THE UNIVERSITY OF CALGARY

THE INFLUENCE OF ENVIRONMENTAL VARIABLES AND HERBICIDE APPLICATION ON THE SOIL MICROBIAL BIOMASS

by

DAVID A. WARDLE

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled THE INFLUENCE OF ENVIRONMENTAL VARIABLES AND HERBICIDE APPLICATION ON THE SOIL MICROBIAL BIOMASS submitted by DAVID A. WARDLE in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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ABSTRACT

Equations for predicting the total soil microbial biomass were evaluated from a statistical viewpoint, and, where necessary, re-determined after correcting for dependency and non-normality of the data used. The fumigation-incubation method of microbial biomass determination depends on values of the $\underline{k}_c$-factor which is the fraction of microbial biomass carbon released within 10 days of fumigation. The three $\underline{k}_c$-values currently in use, i.e. 0.41, 0.45, and 0.50, all have a high degree of associated variability, which is influenced by variability between microbial species and estimates of bacterial:fungal ratios in soil samples. Four methods have been directly or indirectly calibrated against the fumigation-incubation technique, i.e. substrate-induced respiration (S.I.R.), ATP analysis, ninhydrin-extractable nitrogen, and fumigation-extraction. However, when problems associated with dependency and non-normality are corrected for it is uncertain as to whether or not these methods provide a realistic estimate of soil microbial biomass carbon.

Evaluation of methods used for predicting total microbial biomass carbon were performed on soil samples maintained at five different moisture levels for 4 incubation periods. These were amended with ^{14}C-labelled microbial tissue, and fumigation $\underline{k}_c$ values determined. Values of $\underline{k}_c$ were significantly affected by soil moisture content, time of preincubation, and sampling strategy (blocking effects). Microbial biomass for these samples was predicted using fumigation-incubation ($\underline{k}_c$ = 0.41), fumigation-incubation

(different $\underline{k}_c$ value for each sample and treatment), S.I.R. with and without remoistening, and oxygen uptake curves (with fumigation). Little agreement was found between any of the five approaches used, with r^2 values between pairs of methods always being below 0.50. The different approaches also often predicted different responses of the microbial biomass to the soil moisture gradient. The S.I.R. method agreed reasonably well with techniques used to measure only the active microbial biomass, and it is concluded that S.I.R. and related techniques (including selective inhibition) can be used for assessing the dynamics of the active biomass.

In a field study examining the effects of temperature and moisture on S.I.R. and basal respiration determinations, soil moisture was the principal factor involved, especially in conditions of rapid soil moisture fluctuation. Soil temperature was important only when soil moisture content was relatively constant, or when variations of soil moisture were removed using partial correlation analysis.

The response of S.I.R., basal respiration, and inhibition by selective inhibitors in soil to various herbicide applications were determined in laboratory studies and in field plots. In the laboratory study response of biotic variables occurred only at concentrations of 200 ppm glyphosate, 2,4-D, or picloram, which is probably two orders of magnitude higher than what probably occurs in field situations. In field experiments, glyphosate and 2,4-D had little effect on the biotic variables tested compared with the natural dynamics of these variables.

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CHAPTER 1

1. GENERAL INTRODUCTION

The soil microbial biomass is defined as the living microbial component of the soil organic matter, and includes bacteria, fungi, algae, protozoa, and microfauna (Sparling 1985). When the soil microbial biomass is studied, usually only those organisms at the base of the detritivore foodweb are considered, i.e. the bacterial and fungal components (e.g. Anderson and Domsch 1975; Jenkinson and Powlson 1976). In part this is because these two groups are usually responsible for over 90% of the soil respiration (Swift *et al*. 1979; Petersen and Luxton 1982).

The total living microbial biomass has been regarded as consisting of two components, one of which is "active" and readily metabolizes extracellular substrates, and the other which is "inactive" and probably does not (Van de Werf and Verstraete 1987a). The active portion of the microbial biomass is estimated to vary between about 2% and 50% of the total microbial biomass in various soils (Sparling 1985; Van de Werf and Verstraete 1987b). This estimate is based on data from a range of terrestrial ecosystems, derived from microscopical studies (Soderstrom 1979; Clarholm and Rosswall 1980; MacDonald 1980) and measurements of respiration rates (Sparling 1985; Van de Werf and Verstraete 1987b). The inactivity of most of the soil microbial biomass helps to explain its relatively low energy requirements (Jenkinson and Ladd 1981).

The microbial biomass exerts a major influence on soil carbon and nutrient cycling, through both the oxidation of soil organic matter, and the storage of carbon and mineral nutrients (Anderson and Domsch 1980; Jenkinson and Ladd 1981). It also plays an important role in controlling the turnover and mineralization of organic substrates, and serves as a source and sink for mineral nutrients (Paul and Juma 1981; Juma and Paul 1984). Because it regulates the supply of plant nutrients, the soil microbial biomass influences crop (and ecosystem) productivity (McGill et al. 1986).

1.1 METHODS FOR QUANTIFYING THE SOIL MICROBIAL BIOMASS

Quantification of the soil microbial biomass is performed by estimating the content of biomass carbon (for total microbial biomass) in soil samples, and expressing this as a concentration per unit soil weight. The most popular units have been mg biomass C•100 g soil^{-1} (e.g. Jenkinson and Powlson 1976; Anderson and Domsch 1978b) and μg biomass C•g soil^{-1} (e.g. Jenkinson et al. 1979; West et al. 1986).

The earliest methods for quantifying the soil microbial biomass, and those which are most direct, involve microscopical determination of biovolume, and converting this to microbial biomass carbon (Jenkinson and Ladd 1981). However, estimating microbial biomass using direct microscopy involves making numerous assumptions regarding dispersion of microbial tissue, staining efficiency, cell shrinkage, cell density, moisture content, and carbon content (see Jenkinson et al. 1976; Jenkinson and Ladd 1981; Sparling 1985).

Estimating microbial biomass using this technique is also tedious (Martens 1987) making it unsuitable for processing large numbers of samples. Therefore, interest has focussed upon developing and improving other methods that can be used for measuring the size of the soil microbial biomass.

The fumigation-incubation technique (Jenkinson 1966; Jenkinson and Powlson 1976) was developed as an effective and efficient method for quantifying microbial biomass. This method involves measuring the CO_2-C flush from killed microorganisms in soil samples treated with $CHCl_3$ (corrected for basal respiration): the biomass is then estimated by dividing this flush by a $\underline{k}_c$-factor, representing the fraction of killed microbial biomass carbon released as CO_2, usually over ten days. This $\underline{k}_c$-factor is usually determined by measuring the percentage of microbial carbon or carbon-14 in pre-cultured microorganisms released as CO_2 following chloroform fumigation and incubation. Usually the value of $\underline{k}_c$ has been assumed not to vary regardless of soil type and experimental treatment.

The fumigation technique requires ten days of incubation in order to measure the total CO_2-flush and this has led to the development of other methods which provide more rapid biomass estimates. These methods are typically calibrated against fumigation-incubation microbial biomass measurements derived for a number of soil samples, by the means of a regression equation.

The first of these methods is substrate-induced respiration, where the microbial biomass is predicted from the initial microbial response to glucose mixed throughout the soil sample (Anderson and Domsch 1978b). Modifications of the method have been proposed by West and Sparling (1986), West et al. (1986) and Martens (1987). Three biochemical methods for predicting total microbial biomass carbon also have been developed. Two of these have been calibrated against fumigation-incubation biomass estimates, i.e. ATP content (Oades and Jenkinson 1979, Jenkinson et al. 1979) and ninhydrin-extractable nitrogen (Amato and Ladd 1988). The third approach, i.e. the "fumigation-extraction method", involves killing the microbial biomass with $CHCl_3$, followed by extraction and subsequent measurement of carbon in the sample. Attempts have been made to calibrate this method against the fumigation-incubation method (Vance et al. 1987b; Tate et al. 1988), new k_{EC}-factors from cultured or labelled organisms (Tate et al. 1988; Sparling and West 1988a), and substrate-induced respiration (Sparling and West 1988a,b).

While recent emphasis has focused on physiological and biochemical techniques for measuring the total microbial biomass, there has been less interest in measuring the active microbial biomass. The only currently available physiological technique for achieving this is the oxygen uptake curve method of Van de Werf and Verstraete (1987a,b). This method involves adding glucose and minerals to soil samples, and observing the subsequent physiological responses of the microflora. It is assumed that the active

microbial biomass can initiate growth immediately upon glucose addition, while the inactive component cannot.

1.2 INFLUENCE OF ENVIRONMENTAL AND MANAGEMENT FACTORS ON THE SOIL MICROBIAL BIOMASS

The magnitude of the soil microbial biomass appears to be often influenced primarily by soil moisture. Seasonal changes in soil moisture levels are frequently positively correlated with shifts in microbial biomass content (e.g. Clarholm and Rosswall 1980; McGill et al. 1986). Remoistening of soil samples, therefore, usually encourages increments in microbial biomass (Schnürer et al. 1986) unless this is associated with increased compaction (Ross 1987). There is also evidence that a fraction of the soil microbial biomass is killed by drying (Bottner 1985; West et al. 1987). Soil respiratory activity is usually substantially reduced by drier soil conditions, and enhancement of microbial activity due to rewetting of dry soils is well-known (e.g. Adu and Oades 1978; Orchard and Cook 1983). This enhanced respiration due to remoistening is probably due in part to the decomposition of microorganisms killed by earlier drying of the soil (Shields et al. 1974). Soil temperature also can exert an important (though lesser) effect on soil microbial biomass (e.g. McGill et al. 1986) and freeze-thaw cycles appear to affect activity of the microbial biomass (Mathes and Schrifer 1986; Taylor and Parkinson 1988b).

The microbial biomass is often influenced by management factors, and this has been studied most extensively in

agroecosystems. Tillage can reduce microbial (especially fungal) biomass (Sparling 1985; Hendrix et al. 1986; Doran 1987), and direct drilling in the top 5 cm of the soil profile can have similar effects (Lynch and Panting 1980). Cropping sequences also can affect biomass levels (Biederbeck et al. 1984), because of the influences that the quantity and quality of carbon entering the soil system can exert on the soil microbial biomass (Holland and Coleman 1987; Insam et al. 1989). Shifts in the size of the microbial biomass may also be initiated by application of plant residues (Powlson et al. 1987) or fertilizers (Sparling 1985; Curry 1986).

Of particular interest in the present study is the influence of herbicide applications on the soil microbial biomass. In agricultural systems, weeds often pose a major threat to crop yields, and herbicides are the largest class of pesticides used (Somerville 1987). Therefore, there has been considerable interest in the side effects of these chemicals on non-target organisms, including soil microorganisms (See Simon-Sylvestre and Fournier 1979; Greaves and Malkomes 1980). The majority of studies on this subject have focussed on herbicide effects on microbially mediated processes, e.g. nitrification, denitrification, soil respiration, and soil enzyme activity (e.g. Yeomans and Bremner 1987; Helweg 1986; Carlisle and Trevors 1986). However, the effects of herbicides on the size of soil microbial biomass has received little attention. Soulas et al. (1984) found that 2,4-D at levels of 3 - 4 ppm had little effect on the soil microbial biomass. Duah-Yentumi and Johnson (1986) observed little effect of MCPA or simazine on the

microbial biomass, although repeated paraquat applications significantly reduced microbial (probably mostly fungal) biomass. No other studies appear to have investigated herbicide effects on the soil microbial biomass.

1.3 OBJECTIVES

The present study is concerned in part with investigating problems associated with various methods used for quantifying the soil microbial biomass. The influence of certain environmental variables and herbicide application on various measures of microbial biomass size and activity was also investigated on an agricultural field in south-central Alberta, Canada, located 90 km northwest of Calgary. Soil and site details are given in Chapter 2.

A number of physiological and biochemical methods have been developed for quantifying the total soil microbial biomass and Chapter 3 reviews these techniques.

Chapter 4 is concerned with comparing different physiological methods for measuring the active and total soil microbial biomass. This involved artificially adjusting soil samples to a variety of soil moisture levels and determining the microbial biomass for the same samples using a variety of approaches.

The influence of climatic variables on the size and activity of the microbial biomass is considered in Chapter 5. This section presents the results of a field study where soil moisture, temperature, and microbial biomass and activity were monitored through the growing seasons of 1987 and 1988.

The influence of three post-emergence herbicides (picloram, 2,4-D, and glyphosate) on microbial biomass and activity were investigated using soil samples incubated at constant soil moisture and temperature in laboratory conditions. The results are presented and discussed in Chapter 6.

Chapter 7 describes experiments conducted during the growing seasons of 1987 and 1988, to investigate the influence of 2,4-D and glyphosate on soil microbial biomass and activity. The 1987 study investigated the influence of these herbicides on the microbial biomass, compared with the effects of spatial and temporal variation in natural factors. The 1988 study was concerned with detecting whether microbial biomass dynamics and activity were influenced by these herbicides in the presence vs. absence of crop plants and weed flora.

The significance of the results of these investigations are considered in Chapter 8, with regard to reliability of different methods for measuring soil microbial biomass, and whether environmental variables and herbicides exert a significant effect on microbial biomass dynamics.

CHAPTER 2

2. SITE AND SOIL DESCRIPTION

2.1 SITE DESCRIPTION

The site used for the field investigations, and for the collection of soil for the laboratory incubation experiments, is located in south-central Alberta, about 90 km north-northwest of Calgary (Figure 1). The grid-coordinates of the site are 51°30'N, 114°30'W.

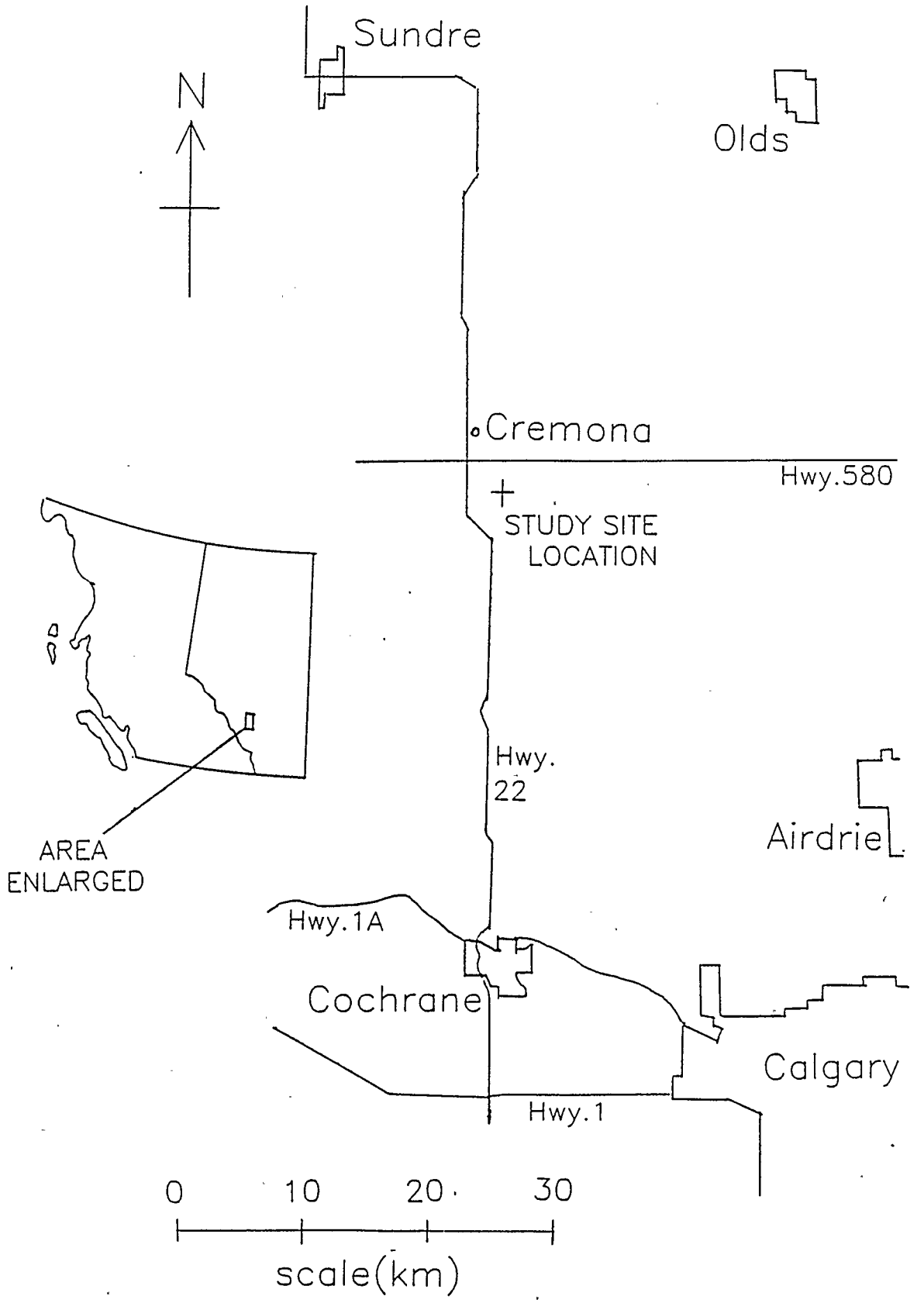
The area has been farmed for approximately the last 100 years, prior to which it was forested. For the five years prior to the experiment it had been under hay pasture (timothy, brome grass and alfalfa).

The nearest climatic stations to the site are located at Cochrane (approximately 40 km south) and Olds (approximately 40 km northeast). Data for the stations for the two seasons during which field work was conducted (1987 and 1988), and the mean of the years 1951 - 1980, are shown in Table 1. Both the 1987 and 1988 seasons were drier than average in spring-early summer, and moister than average during late summer. The district was subjected to a near-record drought in spring 1988, due in part to low snow cover the previous winter (Scholefield and Harvey 1988).

Table 1. Climatic data for Olds and Cochrane for 1988, 1987, and mean of 1951 - 1980.

	1988			1987			1951-1980 average		
	Cochrane	Olds		Cochrane	Olds		Cochrane	Olds	
	Total pptn (mm)	Total pptn (mm)	Mean temp (°C)	Total pptn (mm)	Total pptn (mm)	Mean temp (°C)	Total pptn (mm)	Total pptn (mm)	Mean temp (°C)
Jan	4.0	5.5	-10.1	5.4	6.0	-2.1	27.1	22.1	-13.2
Feb	6.0	9.5	-7.1	2.8	9.1	-2.6	26.4	16.0	-8.5
Mar	22.4	52.3	0.1	20.6	31.0	-3.5	28.7	20.1	-5.3
Apr	11.6	13.4	5.5	19.8	32.1	6.7	48.3	32.7	2.9
May	19.2	10.4	11.8	12.6	13.5	10.9	54.0	55.9	9.3
Jun	79.8	108.9	15.1	36.4	46.0	15.8	87.4	91.5	13.2
Jul	64.0	136.1	15.1	164.0	77.1	15.2	63.1	75.3	15.7
Aug	113.2	72.0	14.4	71.4	93.8	11.8	49.7	70.8	14.7
Sept	43.0	70.8	9.7	28.0	33.8	12.4	42.5	41.7	10.2
Oct	9.8	3.0	6.7	1.0	11.8	5.7	22.4	20.3	5.1
Nov	4.4	3.9	-2.1	4.2	17.5	0.4	20.0	19.0	-3.4
Dec	9.8	22.9	-5.5	7.0	6.4	-3.4	25.2	22.6	-8.8
Whole year	387.6	508.7	4.5	373.2	378.0	5.6	494.8	488.4	2.7

Figure 1. Location of the study site.


Sundre
N
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STUDY SITE
LOCATION
AREA
ENLARGED
Hwy.
22
Airdrie
Hwy.1A
Cochrane
Calgary
Hwy.1
0
10
20
30
scale(km)

2.2 SOIL PHYSICAL AND CHEMICAL DESCRIPTION

The soil used in the present study is a Rego Dark Brown Chernozem (Canada Soil Survey Committee 1978). The A_h horizon is 75 cm deep, and this is followed by a C horizon.

The physical and chemical properties of this soil are given in Table 2. Determinations of these properties were made as follows:

Organic matter content was determined on five replicate oven-dry samples by loss on ignition at 405°C for 24 hours (Nelson and Sommers 1982).

Organic carbon content was determined using a LECO high temperature induction furnace (Nelson and Sommers 1982) on 20 replicate samples.

Soil pH was determined by mixing soil and deionized water at a 1:2 ratio, and leaving the mixture to stand for 30 minutes before measurement. This was performed on 20 replicate samples.

The concentrations of ammonium, nitrate, and phosphate ions were determined in 20 soil samples using a Technicon autoanalyzer following extraction with 1M HCl (for NH_4^+, NO_3^-) or modified Bray solution (PO_4^{3-}) (Technicon Instruments 1976; 1977; 1978).

The soil moisture content corresponding to a matric potential of 33 kPa (= field capacity) was determined for five replicate samples using the pressure-plate method (Cassel and Nielsen 1986; Klute 1986).

Particle-size analysis was performed on four replicate samples using a hydrometer, after first removing organic matter with H_2O_2 (Gee and Bauder 1986).

2.3 SOIL BIOLOGICAL DESCRIPTION

Four discrete soil samples were collected from the study area in September - October 1986 to enable estimation of bacterial, actinomycete, and fungal propagule numbers, and for determination of the principal fungal species in the soil.

Determination of propagule numbers was performed by making a dilution series of 10^{-4}, 10^{-5}, 10^{-6}, and 10^{-7} from 1 g soil in 0.1% peptone solution. For bacterial counts 0.1 ml of each of the 10^{-5}, 10^{-6}, and 10^{-7} dilutions were added to each of 5 nutrient agar spread plates. Dilutions of 10^{-4}, 10^{-5}, and 10^{-6} (0.1 ml) were added to 5 casein agar spread plates for actinomycete counts (Küster and Williams 1964) and 5 malt extract agar spread plates for fungal counts. The densities of these propagules are shown in Table 3.

Assessments of the principal fungal species were performed by mixing 49 g (d.w.) soil with 250 ml water, and separating floating organic fragments and sedimented mineral fragments. Each organic or mineral sample was given 30 sequential 3-minute washes in an apparatus designed to remove microbial propagules from the substrate surface (Bissett and Widden 1972). Forty mineral and 40 organic particles (each 0.5 mm diameter) were placed on malt extract agar plates with 2 particles per plate. Fungal species emerging from each particle were isolated and sorted into taxa. Percentage frequency of each species was determined as the fraction of the total number of particles (i.e. 40) plated for each sample containing that species. Frequencies of the principal fungal species are shown in Table 4.

Table 2 Chemical and physical properties of the soil present in the study site.

	Mean	S.D.
Organic matter content	16.2%	0.66%
Organic carbon content	7.05%	0.85%
pH	5.86	0.20
NH_4^+ -N	13.0 ppm	2.1 ppm
NO_3^- -N	29.2 ppm	11.3 ppm
PO_4^{3-}-P	24.7 ppm	11.6 ppm
soil H_2O at -33 kPa (d.w. basis)	54.6%	3.6%
sand content	52%	1.5%
silt content	30%	1.5%
clay content	18%	0.1%

Table 3. Densities of propagules of bacteria, actinomycetes, and fungi in soil samples from the study site.

	Mean	S.D.
Bacteria (x 10^8 g soil^{-1})	2.15	0.96
Actinomycetes (x 10^6 g soil^{-1})	1.45	0.42
Fungi (x 10^5 g soil^{-1})	8.50	0.10

Table 4. Percentage of plated particles colonized by the principal fungal species isolated in the study site.

Species	Organic particles Mean	Organic particles SD	Mineral particles Mean	Mineral particles SD
Mucor hiemalis Wehmer	17.5	5.0	1.9	2.4
Mortierella alpina Peyronel	48.8	12.5	90.6	1.3
Trichoderma harzianum Rifai	26.9	8.0	55.0	3.5
Arthrinium sphaerospermum (Corda) M.B. Ellis	31.9	8.5	1.9	1.3
Cladosporium cladosporioides (Fres) de Vries	15.0	5.8	0.0	0.0
Fusarium oxysporum Schlecht.	13.8	9.2	8.2	8.0
Penicillium nigricans Bain. ex Thom	13.8	7.2	3.8	3.2
Rhizopus stolonifer (Enrehb. ex Link)	0.6	1.3	7.5	5.4

Total numbers of microarthropods, nematodes, and earthworms were estimated in four hand-weeded plots at each of four sampling times during 1987. Microarthropod numbers were estimated in each of two samples collected from each plot. This was performed using a Macfadyan high gradient extractor (Edwards and Fletcher 1971) in which 20 - 30 g soil samples were simultaneously cooled by water from beneath and heated with a wire heating filament from above; the temperature gradient across these samples was progressively increased over a five day period. Total numbers of mites and Collembola collected from these samples were determined using a stereo-microscope. Nematode numbers were estimated using a Cobb sieving technique (van Gundy 1982) on two samples collected from each plot. Unsieved soil samples (500 g) were mixed with water and progressively sieved with a 125 µm sieve, 53 µm sieve, and twice with a 37 µm sieve. These sieves were backwashed and total nematode numbers in the backwashings determined. Total earthworm numbers were assessed in 0.5 x 0.5 m quadrats (two quadrats per plot) after two applications of 0.275% formaldehyde solution per quadrat added 10 minutes apart (Satchell 1971a). The total number of earthworms which rose to the surface in each plot were counted. Data for these faunal groups for each sampling period are shown in Table 5.

Table 5. Densities of the principal soil faunal groups in the study site.

Date	Collembola No. ($\times 10^4$ m^{-2})[1]		Acari No. ($\times 10^2$ m^{-2})[1]		Nematode No. ($\times 10^4$ m^{-2})[2]		Earthworm No. (m^{-2})	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
June 1	1.09	0.37	12.98	8.33	10.1	5.6	7.5	1.9
June 5	2.10	1.75	5.66	6.33	8.4	3.6	4.5	1.0
June 15	3.27	2.86	5.00	4.32	7.1	2.4	5.0	2.6
July 15	2.78	2.23	3.33	6.66	12.9	4.0	5.0	2.0

[1] Depth = 3 cm only

[2] Depth = 10 cm only

CHAPTER 3

3. A STATISTICAL EVALUATION OF EQUATIONS FOR QUANTIFYING SOIL MICROBIAL BIOMASS

3.1 INTRODUCTION

Following the development of the fumigation-incubation method (Jenkinson and Powlson 1976), a number of methods have been developed to enable more rapid determination of the soil microbial biomass. However, all these methods have been calibrated, directly or indirectly, against the fumigation-incubation method. These calibration procedures typically involve collecting numerous soil samples, measuring microbial biomass carbon using the fumigation method (or another "standardized" method), and the method under study. Bivariate regression or correlation analysis is then used for calibrating the method and providing a measure of the strength of calibration.

The fumigation-incubation method involves determining total microbial biomass carbon according to the following relationship:

$$\text{biomass C} = \frac{CO_2(f) - CO_2(nf)}{\underline{k}_C}$$

where: $CO_2(f)$ and $CO_2(nf)$ = total CO_2-C released for the 10 days following fumigation in fumigated and non-fumigated ("control") soils respectively.

