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SOIL MICROBIAL BIOMASS AND MINERALISATION OF SOIL ORGANIC MATTER AFTER 19 YEARS OF CUMULATIVE FIELD APPLICATIONS OF PESTICIDES

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Summary—The effects of 19 years of cumulative annual field application of five pesticides (benomyl, chlorfenvinphos, aldicarb, triadimefon and glyphosate), applied at, or slightly above, the recommended rates in 2^5 combinations, on soil microbial biomass and the mineralization of soil organic matter were investigated. Soil samples were taken 1 month after the application of benomyl, chlorfenvinphos and aldicarb in April 1992, and again in October 1992, 1 month after the application of triadimefon and glyphosate. The addition of aldicarb caused a significant increase of 7–16% in soil microbial biomass carbon (biomass C), an effect which appeared to be persistent. This effect of aldicarb was not reflected in the mineralization rate of soil organic C, possibly because the measurements of CO_2 evolution showed a greater variation than those of biomass C. Measurement of microbial biomass activity by the substrate-induced respiration method also gave much less precise results than measurements of biomass C by fumigation-extraction. The mineralization of soil organic N to ammonium and then nitrate was mostly unaffected by the pesticide treatments. In the autumn-sampled soil, there was significantly less NH_4-N in the aldicarb-treated soil. It is possible that this was due to immobilization by the increased microbial biomass in these treatments, and did not represent a loss to the soil system. The continuous use of these pesticides, either singly or in combination, therefore had no measurable long-term harmful effects on the soil microbial biomass or its activity, as assessed by C or N mineralization. © 1997 Elsevier Science Ltd

INTRODUCTION

Most studies investigating side-effects of pesticides on soil micro-organisms have involved laboratory-based experiments, often concerned with short-term effects following application of a single pesticide. However, in the field, one or more pesticides may be repeatedly applied to the same soil for many years, which may lead to a build-up of pesticide residues or metabolites, whereby the possibility of damaging effects upon the soil microbial biomass or its activity is much greater (Grossbard, 1971; Greaves, 1979).

Of the relatively few reports that have addressed long-term effects, most have been concerned with field experiments, as it is difficult to maintain laboratory incubations for more than a few months (Greaves, 1979). There have been some papers on the repeated application of a single pesticide to a soil over many years (e.g. Voets *et al.*, 1974; Duah-Yentumi and Johnson, 1986; Beiderbeck *et al.*, 1987), and on the application of several pesticides to a soil over a few years (e.g. Schuster and Schröder, 1990; Jones *et al.*, 1991), but very few on the repeated application of several pesticides over many years (e.g. Grossbard, 1971; Heinonen-Tanski *et al.*, 1985, 1986). Given that repeated application is common in agriculture, it is surprising that there have been few such studies.

In 1973, a field experiment, known as the Chemical Reference Plots, was begun on Long Hoos field at Rothamsted Experimental Station, U.K., in which the effects of the long-term application of three (later increased to five), pesticides on the yield of spring barley was investigated (Bromilow *et al.*, 1996). The experimental treatments were ended after 20 year; the last pesticide applications being given in September 1992 and March 1993. This experiment, therefore, affords a unique opportunity to assess the effects on soil micro-organisms of long-term repeated applications of several pesticides in the field, in a statistically-balanced experimental design.

Measurements of soil microbial biomass have been shown to give early indications of long-term changes in soil organic matter content, long before such changes could be measured by conventional techniques against the background amounts of organic matter already present (Powlson *et al.*, 1987; Saffigna *et al.*, 1989). Here we report the effects of up to 19 years of pesticide application on the size of the soil microbial biomass and its activity,

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measured by the rates of mineralisation of soil organic C and N, in laboratory-based incubations.

MATERIALS AND METHODS

Experimental design

The Chemical Reference Plots were designed as a fully randomized, single-replicate 2^5 factorial experiment. Each of the 32 plots measured 4.57 × 4.06 m, separated by 1.1 and 2.4 m wide paths along the long and short edges, respectively. The pesticides applied were benomyl (methyl 1-(butylcarbamoyl)benzimidazol-2-ylcarbamate) — a benzimidazole fungicide; chlorfenvinphos (2-chloro-1-(2,4-dichlorophenyl)vinyl diethyl phosphate) — an organophosphorus insecticide; and aldicarb (2-methyl-2-(methylthio)propionaldehyde *O*-methylcarbamoyloxime) — a carbamoyloxime insecticide-nematicide. Two other pesticides were later incorporated into the experiment: glyphosate (*N*-(phosphonomethyl)glycine) — a phosphonic acid herbicide, in 1980; and triadimefon (1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butanone) — a conazole fungicide, in 1982. The chemicals were added in the following formulations in 1992: benomyl, Benlate wettable powder [50% active ingredient (a.i.)]; chlorfenvinphos, Birlane granules (10% a.i.); aldicarb, Temik granules (10% a.i.); triadimefon, Bayleton wettable powder (25% a.i.); glyphosate, Gallup soluble concentrate (36% a.i.). The pesticides were applied annually, at slightly greater than normal field application rates (see Fig. 1).

