur et al., 2007). Similar shifts in the microbial community from the more diverse to a less diverse 2,4-D degrading populations was observed in soil microcosm and in field studies with 2,4-D degrading strains of Sphingomonas sp. and Pseudomonas pickettii being the dominant members of the microbial community (Hoben et al., 1994; Ka et al., 1995). In soils, where shift in microbial population was observed from the more diverse population to the less diverse population one wonders how nutrient cycling will be affected. The hypothesis of the present study was that carbon and nitrogen mineralization in field soil will be significantly affected when there is a shift in the microbial community structure. Therefore, this study used 2,4-D as carbon source and applied it at various rates to field soil for ten years with the aim of having a community enriched with 2,4-D degraders and thus shifting the microbial population to a less diverse population and determine the extent that nutrient cycling was affected.

Applying 2,4-D influences the biochemical properties of soil. When Alcaligenes eutrophus JMP134 was inoculated into a nonsterile microcosm amended with 1,000ug of 2,4-D g⁻¹ of soil significant populations of indigenous recipient of transconjugants arose and these contained an 80 kb plasmid similar in size to pJP4 and all degraded 2,4-D. These data show that the plasmid pJP4 was transferred to indigenous soil recipients (Di giovanni et al., 1999). One would expect that communities with long history of high exposure to 2,4-D in the field would have populations adapted to the herbicides and also the possibility of ongoing gene transfer mechanisms. Fifteen years of application of 2,4-D at the rate of 0.95kg/ha, microbial biomass carbon and nitrogen were reduced by 2,4-D treatment, the reduction being more marked when the ester form was used (Rai, 1992). The use of high pesticide concentrations on the soil microbial activity as well as the presence or accumulation of 2,4-D metabolites in soil receiving such high concentrations are not well documented. Thus, Olson and Lindwall (1991) reported that under laboratory conditions, the application of 2,4-D at 2 and 100 times the field rates reduced nitrification by 11% and 71% respectively. At normal field rate of 2,4-D application, nitrification rates were temporarily reduced (Greaves and Malcolm, 1980), whereas ammonification and microbial respiration were unaffected, the activity of urease, acid and alkaline phosphatase were reduced only for a short time (Biederbeck et al., 1987). Lenhard (1954) found that 100 to 1000ppm of 2,4-D decreased dehydrogenase activity as well as the total microbial population in the soil. Rates above 500ppm, caused autolysis of bacteria and decreased nitrogen fixation by Azotobacter.

The objective of this study was to determine the long-term (10 years) field application of 2,4-D at different rates on soil microbial community structure and on carbon and nitrogen mineralization.

MATERIALS AND METHODS

Soil sampling

2,4-D was applied to the 2,4-D Gene Transfer plots of the Kellog Biological Station, Hickory Corners, Michigan, USA. The plots consisted of eight subplots each 3.6 by 9.1m. Each subplot was separated by a buffer zone of 4.5m wide. These subplots were planted with maize during the summer periods and left fallow during winter. The levels of 2,4-D applied to these field plots were 0X (the control plot), 1X (1.1kg/ha, the good agricultural practice), 10X and 100X the normal field application rate for ten consecutive years. Each application rate was replicated twice. At the seventh year of application, the field plots were divided northern side (N-plot) where 2,4-D was applied continuously till the tenth year and a southern section where the application was terminated (S-plot) at the seventh year till the tenth year. Soil
was sampled from the subplots in the Northern plots only for the present study. Ten samples collected randomly from each subplot excluding the border plots and pooled together to form a composite sample for that subplot. The soils were then kept in an ice chest and it was sent to the laboratory. In the Laboratory, the soils were immediately sieved through a 2mm sieve and used for further analysis.

Phospholipid fatty acid analysis
Samples of soil for phospholipid fatty acid analysis (PLFA) were kept frozen at -20°C immediately after sieving the soil through a 2mm sieve. The samples were then sent on dry ice to the Microbial Insight Incorporated at Rockford, Tennessee, USA. The results obtained were analyzed by taking the following into consideration, the sum of PLFAs considered to be predominantly of bacterial origin are i15:0, a15:0, i16:0, 16:1ω7, i17:0, cy17:0, 18:1ω7c and cy 19:0 were chosen as an index of the bacterial biomass (Vestal and White, 1989). The quantity of PLFA 18:2ω6 was used as an indicator of fungal biomass, as it is suggested to be mainly of fungal origin in the soil.