$\underline{k}_c$ = an experimentally determined parameter representing the fraction of biomass C released as CO_2-C for 10 days following fumigation.

Because other physiological and biochemical methods have been calibrated against the fumigation-incubation method, estimation of the value of $\underline{k}_c$ will influence the calibration equations derived for these methods.

The objective of this chapter was to consider critically those equations which have been developed to predict indirectly total microbial biomass carbon from physiological and biochemical information. This involves assessing variability associated with estimates of the parameters used in these equations and determining the suitability of these equations for predicting the size of the microbial biomass. Where violations of statistical assumptions required for the derivation of these equations had occurred, these have been corrected for and new equations have been derived.

3.2 THE $\underline{k}_c$-FACTOR IN THE FUMIGATION-INCUBATION TECHNIQUE

When fumigation-incubation has been employed to measure microbial biomass, the $\underline{k}_c$-factor has been assumed to be invariant across the range of soils and/or treatments used. Typical treatments have included pesticide application (e.g., Duah-Yentumi and Johnson 1986); soil moisture content and rewetting (e.g., Kieft et al. 1987); and the effect of straw incorporation (e.g., Powlson

et al. 1987). However, studies have investigated $\underline{k}_c$-values over a range of conditions, e.g., Vance et al. (1987a) with regard to pH (where very low pH values significantly depressed the $\underline{k}_c$-value) and Ross (1987) with regard to soil moisture (where wet compacted samples had reduced $\underline{k}_c$ values). In experiments where soils have been subjected to a number of treatments, it may be unrealistic to assume that $\underline{k}_c$ values remain invariant, and if a treatment has an observed effect on microbial biomass, then it is likely that decomposition rates of dead microorganisms could also be affected.

$\underline{k}_c$-factors currently in use

Three separate $\underline{k}_c$-factors are commonly in use: 0.50 (e.g. Jenkinson 1976; Sorenson 1987; Bottner 1985); 0.41 (e.g., Anderson and Domsch 1978a; Robertson et al. 1988; Kieft et al. 1987), and 0.45 (e.g., Oades and Jenkinson 1979; Merckx and Martin 1987; Powlson et al. 1987).

(a) $\underline{k}_c$ = 0.50. This figure was first proposed by Jenkinson (1976), based on the decomposition of four fungi (including two yeasts), eight bacteria (including one actinomycete), and one earthworm species, over a 10-day incubation period. All 13 species were given equal weight. This results in twice the weighting being given to bacteria as a group than to fungi. Because fungi appear to be the dominant biomass component in most soils (Satchell 1971b; Shields et al. 1973; Parkinson 1973; Anderson and Domsch 1975) a $\underline{k}_c$-factor heavily weighted in the favour of bacteria is probably

inappropriate. The percentage of fungal carbon released (average of 4 species) was 53.4%; that for bacteria was 48.1%. By varying the fungal:bacterial ratio in the total microbial biomass and by providing a measure of variability (determined using the approach outlined in Snedecor and Cochran 1980: pp. 96-98), the possible range of k_c-factors that may be derived can be determined (Table 6). Appropriate values of k_c determined from this data set could range from below 0.49 to above 0.57.

One species in this data set, Nitrosomonas europaea, had an anomalously low k_c value. This species normally also forms a very small component of the overall bacterial biomass. Recalculation of the overall k_c-factor, with N. europaea excluded from the data set, results in the k_c-factor being elevated slightly although this elevation is not statistically significant at $p = 0.05$ (Table 6). On the basis of a 75:25 fungal:bacterial ratio as proposed by Anderson and Domsch (1975; 1978a), and exclusion of N. europaea, a k_c-factor (from the data of Jenkinson (1976) alone) of 0.53 could be recommended.

(b) $k_c = 0.41$. This value was first proposed by Anderson and Domsch (1978a) and is based on the decomposition of 16 fungal and 12 bacterial species, averaged over four soils. Also the bacterial: fungal k_c-factors were weighted with a ratio of 1:3. However, bacterial:fungal ratios may vary both among treatments and among soils. Also the ratio of 1:3 is derived from bacterial:fungal ratio data using the selective inhibition technique (Anderson and

Table 6. Values for k_c, derived from the data of Jenkinson (1976), corrected for varying bacterial:fungal ratios.

Bacterial:Fungal Ratio	Including *N. europaea*		Excluding *N. europaea*	
	k_c	95% C.I.	k_c	95% C.I.
50:50	.508	[.485, .531]	.523	[.499, .547]
40:60	.513	[.485, .541]	.525	[.496, .554]
30:70	.518	[.482, .554]	.527	[.491, .563]
25:75	.521	[.483, .559]	.528	[.490, .566]
20:80	.523	[.383, .563]	.529	[.489, .569]
10:90	.528	[.484, .572]	.532	[.488, .576]

Domsch 1973; 1975). While selective inhibition (using protein-synthesis inhibitors) provides potentially reproducible results (Domsch et al. 1979) it may not necessarily give an accurate indication of the overall bacterial:fungal ratio. This is because a substantial component of the soil respiratory activity appears not to be influenced by the inhibitors used (West 1986), presumably due to activity of microorganisms that are not undergoing growth at the time of measurement.

In the present study $\underline{k}_c$ values were determined using data for each of the four soils provided by Anderson and Domsch (1978a), and the average of all four soils, for a number of different bacterial:fungal ratios (Table 7). The $\underline{k}_c$-factors for soils I, II and III are not significantly different from each other for most of the given bacterial: fungal ratios while the $\underline{k}_c$-factor for soil IV is significantly lower than all the others for all such ratios. Also, the values for each bacterial: fungal ratio in Table 7 are significantly lower than those of Jenkinson (1976) given in Table 6. When the data from all four soils (Table 7) are considered across the range of bacterial: fungal ratios and the variability in the data and the uncertainty in determining bacterial: fungal ratios are both considered, the appropriate $\underline{k}_c$-factor could be less than 0.33 or over 0.47. However, if one is prepared to assume a bacterial:fungal ratio of 1:3, a $\underline{k}_c$-factor of 0.41 is still probably the most appropriate.

Table 7. Values for $\underline{k}_c$, derived from the data of Anderson and Domsch (1978a), corrected for varying bacterial: fungal ratios, for each of the four soils used and the mean of all four soils.

Bacterial:Fungal Ratio	Soil I $\underline{k}_c$	Soil I 95% C.I.	Soil II $\underline{k}_c$	Soil II 95% C.I.	Soil III $\underline{k}_c$	Soil III 95% C.I.	Soil IV $\underline{k}_c$	Soil IV 95% C.I.	All Soils $\underline{k}_c$	All Soils 95% C.I.
50:50	.414	[.398, .430]	.404	[.386, .422]	.390	[.369, .411]	.330	[.308, .352]	.385	[.325, .445]
40:60	.421	[.405, .437]	.415	[.398, .432]	.402	[.382, .422]	.343	[.321, .365]	.395	[.339, .452]
30:70	.428	[.412, .444]	.425	[.407, .443]	.413	[.392, .434]	.356	[.333, .379]	.406	[.352, .459]
25:75	.431	[.414, .448]	.430	[.411, .449]	.418	[.396, .440]	.363	[.334, .387]	.411	[.359, .462]
20:80	.435	[.417, .453]	.435	[.415, .455]	.424	[.401, .447]	.370	[.345, .395]	.416	[.367, .465]
10:90	.442	[.423, .461]	.445	[.423, .467]	.435	[.410, .460]	.383	[.356, .410]	.426	[.380, .472]

(c) $\underline{k}_c$ = 0.45. This was first introduced by Oades and Jenkinson (1979) and was presumably intended to correct for Anderson and Domsch's (1978a) $\underline{k}_c$-factor of 0.41 for use at 25°C rather than at 22°C. While 0.45 has been recommended as a suitable compromise by other workers (Jenkinson and Ladd 1981; Sparling 1985) the justification for this value remains unclear. It is uncertain as to whether this figure is intended as a compromise between Anderson and Domsch's $\underline{k}_c$ value and that of Jenkinson (1976) adjusted for 25°C, or whether it is assumed (albeit untested) that a 3°C temperature rise would cause $\underline{k}_c$ to increase by nearly 10% during 10 days of incubation.

Variability of $\underline{k}_c$-factors.

The use of a constant $\underline{k}_c$-factor is based on the assumption that decomposition of microbial biomass does not vary between soil types. The small amount of existing evidence suggests that this is incorrect. As outlined earlier, recalculation of the data of Anderson and Domsch (1978a) suggests variability of $\underline{k}_c$ among soils. Nicolardot et al. (1984), using five different microbial species and four different soils, also found that the $\underline{k}_c$ value varied significantly among the soils used, and concluded that the major part of the error associated with microbial biomass determinations arose from the $\underline{k}_c$-factor determination. Values of $\underline{k}_c$ have been determined for only a very narrow range of soil groups and conditions. None of the soils used for determining the three

commonly used k_c values had over 4% organic C, and all but one were arable soils.

For some soil types (e.g. those which are similar to the soils used by Anderson and Domsch 1978a) a k_c value of 0.41 for incubations at 22°C is probably the most appropriate because Anderson and Domsch (1978a) provided the most complete data set, enabling the most realistic statistical assessment. Two smaller studies have also determined k_c values close to 0.41, although this is probably fortuitous (i.e. Nicolardot et al. 1984; and the *in vivo* study of Voroney and Paul 1984).

Oxygen uptake curves and the f_{5d}-factor

Another related method for determining total microbial biomass was developed by Van de Werf and Verstraete (1987c). With this method, both chloroform fumigated and non-fumigated soil are amended with glucose; the total amount of O_2 taken up by each soil is then determined over 5 days. The difference in oxygen uptake between these two treatments is used to determine the total soil microbial biomass after adjustment for several constants. One such constant is f_{5d}, or the fraction of the microbial biomass killed by fumigation which is catabolized over 5 days. This factor is comparable with the k_c-factor of Jenkinson and Powlson (1976). Van de Werf and Verstraete (1987c) determined the f_{5d}-factor for 7 soils after adding suspensions of soil microorganisms (of known microbial C) to soil samples. The range of f_{5d} values across these soils was 0.467 to 0.678 with a mean of 0.551 and 95% confidence interval of

[.481, .621]. The soils used for this determination were relatively heterogeneous, with four soils from under grassland, two from under natural vegetation (mixed herbs) and one from under pine trees. Organic C contents ranged from 0.11% to 6.12%. Recalculation of their data in the present study suggests that the same $\underline{f}_{5d}$ factor is not appropriate for all soils. For example, it appears that organic carbon content of the soil probably influences the value of the $\underline{f}_{5d}$ factor since the value of the Spearman's rank correlation coefficient between soil organic C and $\underline{f}_{5d}$ for the 7 soils is 1.00; $p < 0.01$; $n = 7$.

3.3 CALIBRATION OF DIFFERENT METHODS AGAINST EACH OTHER

Since the development of the fumigation-incubation method a number of separate studies have attempted to calibrate other potential indicators of microbial biomass size against fumigation-incubation using regression and correlation analysis. Four such methods have been developed based on such calibrations, i.e. substrate-induced respiration, ATP analysis, ninhydrin-extractable N analysis, and the fumigation-extraction method. For such calibrations, utilizing type II regression, two assumptions are essential and should be fulfilled. These are:

i) Both X and Y variables must be normally distributed.

ii) All X values must be independent from each other (and all belong to the same homogeneous population); the same applies for all Y values. Individual data points should be randomly extracted from this population (Zar 1974).

In order to fulfill these assumptions and thus produce a reliable calibration curve, appropriate sampling strategies and subsequent analysis of data are first required.

For the construction of a reliable calibration curve, the ecosystem for which the calibration curve is to be constructed and subsequently applied should first be defined, e.g. coniferous forests of North America, or a single wheat field. Several (e.g. >10) independent locations or sites within this region should then be randomly selected, and soil samples collected.

Upon construction of the calibration curve, each location or site within this ecosystem should represent one data point (which may in fact be a mean of many samples within that location). It is normally undesirable to use several data points representing each location, even if the data points are collected at different times of the year or different depths of the soil profile, because of the problems associated with dependency of data points leading to pseudoreplication (Hurlburt 1984). Each location or site should have approximately equal weighting in determining the nature of the calibration curve.

The calibration data also should be checked for discontinuities. If, for example, soils in all the locations have either >8% or <3% organic carbon, then there are also likely to be discontinuities in the size of the microbial biomass, and separate calibration curves should be constructed for each of the two groups. Otherwise X and Y variables will not be normally distributed.

Other checks for normality also should be used. Obvious outliers should be excluded because they can exert a substantial bias on the slope of the calibration curve (Zar 1974). It is inappropriate for example to include data from soils from one location which have four times the microbial biomass of any of the others. If either of the X or Y variables or the regression residuals are not normally distributed (e.g. due to kurtosis or skewness), they also should be transformed (e.g. by $\log_e X$) to satisfy the assumptions of normality (Snedecor and Cochran 1980).

When the calibration curve is constructed, it should be used only for the ecosystem or soil type for which it was developed, and for similar environmental (e.g. soil temperature and moisture) conditions under which the samples used for the calibration curve were collected.

An attempt was made to reanalyze the data sets which have been obtained by the use of the four methods mentioned earlier. Where necessary, this involved correcting for violations of assumptions of normality and independence in the data sets used in each calibration, and recalculating the results. All data were calculated as μg biomass C g^{-1} soil. Assessments of the strength of the various calibrations to allow biomass determinations were also made.

Substrate-induced respiration (S.I.R.)

This method, as first proposed by Anderson and Domsch (1978b) involves incubation of soil samples with glucose in closed

containers and subsequent hourly measurements of evolved CO_2. The initial response of the microflora to glucose was measured in several soils and this was calibrated against values for microbial biomass determined using $CHCl_3$ fumigation (using a value of $\underline{k}_c$ = 0.41 and T = 22°C). This yielded an equation of:

$$Y = 400.4\ X + 3.7\ (r = 0.96)$$

where $Y = \mu g$ biomass C g soil^{-1}

$X = ml\ CO_2\ hr^{-1}\ 100\ g\ soil^{-1}$

n.b. the equation given by Anderson and Domsch (1978b) expressed microbial biomass in units of mg biomass C 100 g soil^{-1}.

However, there appear to be two violations of the assumptions of normality and independence present in this study.

(i) Two highly organic soils were included in the calibration; these had substantially higher microbial biomass values than the other 10, and clearly belonged to a different "population" of soils.

(ii) For the 12 soils studied, anywhere between one and five samples of each soil were used for biomass determinations, leading to unequal weighting of data from the different soils. This resulted in 39 points on the calibration curve.

Therefore, to rectify this problem, these data have been re-analyzed. To do this, data for each soil sample were calculated by measuring off the calibration graph provided in Figure 6 of Anderson and Domsch (1978b), excluding the two highly organic soils, and giving all other samples equal weighting. This resulted in 10 points i.e. one point per sample, yielding a new calibration curve of:

$$Y = 416.7X + 16.2, \; r = 0.82, \; n = 10$$

$$\text{S.E. of slope} = \pm 102.5$$

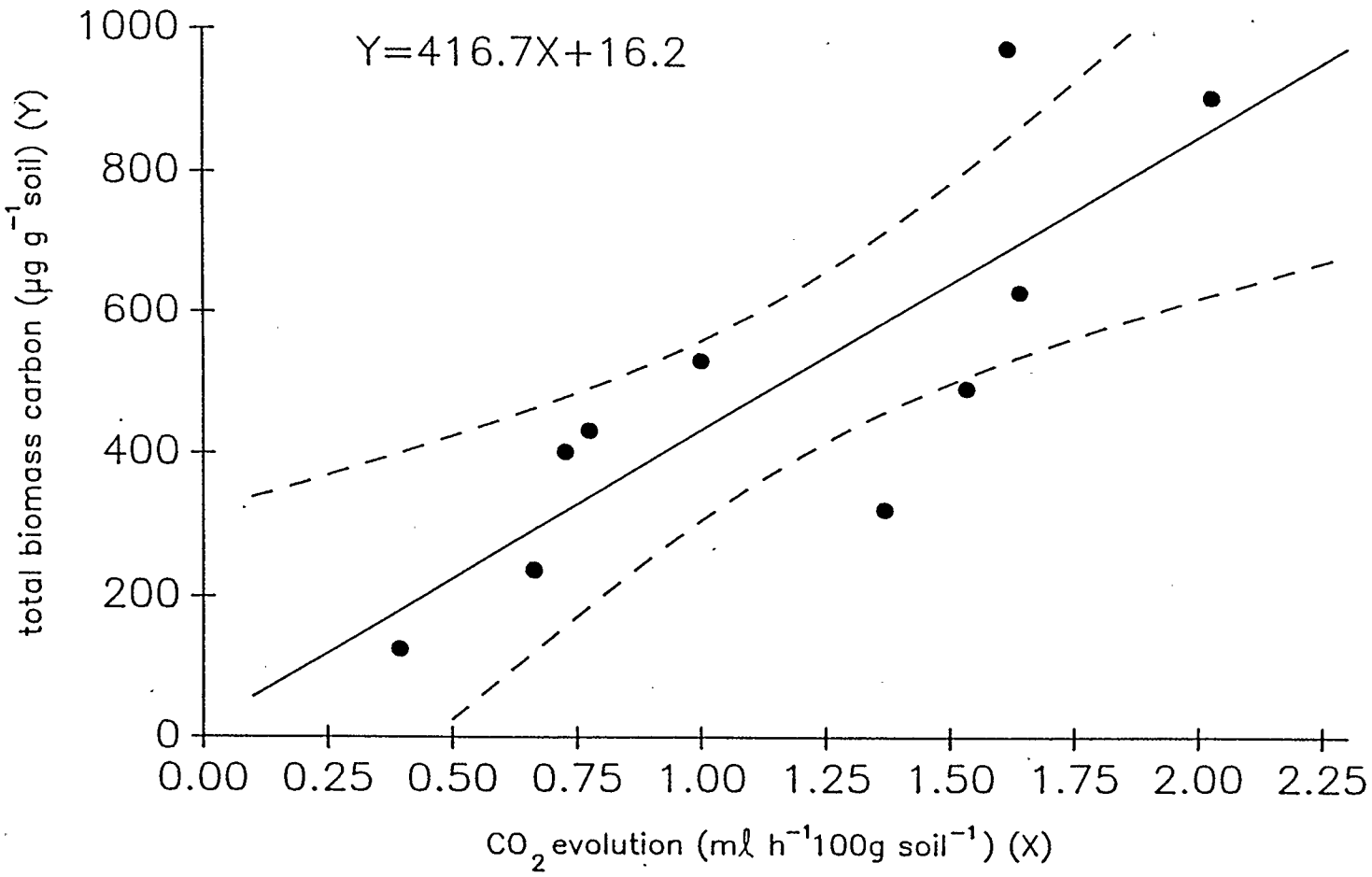
$$\text{S.E. of Y intercept} = \pm 131.0$$

However, the value of r has a 95% confidence interval of [0.360, 0.956]. Therefore, there are insufficient data to determine if a strong relationship between the two methods exists (Figure 2).

Martens (1987) performed a similar calibration of S.I.R. against fumigation-incubation on soils from several locations. The S.I.R. method used by Martens (1987) contained proposed "improvements" to the S.I.R. method of Anderson and Domsch (1978b) by using a continuously flushing aeration train, thus preventing CO_2 dissolving in soil water, especially under high soil pH levels. Based on his data from 22 soils, Martens (1987) then proposed a new equation, which using microbial biomass units of μg C g soil^{-1} is expressed as:

$$Y = 495\ X - 167, \quad r = 0.978.$$

Figure 2. Modified calibration of Anderson and Domsch (1978b) for determining microbial biomass using the substrate-induced respiration technique. Dashed lines represent 95% confidence bands for the expected values of Y from any value of X.


Y=416.7X+16.2
1000
800
600
400
200
0
0.00
0.25
0.50
0.75
1.00
1.25
1.50
1.75
2.00
2.25
total biomass carbon (µg g⁻¹soil) (Y)
CO2 evolution (mℓ h⁻¹100g soil⁻¹) (X)

It was claimed that this equation was different from that of Anderson and Domsch (1978b), but considering the variability associated with both data sets, no statistically significant difference exists.

The calibration was performed on one point per soil used, such that all soils were equally weighted. However, another problem exists in that two "populations" of soils were used to construct the calibration curve. These can be identified as "inorganic" soils (of which there were 16) with organic carbon levels of <3% and microbial biomass C levels of <450 $\mu g\ g^{-1}$; and, "organic" soils (the remaining six) with organic C >7% and biomass C >450 $\mu g\ g^{-1}$. Two separate equations can then be proposed, i.e.,

Inorganic samples: $Y = 234.7\ X + 68.7$, $n = 16$, $r = 0.917$

95% C.I. for $r = [0.773, 0.971]$

S.E. of slope = ± 27.2

S.E. of Y intercept = ± 26.4

Organic samples: $Y = 489.7\ X - 142.7$, $n = 6$, $r = 0.974$

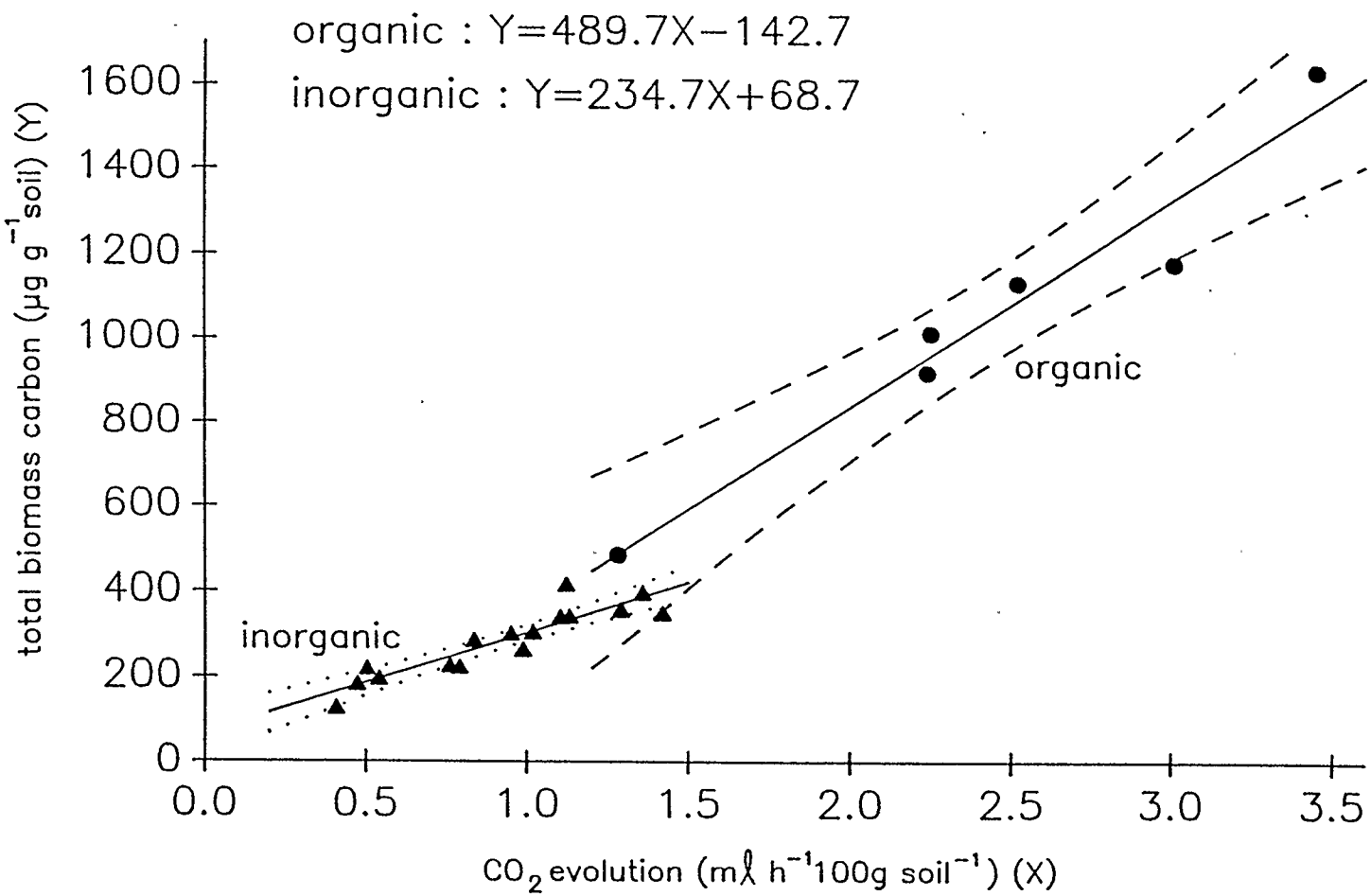
95% C.I. for $r = [0.776, 0.997]$

S.E. of slope = ± 56.8

S.E. of Y intercept = ± 144.7

These two equations are statistically significantly different from each other at $p = 0.001$ (Figure 3), and they provide a more reliable means of estimating microbial biomass in respective groups of soils.

Figure 3. Modified calibration of Martens (1987) for determining microbial biomass C using the substrate-induced respiration technique. Dashed or dotted lines represent 95% confidence bands for the expected values of Y from any value of X.


organic : Y=489.7X−142.7
inorganic : Y=234.7X+68.7
total biomass carbon (µg g⁻¹soil) (Y)
1600
1400
1200
1000
800
600
400
200
0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
CO2 evolution (mℓ h⁻¹100g soil⁻¹) (X)
organic
inorganic

From the data provided by Martens (1987), correlation coefficients were also calculated between fumigation-incubation biomass for these samples, and S.I.R. measured using the non-continuous CO_2-flow method (as used by Anderson and Domsch 1978b). These correlation coefficients were 0.807 and 0.785 for inorganic and organic samples, respectively. However, these correlation coefficients are not statistically significantly lower than those from the calibrations where continuous flow was used. The corresponding equations for non-continuous flow (modified from Martens' 1987 data) are:

$$Y = 145.0\ X + 45.8 \text{ (inorganic samples)}$$

$$Y = 216.4\ X + 382.5 \text{ (organic samples)}$$

Two other equations have also been proposed for the calculation of microbial biomass from substrate-induced respiration measurements. These were based on the non-continuous flow measurements of CO_2 production first outlined by West and Sparling (1986), where soils are incubated in McCartney bottles with a 2:1 weight ratio of concentrated glucose solution:soil (dry weight). One of the equations was derived from a calibration equation based on biovolume-derived microbial biomass C values (West and Sparling 1986), i.e.,

$$Y = 433 \log_{10} W + 5.92,\ r = 0.84$$

$$W = \mu L\ CO_2\ h^{-1}\ g\ soil^{-1}$$

This calibration was based on 14 data points but from only three soils (6, 4, and 4 points from each soil respectively). This does not fulfil the assumption of non-independence amongst X and amongst Y values. Furthermore, the points from each soil form a cluster and effectively only three independent data points are represented.