Pesticide application was split over the year: benomyl, chlorfenvinphos and aldicarb were applied to the seed bed and incorporated by rotovation each spring immediately prior to sowing, while triadimefon and glyphosate were applied in the autumn, usually both on the same day, after the barley had been harvested, and then incorporated by ploughing. All the plots also received other pesticides as standard farm practice. These consisted of late spring applications of herbicides, e.g. mecoprop or bromoxynil, to the growing crop to control broadleaved weeds. The experimental design of the plots is shown in Fig. 1, and the soil characteristics are shown in Table 1.

Sampling and preparation of soil

About 3.5 kg soil (0–10 cm depth) was collected from each plot, from within *ca.* 20–46 cm from the long edges, in the discard strips of the plots, in April 1992 and again in October 1992. Both sampling times were 4 weeks after the appropriate pesticides had been applied. The soil samples were sieved (< 2 mm) and discrete pieces of organic matter (roots, crop residues, insects *etc.*) removed, over about 5 weeks, during which time the soils not undergoing processing were stored at 5°C. Care was taken at all times to ensure that there was no cross-

1 0 CS 0 TR GL	2 BE CS 0 0 0	3 0 CS 0 0 GL	4 BE CS AL TR GL	5 BE 0 AL TR 0	6 BE 0 0 TR GL	7 BE 0 0 0 0	8 0 0 0 TR 0
9 BE CS 0 TR GL	10 0 0 AL 0 0	11 BE 0 0 0 GL	12 BE 0 AL 0 0	13 0 CS AL 0 0	14 BE 0 AL TR GL	15 BE 0 0 TR 0	16 0 CS 0 0 0
17 BE CS 0 TR 0	18 0 CS AL TR GL	19 0 CS 0 TR 0	20 0 0 AL TR 0	21 0 0 AL TR GL	22 0 0 AL 0 GL	23 0 0 0 0 GL	24 0 0 0 0 0
25 0 0 0 TR GL	26 BE 0 AL 0 GL	27 BE CS AL TR 0	28 BE CS AL 0 0	29 BE CS AL 0 GL	30 0 CS AL 0 GL	31 0 CS AL TR 0	32 BE CS 0 0 GL

Fig. 1. Plan of the Chemical Reference Plots field experiment, Long Hoos V, Rothamsted Experimental Station. Treatments from 1973 (cumulative annually): fungicide to seedbed BE benomyl at 4 kg ha^{-1}; insecticide to seedbed CS chlorfenvinphos at 2 kg ha^{-1}; AL aldicarb at 6 kg ha^{-1}; fungicide in autumn TR triadimefon at 0.25 kg ha^{-1} (since 1982 only); herbicide to stubble GL glyphosate at 1.5 kg ha^{-1} (since 1980 only); basal manuring — Nitram *ca.* 435 kg ha^{-1}; crop — spring barley.

Table 1. Characteristics of the spring- and autumn-sampled soil

	Spring	Autumn
pH (H_2O)	6.9	6.9
pH 10 mM $CaCl_2$	6.4	6.3
CEC (meq 100 g^{-1}	14.9	15.1
Organic C (%)	1.36	1.36
Total N (%)	0.143	0.145
Sand (%)	20	
Silt (%)	61	
Clay (%)	19	
Texture	Silty clay loam (Batcombe series)	

contamination of soil between plots. Once prepared, the soil samples were stored at 5°C until use.

For the incubation experiment using the spring samples, 500 g soil was removed from each plot sample, after 10 weeks storage at 5°C, adjusted to 50% of full water-holding capacity (WHC), and equilibrated for 7 days at 25°C over water and soda lime in air-tight metal bins before use. Samples were then removed for microbial biomass analyses, and also weighed out for the incubation experiment.

For the incubation experiment using the autumn samples, the soils were adjusted to 50% WHC immediately after the sieving was completed, and equilibrated as above for 11 days. Samples were then removed for microbial biomass measurements and the incubation experiment, as before.

In both incubation experiments, triplicate soil samples from each plot were incubated in the dark at 25°C, and CO_2 evolution determined after 3, 7, 14, 21, 28, 35, 42, 49 and 56 days, as described by Chander and Brookes (1991). Soil NH_4^+ and NO_3^-, in 0.5 M K_2SO_4 extracts taken after 0 and 56 days incubation, were determined by automatic micro-continous flow analysis (Alpkem, Oregon).