The ratio of fungal and bacterial PLFAs was used as an index of the ratio of fungal/bacterial biomass in the soil.

The microbial community of the various treatments was described by using the PLFA signatures for the following, the normal saturates (Nsats) found in both prokaryotic and eukaryotic kingdoms, Mid chain branched saturates (MidBrSats), branched monoenoic (BrMonos), monoenoics, and terminally branched saturated (Tet. Br.Sat) representative of mostly the Gram positive bacteria and also found in the cell membranes of sulphate reducing bacteria and eukaryotes represented by fungi, protozoa, algae, and the diatoms (18:2 ω6, 18:3 ω3, 20:4 ω6 and 20:5 ω3).

The PLFA stress signatures used in analyzing Gram - negative bacterial community were, cy17:0/16:1ω7c, cy19:0/18:1ω7c, and 16:1ω7t/16:1ω7c and 18:1ω7t/18:1ω7c

Microbial parameter measurements
The potential nitrification rate was determined by the shaken soil –slurry method as described by Hart et al. (1994). Samples that had been optimized with respect to water content, ammonium, aeration and P availability were incubated under laboratory conditions. Fifteen grams of sieved, field – moist soil was put into a 250ml Erlenmeyer flask with 100 ml of combined solution (0.3mM KH2PO4, 0.7mM K2HPO4 and 0.75mM NH4SO4) and the flask covered with a vented cap. All flasks were placed on an orbital shaker and shook at approximately 180rpm for 24h. At 2h intervals, aliquot (5ml) was collected, 1ml of 2M HCl was added and centrifuged. The supernatant was then stored at -20°C and later analyzed for NH4+ and NO3- contents.

Nitrogen mineralization rate
N mineralization was determined following the method as described by Hart et al. (1994). The soil was incubated for at least 100 days at room temperature (25°C) in the dark. Ammonium and nitrate concentrations were determined extracting soil subsamples with 1M KCl at time zero and every tenth day of incubation.

Carbon mineralization
Carbon mineralization was measured by incubating soil for more than 100 days at room temperature in the dark in Mason jars. Accumulated carbon dioxide was collected in plastic vial containing 1M NaOH that was placed near the soil sample in the jar. Every ten days the carbon dioxide released was determined and fresh vial containing 1M NaOH was placed in the Mason jar. The CO2-C evolved at room temperature (28°C) was measured by titrating the CO2 trapped in 1M NaOH with dilute HCl (0.5M) using phenolphthalein as the indicator (Anderson, 1982). Before then, excess BaCl2 had been added to the NaOH solution to precipitate the carbonate as insoluble BaCO3.

Microbial biomass determination by chloroform fumigation
Microbial biomass was determined by the chloro-
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...Brempong roform fumigation incubation method (CFI) as described by Howarth and Paul (1994). Chloroform lysed the living microbial cells in soil sample. The soil samples were then incubated for 10 days in sealed Mason jars. The temporary flush of carbon dioxide was primarily due to the decomposition of microorganisms (Jenkinson, 1966). The microbial biomass C and N were determined.

Statistical analysis
Statistical analysis was performed using the general STATA programme to perform analysis of variance and to test if treatment differences were significant.

RESULTS
Effect of 2,4-D on microbial biomass and microbial respiration
Biomass PLFA was highest in the control (0X plots) and with the increase in 2,4-D application there was a corresponding decrease in the biomass. The 100X plots had the highest reduction in the PLFA biomass. The difference in treatments were statistically significant (p<0.031). Thus within ten years of continuous 2,4-D application, there was a 37% decrease in microbial biomass PLFA for the 100X treatment as compared to the control plot whilst for the 1X plot where the decrease was 8% (Table 1). Similarly, there was 26% decrease in microbial biomass carbon by Chloroform fumigation incubation (CFI) method for the 100X treatment as compared to the control. Values obtained for 0X and 1X were almost the same for the CFI method of determining biomass, treatment differences were not significant with p<0.14. Microbial biomass nitrogen increased with 2,4-D applications thus, 0X had the lowest microbial biomass nitrogen and the highest the 100X treatment. Treatment differences were significant and p< 0.01 (Table 1). Microbial respiration also decreased with 2,4-D application (Table 1). The lowest value was attained by the 100X plots indicating decrease in microbial activity as compared to the control plot (p< 0.1227).