The other equation, proposed by West et al. (1986), was calibrated against fumigation-derived microbial biomass using $\underline{k}_c$ = 0.45, i.e.,

$$Y = 40.92\ W + 12.9,\ r = 0.93$$

This calibration involves 12 points but again, only three soils with 5, 4, and 3 data points from each soil respectively. Again the assumptions required for such analysis are not fulfilled, and the curve is strongly influenced by four points from one sample which form a very tight cluster at the lower end of the slope.

The S.I.R. technique as modified by West and Sparling (1986) has been used for the calibration of data from the direct extraction fumigation technique, with which it shows a relatively high correlation (discussed later). However, data provided by Sparling and West (1988b), when analyzed, show only a correlation coefficient of r = 0.615 between biomass data from the S.I.R. and fumigation-incubation methods for nine pasture soils (95% C.I. for r = [-0.05, 0.914]).

It appears that the best available equations for the calculation of microbial biomass from substrate induced respiration are

those modified from Martens (1987) when a separate relationship is used for soils with 0.8 - 3% organic carbon, and those with 7 - 16% C. No reliable equation exists for determining microbial biomass in soil with a 3 - 7% C content. Also, continuous flushing is probably necessary; especially in soils with high pH. Analysis of the data provided by Martens (1987) shows a significant relationship between soil pH and the difference between S.I.R. values obtained using continuous and non-continuous flow methods. The S.I.R. (continuous flow) method may be unsuitable for dry samples and the modifications proposed by West and Sparling (1986) may overcome problems of glucose remaining unavailable to the microflora in soils with low moisture content. However, more reliable calibrations are required to test this.

ATP content of soil

Oades and Jenkinson (1979) attempted to calibrate soil ATP values with values for microbial biomass determined by the fumigation-incubation technique. They derived a relationship, after fitting the regression through the origin, of:

$$Y = 120\ A,\ r = 0.98,\ n = 11$$

where: A = ATP content of soil ($\mu g\ g^{-1}$ dry weight).

After the incorporation of 6 more data points, this equation was modified (Jenkinson et al. 1979) to:

$$Y = 138\ A,\ r = 0.975,\ n = 17$$

However, in the development of both these equations, data from soils from some locations were weighted more heavily than others; for example, data for soils from the Rothamstead experimental plots provided four of the 17 points on the calibration curve. Two of these soils had much higher microbial biomass than the others, and these values influenced the slope of the calibration curve. By modifying the relationship so that soil from each site provide one point, a new equation can be derived:

$$Y = 120.1\ A + 30.0,\ r = 0.973,\ n = 13$$

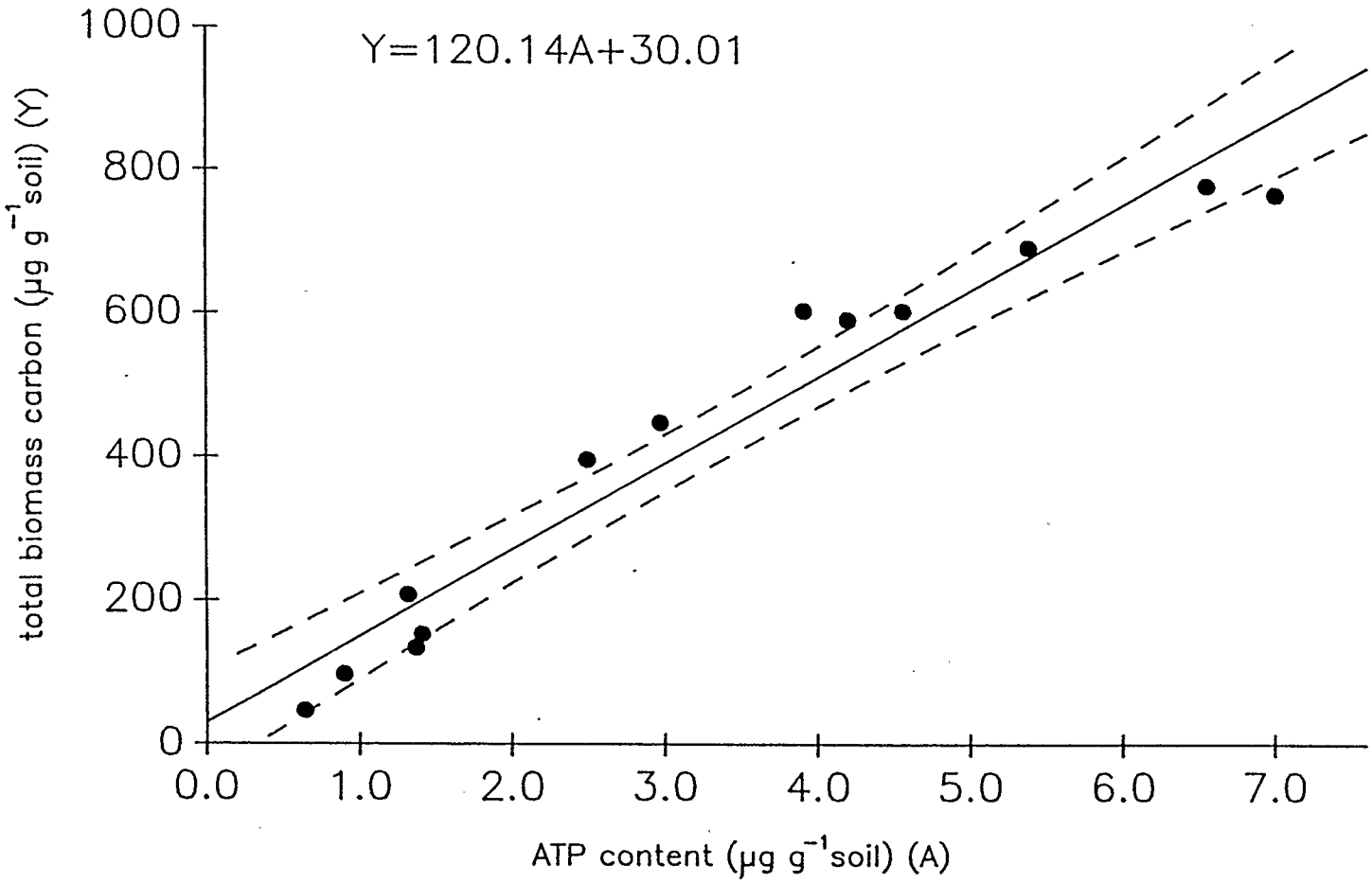
95% C.I. for r:[0.910, 0.992]

S.E. of slope = ± 8.7

S.E. of Y intercept = ± 33.7

This calibration equation is constructed of 13 points, 10 from Australian soils and 3 from English soils. Because the points from each of these two regions were not clearly from different populations with regard to the X and Y variables, they were included in the same equation. The relationship between biomass and ATP content appears to be relatively strong, as indicated by the 95% confidence interval for r and the confidence bands for the curve itself (Figure 4).

Figure 4. Modified calibration of Jenkinson et al. (1979) for determining microbial biomass C using the ATP assay technique. Dashed lines represent 95% confidence bands for the expected value of Y from any value of A.


Y=120.14A+30.01
1000
800
600
400
200
0
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
total biomass carbon (μg g⁻¹soil) (Y)
ATP content (μg g⁻¹soil) (A)

However, the results of other studies suggest that there are conditions under which the relationship between soil ATP content and the microbial biomass may be very different or much weaker. These include storage conditions and seasonal effects (e.g. Ahmed et al 1982; Ross et al. 1981; Sparling et al. 1981), and this is possibly due to the microbial biomass exhibiting differing metabolic states in different environmental conditions (Ross et al. 1981). Jenkinson (1988) notes that ATP analysis is "not strictly a way of measuring either biomass C or N" but is still useful to consider "because of the light it throws on other methods."

Ninhydrin-reactive nitrogen content of soil

Amato and Ladd (1988) detected a significant relationship between the ninhydrin-reactive nitrogen of fumigated soil and the microbial biomass C as detected using the fumigation-incubation technique. They proposed a method of measuring biomass C, based on assays of ninhydrin-active nitrogen for 25 soils that had been pre-incubated for 44 and 66 weeks. The strongest correlation was with soils that had been incubated for 44 weeks, where the relationship derived for microbial biomass was:

$$Y = 24.4\ N - 56,\ r = 0.96$$

where N = ninhydrin-reactive nitrogen ($\mu g\ g^{-1}$ soil) extracted from soils incubated for 10 days after fumigation. However, with the data represented in the calibration graph of Amato and Ladd

(1988), the use of the Wilk-Shapiro statistic (Shapiro and Wilk 1965) in the present study demonstrated that neither the Y nor N variables were normally distributed; this was rectified by the use of $\log_e$ transformations for both variables. Thus, the equation becomes:

$$\log_e Y = 1.335 \log_e N + 2.025$$

or $Y = 7.576N^{1.355}$, $r = 0.954$

95% C.I. for r = (0.903, 0.980)

Parameters ± S.E.:1.335 ± 0.131

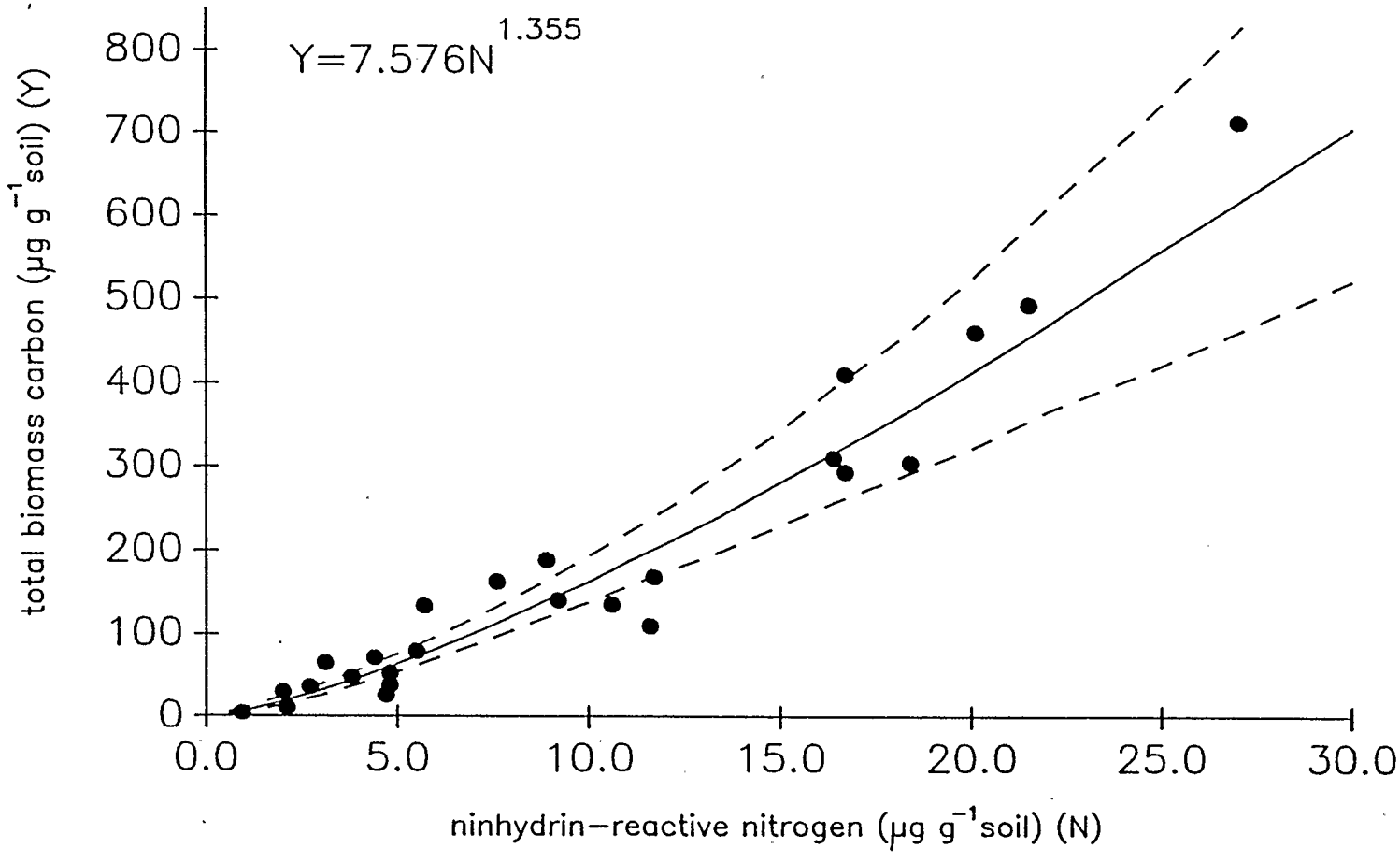
:7.576 ± 1.324

This curve is not very different from the linear form presented by Amato and Ladd (1988), which is easier to apply. However, log-log transformation is necessary for correlation analysis and placement of 95% confidence bands about the predicted values of Y from N (Figure 5). The determination of microbial biomass using this method is clearly appropriate for lower but not higher (>200 μg biomass C g soil^{-1}) values of microbial biomass, as the variability of the predicted values of Y increases as N increases.

Fumigation extraction method

The direct extraction method (Vance et al. 1987b) involves fumigating soil samples with $CHCl_3$ (as for the method of Jenkinson and Powlson 1976) but following this with an extraction of organic

Figure 5. Modified calibration of Amato and Ladd (1987) for determining microbial biomass C using the ninhydrin-extractable N technique. Dashed lines represent 95% confidence bands for the expected value of Y from any value of N.


Y=7.576N^1.355
total biomass carbon (μg g⁻¹soil) (Y)
800
700
600
500
400
300
200
100
0
0.0
5.0
10.0
15.0
20.0
25.0
30.0
ninhydrin–reactive nitrogen (μg g⁻¹soil) (N)

carbon with 0.5 M K_2SO_4; this prevents the need to incubate samples for 10 days.

Vance et al. (1987b) calibrated the fumigation-extraction method against the fumigation-incubation method using ten data points, one of which represented nearly four times more microbial biomass than any of the other nine. Their calibration equation using all 10 points (after fitting the regression through the origin) was:

$$Y = 2.64\ V, \quad r = 0.99$$

where V = flush of extractable C following fumigation ($\mu g\ g^{-1}$ soil). They also provided an equation using only nine points, excluding the outlier point, i.e.

$$Y = 2.78\ V, \quad r = 0.95.$$

They also stated that they could justify leaving that outlier point in the calibration because it had only negligible effects on the regression equation. However, in the present study, it has been excluded from the data set because inclusion of that data point renders the data non-normal. From the data provided in Table 1 in Vance et al. (1987b) the calibration equation then becomes:

$$Y = 3.16\ V - 4.87,\ r = 0.874$$

95% C.I. for r = [0.501, 0.973]

S.E. of slope = ± 0.66

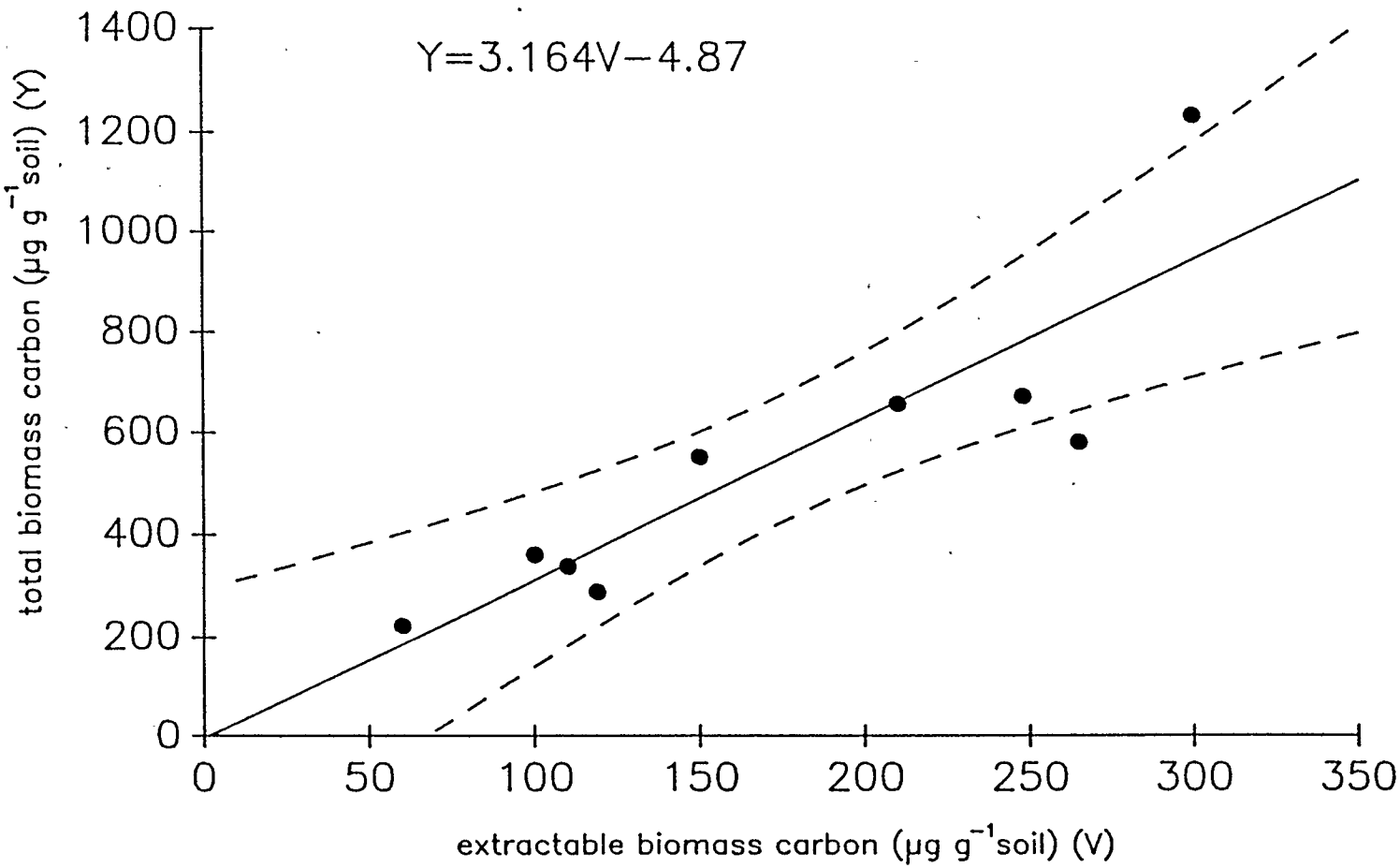
S.E. of Y intercept = ± 126.6

The same results as Vance et al (1987b) could not be obtained upon recalculation of their data. Their study also violates other assumptions of parametric analysis, in that out of the nine points, four were from the Broadbalk experimental plots and, therefore, weight the equation in favour of that region. These four soils all had the highest pH values out of all nine soils. Because there are insufficient soils represented and because the lower limit of the 95% confidence interval for the value of r is very low (explaining only 25% of the variability in the data set), this study itself does not provide conclusive evidence in favour of the extraction technique. The variability associated with the original equation of Vance et al (1987b) using nine data points is shown in Figure 6.

Tate et al. (1988) also attempted to calibrate this method against microbial biomass predicted using the chloroform-incubation technique. From the data set provided, the following relationship can be derived:

$$Y = 1.54V + 314.8,\ r = 0.730,\ n = 24$$

Figure 6. Modified calibration of Vance et al. (1987b) for determining microbial biomass C using the extractable C content of soil. Dashed lines represent 95% confidence bands for the expected value of Y from any value of V.


Y=3.164V−4.87
total biomass carbon (μg g⁻¹soil) (Y)
1400
1200
1000
800
600
400
200
0
0
50
100
150
200
250
300
350
extractable biomass carbon (μg g⁻¹soil) (V)

However, because in some cases up to four data points were used from soils from the same location, this calibration has been redone using the average fumigation incubation C and fumigation-extraction C values for each location. Two data points were excluded from this analysis because these contained 13.3% and 14.6% organic carbon respectively, and were therefore not of the same population as the other soils which were at or below 8.3% organic carbon. The new equation becomes:

$$Y = 1.30\ V + 452.5,\ r = 0.589,\ n = 12$$

This relationship is clearly of insufficient strength to justify use.

Tate et al. (1988) also determined the percentage of microbial carbon added to soil which was extracted by K_2SO_4 following $CHCl_3$ fumigation, for several microbial species. The objective of this was to provide a $\underline{k}_{EC}$ factor (analagous to the $\underline{k}_C$-factor of Jenkinson 1976) indicating the fraction of fumigation-killed biomass which becomes extractable following $CHCl_3$ fumigation. This approach also proved to be unsatisfactory with the values of $\underline{k}_{EC}$ ranging from 0.13 to 0.34 with a 95% confidence interval of 0.203 ± 0.085.

Sparling and West (1988a) also determined $\underline{k}_{EC}$ values for six soils, four with low organic matter levels (<8% C) and two with high organic matter contents (>20% C). These were calculated as the fraction of ^{14}C-labelled glucose incorporated into soil

microbial biomass *in situ* which was extracted following fumigation. For the inorganic soils k_{EC} ranged from 0.25 to 0.38 with a 95% confidence interval of 0.325 ± 0.098. The two organic soils yielded k_{EC} values of 0.29 and 0.38 respectively. These data indicate that there is probably considerable variability amongst k_{EC} values from different soil types.

Two studies have involved calibrating total extractable C following fumigation, against biomass C determined using substrate induced respiration. One of them (Sparling and West 1988a) was used for 26 inorganic soil samples and resulted in an equation of:

$$Y = 1.85\ V + 242,\ r = 0.89$$

However, 10 of the data points used (including all the points at the lower part of the calibration curve) represented soils collected from one agroforestry project and two other locations were also represented by more than one data point. To reduce this bias, the calibration was re-calculated using the average value for each location as one data point. The new equation is:

$$Y = 2.41\ V + 116.5,\ r = 0.917,\ n = 13$$

95% C.I. for r: [0.740, 0.975]

S.E. of slope = ± 0.32

S.E. of Y intercept = ± 102.2

The 95% confidence bands for this equation are shown in Figure 7. This analysis demonstrates that there is probably a strong correlation between S.I.R. and fumigation-extraction estimates of biomass C. The value of r is significantly higher than that obtained by Tate et al. (1988) (i.e., r = 0.583) which was calibrated against fumigation-incubation biomass C. However, it was not found to be significantly higher than the r = 0.874 value of Vance et al. (1987b).

The study of Sparling and West (1988a) also investigated six organic soils (organic C = 24.5 - 41.6%) and this yielded an equation of

$$Y = 4.62V + 226, \; r = 0.99, \; n = 6$$

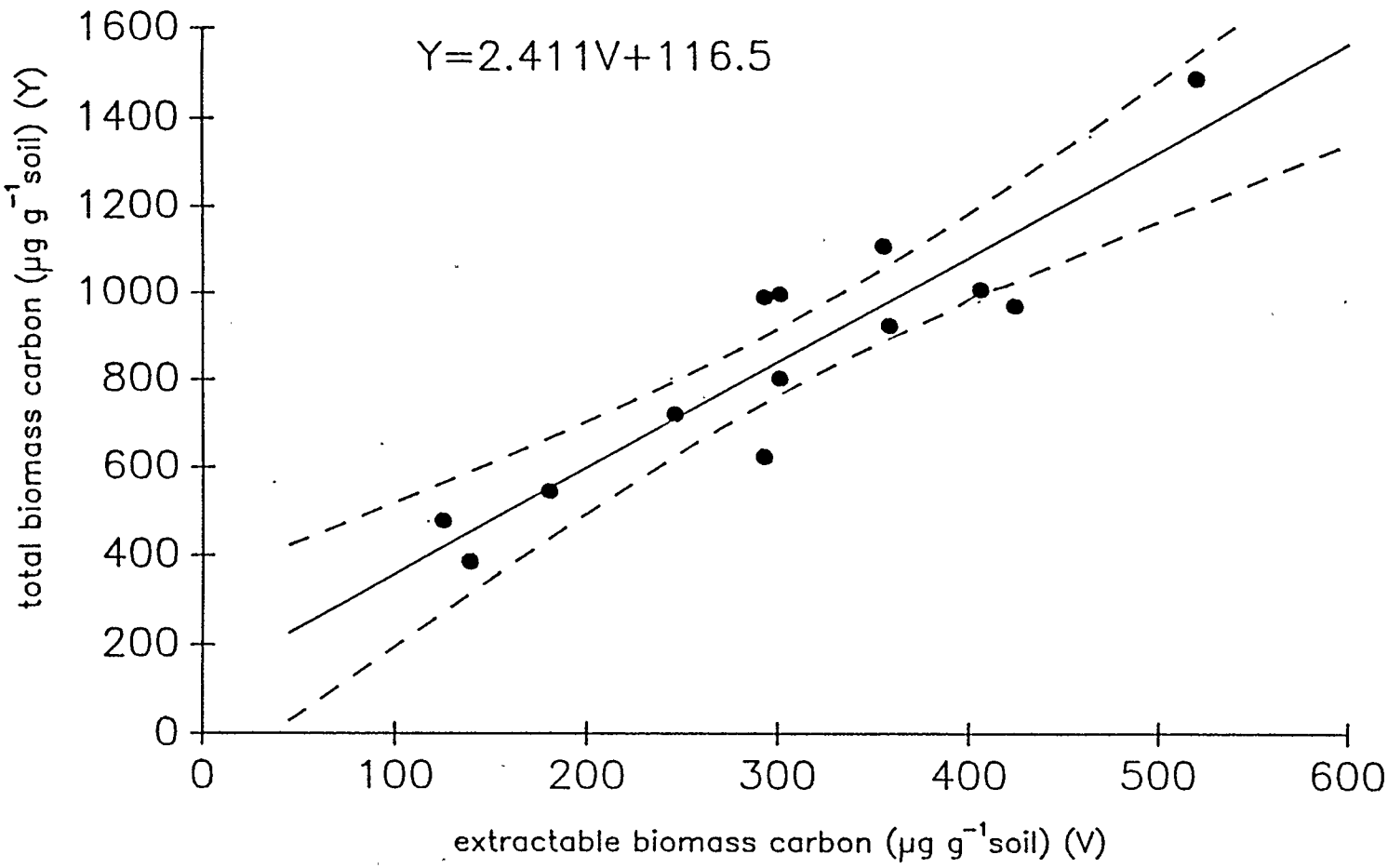
However, one data point represented a microbial biomass value of 2.3 times that of any of the other data points. Furthermore, soils from only four locations are represented because two locations are each represented by two data points.

In the second study (Sparling and West 1988b) the following equation was obtained based on 11 soils.

$$Y = V/0.39 \text{ or } Y = 2.56V, \; r = 0.93$$

However, of these eleven soils, two can be excluded because they are non-pasture soils (the other nine are from pasture systems) and have anomalously low values for the C-flush determined using

Figure 7. Modified calibration of Sparling and West (1988a) for determining microbial biomass C using the extractable C content of soil. Dashed lines represent 95% confidence bands for the expected value of Y from any value of V.