Microbial biomass measurements

Soil microbial biomass C was measured by the fumigation-extraction method (Vance *et al.*, 1987; Wu *et al.*, 1990). Microbial biomass activity was also measured by the substrate-induced respiration (SIR) method of Anderson and Domsch (1978), as modified by Qimei Lin (unpubl. PhD thesis,

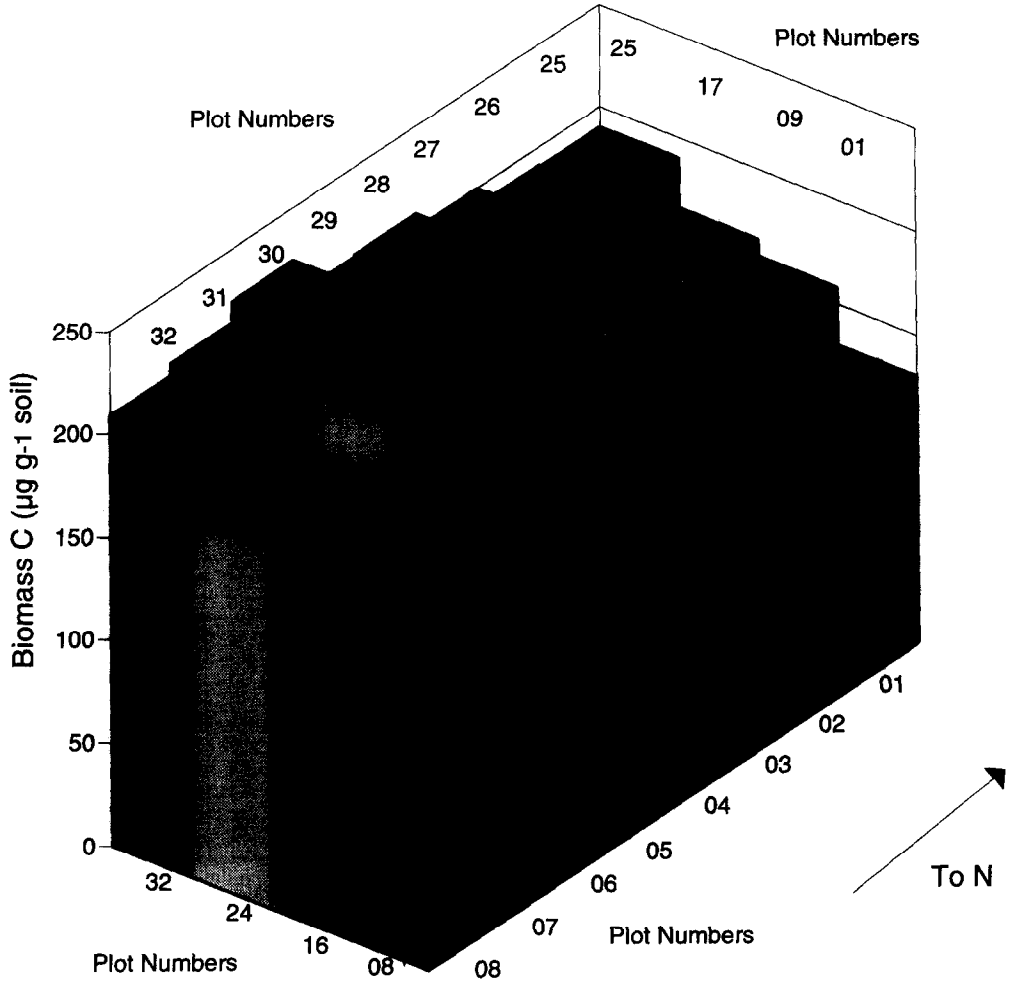

Fig. 2. Microbial biomass C contents of the spring-sampled soil of the Chemical Reference Plots (mean SD = 7.86 $\mu g\ g^{-1}$ soil). Numbers along the x- and y-axes refer to the adjacent plot (*cf.* Fig. 1).

University of Nottingham, 1994), at the beginning of the incubations of the spring- and autumn-sampled soils. Briefly, 30 g portions of moist soil were amended with a 4-to-1 mixture of talc and glucose, to give 6 mg glucose g^{-1} soil. The soils were then incubated at 25°C for 2 h, and the CO_2 concentration in the flask headspace determined by gas chromatography.