Effect of long term 2,4-D application on nutrient cycling
The cumulative CO₂-C productions (carbon mineralization) for the different treatments with time are shown in Fig.1. Carbon mineralization trend with time shows an initially high mineralization rate up to 40 days and this slows down from 40th day onward (Fig. 1). The same trend is observed for all the treatment. Generally carbon mineralization was depressed as 2,4-D application level was increased. Thus the 100X plot recorded lowest carbon mineralization at all time points (Fig. 1). Carbon mineralization rate for the first thirty days was highest for the control treatments (7.5ug CO₂-C/g soil) and this decreased to 6.0ug CO₂-C/g soil with the

<table>
<thead>
<tr>
<th>Treatments Levels of 2,4-D applied</th>
<th>Microbial biomass PLFA pM/g dry soil</th>
<th>Microbial biomass carbon ug C/g soil</th>
<th>Microbial biomass nitrogen ug/g dry soil</th>
<th>Microbial respiration ug/CO₂-C/g dry soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0X</td>
<td>29182.0</td>
<td>122.18</td>
<td>47.92</td>
<td>94.70</td>
</tr>
<tr>
<td>1X</td>
<td>26802.0</td>
<td>121.00</td>
<td>48.73</td>
<td>85.70</td>
</tr>
<tr>
<td>10X</td>
<td>23637.0</td>
<td>117.41</td>
<td>57.07</td>
<td>85.70</td>
</tr>
<tr>
<td>100X</td>
<td>18166.0</td>
<td>89.55</td>
<td>61.11</td>
<td>66.00</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>6277.1</td>
<td>68.71_{(0.01)}</td>
<td>4.257</td>
<td>28.97</td>
</tr>
<tr>
<td>p</td>
<td>p&lt;0.031</td>
<td>p&lt;0.14</td>
<td>p&lt; 0.01</td>
<td>p&lt;0.1227</td>
</tr>
</tbody>
</table>

Table 1: Microbial biomass PLFA, carbon and nitrogen as influenced by continual application of different rates of 2,4-D application
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Fig. 1: Cumulative Carbon mineralization patterns of soils with different 2,4-D application levels

Table 2: Carbon, nitrogen mineralization and nitrification rates as influenced by long term 2,4-D application

<table>
<thead>
<tr>
<th>2,4-D application levels</th>
<th>Carbon mineralization rate within the first 30 days ug C/g soil</th>
<th>Potential nitrification rate</th>
<th>Carbon mineralization at day 30 ug C/g soil</th>
<th>Carbon mineralization at day 70 ug C/g soil</th>
<th>Nitrogen mineralization at day 30 ug N/g soil</th>
<th>Nitrogen mineralization at day 60 ug N/g soil</th>
</tr>
</thead>
<tbody>
<tr>
<td>0X</td>
<td>7.5</td>
<td>4.46</td>
<td>83.36</td>
<td>64.12</td>
<td>6.26</td>
<td>7.53</td>
</tr>
<tr>
<td>1X</td>
<td>6.8</td>
<td>3.27</td>
<td>73.01</td>
<td>59.013</td>
<td>5.56</td>
<td>7.49</td>
</tr>
<tr>
<td>10X</td>
<td>6.7</td>
<td>3.71</td>
<td>72.27</td>
<td>71.34</td>
<td>4.54</td>
<td>8.75</td>
</tr>
<tr>
<td>100X</td>
<td>6.0</td>
<td>4.75</td>
<td>59.42</td>
<td>46.42</td>
<td>4.62</td>
<td>8.31</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>1.8</td>
<td>2.01</td>
<td>6.4016</td>
<td>16.938</td>
<td>2.438</td>
<td>2.948</td>
</tr>
<tr>
<td>p</td>
<td>p&lt;0.10</td>
<td>p&lt;0.60</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0467</td>
<td>p&lt;0.3778</td>
<td>p&gt;0.427</td>
</tr>
</tbody>
</table>

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highest level of 2,4-D application (Table 2). Significant differences in the treatments were observed for carbon mineralization on the 30th day of incubation (p<0.0001) and on seventy days of incubation (p<0.0467) respectively (Table 2), but not on fifty day after incubation (p< 0.1358).