Y=2.411V+116.5
total biomass carbon (µg g^{-1}soil) (Y)
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
extractable biomass carbon (µg g^{-1}soil) (V)

fumigation-incubation or fumigation extraction. Recalculation of the relationship after omitting those two soils yields the equation:

$$Y = 2.68\ V - 44.1,\ r = 0.891,\ n = 9$$

95% C.I. for r = [0.714, 0.997]

S.E. of slope = ± 0.51

S.E. of Y intercept = ± 191.5

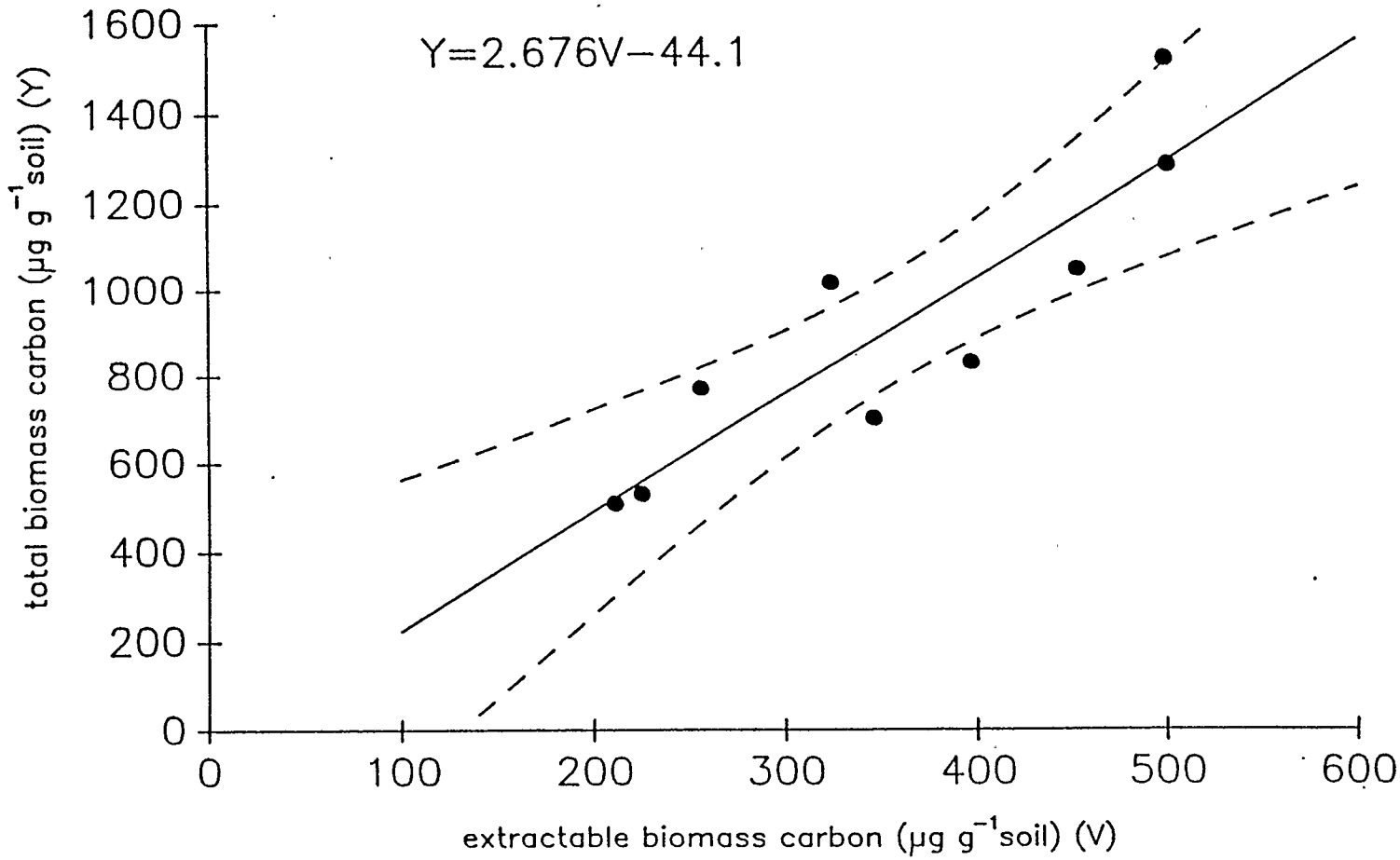
The 95% confidence bands for this equation are shown in Figure 8. This equation is not significantly different from the recalculated equation of Sparling and West (1988a) for inorganic soils.

3.4 DISCUSSION

The performance of four indirect methods of estimating microbial biomass carbon (i.e. S.I.R., ATP content, ninhydrin-extractable nitrogen, and fumigation-extraction) has been assessed in terms of strengths of calibration against other "standardized" methods. This was performed after correcting for problems associated with the violation of assumptions associated with parametric analysis. Other ways of assessing the performance of the various methods are by noting the range of microbial biomass values for which the calibration has been performed, the types of soils tested (especially their organic carbon content), and the validity of the methods against which the technique in use has been calibrated.

The methods described thus far have either directly or indirectly been calibrated against the chloroform fumigation-

Figure 8. Modified calibration of Sparling and West (1988b) for determining microbial biomass C using the extractable C content of soil. Dashed lines represent 95% confidence bands for the expected value of Y from any value of V.


Y=2.676V−44.1
total biomass carbon (μg g⁻¹soil) (Y)
1600
1400
1200
1000
800
600
400
200
0
0
100
200
300
400
500
600
extractable biomass carbon (μg g⁻¹soil) (V)

incubation method. When a weak correlation between two methods is detected this could be due to either technique being a poor indicator of microbial biomass. This could be especially true when fumigation-incubation is used as the standard, because the small amount of evidence which exists suggests that $\underline{k}_c$ does vary amongst soils and because, for the majority of soils (including organic or forest soils), $\underline{k}_c$ has never been determined effectively.

With regard to biomass measurements based on S.I.R., the only calibration equations that can be recommended are the modifications given earlier (Figure 2) to the data provided by Martens (1987). The first of these equations, based on 16 samples, provides a strong relationship for inorganic (0.8-3%C) soils. The second equation relating to the organic (7-16%C) soils is also strong but is based only on six samples. This is the only effective calibration of any type (including fumigation incubation) that has been performed for assessing microbial biomass in organic soils, but this still assumes a constant $\underline{k}_c$-factor of 0.41 in all soils. The $\underline{k}_c$ values in soils with over 4% organic carbon have not been effectively determined and cannot be assumed to be similar to those in less organic (<4%C) soils. Therefore, the equation can, at best, provide only relative figures for microbial biomass in organic soils. No useful calibration has been provided for soils containing 3-7% organic carbon, or for highly organic soils, i.e. over 16% C.

The ATP assay provided a reliable calibration against fumigation-incubation biomass over a large microbial biomass range (40 - 770 $\mu g\ g^{-1}$ C) for soils low in organic matter, using a

modification of the Jenkinson et al. (1979) equation. However, further studies have not proven this technique to be reliable in a wide range of conditions, e.g. Ross et al. (1981), Ahmed et al. (1982).

The ninhydrin extractable nitrogen method of Amato and Ladd (1988) is strongly related to the chloroform-fumigation method for soils with low biomass values and includes soils with lower biomass values than any other method, (i.e., below 10 $\mu g\ g^{-1}$ C). Because the variability of the calibration increases as biomass values increase, it is inadequate for soils with higher biomass values, (e.g., over 200 $\mu g\ g^{-1}$C).

The direct fumigation-extraction method calibrations which have been constructed to date indicate that it may not necessarily be a reliable method for measuring microbial biomass. No effective calibration of this method has been performed against fumigation incubation; this may be a function of either $\underline{k}_C$ or $\underline{k}_{EC}$ values varying between soils. The calibration of fumigation-extraction against substrate induced respiration appears to yield a stronger relationship. However, the S.I.R. equation against which fumigation-extraction has been calibrated is that of West et al. (1986) which not only is calibrated against fumigation-incubation itself but is based on soils from only three locations. Thus, while it is possible that S.I.R. may potentially be a useful method with regard to calibration of other techniques like fumigation-extraction, it is necessary firstly to effectively calibrate S.I.R. As stated earlier, no adequate calibration of substrate induced respiration

has been performed for soils containing 3-7% organic carbon, a range which is typical for many of the soils used in the fumigation extraction calibrations.

It must be emphasized that the aims of this chapter have been to make critical assessments of the sampling programs used in a range of studies of soil microbial biomass and of the calibrations which have been derived for microbial biomass determinations. There has been no attempt to make more detailed comments on the relative methodological advantages and disadvantages of the different techniques which have been dealt with as this is covered, for the physiological methods, in the following chapter.

CHAPTER 4

4. INFLUENCE OF SOIL MOISTURE CONTENT ON SOIL MICROBIAL BIOMASS MEASUREMENTS

4.1 INTRODUCTION

As outlined in section 1.2 the soil microbial biomass appears to be strongly influenced by soil moisture content, with wetting and drying cycles regulating its magnitude. Therefore, there has been recent interest in determining which physiological techniques for quantifying the soil microbial biomass are the most suitable for soils across moisture gradients, and especially for dry soils. The principal technique for investigating soil moisture effects has been the fumigation-incubation method (e.g. Bottner 1985; McGill *et al*. 1986) but the fumigation k_c factor is not necessarily constant across soil moisture gradients (Ross 1987). West and Sparling (1986) modified the substrateinduced respiration technique of Anderson and Domsch (1978b) to enable measurement of soil microbial biomass in dry soils, by adding the glucose substrate to the soils in solution to ensure its dispersal. A conceptually similar approach was taken by West (1986) who modified the selective inhibition method of Anderson and Domsch (1973, 1975) to enable determination of the bacterial:fungal ratio in dry soils, by dissolving the inhibitors prior to addition to the sample. However, there has been no detailed comparison of these various methods for assessing microbial biomass response to soil moisture gradients within a

given soil type. The purpose of the experiments outlined in this chapter was to determine the relative suitability of different methods for quantifying the soil microbial biomass for samples pre-incubated at set moisture levels for predetermined periods of time.

4.2 DETERMINATION OF FUMIGATION $\underline{k}_c$ FACTORS ACROSS A SOIL MOISTURE GRADIENT

4.2.1 INTRODUCTION

In most studies investigating the influence of soil moisture content on the soil microbial biomass, a constant $\underline{k}_c$ value is used for calculating the magnitude of the microbial biomass across all moisture treatments. Therefore, these studies assume that $\underline{k}_c$ is more or less independent of soil moisture influences. The only study to date which has investigated moisture effects on the value of $\underline{k}_c$ is that of Ross (1987) who found that the value of $\underline{k}_c$ was depressed in moist compacted soils, relative to other soils.

The purpose of the experiments outlined in this section was to determine whether the fraction of ^{14}C-labelled bacterial and fungal tissue released as $^{14}CO_2$ (i.e. the $\underline{k}_c$ factor) is influenced by maintaining soil samples over a range of soil moisture contents for a range of preincubation times.

4.2.2 MATERIALS AND METHODS

Growth and harvest of microbial cells

Seven species of fungi were grown, i.e. *Mucor hiemalis*, *Mortierella alpina*, *Trichoderma harzianum*, *Arthrinium sphaerospermum*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, and *Penicillium nigricans*. These were found in preliminary investigations to be the most abundant fungal species in the soil used in this study. Cultures of these fungi were maintained on malt extract agar.

Three 1 g samples of soil were each diluted to 10^{-7} in 100 ml 0.1% peptone solution. A 0.1 ml aliquot of each diluted sample was spread on 5 plates of nutrient agar. Sixteen bacterial (including actinomycete) colonies were randomly selected, and maintained on nutrient agar.

The liquid medium used for growing ^{14}C-labelled microorganisms was based on those of Anderson and Domsch (1978a) and Ross (1987). It consisted (per ℓ water) of 10 g $U^{14}C$-labelled D-glucose (sp. activity 370 Bq mg^{-1} glucose), 1 g NH_4NO_3, 1 g H_2PO_4, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.05 g $CaCl_2$ and 0.02 g $FeCl_3$. In addition, media for fungi and bacteria contained respectively 1 g and 3 g of yeast extract (DIFCO). The media were autoclaved prior to addition of the labelled glucose. The bacterial and fungal colonies were cultured in 50 ml liquid medium in 125 ml Erlenmeyer flasks, on a culture shaker at 22°C, until harvest.

Fungi were harvested, using filtration, at the late linear phase, as determined by prelimary experimentation. They were rinsed with deionized water, air-dried and ground to 0.5 mm using a mortar

and pestle. The bacteria were harvested at the late logarithmic growth phase by centrifugation at 15,000 rpm for 20 minutes. These were then air-dried, rinsed, air-dried again, and ground to 0.5 mm. All the fungal material was then mixed together, as was all the bacterial tissue.

Determination of ^{14}C content of tissue

For both the bacterial and fungal tissue, six replicate 50 mg subsamples were dissolved using 1 ml of the tissue solvent Protosol (Du Pont). This was left for 2 days, and 15 ml of the scintillation fluid BIOFLUOR (Du Pont) was added. This was left for a further day and the ^{14}C content was determined using a liquid scintillation counter (2200CA Tricarb Packard).

Pretreatment of soil samples

Six soil samples were passed through a 4 mm sieve. Each sample was divided into 20 subsamples (each 15 g.d.w.) i.e. 5 moisture levels (15, 25, 35, 45, and 55% H_2O on a dry weight basis) x 4 preincubation times (6 hours, and 3, 10, and 30 days). The moisture levels used have been found to be the normal range of moisture levels in the field soil during the growing season. The above procedure was performed twice, once for each of the bacterial and fungal $\underline{k}_c$ determinations described below.

Determination of ^{14}C release from tissue following fumigation

At the end of the 4 incubation times, each subsample of soil was amended with 40 mg of either bacterial or fungal tissue, and thoroughly mixed. This was then fumigated with chloroform (alcohol-free) for 18-24 hours (Jenkinson and Powlson 1976). Subsequently the soil was remoistened to 55% (d.w. basis) moisture content (= field capacity) and reinoculated with 1.67 g.d.w. soil so as to provide a 9:1 ratio of fumigated soil to re-inoculant (Chapman 1987). The subsamples were incubated in airtight Qorpak jars (500 ml) together with a 50 ml beaker containing 15 ml of 1.0M NaOH. After 10 days, 1.5 ml NaOH was removed from each beaker and added to 15 ml BIOFLUOR scintillation fluid. The $^{14}CO_2$-C content of each sample was then determined using liquid scintillation counting.

Data analysis

For each subsample, k_c factors were determined separately for fungi and bacteria, as a ratio of the amount of $^{14}CO_2$-C evolved from the tissue in 10 days to the total ^{14}C content of the tissue at the beginning of the incubation.

In order to test whether the k_c factors were influenced by the treatments used in the present study, data were analyzed using 3-way analysis of variance, testing for the blocking, moisture, and time of preincubation effects, as well as the time x moisture interaction. When significant moisture effects were detected. Tukey's multiple comparison analysis was performed within each

preincubation time across the soil moisture gradient following two-way ANOVA (moisture content and blocking).

4.2.3 RESULTS

For the 120 fungal $\underline{k}_c$ factors determined, the values ranged from 0.219 to 0.449, with a mean of 0.365. This $\underline{k}_c$-factor was significantly influenced by soil moisture, time of preincubation, and blocking, although time effects were the strongest (Tables 8,12). The $\underline{k}_c$ factors for the 15% moisture treatment were always significantly lower than for the 35% treatment, as well as below the 45% treatment (10 days) and 55% treatment (6 hours). The differences were, however, not large. The 25, 35, 45, and 55% moisture treatments were never significantly different from each other. Time of preincubation exerted strong effects on the $\underline{k}_c$ factor, with soil incubated for 30 days having $\underline{k}_c$ factors well below the other preincubation times. The results suggest that the $\underline{k}_c$ factors vary between soils of different moisture content, between soils incubated at a fixed moisture content for different time periods, and between different samples of the same soil type. There was no significant interaction between incubation time and soil moisture content, indicating that moisture effects are essentially similar regardless of incubation time.

The values for the 120 bacterial $\underline{k}_c$ factors were slightly higher than those for the fungi. The factors ranged from 0.243 to 0.484 with a mean of 0.398. However, the treatment effects were essentially similar to those for the fungi (Tables 9,12). The $\underline{k}_c$

Table 8. Fungal k_c factors determined for 5 moisture levels and 4 preincubation times.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	.358a	.379ab	.400b	.390ab	.403b
3 days	.379a	.396ab	.411b	.389ab	.382ab
10 days	.335a	.352ab	.378b	.371b	.358ab
30 days	.296a	.319ab	.350b	.328ab	.316ab

Within each row, numbers followed by different letters are significantly different at p=0.05.

Table 9. Bacterial k_c factors determined for 5 moisture levels and 4 preincubation times.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	.402a	.404ab	.422ab	.421ab	.435b
3 days	.391a	.415ab	.435b	.421ab	.440ab
10 days	.358a	.417b	.391ab	.396ab	.400b
30 days	.347a	.357ab	.389b	.370ab	.346ab

Within each row, numbers followed by different letters are significantly different at p=0.05.

values for the 15% treatment were always significantly lower than for at least one of the other moisture treatments but these differences were usually relatively small. The 25, 35, 45, and 55% moisture treatments were never significantly different from each other. Increasing time of preincubation reduced the $\underline{k}_c$ factor for all moisture contents, with 30 day samples having $\underline{k}_c$ factors that were the lowest. The $\underline{k}_c$ factors varied significantly across soil moisture contents, different preincubation periods, and different samples.

When microbial $\underline{k}_c$ factors were determined for the microbial biomass as a whole, essentially similar trends were found. Using a constant bacterial:fungal ratio of 25:75 (Anderson and Domsch 1975, 1978a), $\underline{k}_c$ values of the 120 subsamples ranged from 0.250 to 0.450 with a mean of 0.373. Essentially similar treatment effects were found as for the bacterial and fungal $\underline{k}_c$ factors (Tables 10,12). The 15% moisture treatment was significantly different to the 55% one at 6 hours, and to the 35% treatment at days 3, 10, and 30. Again the 25, 35, 45, and 55% treatments were not significantly different from each other. The $\underline{k}_c$ factor was significantly reduced by prolonged preincubation times and also influenced by blocking (Table 12).

When a separate bacterial:fungal ratio was determined for each subsample a very similar pattern was found. Bacterial: fungal ratios were determined separately for each of the 120 subsamples using the selective inhibition technique of Anderson and Domsch (1973, 1975) in which 10,000 ppm streptomycin in sulphate and 15,000 ppm actidione were added to inhibit glucose-stimulated

respiration of bacteria and fungi, respectively. These ratios are presented in Section 4.4.3. Although selective inhibition does not appear to inhibit much of the microbial respiration even when both inhibitors are added together (West 1986), these values are used as an indicator in the present study to determine what effects variation in bacterial:fungal ratios might exert on the factor. Using $\underline{k}_c$ factors based on separate ratios for each subsample, $\underline{k}_c$ values ranged from 0.246 to 0.448 with a mean of 0.374. Virtually identical trends were found by using varying bacterial:fungal ratios compared with using a constant ratio of 25:75, with regard to moisture effects, incubation time, and blocking (Tables 11,12). For all 120 subsamples, the $\underline{k}_c$ factor determined using a ratio of 25:75 never differed by more than 0.007 from the factors determined using a varying ratio.

4.2.4 DISCUSSION

The fumigation-incubation technique assumes that the $\underline{k}_c$ factor is constant for all soil samples and treatment effects (Jenkinson and Powlson 1976; Parkinson and Paul 1981). In the present study, this was not found to be the case. With regard to soil moisture content, relatively dry soil (15%) displayed reduced decomposition of added microorganisms even when incubated at 55% moisture for the 10 days following fumigation like all the other treatments. Soil moisture contents of 10-20% were typical for this soil in the field during June and July 1988; during such conditions microbial biomass would be underestimated by using a constant $\underline{k}_c$

Table 10. Microbial k_c factors determined for 5 moisture levels and 4 preincubation times, assuming a bacterial-fungal ratio of 25:75.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	.370a	.385ab	.405ab	.397ab	.411b
3 days	.382a	.400ab	.417b	.397ab	.396ab
10 days	.341a	.370ab	.381b	.377ab	.368ab
30 days	.309a	.328ab	.360b	.338ab	.324ab

Within each row, numbers followed by different letters are significantly different at p=0.05.

Table 11. Microbial k_c factors determined for 5 moisture levels and 4 preincubation times, using a separately determined bacterial: fungal ratio for each subsample.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	.369a	.385ab	.406ab	.398ab	.411b
3 days	.381a	.401ab	.416b	.398ab	.396ab
10 days	.341a	.371ab	.381b	.378ab	.371ab
30 days	.312a	.329ab	.361b	.334ab	.323ab

Within each row, numbers followed by different letters are significantly different at p=0.05.

Table 12. Relative importance of treatment effects on data presented in Tables 8-11.

Treatment Effect	Fungal k_c factor		Bacterial k_c factor		[2] Microbial k_c factor		[3] Microbial k_c factor	
	% of S.S.[1]	F	% of S.S.	F	% of S.S.	F	% of S.S.	F
soil moisture (A)	12.7	11.7***	9.5	6.7***	13.4	17.2***	14.0	18.1***
time of pre-incubation (B)	51.6	63.5***	35.8	33.6***	55.6	95.1***	54.8	94.2***
A x B interaction	2.9	0.9 ns	8.2	1.9*	2.8	1.2 ns	3.1	1.3 ns
blocking effect	7.5	5.5***	12.7	7.2***	10.0	10.3***	9.8	10.1***
unexplained	25.5		33.8		18.3		18.2	

[1] Percentage of total sums of squares in data set explained by each effect.
[2] k_c-factor using bacterial:fungal ratio of 25:75 (Table 3).
[3] k_c-factor using bacterial:fungal rate separately determined for each sample (Table 4).

*,**,*** = effect is significant at p=0.05, p=0.01, and p=0.001 ns = effect not significant at 0.05.

factor relative to soils with a more optimal moisture status. The depression of the $\underline{k}_c$ factor caused by low moisture levels was not large, causing it to be reduced by less than 0.080 (relative to optimal moisture conditions) in all cases. Nevertheless, this could exert an effect on the results of studies conducted across a gradient of moisture contents.

Because incubation conditions (including the moisture status) for the ten days following fumigation were identical for all soils, there is no obvious explanation as to why microorganisms in soils preincubated at 15% of soil moisture (even for only 6 hours) should demonstrate a reduced $\underline{k}_c$ factor relative to other treatments. It is most likely, however, that soils at 15% moisture are in some way physically or chemically different from those at other moisture levels, and even when they are brought up to 55% moisture these differences are maintained for some time. Presumably this results in the decomposition of added substrates being reduced over 10 days.

The present study found the reverse trend to that found by Ross (1987) with regard to soil moisture effects. In that study, higher soil moisture contents (above 60% of field capacity) resulted in reduced mineralization of added microbes, especially if moister soils had been smeared and compacted, $\underline{k}_c$ values were also reduced if soils were preincubated at 60% F.C. for 7 days. In contrast, mineralization of microorganisms in the present study was not significantly reduced by higher moisture levels, probably because

smearing or compacting was not observed to occur even at the highest moisture level, which represented field capacity.

In the present study, there was considerable variation in k_c between different soil samples. This can be explained in terms of chemical or physical differences amongst the six samples (or blocking units) used in this study. Nicolardot et al. (1984) demonstrated that different soil types can have different microbial k_c factors. Variations in soil pH may also be important especially if soils of low pH are considered (Vance et al. 1987a; Jenkinson 1988). The present study suggests that there are also important variations in k_c between replicate samples collected within one soil type, as indicated by the blocking effect.

Comparison of the bacterial and fungal k_c factors indicates that a higher percentage of bacterial than fungal carbon is released as CO_2 over 10 days. This was observed across all treatments, i.e. 36.5% of fungal ^{14}C was released vs. 39.8% of bacterial ^{14}C (mean of all 120 samples). Whether these differences are truly significant or not is unclear because tissue from all 7 fungal species were pooled as was tissue from all 16 bacterial colonies, preventing assessment of variability amongst species within each of the two microbial groups. In contrast, Anderson and Domsch (1978a) found that fungal k_c factors were significantly higher than bacterial k_c factors (43.7% vs 33.3%).

In the present study varying the bacterial:fungal ratios did not greatly influence the microbial k_c factors compared with using a constant ratio of 25:75 (Table 10 vs. Table 11). This is

partly because the bacterial and fungal $\underline{k}_c$ factors were not very different from each other, nor did the bacterial/fungal ratio differ greatly from a 25:75 ratio (Section 4.4.3). If the soils in the study had a ratio which differed greatly from 25:75, then differences between Table 10 and Table 11 also would have been greater.

The use of a constant $\underline{k}_c$ factor may also be problematic due to differences in microbial species composition between different treatments. Data provided by Anderson and Domsch (1978a) indicates that mineralisation of the carbon in some fungal species in some soils was nearly twice as great, over 10 days, as that of other species. For the bacteria in that study, some species decomposed over five times faster than other species during that time. It is possible that microbial community structure may affect the $\underline{k}_c$-factors of different soils or treatments, because some conditions may encourage microbial species which decompose faster in 10 days than other conditions. Therefore, adding microbial tissues of the same species composition to different soils may not accurately reflect what happens to the native soil microbial biomass following fumigation, although there is no effective way to test this.

Values of $\underline{k}_c$ determined in different studies have varied substantially and this can be explained in part by the methods used for determining them. Jenkinson (1976) found a $\underline{k}_c$ factor of 0.50, based on equal weighting of each of the 8 bacteria, 4 fungi, and one earthworm species used in their study. Ross (1987) obtained variable and often high $\underline{k}_c$ values, ranging from 0.32 to 0.68. Neither of these two studies used labelled (^{14}C) microbial

tissue. Therefore, it is unclear as to whether CO_2-C released from fumigated soil amended with microbial tissue (over and above that released from fumigated control soil) came only from microbial tissue or from native organic carbon in the soil due to priming action caused by adding microbial tissue. In the present study an approach such as this would have been unsuitable because extensive priming action has been previously observed in soils from the same location upon addition of other substrates, e.g. herbicides (see chapter 6).

Other studies concerned with determining $\underline{k}_c$ factors, have used ^{14}C-labelled microorganisms, so any recovered $^{14}CO_2$-C is therefore of microbial origin. This includes the determinations by Anderson and Domsch (1978a) of a $\underline{k}_c$ factor of 0.41. Nicolardot _et al_. (1984) obtained values of $\underline{k}_c$ of 0.35 to 0.49, based on a seven-day incubation. Vance _et al_. (1987a) determined $\underline{k}_c$ values of 0.30 and 0.45 for soils of pH below 4.5 and above 4.5, respectively. In the present study the range extended from 0.25 to 0.44. One _in situ_ study using ^{14}C-labelled glucose has also been performed (Voroney and Paul 1984). In that investigation, ^{14}C was added to the soil, and that carbon which was immobilized in the soil was all assumed to be incorporated into the soil microbial biomass. The fraction of this immobilized ^{14}C released as CO_2 following fumigation was determined, and this yielded a $\underline{k}_c$ factor of 0.41.