All measurements were done using triplicate soil portions and are expressed on an oven-dry soil

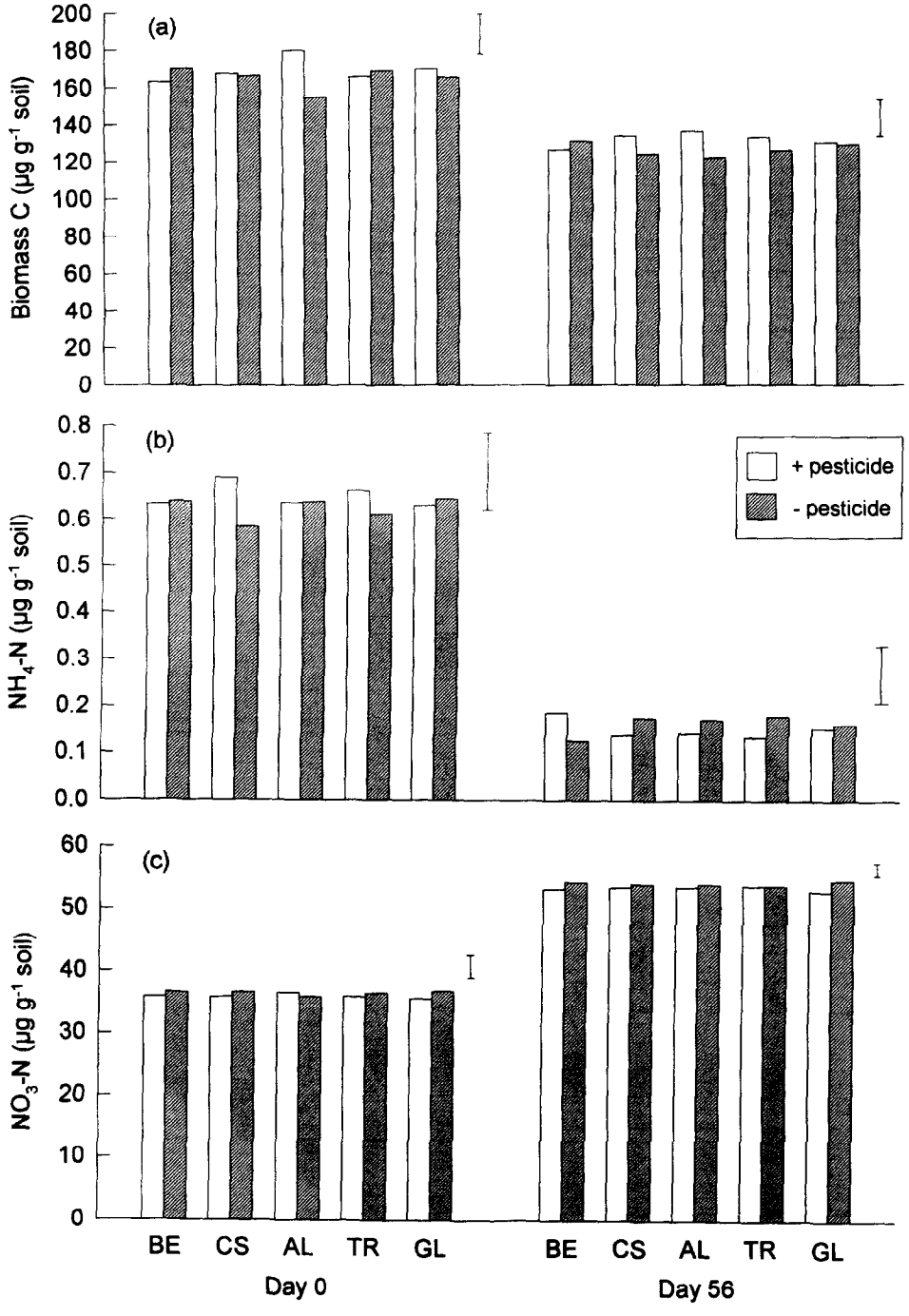

Fig. 3. (a) Microbial biomass C contents; (b) ammonium-N contents; and (c) nitrate-N contents, of benomyl (BE), chlorfenvinphos (CS), aldicarb (AL), triadimefon (TR) and glyphosate (GL)-treated spring-sampled soil after 0 and 56 days incubation. Bars = L.S.D. (P = 0.05).

basis (105°C, 24 h). Statistical analyses were carried out using Genstat 5, Release 3.1 (ANOVA) and SigmaPlot for Windows (linear regression).

RESULTS AND DISCUSSION

Incubation of spring-sampled soil

Microbial biomass. The microbial biomass C content of the soil from each plot is shown in Fig. 2. There was a gradual decrease in biomass C across the plots, from west to east, presumably related to changes in soil texture or some other characteristic. These data cannot be used to interpret the effects of the pesticide treatments, and all subsequent results will refer to data following analysis of variance.

The main effects of the pesticide treatments (i.e. the meaned effects of each pesticide measured over the different amounts of the other pesticides) on microbial biomass C in the spring-sampled soil are shown in Fig. 3 (a). The addition of aldicarb had the greatest effect, significantly increasing biomass C by about 16% ($P < 0.05$). Jones *et al.* (1991) also reported increased microbial biomass in aldicarb-treated sandy loam soils in the field, although at lower doses than were applied here. They found that the maximum increase was caused by applications of 1.4 kg ha^{-1}, while at the maximum rate of aldicarb, 4.0 kg ha^{-1}, the microbial biomass was not significantly different from the control soil. The rate of aldicarb applied to the Chemical Reference Plots, 6.0 kg ha^{-1}, was much greater than in the experiment of Jones *et al.* (1991), but still caused a significant increase in microbial biomass, perhaps because of the difference in soil texture.