Nitrogen mineralization was also depressed with 2,4-D application, thus the control was slightly higher than the rest of treatments (Fig. 2). The rest of the other treatments had similar values at any time point. Nitrogen mineralization was highest at the control treatment plot and was depressed for the 100X treatment even though the effect was not significant (p<0.37) by the 30th day of incubation. By the 60 day of

Fig. 2: Cumulative nitrogen mineralization patterns of soils with different 2,4-D application

incubation, 10X treatment had the highest mineralization rate even though the treatment differences was not significant (p<0.43). There was significant difference in treatments for nitrogen mineralization for the 70 days of incubation p< 0.025. 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 100X plots.

The 100X treatment had the highest potential maximum nitrification rate of 4.57ug/g soil followed by the 0X whilst 1X and 10X had relatively the same rates (Table 2).

**Microbial community as assessed by** the **various PLFA and stress signatures** Decreases in bacterial PLFA was observed with 2,4-D application, treatment differences were significant, p <0.01 (Table 3). Similarly, fungal PLFA decreased with increased in 2,4-D application, p<0.67. The 10X treatment recorded a lower fungal PLFA value than the 100X treatment. The fungi/bacteria ratio decreased with 2,4-D application. With 2,4-D application, decreases in the following PLFA signatures were observed, Monos, Br Monos, and some of the eukaryote especially in the 100X treatment plots as compared to the control treatment (Fig. 3). Increases in the PLFA signatures were also observed in the N sats, Mid Br Sats with 2,4-D application. No major shift in microbial community was observed with the use of PLFA signature as 2,4-D was applied (Fig. 3).

Table 3: Effect of 2,4-D on diversity and evenness of microbial communities

<table>
<thead>
<tr>
<th>2,4-D application levels</th>
<th>Bacterial PLFA pM/g soil</th>
<th>Fungal PLFA pM/g soil</th>
<th>Fungi/ Bacteria ratio</th>
<th>Shannon-Weiner Diversity Index</th>
<th>Jaccard Evenness</th>
</tr>
</thead>
<tbody>
<tr>
<td>0X</td>
<td>11,753.68</td>
<td>1,368.73</td>
<td>0.11645</td>
<td>1.654</td>
<td>0.923</td>
</tr>
<tr>
<td>1X</td>
<td>10827.58</td>
<td>694.26</td>
<td>0.0641</td>
<td>1.672</td>
<td>0.933</td>
</tr>
<tr>
<td>10X</td>
<td>9739.814</td>
<td>451.88</td>
<td>0.0463</td>
<td>1.5337</td>
<td>0.856</td>
</tr>
<tr>
<td>100X</td>
<td>7208.29</td>
<td>488.16</td>
<td>0.0677</td>
<td>1.678</td>
<td>0.94</td>
</tr>
<tr>
<td>LSD (0.05)</td>
<td>20831</td>
<td>29.45</td>
<td>0.248</td>
<td>0.567</td>
<td>1.21</td>
</tr>
<tr>
<td>p&lt; 0.01</td>
<td>p&lt;0.67</td>
<td>p &lt; 0.98</td>
<td>p&lt;0.37</td>
<td>p &lt;0.17</td>
<td></td>
</tr>
</tbody>
</table>

The Shannon –Weiner Diversity recorded almost the same value of diversity of 1.6 for 0X, 1X and the 100X treatments. The 10X treatment had a lower value (1.533) than all the others, p<0.37. Similarly, the Jaccard evenness index recorded a lower value for the 10X than the rest of the treatments, p<0.17 (Table 3). Thus to a considerable extent the microbial communities in all the treatment plots were even.