The chemical and physical nature of microbial tissue added to soil may also influence $\underline{k}_c$ values. A significant portion of the soil microbial biomass is limited by energy supply and therefore inactive (Jenkinson and Ladd 1981; Van de Werf and Verstraete 1987b).

In contrast, added microbial tissues are grown in nutrient-amended glucose solutions (e.g. see media recipes given by Anderson and Domsch 1978a; Nicolardot et al. 1984), and because they are harvested in the log or linear growth phases they are mostly active and not nutrient limited. Influence of the age of cultures of Fusarium oxysporum has been demonstrated to influence k_c-factor determinations, with older cultures containing less cytoplasm also having lower k_c values (Ross et al. 1987). Tissue preparation may also influence the k_c factor obtained. Tissues have been lyophilized in the studies of Ross (1987), Vance et al. (1987a), and Nicolardot et al. (1984). They have either been fine ground (e.g. Jenkinson 1976; Ross 1987) or added live in pieces (fungi) and liquid suspensions (bacteria) (Anderson and Domsch 1978a) prior to chloroform-fumigation. In the present study the tissues were air-dried, finely ground but possibly still partially alive prior to fumigation.

Because methods used to determine k_c factors probably do not accurately represent ecological reality, experimentally determined k_c factors can at best provide an indirect approximation of the fraction of microbial biomass carbon released for 10 days following fumigation. Furthermore, k_c values can vary between different samples, and are influenced by soil moisture and preincubation time as found in the present study. Other studies have also indicated that such factors may vary between soil types, soils of different pH's and soils with different species composition. Therefore, there is little evidence to suggest k_c values remain invariant between different soils and between different treatments.

4.3 COMPARISON OF PHYSIOLOGICAL TECHNIQUES FOR ESTIMATING THE MAGNITUDE OF THE SOIL MICROBIAL BIOMASS

4.3.1 INTRODUCTION

Three physiological techniques have been developed to quantify total soil microbial biomass, i.e. the fumigation-incubation method (Jenkinson and Powlson 1976), substrate-induced respiration (S.I.R.) (Anderson and Domsch 1978b) and the oxygen uptake method (Van de Werf and Verstraete 1987c). Strong correlations between fumigation-incubation and S.I.R. biomass values have been detected in certain studies (e.g. Anderson and Domsch 1987b, West and Sparling 1986; West et al. 1986; Martens 1987) but these studies were performed on several soil types with a wide range of biomass values. Also, the strengths of these relationships are questionable when the data used are reanalyzed (see Section 3.3). Van de Werf and Verstraete (1987c) also found a strong relationship between microbial biomass values determined using fumigation-incubation and the oxygen uptake methods, but this again involved soil types of a vastly different range of microbial biomass values. Although many studies have used one of these methods to compare effects of different treatments within a single soil type (e.g. Anderson et al. 1981; Duah-Yentumi and Johnson 1986) there have been few studies concerned with how these different methods agree with each other within a soil type across a range of treatments.

The purpose of the experiments outlined in this section was to compare the predicted relative values of the soil microbial biomass, using the three methods outlined above, on soil samples preincubated across a range of soil moisture contents for a range of time intervals. Therefore, it could be determined whether different physiological methods for estimating soil microbial biomass predict the same relative trends with regard to soil moisture gradients and whether these measurements change when soil samples are maintained at these moisture levels for differing time periods.

4.3.2 MATERIALS AND METHODS

Sample preparation

Methodologies for sample preparation were the same as those described in Section 4.2.2 Six soil samples were passed through a 4 mm sieve and each was adjusted to 5 separate moisture levels i.e. 15, 25, 35, 45, and 55% H_2O (dry weight basis). For each moisture level soil was preincubated at 22°C for each of four time periods, i.e. 6 hours, 3 days, 10 days, and 30 days. For each subsample, microbial biomass was predicted using three methods, i.e. fumigation-incubation (Jenkinson and Powlson 1976), substrate-induced respiration (Anderson and Domsch 1978b), and oxygen uptake curves (Van de Werf and Verstraete 1987c).

Fumigation of soil subsamples

For each subsample, two 80 g.d.w. subsamples were removed after each incubation time. One of these was fumigated with alcohol-free chloroform for 18 - 24 hours, while the other was not. Each of the pair of subsamples was then remoistened to 55% soil moisture (dry weight basis), and reinoculated with 8.89 g.d.w. soil, so as to provide a ratio of fumigated to non-fumigated soil of 9:1 (Chapman 1987; Van de Werf and Verstraete 1987c). For each of the fumigated and non-fumigated soils two 16.67 g.d.w. subsamples (i.e. 15 g original soil and 1.67 g inoculum) were removed and incubated as for the Jenkinson and Powlson (1976) method. A further two 11.11 g.d.w. subsamples (i.e. 10 g original soil and 1.11 g inoculum) were removed for biomass determination by the Van de Werf and Verstraete (1987c) method.

Fumigation-incubation technique

Each 16.67 g.d.w. subsample was incubated in an airtight 500 ml Qorpak jar, together with a 50 ml beaker containing 20 ml 1M NaOH. After ten days the total CO_2-C content of each beaker was determined using double-endpoint titration with 0.1 M HCl from pH 8.3 to 3.7 (Parkinson and Paul 1981). Microbial biomass was then determined using:

$$B = \frac{F-NF}{k_c}$$

where B = microbial biomass C ($\mu g\ g^{-1}$ soil)

F = total CO_2-C from fumigated soil ($\mu g\ g^{-1}$ soil)

NF = total CO_2-C from non-fumigated soil ($\mu g\ g^{-1}$ soil)

$\underline{k}_c$ = fraction of microbial biomass mineralized in 10 days.

The biomass for each subsample was calculated twice, using both $\underline{k}_c$ = 0.41 (Anderson and Domsch 1978a), and the $\underline{k}_c$ values obtained for each subsample in the previous section (i.e. Table 4.2.3).

Oxygen uptake curves

Each 11.1 g.d.w. subsample of fumigated and non-fumigated soil was amended with the compounds proposed by Van de Werf and Verstraete (1987c), i.e. glucose monohydrate 20.6 mg; yeast extract 5.16 mg; NH_4Cl 7.74 mg; $MgSO_4 \cdot 7H_2O$ 2.06 mg; and KH_2PO_4 1.72 mg. These were then placed in flasks (Parkinson and Coups 1963) which were attached to a Gilson respirometer. The total amount of oxygen taken up by the sample was measured for 10 minutes every 1 - 2 hours for the first 20 hours, and approximately every 4 - 8 hours after that. This was continued until the end of 3 days when the difference in oxygen uptake between fumigated and non-fumigated subsamples was negligible.

The total quantity of oxygen taken up during the three days by fumigated and non-fumigated soils was subsequently determined by plotting oxygen uptake rate against time, and fitting a polynomial (3rd to 6th order) to the curve. The total microbial biomass C was then determined by substituting these polynomials into the equation

given by Van de Werf and Verstraete (1987c) and integrating them, i.e.

$$B = 1.5Y \left(\int_0^{72} P_F dt - \int_0^{72} P_{NF} dt\right)$$

where Y = fraction of C in microbial biomass (d.w.basis). The method as proposed by Van de Werf and Verstraete (1987c) estimates only the total microbial biomass. Microbial biomass C was estimated using Y = 0.47 (Jenkinson and Ladd 1981)

P_F = and P_{NF} = polynomial approximating oxygen uptake rate for fumigated and non-fumigated soils respectively ($\mu g\ O_2\ h^{-1}\ g\ soil^{-1}$)

t = time (hours)

$\int_0^{72} P_F dt$ and $\int_0^{72} P_{NF} dt$ = total amount of oxygen taken up over 72 hours by fumigated and non-fumigated soils respectively ($\mu g\ O_2^{-1}\ g\ soil^{-1}$).

Substrate-induced respiration

Two 30 g.d.w. subsamples were measured for each of the same 120 subsamples described above (i.e. 6 replicates x 5 moisture contents x 4 incubation times). Substrate-induced respiration was determined using one of the subsamples, by adding 6000 ppm anhydrous glucose, and observing the rate at which CO_2-C was evolved (Anderson and Domsch 1978b), usually within 4 hours following

glucose addition. The second subsample was remoistened to 55% by spraying with glucose solution (6000 ppm) using an atomiser, except for the 55% treatments when glucose powder was mixed throughout the sample. Substrate-induced respiration was determined in this case also. In all cases the rate of CO_2-C evolution was determined at 22°C using an infrared gas analyzer with a flow rate of 286 ml min^{-1}.

Data analysis

The various estimates for microbial biomass were all analyzed using 3-way ANOVA, testing for the following effects: moisture content, incubation time, blocking and the moisture x time interaction. When significant moisture effects were detected, multiple comparison analyses were performed within each incubation time across the soil moisture gradient using Tukey's test, following two-way ANOVA (moisture content and blocking).

The percentage of variation shared between pairs of methods for the same 120 subsamples was determined using r^2 analysis.

4.3.3 RESULTS

Fumigation-incubation technique

There was a significant effect of soil moisture content on the soil microbial biomass when a constant $\underline{k}_C$ factor of 0.41 was used (Tables 13,19). Microbial biomass calculated using the fumigation-incubation technique was found to be maximal at 25 - 45% moisture. The biomass for the 15% treatment was always lower than

Table 13. Soil microbial biomass (μg biomass C•g soil^{-1}) determined for soils at 5 moisture levels and 4 preincubation times, using a $\underline{k}_C$ value of 0.41.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	806a	889ab	909b	888ab	858ab
3 days	809a	970c	974c	916bc	835ab
10 days	815a	1010c	973bc	964bc	887ab
30 days	726a	866bc	924c	905c	801ab

Within each row, numbers followed by different letters are significantly different at p = 0.05.

Table 14. Soil microbial biomass (μg biomass C•g soil^{-1}) determined for soils at 5 moisture levels and 4 preincubation times, using a separate $\underline{k}_C$ value for each subsample.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	893a	947a	920a	917a	856a
3 days	868a	994b	958ab	946ab	865a
10 days	980a	1119a	1047a	1048a	988a
30 days	963a	1082a	1052a	1098a	1014a

Within each row, numbers followed by different letters are significantly different at p = 0.05.

for some of the 25 - 45% treatments, but never differed significantly from that of the 55% treatment.

When a separate $\underline{k}_c$ factor was used for each soil sample (i.e. $\underline{k}_c$ factors in Table 11) there was also a detectable effect of soil moisture (Tables 14,19), but the only preincubation time for which this was significant was at day 3 when the 25% treatment had a higher biomass than the 15% or 55% treatments.

The time of preincubation also had a significant effect on soil microbial biomass. When a constant $\underline{k}_c$ factor was used, the calculated microbial biomass was highest at days 3 and 10 across all moisture levels. However, when a varying $\underline{k}_c$ factor was used, microbial biomass estimates increased at each preincubation time and were maximal at day 30.

There was no significant interaction between soil moisture and time of preincubation. This indicated that any moisture response which may have occurred was not influenced by time. Therefore, if there was any effect of varied soil moisture levels on soil microbial biomass, it probably occurred within six hours of adjustment of the soil moisture content.

Oxygen uptake curves

The oxygen uptake method predicted that there was a significant effect of soil moisture on the total microbial biomass (Tables 15 and 19) with biomass increasing with increasing soil moisture. The 55% soil moisture treatment always had the highest measured microbial biomass. Time effects were also significant,

with measured microbial biomass observed to decline with increasing preincubation time. Again there was no significant interaction between soil moisture content and time of incubation.

Substrate-induced respiration

Substrate-induced respiration was found to be strongly influenced by soil moisture content when samples were measured at the moisture content at which they were preincubated (Tables 16,19). The 15% and 25% moisture samples were always significantly lower than the other three treatments. When soils were rewetted to 55% moisture immediately prior to measurement S.I.R. was not observed to vary significantly with (preincubation) moisture content, except at day 3 when S.I.R. was significantly higher at 55% moisture than either 15% or 35% moisture (Tables 17,19).

All measurements of S.I.R. were significantly reduced by increasing the length of preincubation time, except for the 15% treatment in Table 16.

There was a significant but slight interaction between soil moisture content and time of incubation before measurement, but this was only significant where samples had not been remoistened prior to determination.

4.3.4 DISCUSSION

Three different methods (and two variants of two of the methods) have been used to predict whether microbial biomass is influenced by soil moisture content and length of preincubation

Table 15. Soil microbial biomass (μg biomass C•g soil^{-1}) determined for soils at 5 moisture levels and 4 preincubation times, using the oxygen uptake curve method.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	821a	900ab	995ab	979ab	1121b
3 days	700a	792a	839ab	982bc	1088c
10 days	740a	839ab	955bc	972c	1109d
30 days	528a	572a	651a	620a	818b

Within each row, numbers followed by different letters are significantly different at p = 0.05.

Table 16. Substrate-induced respiration (μg CO_2•C•h^{-1}•g soil^{-1}) determined for soils at 5 moisture levels and 4 preincubation times.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	2.2a	8.7b	13.2c	15.0cd	15.2d
3 days	2.1a	7.7b	12.3c	14.2cd	16.0d
10 days	1.1a	7.9b	10.8c	13.1d	13.8d
30 days	1.6a	6.6b	10.5c	11.8cd	12.4d

Within each row, numbers followed by different letters are significantly different at p = 0.05.

time. They did not all predict the same responses of the soil microbial biomass to variations in soil moisture. Data from the use of the fumigation-incubation method (constant $\underline{k}_c$) predicted reduction of microbial biomass by both high and low moisture levels. Fumigation-incubation (varying $\underline{k}_c$) suggested no real effect of soil moisture except at day 3 when 25% soil moisture emerged as the optimum. Data from the oxygen uptake method and S.I.R. (non-remoistened) both predicted that increasing moisture content would cause microbial biomass to increase, although the trends were more dramatic in the latter of the two cases. Data for S.I.R. (remoistened samples) predicted that microbial biomass would be unaffected by the level of soil moisture except at day 3 when 55% moisture emerged as the optimum.

While these approaches all predicted different moisture responses of the soil microbial biomass, all methods, except for fumigation-incubation (varying $\underline{k}_c$), predicted reduction of microbial biomass with increasing preincubation time. No method showed a strong time x moisture interaction, suggesting that any moisture responses detected within a given method were approximately similar regardless of preincubation time. That is, if microbial biomass was affected by varying soil moisture content, then these effects occurred within the first 6 hours following moisture adjustment.

Other data collected in the present study indicate that a significant portion of the microbial biomass was killed by drying. When the results of the non-fumigated soils used in the fumigation-incubation method are considered, the total CO_2-C released for 10

days after remoistening to 55% was much higher in those samples preincubated at lower moisture levels (Table 18,19). Because the basal respiration of those remoistened soils was highest in the 15% treatment, it follows that there was more available substrate in the form of killed microorganisms at that moisture level. Also there was a significant "moisture x time of preincubation" interaction, because the differences between the 15% treatment and the other four treatments became greater with greater preincubation time.

The above information indicates that only a small portion of the biomass was killed when soils were preincubated at the 15% moisture for 6 hours or 3 days, while considerably more was killed if soils were maintained at that moisture status for 30 days. A similar trend was observed in the non-fumigated soils when oxygen uptake following remoistening to 55% moisture was determined (Section 4.4.3). Using the data given in Table 17, a strong reduction in microbial biomass at low soil moisture levels could be predicted, and this reduction would be more dramatic with increasing preincubation time. Only two methods predicted that microbial biomass was killed or reduced by soil moisture limitation (relative to all other moisture levels) i.e. oxygen uptake curves and S.I.R. without remoistening. Neither of these methods predicted that microbial biomass was reduced by moisture limitations in a more extensive manner with increasing preincubation time.

The 120 biomass estimates made using each method show very little agreement with each other, even relatively. The coefficient of determination, r^2, was used to determine the percentage of

Table 17. Substrate-induced respiration ($\mu g CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) determined for soils at 5 moisture levels and 4 preincubation times with samples remoistened prior to determination.

Time of preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	14.8a	15.0a	15.1a	15.0a	15.2a
3 days	13.6a	14.1ab	14.0a	14.1ab	16.0b
10 days	13.7a	14.2a	13.2a	13.2a	13.8a
30 days	11.5a	12.3a	11.8a	11.4a	12.8a

Within each row, numbers followed by different letters are significantly different at p = 0.05.

Table 18. $CO_2\text{-}C$ release ($\mu g\ C \cdot g\ soil^{-1}$) from soil samples over 10 days following adjustment to 55% moisture content.

Time of Preincubation	Soil Moisture Content (d.w. basis) 15%	25%	35%	45%	55%
6 hours	2.76a	2.43ab	2.36b	2.54ab	2.48ab
3 days	2.74a	2.32b	2.41b	2.58ab	2.53ab
10 days	2.58a	2.02b	1.81b	1.91b	1.95b
30 days	2.86a	2.10b	1.86b	1.82b	1.76b

Within each row, numbers followed by different letters are significantly different at p = 0.05.

Table 19. Relative importance of treatment effects on data presented in Tables 13 - 18.

Treatment effect	F.I.[2] % of SS[1]	F	F.I.[3] % of SS	F	Oxygen Uptake % of SS	F	S.I.R.[4] % of SS	F	S.I.R.[5] % of SS	F	CO_2-C[6] % of SS	F
Soil moisture (A)	31.0	31.5***	10.0	7.4**	28.3	29.1***	89.7	687.9***	3.4	4.3**	23.3	24.9***
Time of pre-incubation (B)	9.0	11.9***	20.6	20.4***	35.3	48.2***	3.4	35.0***	36.8	62.5***	22.2	31.6***
A x B interaction	4.0	1.3 n.s.	1.8	0.4n.s.	1.8	0.6n.s.	1.0	2.6**	3.4	1.5n.s.	8.5	3.0**
Blocking effect	31.1	24.7***	35.0	21.3***	11.3	9.4***	2.8	17.2***	37.7	38.5***	23.7	20.2***
Unexplained	24.0		31.8		23.1		3.1		18.6		22.3	

[1] Percentage of total sums of squares in data set explained by each efffect.
[2] Fumigation-incubation using $\underline{k}_C$ = 0.41.
[3] Fumigation-incubation using a different $\underline{k}_C$ value for each subsample.
[4] Substrate-induced respiration.
[5] Substrate-induced respiration with samples remoistened prior to determination.
[6] Total CO_2•C released for 10 days following remoistening to 55%.

*, **, *** = effect is significant at p = 0.05; p = 0.01, and p = 0.001 respectively.
n.s. = effect not significant at p = 0.05.

variability shared between pairs of methods (Table 20). The percentage of variability shared between pairs of method was always very low, i.e. always below 50%, and often below 15%. The highest r^2 value, i.e. between the two fumigation-incubation determinations (r^2 = 0.436), is explicable in terms of both methods using the same "fumigated-non-fumigated" respiration values. Therefore, of all the methods used here to measure microbial biomass, at most only one method is an accurate predictor of microbial biomass across the soil moisture gradient.

The fumigation-incubation method yielded different results when a constant $\underline{k}_c$ factor was used compared with those obtained using a separate $\underline{k}_c$ factor determined for each subsample. Comparison between Tables 13 and 14 suggest that while differences are not large, treatment effects may be erroneously detected by using a constant $\underline{k}_c$ factor. If a null hypothesis is used which states that soil moisture has no effect on soil microbial biomass, then this would have been rejected at the 5% level for all 4 preincubation times, when a constant $\underline{k}_c$ factor (e.g. 0.41) was used. However, if a different $\underline{k}_c$ factor was used for each sample, the null hypothesis would only have been rejected at day 3. The absolute biomass figures were larger when $\underline{k}_c$ values determined in Section 4.2 were used rather than a constant $\underline{k}_c$ value because these factors were usually smaller than 0.41.

There is no obvious explanation as to why the soil moisture response using the oxygen uptake method was so different from that observed using the fumigation-incubation technique, especially since

Table 20. Values of the coefficient of determination, r^2, between pairs of methods used to assess microbial biomass responses to soil moisture, for 120 samples.

	CO_2 release from non-fumigated soil (Table 18)	S.I.R. (remoistened samples) (Table 17)	S.I.R. (Table 16)	Oxygen uptake curves (Table 15)	Fumigation-incubation (varying k_c) (Table 14)
Fumigation-incubation (k_c=0.41) (Table 13)	.038	.130	.114	.082	.436
Fumigation-incubation (Varying k_c) (Table 14)	.096	.003	.001	.011	
Oxygen uptake curves (Table 15)	.001	.266	.302		
S.I.R. (Table 16)	.049	.122			
S.I.R. (remoistened samples) (Table 17)	.169				

both techniques involve measurement of total respiratory differences amongst fumigated and non-fumigated soils. It appears likely that the ratio of CO_2 release to O_2 uptake varies between soils of different moisture contents after fumigation. This could be due to the $\underline{f}_{5d}$ factor of Van de Werf and Verstraete (1987c) varying amongst treatments. This factor represents the fraction of added microbial tissue catabolized over 5 days and is analogous to the $\underline{k}_c$ factor for the fumigation-incubation method. If, in the present study, the $\underline{k}_c$ factor was influenced by soil moisture content then it is likely that the $\underline{f}_{5d}$ factor was also influenced.

The magnitude of the microbial biomass measured using oxygen uptake curves is similar to, although slightly less than, that measured using fumigation-incubation. This is in contrast to the comparison between the two methods by Van de Werf and Verstraete (1987c) where microbial biomass measured using oxygen uptake curves was typically 1.4 times that measured using fumigation-incubation.

The substrate-induced respiration method predicted different responses of the microbial biomass to soil moisture, dependent upon whether soils were rewetted prior to measurement, and this is most probably attributable to glucose not dissolving in the driest soil samples. West and Sparling (1986) recognized the value of determining S.I.R. on soils adjusted to the same soil moisture content. In the present study this was achieved by spraying drier soils with glucose solution while simultaneously bringing them up to 33 kPa moisture tension.

Substrate-induced respiration may also be a poor predictor of total microbial biomass because different components of the microbial biomass may respond differentially to an added substrate. Van de Werf and Verstrate (1987a,b) suggest that only soil microorganisms which are actively metabolizing will initiate growth immediately upon glucose addition, and will therefore be responsible for glucose mineralization. These microorganisms make up between 2.4% and 27.2% of the total microbial biomass, and this is dependent on soil conditions (Sparling 1985). Anderson and Domsch (1978b) recognized that only part of the microbial biomass would respond initially to added substrate, yet calibrated their method (S.I.R.) against the fumigation-incubation method which measures total microbial biomass. The method proposed by Van de Werf and Verstraete (1987a,b) for determining active microbial biomass involves assessing initial respiratory response to added glucose monohydrate and nutrients and is directly comparable with the method of Anderson and Domsch (1978b) because both involve detecting the immediate response of the active microbial biomass to added glucose. Because S.I.R is more likely to be related to the active rather than the total soil microbial biomass, and because the proportion of the total microbial biomass which is active is highly variable, it is reasonable to expect S.I.R. to correlate poorly with fumigation-incubation, especially within a given soil type.

Previous studies have shown significant relationships between S.I.R. and fumigation-incubation (e.g. Anderson and Domsch 1978b; West _et al_. 1986; Martens 1987; Vekemans _et al_. 1989)

although the strengths of some of these relationships is questionable (Section 3.3.1). Van de Werf and Verstraete (1987c) found a strong relationship between fumigation-incubation and oxygen uptake curve predictions of microbial biomass. However, all these comparison between methods have involved relating these method to each other over a number of soil types and therefore a very wide range of microbial biomass values. In contrast, the present study has involved comparison of methods within a single soil type, where natural variability between data points is not as great as between soil types. This indicates that most methods may be adequate for predicting microbial biomass across soil moisture gradients when several soil types are compared in the same study, but not when the range of values is narrower, e.g, within a soil type or within a study area. In the latter instances, soil chemical, physical, and biological factors may influence even relative measures of microbial biomass.

4.4 METHODS FOR STUDYING THE ACTIVE COMPONENT OF THE SOIL MICROBIAL BIOMASS

4.4.1 INTRODUCTION

As outlined in Section 1.1 the soil microbial biomass has been regarded as consisting of two components, one of which is "active" and readily metabolizes extracellular substrates, and the other which is "inactive" and probably does not. While recent emphasis has been focussed on physiological techniques for measuring

total microbial biomass (e.g. Jenkinson and Powlson 1976; Anderson and Domsch 1978b; Parkinson and Paul 1982; Martens 1987), there has been less interest in measuring the active component. The only currently available physiological technique for achieving this is that of Van de Werf and Verstraete (1987a,b). This method involves adding glucose and minerals to soil samples and observing the physiological responses (oxygen uptake) of the microflora. It is assumed that the active microbial biomass can initiate growth immediately upon glucose addition while the inactive component cannot. The substrate-induced respiration technique of Anderson and Domsch (1978b) also assumes this but their technique is calibrated against the fumigation-incubation method of Jenkinson and Powlson (1976), which measures both active and inactive components of the soil microbial biomass.

The selection inhibition technique (Anderson and Domsch 1973, 1975, modified by West 1986) aims to determine the ratio of bacterial to fungal biomass in soil samples. However, even when bacterial and fungal inhibitors are added simultaneously to glucose-amended soil, there is a substantial proportion of the total (substrate-induced) respiration which is not inhibited by these compounds. This appears to be irrespective of the amounts of inhibitors added once certain critical quantities have been applied. The level of inhibition of S.I.R. caused by an antifungal inhibitor and an antibacterial inhibitor together is usually less than 50% (see data of Anderson and Domsch 1975; West 1986). Therefore, it appears that there is a component of the microbial biomass for which

activity is not suppressed by these inhibitors. If the selective inhibition technique is used to determine bacterial:fungal ratios then it must be assumed that the ratio of bacteria to fungi in the non-sensitive fraction of the soil microbial biomass is the same as for the sensitive component. There is little evidence to support this assumption (West 1986).

The active component of the total soil microbial biomass can be defined as that which can initiate growth (i.e., protein synthesis) immediately upon addition of glucose (see Van de Werf and Verstraete 1987a). The selective inhibitors proposed by Anderson and Domsch (1973) are both protein-synthesis inhibitors. Streptomycin sulphate inhibits bacterial (70s) ribosomes while actidione inhibits fungal (80s) ribosomes (Jacoby and Gorin 1967, Obrig et al. 1971). Inhibition of protein synthesis translates into an inhibition of substrate-induced respiration. It therefore follows that the growth of those organisms which are undergoing instant proliferation and enhanced protein synthesis upon glucose addition will be inhibited by protein synthesis inhibitors. The degree to which S.I.R. is inhibited in these organisms will be related to the extent to which protein synthesis and growth is occurring when an unlimited quantity of substrate is present. This explains why studies outlined in chapter 6 indicate that increases in the absolute level of inhibition by actidione and/or streptomycin occur only when microbial biomass rapidly increases.