There were some statistically significant ($P < 0.05$) higher-order interactions, between benomyl and chlorfenvinphos, and between triadimefon and glyphosate. The addition of benomyl in the absence of chlorfenvinphos caused a decrease in biomass C of 15.2%, from *ca.* 180 to 152 $\mu g\ g^{-1}$ soil. The addition of glyphosate in the absence of triadimefon caused an increase in biomass C of 16.2%, from *ca.* 156 to 181 $\mu g\ g^{-1}$ soil, while the addition of triadimefon in the absence of glyphosate caused an increase in biomass C of 11.3%, from *ca.* 156 to 173 $\mu g\ g^{-1}$ soil.

The main effects on biomass C after 56 days incubation are also shown in Fig. 3 (a). The effects of the pesticides lessened over the course of the incubation, and none of the main effects or higher-order interactions were significant at the 5% level. The grand mean of the microbial biomass C contents declined by 23.6% during the incubation, from *ca.* 167 to 127 $\mu g\ g^{-1}$ soil.

There were no significant differences ($P = 0.05$) between any of the pesticide treatments as measured by SIR, either as main effects or higher-order interactions (data not shown). Regression analysis of the mean SIR values of the plot samples with the corresponding mean microbial biomass C values showed a very low correlation between the two variables ($r = 0.33$). This was due to all the soil samples having very similar SIR rates, while there was a gradual increase in the soil microbial biomass content from plots 1–8 and 25–32 (Fig. 2). No attempt was made to convert the SIR rates to microbial biomass C, as calibration of the SIR method is problematic (Wardle and Parkinson, 1990).

Mineralisation of soil organic matter

There were no significant differences ($P = 0.05$) between the main effects of the pesticides on CO_2 evolution over the 56 day incubation, with one exception on day 42, when the mean CO_2 evolved from the triadimefon-treated soils was slightly higher than that evolved from the non-treated soils (Table 2). This difference was very small, however (about 8%), and essentially, the soil respiration rates were the same. There were several statistically significant ($P < 0.05$) higher-order interactions between the various treatments during the incubation, for differences of about 15% (±6) on average, but these were sporadic and inconsistent, and were probably of no consequence.

The main effects of the pesticides on ammonium- and nitrate-N dynamics over the 56 day incubation are shown in Fig. 3 (b) and (c), respectively. There were no significant differences between the soil NH_4-N contents of any of the treatments, nor were any of the 2- or 3-factor interactions statistically

Table 2. Cumulative CO_2 evolution from the benomyl (BE), chlorfenvinphos (CS), aldicarb (AL), triadimefon (TR) and glyphosate (GL)-treated spring-sampled soil

Day	CO_2-C evolved from soil (pesticide main effects) ($\mu g\ g^{-1}$ soil)										L.S.D. ($P = 0.05$)
	+BE	−BE	+CS	−CS	+AL	−AL	+TR	−TR	+GL	−GL	
3	14.9	15.3	15.4	14.8	15.7	14.5	15.4	14.8	15.0	15.2	1.43
7	29.9	30.3	30.7	29.4	30.5	29.6	30.6	29.6	29.9	30.2	1.04
14	51.5	52.5	52.7	51.3	52.3	51.8	52.9	51.1	51.5	52.5	1.98
21	70.2	71.9	71.5	70.7	71.1	71.0	72.1	70.1	70.2	71.9	1.65
28	86.4	88.0	87.2	87.2	86.9	87.5	88.6	85.8	85.9	88.5	1.53
35	102.7	105.1	103.3	104.4	103.1	104.6	105.3	102.4	101.8	105.9	1.55
42	117.7	120.4	118.4	119.6	118.3	119.8	121.1	116.9	116.7	121.4	1.24
49	132.0	135.0	132.9	134.2	132.5	134.5	135.9	131.1	131.1	135.9	1.65
56	144.4	147.5	145.3	146.7	145.1	146.9	148.5	143.5	143.7	148.3	1.05

significant ($P = 0.05$), at day 0 or day 56. There were also no significant differences in the soil NO_3-N contents of any of the treatments at day 0. However, at day 56 there were a number of significant ($P < 0.05$) higher-order interactions, between benomyl and aldicarb; between benomyl, triadimefon and glyphosate; and between chlorfenvinphos, triadimefon and glyphosate. These interactions involved differences of around 10%, but were probably not of any consequence.