To assess the physiological status of the Gram negatives in the microbial community structure, the following PLFA ratios were used, cy17:0/16:1ω7c, cy 19:0/18:1ω7c, 16:1ω7t/16:1ω7c and 18:1ω7t/18:1ω7c. With increase in 2,4-D application, there was a corresponding increase in the ratio value cy17:0/16:1ω7c such that treatment 100X had almost twice higher value than the 0X treatment (p<0.076) indicating that the cells in 100X were stressed and entering the stationary phase. A similar trend was observed for the cy 19:0/18:1ω7c (p<0.03511). With increase in 2,4-D application, the ratio 16:1ω7t/16:1ω7c remained the same for treatments 0X, 1X and 10X (Table 4). It was the 100X treatment that the ratio increased (p<0.068). There was a corresponding increase in the 18:1ω7t/18:1ω7c from 0X through 10X with increase in 2,4-D application, 100X had a lower value (p<0.532).
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**DISCUSSION**

Effect of 2,4-D on microbial biomass and microbial respiration

Four different rates of 2,4-D application were made to soil for ten years. Among the treatments, was the 100X and the normal rate of 2,4-D application. Such high rates of 2,4-D are not applicable to agricultural soils but can be applicable at accidental spillage site, 2,4-D disposal site or at the herbicide manufacturing centre. 2,4-D being applied to soil is a source of organic carbon and organic nitrogen to heterotrophs especially.

The decrease in the microbial biomass as indicated by both PLFA and by chloroform fumigation shows that applying 2,4-D continuously kills some of the microbial population presuma-

**Table 4: PLFA stress signatures to assess Gram negatives in the microbial community structure of the various treatments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>cy17:0/16:1w7c</th>
<th>cy19:0/18:1w7c</th>
<th>16:1w7t/16:1w7c</th>
<th>18:1w7t/18:1w7c</th>
</tr>
</thead>
<tbody>
<tr>
<td>0X</td>
<td>0.62</td>
<td>0.725</td>
<td>0.70</td>
<td>0.165</td>
</tr>
<tr>
<td>1X</td>
<td>0.47</td>
<td>0.55</td>
<td>0.70</td>
<td>0.30</td>
</tr>
<tr>
<td>10X</td>
<td>0.59</td>
<td>0.875</td>
<td>0.70</td>
<td>0.33</td>
</tr>
<tr>
<td>100X</td>
<td>1.035</td>
<td>1.130</td>
<td>0.105</td>
<td>0.065</td>
</tr>
<tr>
<td>LSD(0.05)</td>
<td>0.739</td>
<td>0.468</td>
<td>0.932</td>
<td>1.13</td>
</tr>
<tr>
<td>p</td>
<td>p&lt;0.076</td>
<td>p&lt;0.03511</td>
<td>p&lt;0.068</td>
<td>p&lt;0.532</td>
</tr>
</tbody>
</table>

**Fig. 3:** Microbial community structure of soils described by using the PLFA signatures after 2,4-D application. (Numerals in charted areas show the percentage of PLFA signature for a particular community.)

Table 4: PLFA stress signatures to assess Gram negatives in the microbial community structure of the various treatments
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bly the sensitive ones such as the, monoenoics –being mostly the Gram negatives, branched monoenoics–commonly found in obligate anaerobes such as the sulphate reducing bacteria, and some components of the eukaryote. Corresponding increases in PLFA signatures such as Terminally Branched saturates, Mid-chain branched saturate, Branched Saturates, Normal saturates in the 100X plots i.e the Gram positive, some sulphate reducing bacteria, actinomycetes and some eukaryotes observed indicate that such organisms used 2,4-D as carbon and energy sources and the microbial community structure shifting to their favour even though the shift in the microbial community was not dramatic. The increase in some anaerobic microorganisms in the higher 2,4-D treated plot suggest anaerobic condition prevailing in that plot thus favouring the prevalence of sulphate reducing bacteria. Biomass determination by PLFA was more sensitive in capturing higher percentage of the microbial population affected by the 2,4-D than the chloroform fumigation method (Robie et al., 1989).