The purposes of the experiments outlined in this section were (i) to determine whether active (rather than total) microbial

biomass could be predicted for soils held at different moisture levels for varying lengths of times, and (ii) to determine whether the amount by which S.I.R. is inhibited by actidione and streptomycin could be used to provide a relative measure of the active microbial biomass (both bacterial and fungal), i.e. that which undergoes growth immediately upon glucose addition.

4.4.2 MATERIALS AND METHODS

Sample preparation

The sampling procedure and sample preparation were the same as that described in Sections 4.2 and 4.3. This involved incubating soil samples at each of 5 separate moisture levels, i.e. 15, 25, 35, 45, and 55% H_2O. For each moisture level, soil was preincubated at 22°C for each of 4 preincubation times, i.e. 6 hours, and 3, 10, and 30 days.

Oxygen uptake curves

These were obtained in the manner described in Section 4.3.2, except that oxygen uptake was measured for 10 hours only, and only for non-fumigated soil samples amended with glucose medium (Van de Werf and Vestraete 1987b,c). The total active microbial biomass C was then determined using the following modification of the equation given by Van de Werf and Verstraete (1987b):

$$B = 0.778\ Y\ (\int_0^{10} P_R dt)$$

where Y = fraction of C in microbial biomass (d.w. basis). The method as proposed by Van de Werf and Verstraete (1987a,b) estimates only the total active microbial biomass. Microbial biomass C was estimated using Y = 0.47 (Jenkinson and Ladd 1981).

P_R = polynomial approximating oxygen uptake rate ($\mu g O_2 h^{-1}$ g $soil^{-1}$).

t = time (hours) following glucose amendment.

($\int_0^{10} P_R dt$) = total amount of oxygen taken up by soil in 10 hours ($\mu g O_2$ g $soil^{-1}$). The total amount of oxygen taken up at earlier time intervals was also determined, by integrating the rate of polynomial between 0 and 2, 4, 6, and 8 hours.

Selective inhibition

For each soil subsample, 8 determinations were made. For four of these determinations, 30 g.d.w. soil was used and each subsample was amended as follows.

A: 6000 ppm glucose only (value obtained from Section 4.3)

B: 6000 ppm glucose + 10 000 ppm streptomycin sulphate

C: 6000 ppm glucose + 15 000 ppm actidione (cycloheximide).

D: 6000 ppm glucose + 10 000 ppm streptomycin sulphate + 15 000 ppm actidione.

The rate of CO_2 evolution was subsequently determined continuously using an infra-red gas analyzer with a flow rate of 283 ml min^{-1}. The levels of inhibitors used represent the minimum quantity required to provide maximum inhibition of CO_2 evolution as determined in preliminary experiments. Each determination was made when the sum of respiratory inhibition caused by each inhibitor was closest to that of both inhibitors together.

For the other four determinations, the above procedure was repeated except that the soil subsamples were all brought up to 55% soil moisture content by spraying glucose solution (6000 ppm glucose) throughout the soils using an atomiser.

For all 8 subsamples the following determinations were subsequently made:

(i) inhibition of S.I.R. by streptomycin sulphate, i.e. A-B

(ii) inhibition of S.I.R. by actidione, i.e. A-C

(iii) inhibition of S.I.R. by actidione and streptomycin sulphate added separately, i.e. 2A-B-C.

(iv) inhibition of S.I.R. by actidione and streptomycin sulphate added together, i.e. A-D

(v) bacterial:fungal ratio, i.e. A-B:A-C (Anderson and Domsch 1975).

Data analysis

The various estimates for active microbial biomass C were all analyzed using 3-way ANOVA, testing for the following effects: moisture content, incubation time, blocking and the moisture x time interactions. When significant moisture effects were detected, multiple comparison analysis was performed within each incubation time, across the soil moisture gradient, using Tukey's test, following two-way ANOVA (moisture content and blocking). The percentage of variation shared between different approaches for assessing microbial biomass for the same 120 subsamples was determined using r^2 analysis.

4.4.3 RESULTS

Oxygen uptake curves

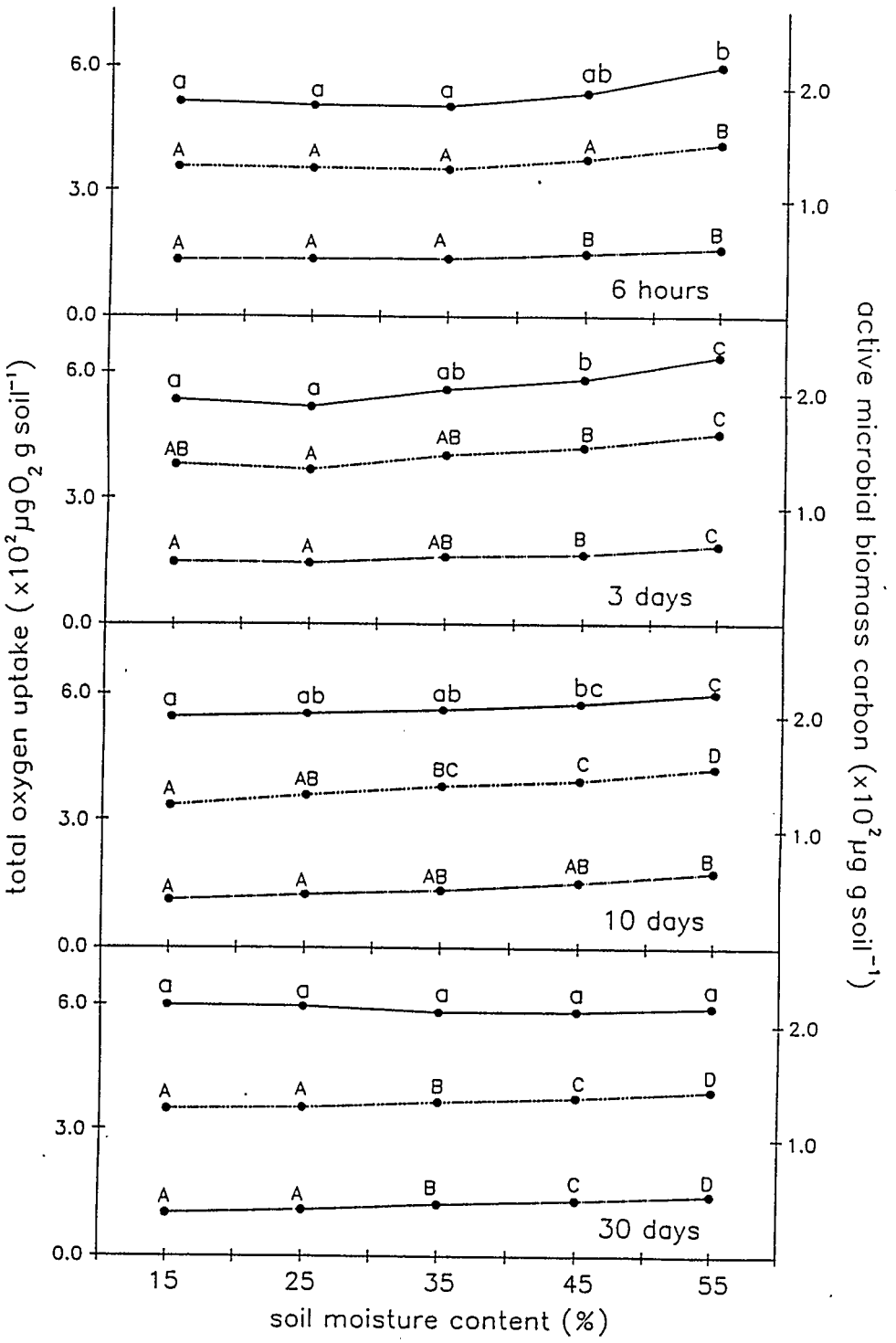
The active microbial biomass, calculated using the equation of Van de Werf and Verstraete (1987b), increased with increasing soil moisture at 6 hours, 3 days and 10 days, but not at 30 days incubation time (Figure 9). This means that when soils were amended with glucose monohydrate medium and remoistened to 55% moisture, drier soils took up less O_2 in 10 hours than moister ones, except at day 30. The ANOVA data showed significant interactions between "time of preincubation" and "moisture content" for the results of total O_2 uptake over 10 hours (Table 21). This suggests that the

Table 21. Relative importance of treatment effects on data presented in Figs. 9-13.

		Soil Moisture (C)		Time of pre-incubation (D)		C X D Interaction		Blocking		Unexplained
		% of SS[1]	F	% of SS	F	% of SS	F	% of SS	F	% of SS
O_2 uptake	2 hr	32.9	41.9***	25.5	43.3***	3.9	1.6 ns	19.0	19.3***	18.7
	4 hr	32.6	27.9***	21.1	24.1***	1.7	0.5 ns	16.8	11.5***	27.6
	6 hr	37.4	25.2***	12.8	11.4***	4.8	1.1 ns	9.7	5.2***	35.8
	8 hr	29.7	15.2***	9.6	6.5***	6.6	1.1 ns	7.7	3.2*	46.4
	10 hr	22.5	13.8***	16.2	13.3***	10.8	2.2*	11.8	5.8***	38.7
S		70.8	101.1***	3.3	6.3***	2.4	1.2 ns	6.8	7.8***	16.6
S(R)		4.9	3.5*	4.9	4.7**	6.5	1.6 ns	50.5	28.9***	33.2
A		83.0	286.5***	3.2	14.8***	3.3	10.0***	3.6	3.8***	6.9
A(R)		15.0	10.6***	17.8	16.8***	11.3	2.7**	22.0	12.4***	33.7
B		83.6	342.2***	4.6	25.1***	3.0	4.1***	3.0	9.9***	5.8
B(R)		6.6	5.3***	24.4	26.1***	9.3	2.5**	30.0	19.3***	29.6
S:A		0.4	0.1 ns	2.1	0.8 ns	11.4	1.1 ns	6.7	1.6 ns	79.4
S(R):A(R)		5.3	2.6*	8.3	5.5**	9.1	1.5 ns	29.6	11.8***	47.8

[1] Relative of total sums of squares in data set explained by each effect.
A = inhibition of S.I.R. by actidione
S = inhibition of S.I.R. by streptomycin
B = inhibition of S.I.R. by actidione and streptomycin added together
S:A = bacterial:fungal ratio
R = sample remoistened prior to determination
*, **, *** = effect is significant at p=0.05, p=0.01, and p=0.001, respectively.
ns = effect not significant at p=0.05

Figure 9. Total oxygen taken up by amended soils in first two hours (dash-dot lines), six hours (dash-dot-dot lines) and ten hours (solid lines). The 10 hour result is substituted into the equation of Van de Werf and Verstraete (1987b) to provide a measure of active microbial biomass.


total oxygen uptake (x10²µgO₂ g soil⁻¹)
active microbial biomass carbon (x10² µg g soil⁻¹)
soil moisture content (%)
6 hours
3 days
10 days
30 days
0.0
3.0
6.0
1.0
2.0
15
25
35
45
55

magnitude of active microbial biomass was initially influenced by soil moisture content while later it was not.

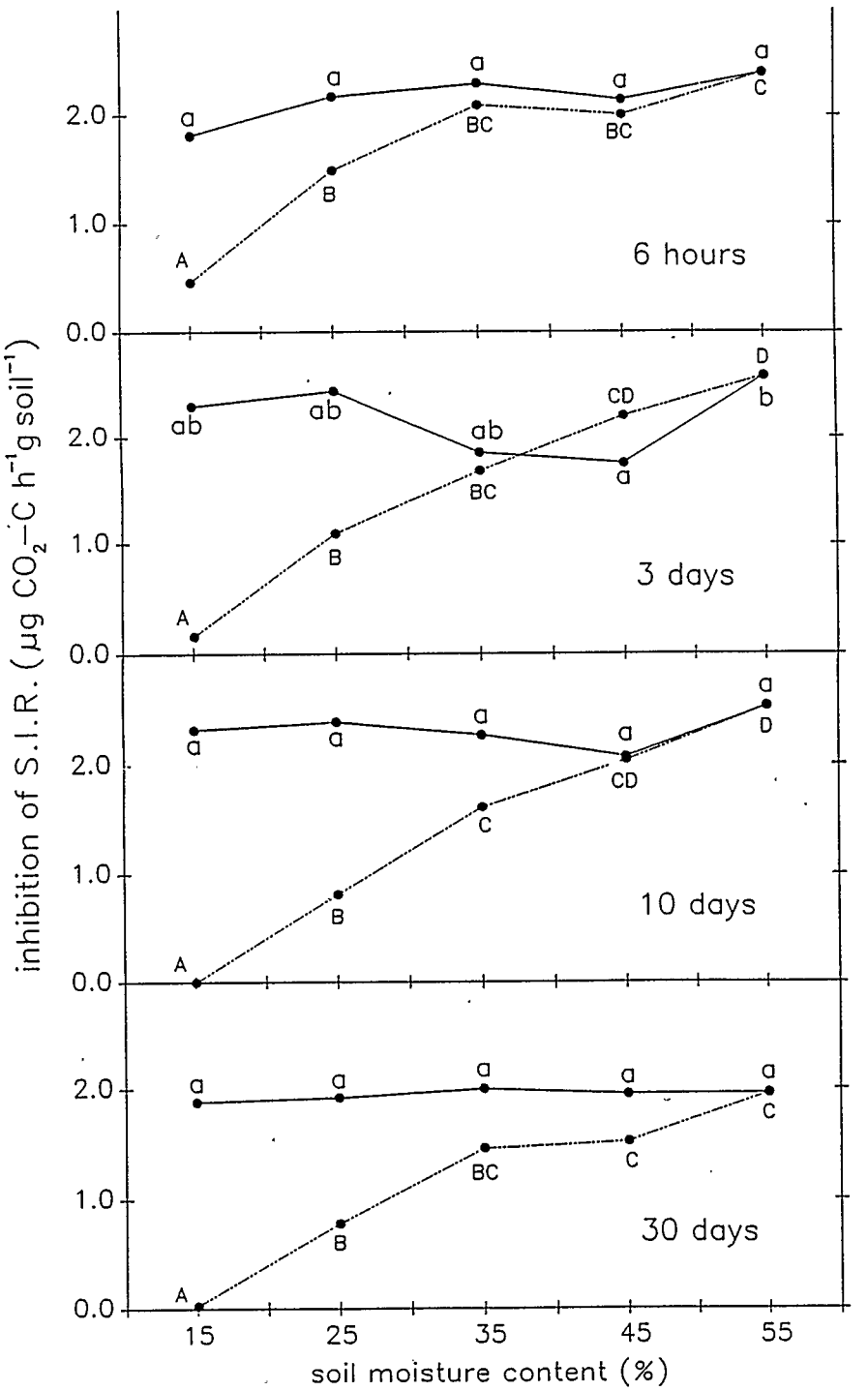
Although at day 30 the 10 hour total O_2 uptake data were not significantly influenced by soil moisture content, the total amount of oxygen taken up after 2, 4, and 6 hours after glucose amendment increased with increased moisture content. The total amount of oxygen taken up between 6 and 10 hours was highest for the driest soils at day 30; this was also true to a lesser extent for the 10 day results. For both the day 10 and 30 results, increasing responses to increasing soil moisture levels only occurred within 2 hours following glucose monohydrate addition.

Regardless of the time of measurement of oxygen uptake after glucose addition, soil moisture content, time of preincubation, and blocking effects all explained a significant proportion of the variability in the data set (Table 21).

Selective inhibition

Soil moisture content exerted a statistically significant but weak influence on the inhibition of S.I.R. by streptomycin, when samples were remoistened to 55% immediately before respiration measurements (Figure 10, Table 21). The driest and moistest samples never differed significantly. In contrast, the magnitude of S.I.R. inhibition by streptomycin was greatly reduced by lower moisture levels, when soil samples were not remoistened. In both remoistened and non-remoistened soils, time of preincubation exerted a significant

Figure 10. Inhibition of substrate-induced respiration by streptomycin sulphate, in soils remoistened to -33 kPa (solid lines) and soils at original moisture level (dash-dot-dot lines).


inhibition of S.I.R. (µg CO2–C h-1 g soil-1)
6 hours
3 days
10 days
30 days
0.0
1.0
2.0
15
25
35
45
55
soil moisture content (%)

although weak effect on streptomycin-induced inhibition. Blocking had a weak effect on non-moistened samples and a very strong one when samples were remoistened.

An essentially similar trend was observed when actidione was added (Figure 11, Table 21). When samples were remoistened prior to measurement, slight but statistically significant effects of soil moisture on inhibition of S.I.R. by actidione were observed. Within each preincubation time, this inhibition was largely independent of soil moisture status in remoistened soils, except that at 3 and 10 days the 45% moisture treatments were inhibited by actidione somewhat less than were the other moisture treatments at days 3 and 10. When soils were not remoistened, inhibition of S.I.R. by actidione increased as soil moisture increased. For both remoistened and nonremoistened soils, statistically significant but relatively weak effects were noted for blocking, time of preincubation, and the time x moisture interaction.

The same trends were also noted when actidione and streptomycin were added together (Figure 12, Table 21). Inhibition caused by both compounds was independent of soil moisture content when soils were remoistened, except at day 3 when the 55% treatment had higher values than the other 4 treatments. When soils were not remoistened, inhibition by both compounds was strongly related to soil moisture. Blocking, time of preincubation, and time x moisture interactions were all significant, although they explained a higher percentage of variation when soils were remoistened prior to measurement.

Figure 11. Inhibition of substrate-induced respiration by actidione, in soils remoistened to -33 kPa (solid lines) and soils at original moisture level (dash-dot-dot lines).

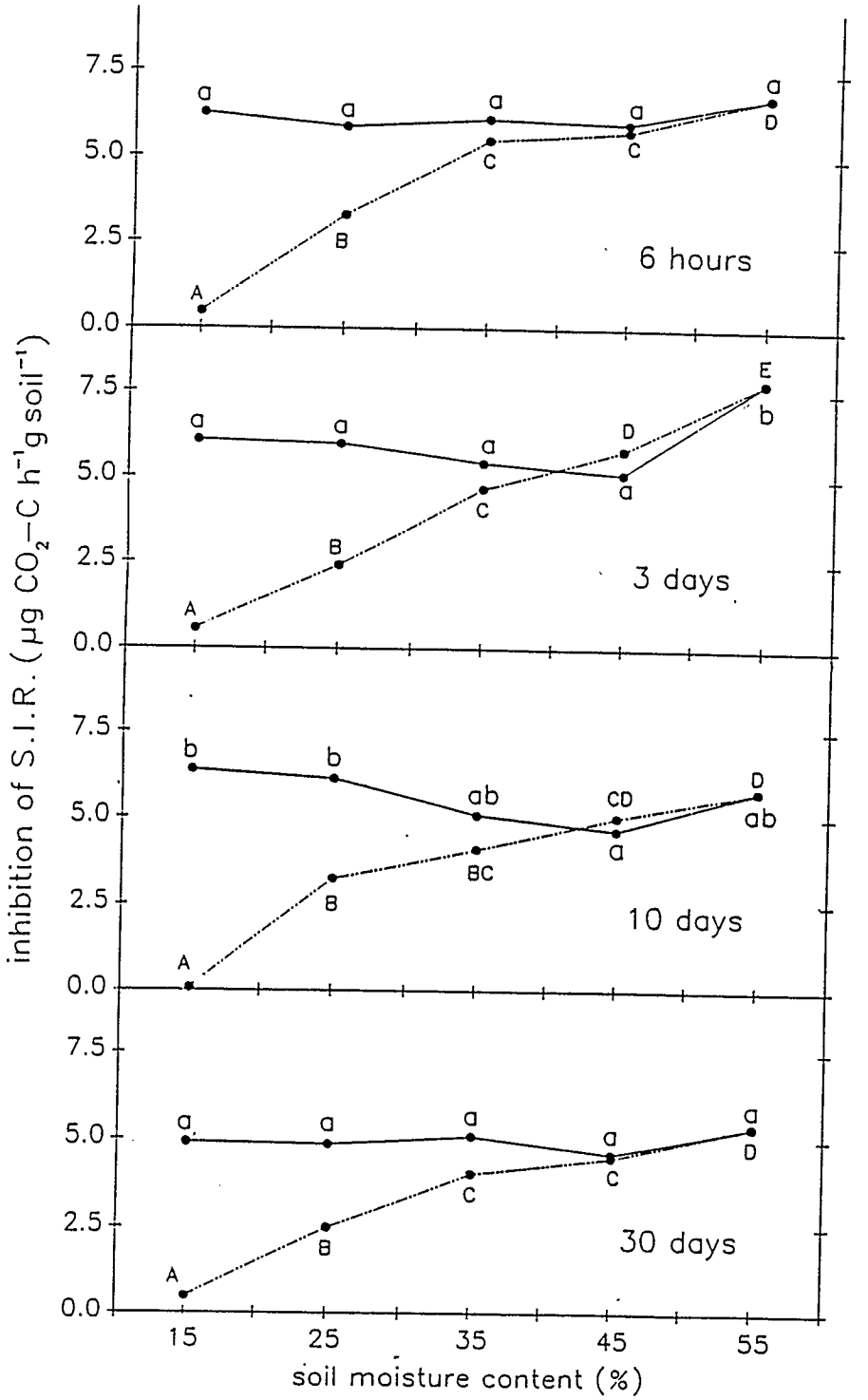
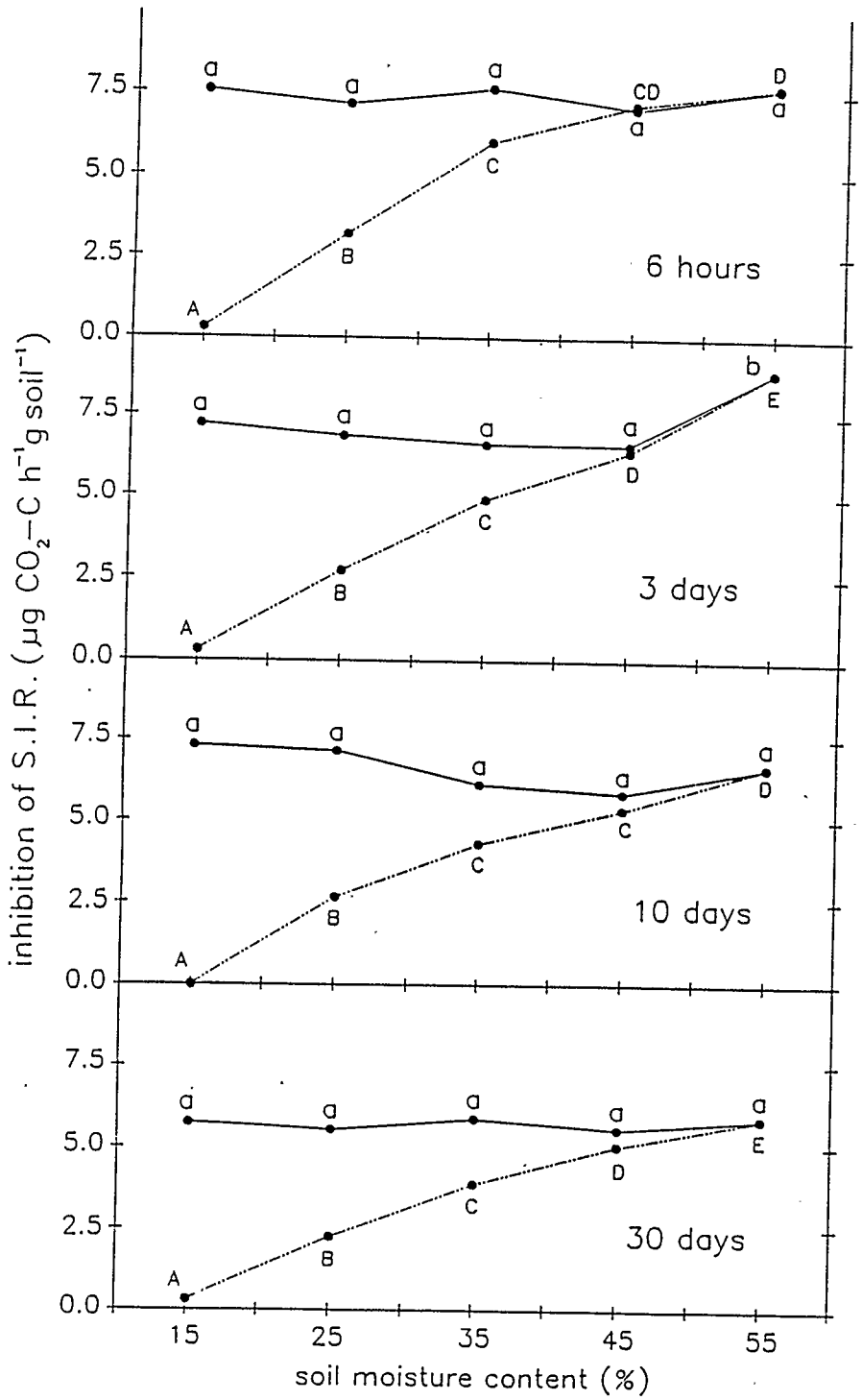

inhibition of S.I.R. (µg CO2–C h-1 g soil-1)
7.5
5.0
2.5
0.0
6 hours
3 days
10 days
30 days
15
25
35
45
55
soil moisture content (%)

Figure 12. Inhibition of substrate-induced respiration by actidione and streptomycin sulphate added in combination, in soils remoistened to -33 kPa (solid lines) and soils at original moisture level (dash-dot-dot lines).


inhibition of S.I.R. (µg CO_2–C h^{-1} g $soil^{-1}$)
7.5
5.0
2.5
0.0
6 hours
3 days
10 days
30 days
15
25
35
45
55
soil moisture content (%)

4.4.4 DISCUSSION

For the oxygen uptake curve method, the results suggested that the active microbial biomass was maximal at higher moisture contents, but only for preincubation periods of 6 hours or 3 days (and to a lesser extent, 10 days). However, for the day 10 and 30 results, there was higher oxygen uptake in the driest soils between hours 6 and 10 following addition of glucose medium. This is most likely due to decomposition of microbial biomass killed by drying. Results presented in the previous section (especially Table 18) indicated that soils preincubated at low moisture contents had higher basal respiration rates upon rewetting, when compared with soils preincubated at higher moisture contents. These differences were larger with 10 and 30 day preincubation periods, compared with shorter (6 hour and 3 day) preincubations. This was attributable to the flush of decomposition of organisms killed by drying. Flushes of respiratory activity caused by killed microorganisms (e.g. by chloroform) are known, from detailed preliminary studies involving the measurement of CO_2 evolution from this soil, to begin at 4 - 6 hours. Therefore, when a soil preincubated at a low moisture content for a number of days is remoistened and amended with glucose monohydrate, enhanced oxygen uptake is attributable initially to oxidation of glucose (0 - 4 hours) and later to decomposition of microbial biomass killed by drying (4 - 10 hours). Therefore, because the method of Van de Werf and Verstraete (1987a,b) assumes that all oxygen taken up in the first 10 hours is used to metabolize only those added compounds with a known biological oxygen demand

(B.O.D.), that technique is unsuitable for determining effects of soil moisture on the active microbial biomass, especially in very dry soils with a significant content of dead microbial biomass.