Thus, none of the pesticide treatments affected the rates of ammonification of soil organic N, or nitrification of the ammonium produced. The grand mean NH_4-N content of the soils decreased by

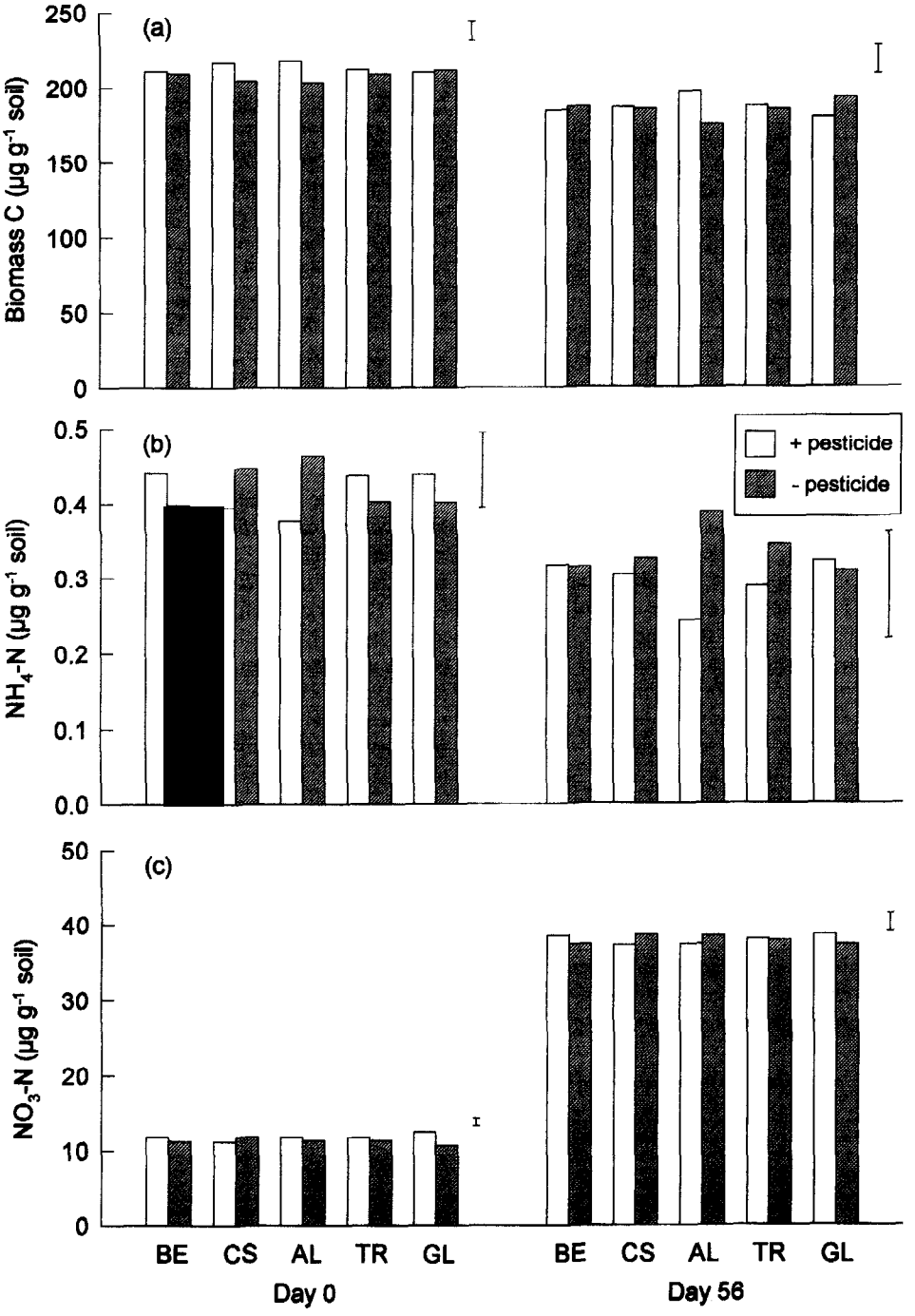

Fig. 4. (a) Microbial biomass C contents; (b) ammonium-N contents, and (c) nitrate-N contents, of benomyl (BE), chlorfenvinphos (CS), aldicarb (AL), triadimefon (TR) and glyphosate (GL)-treated autumn-sampled soil after 0 and 56 days incubation. Bars = L.S.D. ($P = 0.05$).

75.5% over the 56 days incubation, from *ca.* 0.64 to 0.16 $\mu g\ g^{-1}$ soil, while the NO_3-N content increased by 48.4%, from *ca.* 36.19 to 53.72 $\mu g\ g^{-1}$ soil, over the same period.