When 2,4-D is applied at high rates there is the accumulation of dichlorophenol (Newman, 1947) that are known as classic cytochrome uncouplers and are not always readily used as sole carbon source because of the toxicity at low substrate concentration (Radehaus and Schmidt, 1992). 2,4-dichlorophenol has been identified as a soil degradation product of 2,4-D (Smith, 1989). Also, chlorides are released during 2,4-D degradation and this can be toxic to microbial activity. The corresponding decrease in microbial respiration with increase in 2,4-D application indicates a reduction in the microbially related activities in soil leading to the reduction in the rate of herbicide degradation which might be injurious to some soil microorganisms. Increase in microbial biomass nitrogen with increase in 2,4-D application i.e. from 0X through to 100X treatments shows that, the dimethylamine form of the 2,4-D was used as an organic nitrogen source by the microbial community and it can be mineralized to inorganic nitrogen forms. In the 100X plots, since a higher rate of 2,4-D was applied, more of the dimethylamine was available as organic N for microbial use hence higher microbial nitrogen biomass was observed. This form of 2,4-D is 20% N by weight and organic N released could stimulate growth of the nitrifier population. 2,4-D in the dimethylamine formulation being neutralized by the dimethylamine could not have changed the soil pH appreciably through the continual use as compared to the technical grade of 2,4-D (Ou et al., 1978) to significantly affect the microbial community.

Effect of long term 2,4-D application on nutrient cycling

Carbon mineralization was high for the first 30 days indicating the degradation of easily decomposable organic substrate and thence that of resistant organic substrate. Active populations include primary decomposers (bacteria, actinomycetes and fungi) as well as secondary feeders that are involved in carbon mineralization (Tate, 1990). Therefore high 2,4-D application significantly affected some of these organisms and thence affecting carbon mineralization.

The absence of any significant effect of 2,4-D concentration on nitrogen mineralization shows that over the many years of 2,4-D application, the major groups of organisms within the soil microflora involved in nitrogen mineralization have 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 (Fryer, 1981; Greaves, 1979; Wardle and Parkinson, 1990; Teater et al., 1958). The increase in nitrification 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. The slightly higher nitrification rate observed for the 100X treatment plot might also be due to the effect of higher organic N added through the usage of dimethylamine form of 2,4-D.

Microbial community as assessed by the various PLFA and stress signatures

Even though 2,4-D was applied at different
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rates, the Shannon Weiner Diversity Index was similar for all the treatments except the 10X that had a slightly lower value indicating that the other three treatments have similar ability to withstand disturbances in the ecosystem. The lower fungal biomarker in the 10X plot than in the 100X plots might have accounted for such an observation showing that the filamentous fungi are more sensitive than the single celled fungi in that plot. The use of this index does not give the species or the genetic diversity in the different treatment plots.

Increase of the cis fatty acid to the trans fatty acid especially in the 100X treatment is associated with the stresses that could be due to organic compound toxicity (Heipieper et al., 1992) as 2,4-D breaks down in the treatment plots, starvation (Guckert et al., 1986), osmotic stress etc. that specifically result in increase in membrane fluidity. Similarly, increased in the cis unsaturated fatty acid to their monoenoic precursor in the 100X treatment plots help to maintain a functional living membrane by minimizing the membrane lipid losses or changes in membrane fluidity owing to cellular degradation during stress condition. Stress condition such as pesticide application reportedly increased the abundance of Gram-negative bacteria PLFA with concomitant decrease in Gram-positive bacterial PLFA (Zelles et al., 1994 and Frogestard et al., 1993). Greater survival of Gram-negative bacteria under stress conditions could be attributed to the presence of the cyclo fatty acids in their membrane and the outer polysaccharide layer which can counteract with stress (Guckert et al., 1986).

CONCLUSION
Applying 2,4-D at high rates to soil for a period of years could affect the soil fertility. This was because, carbon mineralization was significantly affected, portions of the microbial community were killed and the PLFA of the Gram-negative bacteria especially in the higher 2,4-D treated plots indicate stressed. There was no dramatic shift in the microbial community such that diversity of the microbial communities of the different treatments were different indicating the resilience of the soil ecosystem to perturbation. Carbon mineralization i.e. the conversion of organic carbon to the inorganic form was affected significantly, but not nitrogen mineralization and nitrification as initially hypothesized. Probably by continually applying such concentrations for a long time (40 years) that might be realized.

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REFERENCES


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