A variation of the method proposed by Van de Werf and Verstraete (1987b) was used in the present study. Van de Werf and Verstraete (1987b) proposed incubating soils at a moisture content of 3/4 field capacity for 10 days prior to biomass determination, presumably to allow the samples to equilibrate. However, because it is likely that the magnitude of the active microbial biomass will change to a new value during these ten days, this step was omitted. That is, by preincubating soils at five different moisture levels for the 4 preincubation times used in the present study, then adjusting them to 3/4 F.C. for 10 days prior to biomass measurement, the magnitude of the active microbial biomass for each sample would probably change over those 10 days, and converge to a common value.

The total amount of oxygen taken up in the first 4 or 6 hours following glucose amendment may be a more appropriate indicator of active microbial biomass than the 10 hour value, when moisture gradients are being considered. This is despite the observations of Van de Werf and Verstraete (1987b) who only found strong correlations between total oxygen uptake after 10 hours, and active microbial biomass estimated using microbial metabolic coefficients. Total oxygen uptake over a shorter time period did not predict active microbial biomass as accurately in their study. Oxygen uptake has also been used for providing a measure of microbial biomass

by Beck (in Domsch et al. 1979), and Beck (1984), but this involves considering only that oxygen taken up shortly after glucose addition.

When total oxygen uptake for 4 - 6 hours following glucose amendment is considered, the method becomes conceptually similar to Anderson and Domsch's (1978b) technique of substrate-induced respiration. In Section 4.3 the use of the S.I.R. method involved determining the respiratory response of the soil microbial biomass to an optimal level of glucose after about 4 hours following glucose amendment.

The coefficient of determination, r^2, was determined between the S.I.R. values (Section 4.3: Tables 16, 17) and total oxygen uptake at different times after glucose monohydrate amendment, for the 120 samples. These analyses are shown in Table 22. Very weak values of r^2 were found between total O_2 uptake at either 2, 4, 6, 8, or 10 hours, and S.I.R. (remoistened samples). Stronger r^2 values were found between S.I.R. (non-remoistened) and O_2 uptake initially after amendment, i.e., 2, 4, or 6 hours. These r^2 values were highly significantly above 0 although they still involved only about 25% of total variability being shared between the pairs of data sets which were compared with each other. Because it was suggested in Section 4.3.4. that S.I.R. is more likely to measure active (rather than total) microbial biomass and because S.I.R. values are more strongly correlated with total O_2 uptake at 2, 4, and 6 hours (compared with 8 or 10 hours), then the total amount of oxygen taken up before 6 hours is more likely to provide a suitable relative measure of active microbial biomass than that at 10 hours.

Table 22. Values of the coefficient of determination, r^2, between potential predictors of active microbial biomass for 120 samples.

	O_2 uptake					S.I.R.	
	2 hr	4 hr	6 hr	8 hr	10 hr		(R)
S.I.R.	.267	.249	.238	.145	.073	-	.114
S.I.R. (R)	.082	.058	.024	.000	.002	.114	-
A	.262	.248	.244	.153	.095	.919	.130
A(R)	.037	.030	.013	.003	.000	.023	.612
S	.209	.194	.193	.127	.069	.835	.154
S(R)	.006	.012	.004	.000	.010	.036	.360
A + S	.257	.241	.238	.152	.091	.927	.138
A(R) + S(R)	.012	.007	.004	.001	.002	.036	.704
B	.285	.269	.268	.169	.096	.933	.145
B(R)	.027	.164	.005	.000	.001	.191	.627

A = inhibition of S.I.R. by actidione

S = inhibition of S.I.R. by streptomycin

B = inhibition of S.I.R. by actidione and streptomycin added together

R = sample remoistened prior to determination

The arguments presented in Section 4.3.4 as well as those presented here suggest that S.I.R. may provide a suitable means to determine relative active microbial biomass. Meanwhile the use of selective inhibition may provide a relative measure of the active microbial biomass (both bacterial and fungal) in which active protein synthesis is occurring at the time of measurement of S.I.R. If the assumption is made that the inhibition of protein synthesis is proportionally related to the growth occurring at that time, then this growth should be related to the active microbial biomass. An r^2 analysis was performed across the 120 samples between pairs of methods which may have potential in predicting active microbial biomass (Table 22), using data collected from oxygen uptake curves, S.I.R., and selective inhibition. For non-remoistened samples S.I.R. was found to be very strongly related to total inhibition of S.I.R. by selective inhibitors either in combination or separately, with r^2 values always above 0.80. For remoistened samples, S.I.R. was relatively strongly related to inhibition by selective inhibitors (with remoistening), with r^2 values always above 0.60 except for streptomycin ($r^2 = 0.36$). However, these r^2 values may all be somewhat elevated because the S.I.R. measurement was one of the components used in the determination of selective inhibition. When results for selective inhibition were compared with those of total O_2 uptake, the only r^2 values which were significantly above 0 were between inhibition of S.I.R. by various inhibitors, and total oxygen uptake within 2, 4, or 6 hours. These r^2 values were typically above 0.20.

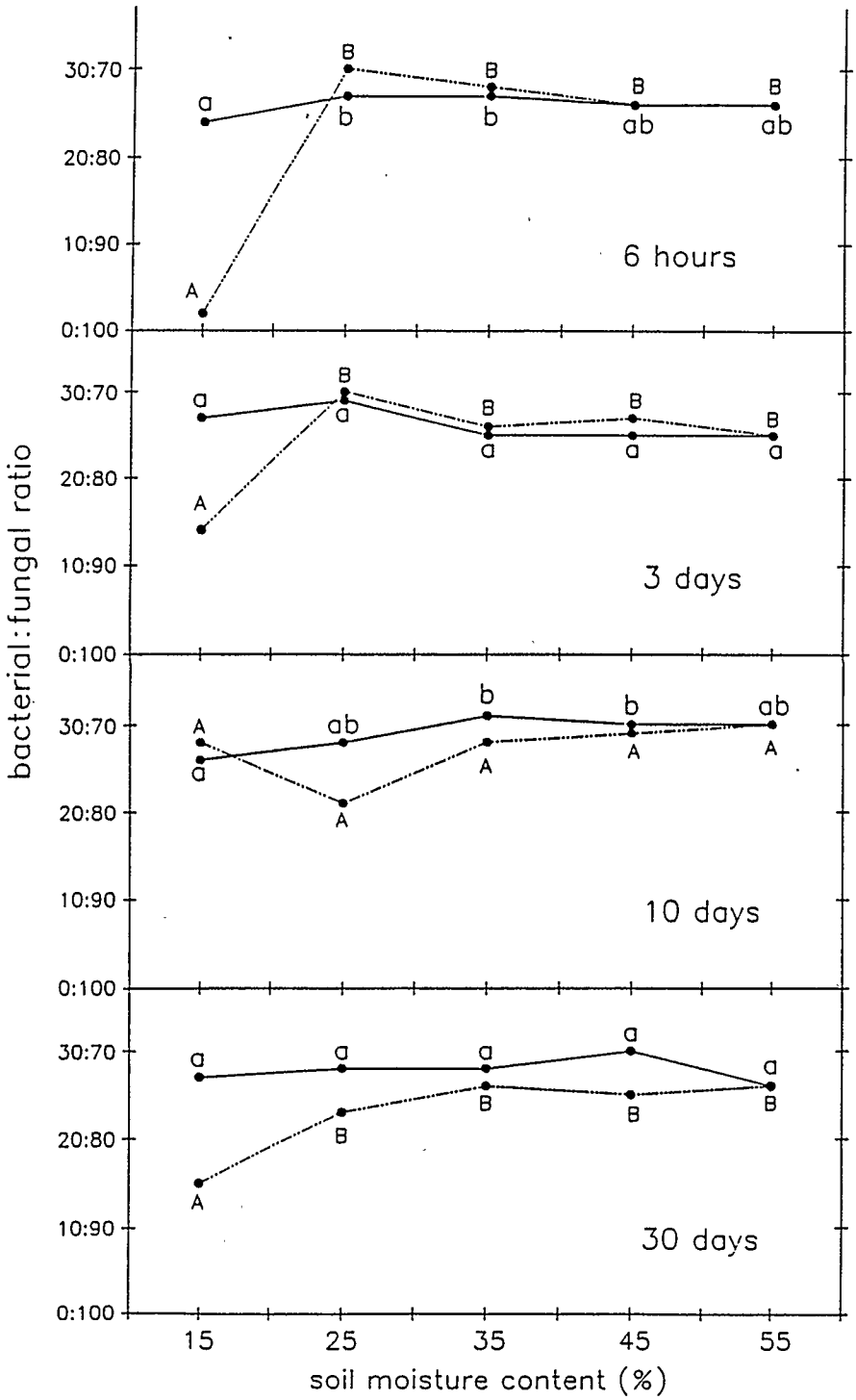
When active microbial biomass is investigated using S.I.R. or selective inhibition, very different results are obtained depending on whether or not soils are remoistened prior to measurement. West and Sparling (1986) and West (1986) recommend adding glucose and inhibitors to dry soil in a solution because otherwise they remain unavailable to the soil microbial biomass and because respiration is restricted in dry soils. However, because the present study recommends that S.I.R. and selective inhibition are more appropriate for assessing active rather than total microbial biomass, remoistening may not be appropriate for the following reasons. When a soil is maintained at a limiting moisture level (e.g. 15%) most of the soil microbial biomass is inactive and is therefore incapable of oxidizing glucose or initiating protein synthesis upon glucose addition. Remoistening a dry sample immediately activates a significant portion of the inactive microbial biomass because moisture is no longer limiting. This is coupled with increased activity of the active microbial biomass upon rewetting. This was demonstrated to be immediate through a preliminary experiment in which 5 soil samples were dried to 15% moisture content and preincubated for 1 day. Subsamples of each sample were then remoistened to 55% and this caused basal respiration to increase by 453% (95% C.I. = [305%, 655%]) in 1 hr or 687% (95% C.I. = [328%, 1345%]) in 4 hrs (n.b. these confidence intervals were calculated from $\log_e X$-transformed data). Therefore, for assessing active microbial biomass in dry soils remoistening immediately prior to measurement may be inappropriate even though not all of the glucose or inhibitor added dissolves immediately at low moisture levels.

One surprising observation in the present study was that the data on initial O_2 uptake by soils remoistened and amended with glucose were not significantly correlated with data from either S.I.R. or selective inhibition following remoistening, while oxygen uptake vs. S.I.R. or selective inhibition (non-remoistened samples) did provide significant correlations. There is no obvious explanation for this. However, when glucose monohydrate is added to soil in the oxygen uptake curve method, the quantities are below the levels required to saturate the microbial biomass, such as happens in the S.I.R. or selective inhibition methods. When remoistening is used in conjunction with two methods, one of which involves adding saturating levels of glucose, and the other involves adding lower amounts, then different results may occur.

Selective inhibition can be used to provide a bacterial: fungal ratio, but this ratio is for only those organisms undergoing protein synthesis following glucose addition. Therefore, this method gives data on the active (rather than the total) microbial biomass. Using this approach, the ratio of active bacterial to active fungal biomass was found in the present study to be largely unaffected by varying soil moisture content regardless of whether or not rewetting was performed prior to determination of this ratio (Figure 13, Table 21). The ratio was consistently between 20:80 and 30:70, except for the 15% soil moisture treatment (non-remoistened samples) where the ratio was highly variable and sometimes substantially lower (e.g., 2:98). At that moisture level, the magnitude of inhibition of S.I.R. by both streptomycin and actidione was so low

that accurate determination of this inhibition was sometimes difficult, and sometimes these inhibitions were measured as being slightly negative. At this moisture level, protein synthesis by both bacteria and fungi is extremely low and less accurately quantified.

Figure 13. Bacterial:fungal ratios for soils remoistened to -33 kPa (solid lines) and soils at original moisture level (dash-dot-dot lines).


bacterial:fungal ratio
30:70
20:80
10:90
0:100
6 hours
3 days
10 days
30 days
15
25
35
45
55
soil moisture content (%)

CHAPTER 5

5. INTERACTIONS BETWEEN MICROCLIMATIC VARIABLES AND THE SOIL MICROBIAL BIOMASS

5.1 INTRODUCTION

The dynamics of the soil microbial biomass is known to be strongly influenced by changes in soil moisture content and soil temperature (McGill et al. 1986; Kieft et al. 1987). Wetting-drying cycles have been implicated as having a considerable influence in regulating the rate of microbial biomass turnover (Sorenson 1974; Bottner 1985). Rewetting dried soils can cause a rapid enhancement in microbial activity (Lund and Goksoyr 1980; Taylor and Parkinson 1988a) which may be due in part to a flush of decomposition of those microbes killed by drying (Shields et al. 1974). Because change in microclimatic variables is important in regulating microbial biomass turnover, they are therefore important in regulating availability of nutrients immobilized by the soil microbial biomass (Anderson and Domsch 1980).

The purpose of the experiments outlined in this chapter was to investigate changes in relative microbial biomass and respiration in the site described in Chapter 2, and to relate these changes to fluxes in soil temperature and moisture. Because rapid shifts in environmental conditions are considered important in regulating the size (and therefore turnover) of the microbial biomass, emphasis was

placed on frequent sampling especially during periods of rapid fluctuations in soil microclimatic conditions.

5.2 MATERIALS AND METHODS

Plot Details

Within the study site, two plots (each approximately 8 x 8 m) were set up in 1987, and two in 1988. Each plot was regarded as a separate entity, and data from samples collected within one plot were not incorporated with data from other plots. Thus problems associated with pseudoreplication (see Hurlburt 1984) were avoided. The plots were tilled to 15 cm depth at least 7 days prior to the start of the studies in 1987 and 1988. Agricultural crops were not grown on the plots, and weeds were removed by hand weekly.

Sample and Data Collection

During 1987, two random soil samples were collected from each plot every 3 - 5 days, between April 30 and September 31 inclusive. Each sample was approximately 15 x 15 x 15 cm. The same, but somewhat less frequent, sampling procedure was followed between May 17 and August 27, 1988. In 1988 sampling was typically before and after rainfall events; this approach was based on 1987 data which suggested that fluxes in soil moisture were the dominant factor influencing substrate-induced respiration and basal respiration, rapidly following changes in soil moisture. Because of the low

spatial heterogeneity within plots, two samples were considered to be sufficient to detect important trends which might occur.

Each sample was sieved (4 mm) as soon as possible after collection, and the following determinations made:

a. Microbial substrate induced respiration, determined after the addition of 6000 ppm glucose to 30 g.d.w. soil samples, using the approach outlined in Section 4.3.2.

b. Basal respiration, determined using CO_2 evolution rates from soil samples of 60 g (d.w.) (see Anderson 1982). Respiration measurements were made when the rate of CO_2 evolution was found to be constant.

c. Basal respiration:substrate induced respiration ratio (i.e. a ÷ b, above). This was used to provide a relative measure of the respiration:biomass ratio, as proposed by Odum (1969, 1985).

d. Soil gravimetric moisture content, on 20 g (net weight) soil, after drying at 105°C for 24 hours.

Also, throughout the experimental period, one thermograph was placed in each plot, which continuously measured soil temperature at a depth of 10 cm, recording the data separately for each of 3 randomly located probes.

Data Analysis

For each plot, correlation analysis was used to determine the strength and significance of relationships between the microbial and climatic variables by treating each sampling period as an independent data point. Data transformations were used to fulfil the assumptions of normality for parametric analyses, if required.

The variables were used as follows:

(i) microbial variables:

SIR: microbial substrate induced respiration, in $\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$

DSIR: percent change in SIR between the present (n) and previous (n - 1) sampling period, i.e. $[(SIR_n - SIR_{n-1})/SIR_{n-1}] \times 100$

The values were then subjected to the following transformations:

1987 data: $\log_e(X/100 + 1)$

1988 data: $\sqrt{[(\log_e X/100 + 1) + 1]}$

BRES: microbial basal respiration in $\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$

DBRES: percent change in RESP between the percent (n) and previous (n - 1) sampling period, i.e. $[(BRES_n - BRES_{n-1})/BRES_{n-1}] \times 100$

These values were then subjected to the same transformations as for the DSIR data.

RAT: ratio of basal respiration to substrate induced respiration

DRAT: percent change of RAT between present (n) and previous (n - 1) sampling periods, i.e. $[(RAT_n - RAT_{n-1})/RAT_{n-1}] \times 100$

(ii) Climatic variables:

WAT: percentage of water in the soil on a dry-weight basis

DWAT: percentage change in water between present (n) and previous (n - 1) sampling periods, i.e. $[(WAT_n - WAT_{n-1})/WAT_{n-1}] \times 100$ This data was then transformed by the $\log_e(X/100 + 1)$ transformation.

Soil temperature data were divided into five variables, i.e. the percentage of time in the previous 72 hours that the temperature was in each of five classes: <5°C, 5-10°C, 10-15°C, 15-20°C, and >20°C. Because the data for these variables was inherently non-normal, they were rank-transformed. The rank-transformed variables were then assigned the following names: T(<5), T(5-10), T(10-15), T(15-20), and T(>20), for each of the five classes.

To summarize the entire temperature data in terms of fewer variables, principal components analysis was performed on the sampling time x temperature class matrix. The variables representing the first and second principal axes (explaining most of the variation) were assigned the variable names PCI and PCII.

5.3 RESULTS AND DISCUSSION

Basal Respiration

The magnitude of the soil basal respiration through the experimental period is shown in Figure 14 for 1987 and Figure 15 for 1988. The pattern of changes in respiration appear to follow the changes in soil moisture, with rapid wetting/drying periods leading to rapid shifts in basal respiration.

Correlation analysis between basal respiration and the microclimatic variables is shown in Table 23. Significant correlations were found between basal respiration (BRES) and changes in soil moisture (DWAT) in all cases; correlations with absolute soil moisture content (WAT) were, however, weak and usually not significant. In all but one instance, the value of r between BRES and DWAT remained significant, even when variation in the soil temperature variables (e.g. PCI), were held constant using partial correlation.

Significant negative correlations were found between BRES and T(<5) for both plots 1 and 2 (May 1 - September 25). T(5-10) and BRES were significantly negatively correlated for plot 1 (May 1 - September 25) and positively correlated for plots 3 and 4. Significant negative correlations were found between BRES and T(10-15) for plot 1 (May 1 - September 25) while for plot 2 (June 15 - August 31) negative correlations occurred between BRES and T(15-20) and T(>20). For plot 4, BRES and T(>20) were also significantly negatively correlated. It is likely that negative correlations between the highest temperatures (in plots 2 and 4) and BRES can be attributed to the role of soil moisture as the driving force in the system; i.e. rainfall events simultaneously enhancing microbial activity while depressing the soil temperature. When variations in DWAT were corrected for through partial analysis, significant negative correlations occurred between low temperature variables and BRES for plot 1, and a positive correlation between T(10-15) and BRES also occurred. However, for plot 2 (June 15 - August 31) a significant negative partial correlation coefficient was detected

Table 23. Values of Pearsons correlation coefficient, r, between values for basal respiration (BRES) and climatic variables.

Independent variables(s)[1]	Plot number and date (inclusive) 1 May 1- Sept 25 1987	1 June 15- Aug 31 1987	2 May 1- Sept 25 1987	2 June 15- Aug 31 1987	3 May 26- Aug 27 1988	4 May 26- Aug 27 1988
DWAT	.453**	.687**	.329*	.607**	.715**	.820**
[DWAT]	.570**	.664**	.302	.590**	.701**	.755**
T(<5)	-.346*	-	-.402**	-	-	-
T(5-10)	-.321*	.375	-.001	.397	.454*	.543*
T(10-15)	.434**	.304	.250	.206	.078	.255
T(15-20)	.296	-.409	-.125	-.549**	-.404	-.368
T(>20)	.199	-.418	-.198	-.453*	-.265	-.470*
[T(<5)]	-.350*	-	-.426**	-	-	-
[T(5-10)]	-.362*	.327	-.001	.476**	.178	.265
[T(10-15)]	.443**	.160	.226	-.130	.292	.346
[T(15-20)]	.313	-.408	-.087	-.522*	-.326	-.258
[T(>20)]	.155	-.387	.179**	-.398*	-.326	-.461
PCI	.370*	.451*	.443**	.520*	.391	.478*
[PCI]	.567**	.397	.426**	.497*	.148	.410
DWAT,PCI	.564**	.744**	.519**	.724**	.734**	.817**

[1] Square bracket around climatic variable names indicate that partial correlation analysis was used, after holding DWAT constant (temperature variables) or PCI (moisture variable).

*,** indicates that correlation coefficient is significantly different to 0 at p=0.05 and p=0.01 respectively.

Figure 14. Measurements of microclimatic and microbial variables during the 1987 field season. Solid lines = plot 1; dash-dot-dot lines = plot 2.

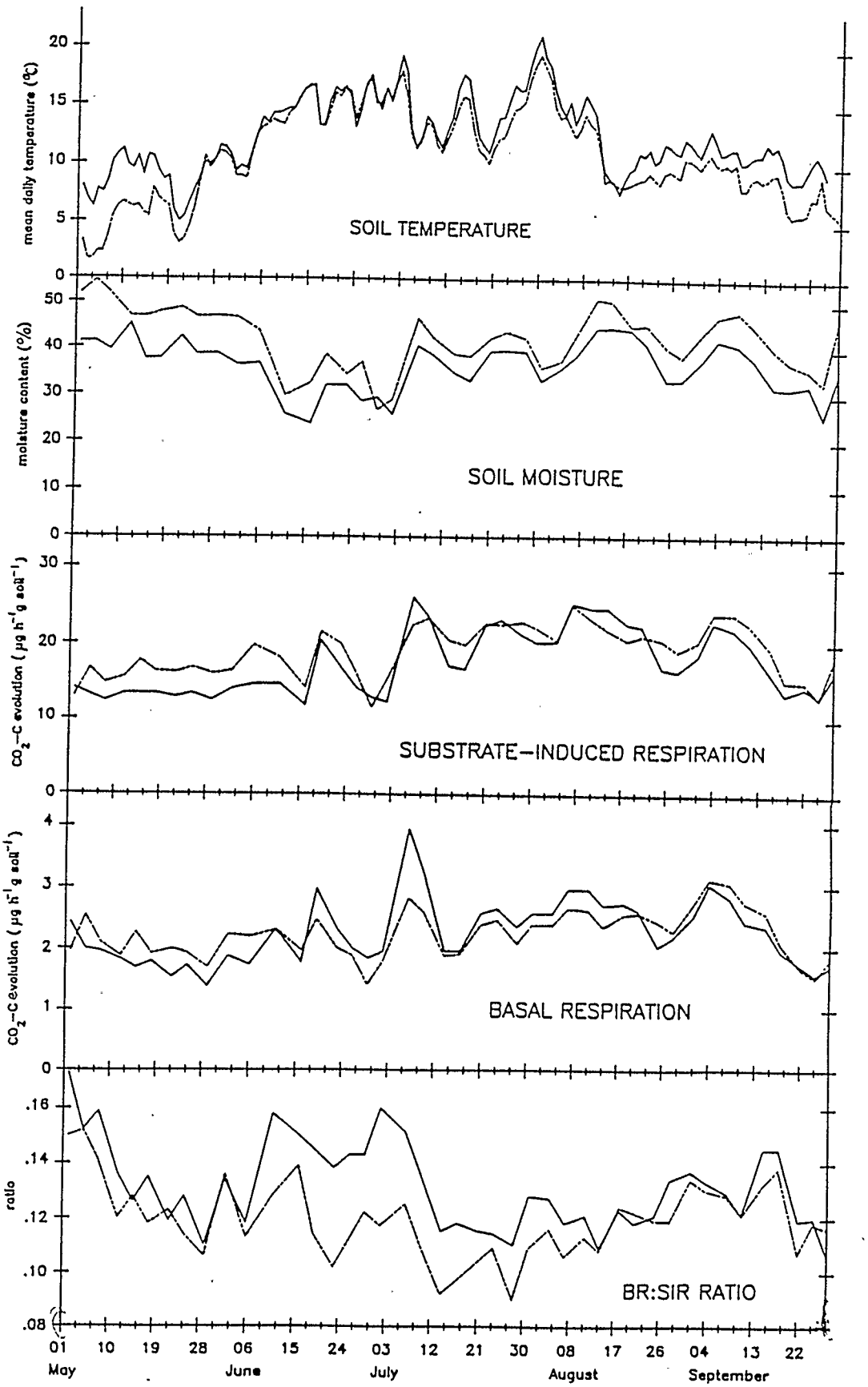
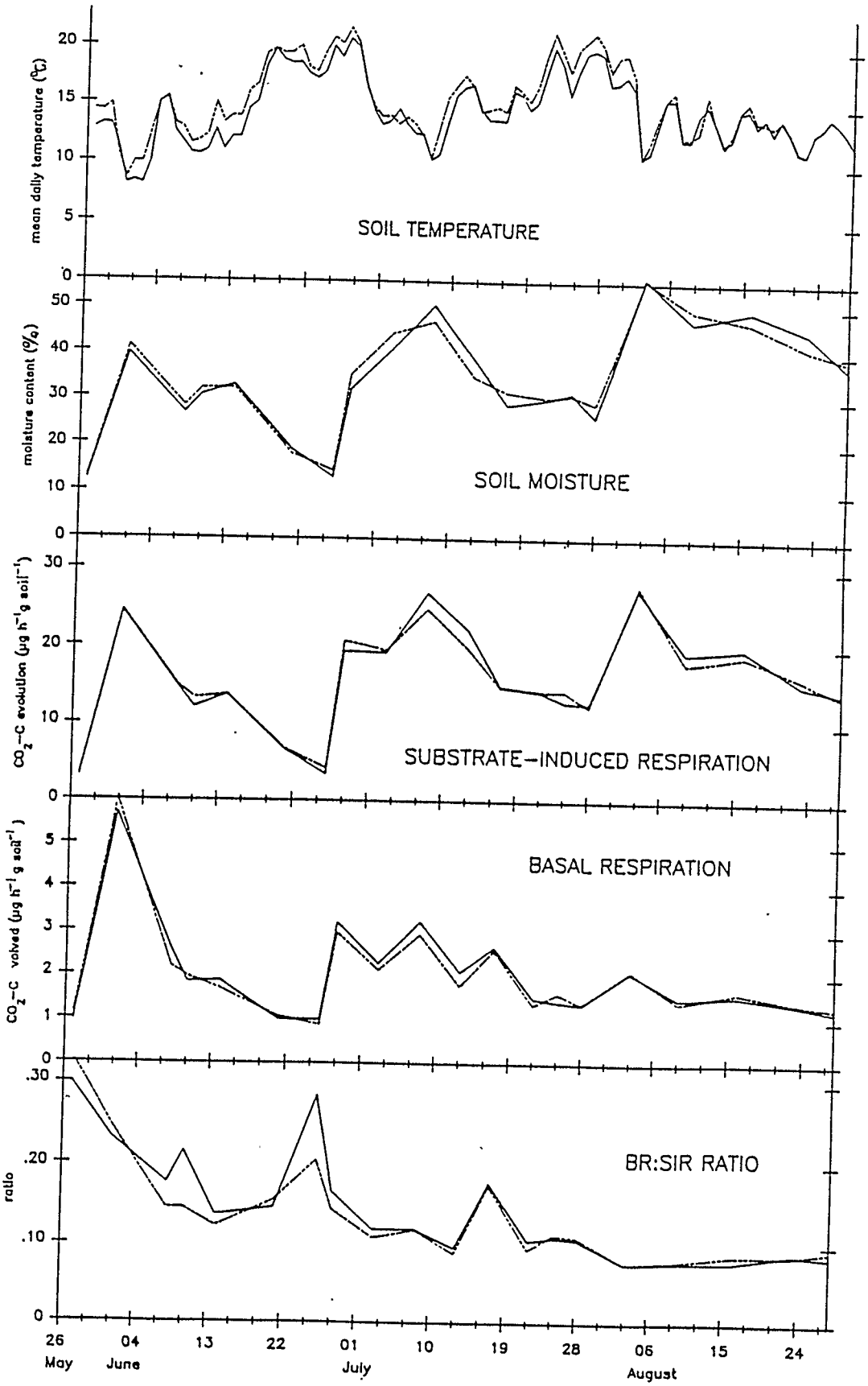

mean daily temperature (°C)
SOIL TEMPERATURE
moisture content (%)
SOIL MOISTURE
CO_2–C evolution (µg h^{-1} g $soil^{-1}$)
SUBSTRATE–INDUCED RESPIRATION
CO_2–C evolution (µg h^{-1} g $soil^{-1}$)
BASAL RESPIRATION
ratio
BR:SIR RATIO
01 10 19 28 06 15 24 03 12 21 30 08 17 26 04 13 22
May
June
July
August
September

Figure 15. Measurements of microclimatic and microbial variables during the 1988 field season. Solid lines = plot 3; dash-dot-dot lines = plot 4.


mean daily temperature (°C)
SOIL TEMPERATURE
moisture content (%)
SOIL MOISTURE
CO_2–C evolution (µg h^{-1} g soil^{-1})
SUBSTRATE–INDUCED RESPIRATION
CO_2–C valved (µg h^{-1} g soil^{-1})
BASAL RESPIRATION
ratio
BR:SIR RATIO
26 May
04 June
13
22
01 July
10
19
28
06 August
15
24

between BRES and T(5-10), and a positive one for BRES and T(15-20). There is, therefore, apparently a positive influence of high temperature on BRES in plot 1 (May 1 - September 25) while during a portion of the same season, there was an apparent negative effect of high temperature (or positive effect of low temperature) on BRES in plot 2 (June 15 - August 31). There is no obvious explanation for this, but in the drier portions of the summer it is possible that there is another factor (other than soil moisture) which is associated with temperature, that causes enhanced respiration at lower temperatures, e.g. that temperatures at 5 - 10°C may directly or indirectly kill a small component of the soil microbial biomass, thus enhancing the respiration of the surviving microorganisms through enhanced substrate availability in the form of dead microbes.