Incubation of autumn-sampled soil

Microbial biomass. The effects of the main treatments on soil microbial biomass C contents at day 0 are shown in Fig. 4 (a). The overall mean biomass C content of the plots had increased by 47.1% compared to the initial measurements of the spring-sampled soil, from 143 to 210 $\mu g\ g^{-1}$ soil. This increase was presumably mainly caused by inputs into the soil of labile organic substrates (e.g. root exudates, sloughed cells) from the growing barley crop and, to a lesser extent, from weeds. Once again, the addition of aldicarb caused a significant increase in biomass C, although only of about 7%, while the other pesticides had no significant effect ($P = 0.05$). There was only one statistically significant ($P < 0.05$) higher-order interaction, between chlorfenvinphos, triadimefon and glyphosate. The addition of chlorfenvinphos in the presence of one and the absence of the other of the two pesticides caused an increase in biomass C of about 16% (*ca.* 33 $\mu g\ g^{-1}$ soil).

The main effects on biomass C after 56 days incubation are also shown in Fig. 4 (a). Unlike the spring-sampled soil, the effect of aldicarb was still significant at the end of the incubation ($P < 0.05$), the biomass C content of the treated soil being about 12% greater than that of the untreated soil. However, the 3-factor interaction between chlorfenvinphos, triadimefon and glyphosate was no longer significant at the 5% level at day 56. The gradual decrease in the number of higher-order interactions in the biomass C measurements over the course of the incubations, and in the autumn compared to the spring samples, indicate that there were probably no long-term effects of the combinations of the various pesticides, although there may have been some short-term ones.

The grand mean of the microbial biomass C content declined by 11.8% during the incubation, from *ca.* 210 to 186 $\mu g\ g^{-1}$ soil. This was exactly half as much as the decline in the spring-sampled soil, presumably because the autumn-sampled soil contained more labile organic matter, derived from the barley crop.

It is likely that the increase in microbial biomass C in the aldicarb-treated soil was a direct consequence of the chemical's effect on the spring barley. Between 1974 and 1983, the mean yield of barley in the aldicarb-treated soil was 5.17 t ha^{-1}, compared to 4.86 t ha^{-1} in the non-treated soil. In the second decade of the experiment, from 1984 to 1993, the mean yields were 5.21 and 5.09 t ha^{-1}, respectively (R.H. Bromilow, pers commun.). The increase in yield was presumably due to the effect of aldicarb on plant-parasitic nematodes and other soil pests, while the difference between the first and second decades was probably caused by increasing adaptation to and mineralization of the chemical by the soil microflora (Suett and Jukes, 1988; Bromilow *et al.*, 1995). There were no significant effects on crop yield caused by the other pesticides (Bromilow *et al.*, 1995).

The Rothamsted carbon turnover model (RothC-26.3) was used (Jenkinson and Coleman, 1994) to calculate how much of an increase in annual input of plant debris would be needed to produce an increase of 12% (the observed value) in the biomass contents of the aldicarb treated plots after an experimental period of 20 year. The increased input thus calculated was 14%, rather larger than the measured increase in the harvested yield, a mean of 4.9% over the 20 years. It is possible that the increase in the unharvested parts of the aldicarb-treated crop (in roots, stubble etc.) was greater than the increase in harvested yield but the inherent errors in biomass measurements makes such speculation unproductive. However, the effect of aldicarb on the soil microbial biomass does indicate that its continuous use would lead to an increase in soil organic matter content in the long term (Powlson *et al.*, 1987; Saffigna *et al.*, 1989).

In contrast to the spring-sampled soil, statistical analysis of the SIR data gave similar results to biomass C, although only the chlorfenvinphos, triadimefon and glyphosate interaction was significant at the 5% level (data not shown). The linear correlation between SIR and biomass C was also much stronger than in the spring-sampled soil ($r = 0.83$). The autumn-sampled soil was conditioned for 11 days compared to 7 for the spring-sampled soil. It may be that the latter was not long enough for the soil microflora to stabilise for the purposes of the SIR method, while the fumigation-extraction method was more robust. While the SIR results from the autumn-sampled soil were a marked improvement on the earlier incubation, the fumigation-extraction method still gave more precise results.

Mineralisation of soil organic matter

The main effects of the pesticides on CO_2 evolution over the 56 day incubation are shown in Table 3. Approximately 76% more CO_2-C was evolved overall from the autumn-sampled soil compared to that from the spring-sampled soil, presumably reflecting the larger microbial biomass and readily decomposable organic matter inputs from the crop. There were no significant differences ($P = 0.05$) between the main effects over the incubation, except for a small decrease caused by glyphosate at day 3. The decreases on day 7 and day 28 caused by glyphosate were not quite significant at this level ($P < 0.06$), but help to account for the

Table 3. Cumulative CO_2 evolution from the benomyl (BE), chlorfenvinphos (CS), aldicarb (AL), triadimefon (TR) and glyphosate (GL)-treated autumn-sampled soil

Day	CO_2-C evolved from soil (pesticide main effects) ($\mu g\ g^{-1}$ soil)										L.S.D. ($P = 0.05$)
	+BE	−BE	+CS	−CS	+AL	−AL	+TR	−TR	+GL	−GL	
3	20.4	19.8	20.4	19.9	20.2	20.1	20.1	20.2	19.2	21.1	1.59
7	46.9	45.9	46.0	46.8	46.4	46.4	46.0	46.8	44.1	48.7	2.64
14	89.6	86.4	86.4	89.7	87.6	88.5	87.5	88.6	84.0	92.1	5.78
21	122.1	120.2	118.5	123.9	120.2	122.2	120.6	121.7	115.7	126.6	4.55
28	150.1	148.2	146.2	152.1	147.8	150.5	148.6	149.7	142.5	155.8	2.48
35	181.4	179.7	176.9	184.1	178.9	182.1	180.0	181.0	173.0	188.0	3.03
42	207.4	204.7	202.7	209.5	204.1	208.1	205.2	207.0	197.9	214.3	2.43
49	233.7	229.9	228.7	234.9	229.0	234.7	231.1	232.5	223.2	240.4	2.49
56	259.0	254.2	253.0	260.2	253.3	259.9	255.7	257.5	247.7	265.5	1.36

difference between the + and − glyphosate treatments at day 56. Less CO_2-C was consistently evolved from the glyphosate-treated soil compared to the non-treated soil, by about 2–3 $\mu g\ g^{-1}$ soil at each sample time over most of the incubation. However, the somewhat large standard errors of the differences of the means meant that most of the apparent inhibitions by glyphosate were not statistically significant. There were again a number of significant ($P < 0.05$) 2- and 3-factor interactions over the incubation, although fewer than in the spring-sampled soil. These were also inconsistent and bore no relation to the higher-order interactions in the earlier incubation, and were probably of no consequence.

One slightly puzzling aspect of the CO_2 evolution in both incubation experiments, is that the statistically-significant increase in microbial biomass C caused by the addition of aldicarb was not reflected in the soil respiration rates. It may be that the measurement of CO_2 evolution was not sufficiently sensitive to detect what would have been relatively small differences between treatments, probably due to the larger errors in the CO_2 measurements compared to those of the soil microbial biomass C.

The main effects of the pesticides on NH_4- and NO_3-N dynamics over the 56 day incubation are shown in Fig. 4 (b) and (c), respectively. In contrast to the spring incubation, there was a significant decrease in the NH_4-N contents of the aldicarb-treated soil compared to the non-treated soil at day 56 ($P < 0.05$), of about 37%, although the difference in quantity was small. There was also a similar effect at day 0, although it was less marked in size and statistical significance ($P = 0.075$). This may be due to the uptake of NH_4^+ by the larger microbial biomass in the aldicarb-treated soil. This effect was not noticeable in the spring-sampled soil, but the effect of aldicarb in the earlier incubation decreased over time, which was not the case in the autumn-sampled soil. There was a statistically significant interaction between chlorfenvinphos and glyphosate at day 0 ($P < 0.05$), but this had disappeared by day 56, and was probably of no importance.

Unlike the spring-sampled soil, there was a significant effect on the NO_3-N content of the glyphosate-treated soil at day 0, which was about 18% greater than that of the non-treated soil ($P < 0.01$). There were also two significant 3-factor interactions between benomyl, chlorfenvinphos and triadimefon, and between chlorfenvinphos, aldicarb and glyphosate. None of these treatment effects were apparent at the end of the incubation.

The grand mean of the NH_4-N contents of the soils decreased by only 24% over the 56 days incubation, from 0.42 to 0.32 $\mu g\ g^{-1}$ soil, while the NO_3-N content increased by 3.278-fold from *ca.* 11.55 to 37.85 $\mu g\ g^{-1}$ soil over the same period. Thus, the total NH_4-N and NO_3-N contents were significantly less than in the spring-sampled soils, while the relative changes over the 56 days were significantly less and greater for NH_4^+ and NO_3^-, respectively, compared to the spring incubation. This was probably due to the increased microbial biomass and readily decomposable organic matter content of the autumn-sampled soil.

Overall, the application of these pesticides to the same sites, for up to 19 years, had very little effect on the microbial variables measured. The one consistent significant side-effect on the soil microbial biomass, caused by the addition of aldicarb, was actually of a beneficial nature. Although there were several statistically-significant 2- and 3-factor interactions between the pesticides, they were inconsistent over the course of the incubations and between the various variables measured, and could not be considered as real effects. Thus, it would seem that the continuous use of these pesticides at, or slightly above, the recommended rates, singly or in combination, had no long-term harmful effects on the soil microbial biomass or its activity.

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