Significant relationships occurred between PCI and BRES in plots 1, 2, and 4; and these remained significant in plots 1 and 2 when variations in DWAT were held constant using partial correlation. Also, for plots 1 and 2 significant enhancements of r occurred when both PCI and DWAT were correlated together against BRES rather than when each variable was used alone.

With regard to changes in basal respiration (DBRES) significant correlation occurred with DWAT regardless of whether the variations in soil temperature (i.e. PCI) were held constant (through partial correlation) or not (Table 24). With regard to temperature, DBRES was significantly correlated only with T(5-10) for plot 4 and with PCI for plot 3, and only when partial analysis correcting for DWAT was not used. These coefficients were

Table 24. Values of Pearsons correlation coefficient, r, between values for changes in basal respiration (DBRES) and climatic variables.

	Plot number and date (inclusive)					
	1	1	2	2	3	4
Independent variables(s)[1]	May 1-Sept 25 1987	June 15-Aug 31 1987	May 1-Sept 25 1987	June 15-Aug 31 1987	May 26-Aug 27 1988	May 26-Aug 27 1988
DWAT	.438**	.791**	.547**	.718**	.792**	.828**
[DWAT]	.441*	.787**	.535**	.702**	.719**	.810**
T(<5)	-.116	-	-.143	-	-	-
T(5-10)	-.131	.113	-.063	.090	.287	.464*
T(10-15)	.205	.120	.160	.269	-.231	.093
T(15-20)	.103	-.234	-.118	-.320	-.089	-.196
T(>20)	.057	-.034	-.134	-.223	-.064	-.273
[T(<5)]	-.157	-	-.173	-	-	-
[T(5-10)]	-.152	-.086	-.073	-.097	-.010	.231
[T(10-15)]	.191	-.174	.167	-.135	-.141	-.027
[T(15-20)]	.102	-.161	-.055	-.218	.098	.205
[T(>20)]	-.003	.217	-.102	-.076	-.043	-.149
PCI	.150	.149	.236	.250	.493*	.298
[PCI]	.137	.082	.195	.135	.080	-.055
DWAT,PCI	.525**	.792**	.572**	.723**	.789**	.829**

• Legend as for Table 23.

presumably positive because of the effects of increasing DWAT simultaneously depressing soil temperature while promoting DBRES. These coefficients are not significant when variation in soil moisture is held constant. Changes in soil moisture content appear to be the only variable which causes short-term shifts in basal respiration.

Substrate-Induced Respiration

The magnitude of subtrate-induced respiration (SIR) used in this study as a relative measure of microbial biomass (Anderson and Domsch 1978b), is shown for 1987 in Figure 14 and 1988 in Figure 15. For the 1987 data, it appears that the relative microbial biomass remained relatively constant until mid-June, then fluctuated somewhat more rapidly, apparently following fluctuations in soil moisture content during wetting and drying cycles. In 1988, the relative microbial biomass fluctuated very rapidly, and paralleled fluctuations in soil moisture content.

Correlation analysis between SIR and the microclimatic variables is shown in Table 25. Significant correlations were found between soil moisture content (WAT) and SIR in all plots regardless of whether or not the main temperature variable (i.e. PCI) was held constant using partial correlation analysis. Correlations were strongest during periods of rapid wetting and drying, i.e. June 15 to August 31, 1987 for plots 1 and 2; and the entire 1988 season for plots 3 and 4.

Relationships between the various temperature variables and MSIR were unpredictable when WAT variations were not corrected for

Table 25. Values of Pearsons correlation coefficient, r, between values for substrate induced respiration (SIR) and climatic variables.

	Plot number and date (inclusive)					
	1	1	2	2	3	4
Independent variables(s)[1]	May 1-Sept 25 1987	June 15-Aug 31 1987	May 1-Sept 25 1987	June 15-Aug 31 1987	May 26-Aug 27 1988	May 26-Aug 27 1988
WAT	.484**	.860**	.568**	.921**	.866**	.910**
[WAT]	.563**	.799**	.533**	.901*	.890**	.881**
T(<5)	-.305	-	-.417**	-	-	-
T(5-10)	-.217	.470*	.023	.372	.408	.554**
T(10-15)	.360*	.233	.374*	.317	.185	.293
T(15-20)	.199	-.512*	-.046	-.424*	-.271	-.380
T(>20)	.134	-.531*	-.294	.546**	.363	.350
[T(<5)]	-.492**	-	-.767**	-	-	-
[T(5-10)]	-.570**	.001	-.273	-.363	-.126	-.149
[T(10-15)]	.454**	-.159	.549*	.325	-.309	.129
[T(15-20)]	.557**	.003	.402	.260	-.126	-.073
[T(>20)]	.387*	-.078	.074	.170	.269	.111
PCI	.276	.533*	.558**	.504*	.182	.457*
[PCI]	.600**	.068	.521**	.273	.289	.104
DWAT,PCI	.707**	.861**	.712**	.927**	.897**	.907**

[1] Square bracket around climatic variable names indicate that partial correlation analysis was used, after holding WAT constant (temperature variables) or PCI (moisture variable).

*,** indicates that correlation coefficient is significantly different to 0 at p=0.05 and p=0.01 respectively.

using partial correlation. Significant negative correlations were found in plot 2 for T(<5) (May - September 25) and in plots 1 and 2 for T(15-20) and T(>20), during June 15 - August 31. Significant positive correlations were detected for T(5-10) for plots 1 and 4, and T(10-15) for plots 1 and 2. As for the BRES data, negative correlations between high temperatures and SIR (or, sometimes, positive correlations between relatively low temperatures and SIR) are best explained in terms of soil moisture relationships; i.e. during the warmer periods of the summer moisture is often limiting and relatively dry periods are associated with higher temperatures. Rainfall, therefore, causes a rapid increase in soil moisture, with usually a drop in soil temperature, and increased MSIR. This is borne out further using partial correlation analysis, controlling for soil moisture variation. When this is done, all significant values for r between MSIR, and T(<5), or T(5-10) are negative; all for T(10-15), T(>20), or T(20>25) are positive.

When variations in WAT are held constant using partial correlation, the relationships between the various temperature variables and PCI (or PCII) are stronger for plots 1 and 2 when the whole season is considered, rather than only for the drier warmer months. This is presumably because when the periods May 1 - June 14 and September 1 - September 25 are included (as well as June 15 - August 31) in the analysis, overall microbial biomass fluctuations are less driven by soil moisture, and soil temperature becomes relatively more important in comparison with WAT as a force influencing the soil microbial biomass. In these two cases (c.f. the other 4)

the value of r when both PCI and WAT are included is considerably higher than when WAT only is used.

Correlation analysis between the microclimatic variables and relative change in the microbial biomass (DSIR) are shown in Table 26. Significant correlations were found between changes in soil moisture (DWAT) and DSIR in all cases, whether or not variation in soil temperature (i.e. PCI) was controlled using partial correlation. No significant correlations were found between DSIR and WAT. With regard to temperature variables, few significant correlations occurred. There was a significant correlation between DSIR and T(5-10) in plot 4, and between DSIR and PCI in plot 3, but these were only significant when partial correlation (correcting for DWAT) was not used. This indicates that any relationships between lower soil temperatures and changes in soil microbial biomass were merely associated with improved soil moisture status. Short term changes in DSIR are influenced primarily by changes in DWAT, with variations in DWAT explaining between 40.0% and 88.9% of the variation in DSIR. Because the temperature variables used in this study (or other temperature variables used in preliminary analyses of this data but not reported here) typically did not correlate well with DSIR but did correlate well with SIR, soil temperature (unlike soil moisture) influences microbial biomass only in the long term (e.g. a few days).

If the SIR method can be presumed to provide relative measures of the total soil microbial biomass (Anderson and Domsch 1978b), the turnover time can be determined for this biomass. Fogel

Table 26. Values of Pearsons correlation coefficient, r, between values for changes in substrate induced respiration (DSIR) and climatic variables.

	Plot number and date (inclusive)					
	1	1	2	2	3	4
Independent variables(s)[1]	May 1-Sept 25 1987	June 15-Aug 31 1987	May 1-Sept 25 1987	June 15-Aug 31 1987	May 26-Aug 27 1988	May 26-Aug 27 1988
DWAT	.718**	.878**	.638**	.714**	.955**	.882**
[DWAT]	.709**	.860**	.630**	.712**	.943**	.893**
T(<5)	-.130	-	-.084	-	-	-
T(5-10)	-.018	.220	-.074	-.009	.309	.458*
T(10-15)	.198	.254	.213	.317	-.180	.144
T(15-20)	.026	-.284	-.017	-.153	-.187	-.358
T(>20)	.030	-.127	-.057	-.082	-.018	-.220
[T(<5)]	-.214	-	-.111	-	-	-
[T(5-10)]	-.109	.083	-.092	-.044	-.287	.211
[T(10-15)]	.270	.017	.180	-.054	-.026	.059
[T(15-20)]	.076	-.280	.088	.027	.042	-.060
[T(>20)]	-.063	.117	.002	.131	.090	-.034
PCI	.080	.085	.181	.106	.514*	.336
[PCI]	.130	.260	.122	.076	.288	.104
DWAT,PCI	.712**	.879**	.645**	.716**	.960**	.891**

• Legend as for Table 23.

and Hunt (1979) and McGill et al. (1986) have provided equations for determining turnover time of various soil microbial components. In this study, microbial biomass turnover was determined by using a modification of these relationships, i.e.

$$T = \frac{N\bar{X}}{\sum_{i=2}^{j} (/X_i - X_{i-1}/)}$$

where N = time over which S.I.R. measurements are determined (days)

j = number of measurements of S.I.R. made during time N

X = substrate-induced respiration ($\mu g\ CO_2\text{-}C \cdot h^{-1}\ g\ soil^{-1}$)

$\bar{X}$ = mean S.I.R. during time N ($\mu g\ CO_2\text{-}C \cdot h^{-1}\ g\ soil^{-1}$)

T = turnover time of the microbial biomass (days)

The turnover times of the microbial biomass for each plot are shown in Table 27. For the 1987 data the turnover time of the microbial biomass was approximately 50% higher when the whole experimental period was considered than for only June 15 - August 31 when wetting-drying cycles were more rapid. In 1988, the turnover time was considerably shorter, indicative of very rapid fluctuations in the microbial biomass caused by rapid wetting-drying cycles.

The size and turnover of the soil microbial biomass is well known to be influenced by microclimatic factors, especially soil moisture and wetting/drying cycles. Bottner (1985) has demonstrated reductions of microbial biomass caused by artificial drying of soil samples. Schnürer et al. (1986) noted changes in both fungal and

Table 27. Turnover time of the soil microbial biomass during 1987 and 1988 in four experimental plots.

Plot number and date (inclusive)	Turnover time (days)
1 (May 1-Sept 25, 1987)	30.9
1 (June 15-Aug 31, 1987)	20.0
2 (May 1-Sept 25, 1987)	45.3
2 (June 15-Aug 31, 1987)	30.6
3 (May 26-Aug 27, 1988)	15.2
4 (May 26-Aug 27, 1988)	13.9

bacterial components following wetting and drying treatments of field plots. McGill et al. (1986) determined that between 33% and 69% of the variability in microbial biomass dynamics over two seasons could be explained in terms of soil moisture or temperature dynamics. The general trends observed in the present study are in agreement with this, i.e. that microclimatic variations, and primarily soil moisture fluxes, appear to be the driving force with regard to the size of the soil microbial biomass. In the present study, the turnover time of the soil microbial biomass is considerably lower than that determined by either Jenkinson and Ladd (1981) (i.e. 1.7 - 2.5 years) or McGill et al. (1986) (i.e. 0.2 - 3.9 years). One possible reason for this difference is that the wetting drying cycles recorded in the present study were frequent and extreme. Also, microbial biomass determinations were made relatively frequently, i.e. every 3 - 5 days in 1987, or before and after rainfall events in 1988. Therefore, short term fluctuations of microbial biomass could be followed closely. Another possibility is that the use of different methods for measuring the microbial biomass may yield different results. The chloroform incubation technique (Jenkinson and Powlson 1976), as used by McGill et al. (1986), assumes that the decomposition ($\underline{k}_c$) factor of the microbial biomass following fumigation remains invariant of the soil moisture content prior to measurement, possibly influencing the results of (relative) microbial biomass determinations. Conversely, with substrate induced respiration, it is likely, especially for extremely dry samples (such as occurred occasionally for the 1988

samples), much of the added glucose did not dissolve and thus remained unavailable to the soil microflora, thus resulting in an underestimate of the microbial biomass and the turnover time. Because the active component of the microbial biomass is more likely to respond to glucose addition than the inactive component (van de Werf and Verstraete 1987) S.I.R. measurements may also be biased towards the active component. If part of the inactive microbial biomass is activated upon rewetting, and is inactivated upon drying, the turnover of the total microbial biomass may be estimated to be more rapid than it actually is.

In the present study the soil biomass appeared to be killed by drying and promoted by rewetting. The microorganisms killed by the drying process are decomposed and this substrate is both released as enhanced respiration and used to build new microbial biomass. This is a well-documented phenomenon and enhancement of soil respiration by rewetting dry soils is also documented by Adu and Oades (1978), Lund and Goksoyr (1980), and Orchard and Cook (1983). That the enhanced respiration is derived from decomposing microbial cells has been demonstrated using ^{14}C studies, e.g. Shields et al. (1974); Sorenson (1974).

Basal Respiration:Substrate-Induced Respiration Ratio

Part of Odum's (1969, 1985) theory of ecosystem succession predicts that ecosystems which have been subjected to disturbance have a higher respiration-biomass ratio than those which have not. This approach has been applied to soil ecosystems (Anderson and

Domsch 1985, Insam and Domsch 1988) using a microbial basal respiration:microbial biomass ratio. Because SIR gives a relative measure of microbial biomass, in the present study the ratio of basal respiration to substrate-induced respiration (RAT) could be used to provide a relative measure of the respiration:biomass ratio.

The magnitude of RAT over time is shown for 1987 in Figure 14 and 1988 in Figure 15. This ratio does not show such rapid fluctuations as found for BRES and SIR.

Correlation analysis demonstrated that RAT is significantly negatively correlated with WAT regardless of whether or not temperatures variables were held constant (Table 28). This suggests that RAT is enhanced by low soil moisture conditions. This is most probably because in dry soil systems, much of the microbial biomass has been killed through the drying process, and those microbes which survive have a readily available supply of substrate in the form of killed microorganisms. Conservation of carbon resources is not as important (carbon is not as limiting) as in a more "stable" (persistent) system and a disproportionately large amount of C is released as CO_2, hence enhancing the value of RAT.

Soil moisture effects on RAT were strongest during drier periods, i.e. June 1 - August 31, 1987 (plot 2) and the 1988 season (plots 3 and 4), especially when partial analysis was used to hold variations in soil moisture constant. For each of plots 2, 3, and 4, positive correlations were detected between RAT and T(5-10). Negative correlations were found between RAT and T(15-20) in plots 2 and 3, and between RAT and T(>20) in plot 4. For those three plots

PCI was significantly correlated with RAT when variations in WAT were held constant. This indicates that in dry conditions lower temperatures create conditions of disturbance which enhance the BRES:SIR ratio and contributes to a less "stable" (or less persistent: Richards 1987) ecosystem. The least stable combination of climatic variables would therefore be low temperature, low moisture conditions.

Table 28. Values of Pearsons correlation coefficient, r, between values for the basal respiration:substrate induced respiration ratio (RAT) and climatic variables.

	Plot number and date (inclusive)					
	1	1	2	2	3	4
Independent variables(s)[1]	May 1-Sept 25 1987	June 15-Aug 31 1987	May 1-Sept 25 1987	June 15-Aug 31 1987	May 26-Aug 27 1988	May 26-Aug 27 1988
WAT	-.411	-.658**	-.717**	-.830**	-.620**	-.621**
[WAT]	-.383*	-.646**	-.703**	-.866**	-.765**	-.727**
T(<5)	-.020	-	-.093	-	-	-
T(5-10)	-.187	-.315	-.200	-.276	.111	.017
T(10-15)	.048	-.050	.031	-.097	-.214	-.113
T(15-20)	.194	.347	.237	.228	-.062	-.025
T(>20)	.120	.327	.371*	.381	.144	.060
[T(<5)]	.185	-	.205	-	-	-
[T(5-10)]	.096	.097	.099	.462*	.696**	.692**
[T(10-15)]	-.065	.101	-.090	.143	.106	.164
[T(15-20)]	-.092	-.108	-.289	-.495*	-.451*	-.285
[T(>20)]	-.076	-.118	-.114	-.367	-.355	-.464*
PCI	.148	.325*	.203	.306	.374	.167
[PCI]	.129	.131	.032	.518*	.574*	.486*
DWAT,PCI	.385**	.692**	.718**	.879**	.813**	.727**

• Symbols as for Table 25.

CHAPTER 6

6. INFLUENCE OF POST-EMERGENCE HERBICIDES ON THE SOIL MICROBIAL BIOMASS

6.1 INTRODUCTION

As outlined in Chapter 1 the soil microbial biomass plays an important role in the soil ecosystem by acting as a labile pool of nutrients and by regulating plant-available nutrient levels. Applications of agricultural chemicals may therefore be important in influencing the microbial biomass, especially considering the extensive use of chemicals in agricultural practice (Somerville 1987). Therefore, there has been extensive literature published on the effects of pesticides (especially herbicides) on processes controlled by the microbial biomass (e.g. see reviews by Simon-Sylvestre and Fourner 1980; Goring and Laskowski 1982). However, only two studies (i.e. Soulas et al. 1984 and Duah-Yentumi and Johnson 1986) have utilized physiological approaches to quantify the effects of herbicide additions on the magnitude of the soil microbial biomass. A further study (Anderson et al. 1981) monitored shifts in the soil microbial biomass and bacterial:fungal ratio following fungicide addition.

The purpose of the present investigation was to assess the influence of three post-emergence herbicides on the activity and relative size of the soil microbial biomass, and the bacterial and fungal components of this biomass, in controlled laboratory

conditions. The three herbicides used were picloram, 2,4-D, and glyphosate, selected because they represent a range of chemical structures and degrees of persistence.

6.2 MATERIALS AND METHODS

Preparation of Samples

For each of the three herbicides used, four soil samples were randomly collected and passed through a 4 mm sieve. From each sample, four 1.5 kg (dry weight) subsamples were taken. Each sub-sample was treated with a different herbicide concentration i.e. 0, 2, 20, and 200 ppm. The herbicides were added in solution to the soil by spraying and mixing; the soil sub-samples were then sprayed with deionized water to bring them to 55% moisture (d.w.) (-33 kPa). The treated subsamples were incubated in plastic bags (tops not sealed) at 22°C in the dark. Samples of soil were removed from each bag for data collection at 8 hrs, and 1, 3, 9, and 27 days after herbicide application. These times had been shown by preliminary experimentation to be the most suitable for monitoring any microbial biomass and activity changes that were likely to occur. Day 0 data were also collected, immediately prior to herbicide application.

At each sampling time the following measurements were made from subsamples of each bag of soil:

(i) Basal respiration determined as the rate of CO_2-C evolution from 60 g.d.w. samples. The method used was exactly as outlined in Section 5.2.

(ii) Substrate-induced respiration (S.I.R.), determined after addition of 6000 ppm glucose. The methodology used was the same as that in Section 4.3.2.

(iii) Basal respiration:substrate-induced respiration ratio, i.e. the ratio of (i) to (ii).

(iv) Inhibition of S.I.R. by the selective inhibitors streptomycin (10000 ppm addition) or actidione (15000 ppm addition) using the methodology outlined in Section 4.4.2.

All respiration measurements were collected using an Infrared Gas Analyzer with a flow-rate of 0.178 ℓ min^{-1}.

Statistical Analysis

Unless otherwise stated, all data were analyzed using three-way ANOVA testing for the following effects: herbicide (A), time after application (B), blocking (C), and the A x B interaction. Data were transformed using the $\sqrt{x}$ or $\log_e x$ transformation if this was required for the data to fulfil the assumptions of normality and homogeneity of variances. When significant herbicide effects were detected following the 3-way analysis (after blocking

and time effects were accounted for), multiple comparisons were performed using Tukey's test (following two-way ANOVA accounting for blocking) across herbicide concentrations within each sampling time.

6.3 RESULTS AND DISCUSSION

Basal Respiration

For each herbicide treated soil tested, the 200 ppm treatment had significantly higher respiration levels than the 0 ppm control (Table 29). This lasted until day 9 for 2,4-D and picloram, and day 3 for glyphosate. In no instance did the 2 ppm or 20 ppm treatments show higher respiration than the controls. The 3-way ANOVA indicated that time after application and time x herbicide effects were both significant for all three herbicides. This is because changes in microbial activity occurred throughout the experiment for at least some treatments, and that differences between herbicide (200 ppm) and control treatments were transitory. In other studies both enhancement or reduction of CO_2 evolution have been observed following herbicide application, this being dependent on the herbicide and soil used. In contrast to the present study, Schinner et al., (in Curry 1986) and Malunchuk and Joyce (1983) both observed inhibition of CO_2 evolution caused by 2,4-D, presumably in soils with a different spectrum of microbial species than the soil used in this study. Glyphosate has been observed to cause either stimulation or inhibition of soil

Table 29. CO_2 evolution (in $\mu g CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) under various herbicide regimes. For each herbicide, values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Herbicide Concentration (ppm)	Time after application (days) 1/3	1	3	9	27
a. 2,4-D					
0	2.33 a	2.05 a	1.96 a	1.75 a	1.75 a
2	2.75 a	2.60 a	2.24 a	2.23 a	2.18 a
20	2.75 ab	2.39 ab	2.14 a	1.69 a	1.79 a
200	3.96 b	3.57 b	3.26 b	3.36 b	2.25 a
b. Picloram					
0	1.57 a	1.27 a	1.20 a	1.17 a	0.89 a
2	1.82 a	1.67 a	1.29 ab	1.26 a	0.96 a
20	1.91 a	1.77 a	1.40 ab	1.26 a	0.90 a
200	2.12 b	2.00 b	1.69 b	1.80 b	0.82 a
c. Glyphosate					
0	1.68 a	1.56 a	1.32 a	1.06 a	0.78 a
2	1.82 a	1.63 a	1.34 a	1.13 a	0.82 a
20	1.89 a	1.69 ab	1.33 a	1.08 a	0.83 a
200	2.47 b	2.36 b	2.67 b	1.29 a	0.80 a

(a) 2,4-D — Day 0 respiration = 2.39 $\mu g CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
ANOVA results (following $\log_e$ x transformation): Herbicide (A): F = 39.26***; Time after application (B):F = 11.9***; Blocking: F = 3.73*; A x B interaction: F = 3.05**.

(b) Picloram — Day 0 respiration = 1.61 $\mu g CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
ANOVA results: Herbicide (A): F = 23.8***; Time after application (B): F = 44.6***; Blocking: F = 10.7***; A x B interaction: F = 2.80**.

(c) Glyphosate — Day 0 respiration = 1.63 $\mu g CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
ANOVA results: Herbicide (A): F = 5.37**; Time after application (B): F = 63.9***; Blocking: F = 74.7***; A x B interaction: F = 7.13***.

respiratory activity, depending on the soil and herbicide concentration used. (Grossbard 1985; Carlisle and Trevors 1986).

The enhancement of CO_2-C release from the 200 ppm treatments for each herbicide (c.f. controls) during the first nine days was, in each case, significantly more than the total amount of herbicide-C added in the treatment (Figure 16). This indicates that at least some of the enhanced CO_2 production must have arisen from sources other than direct microbial decomposition of the herbicide. This suggests that each of the herbicides caused a "priming" effect, by stimulating at least some of the soil microbial community to such an extent that extra indigenous carbon was released as CO_2 (see Stevenson 1986). At least part of this indigenous carbon may be released from the decomposition of microbial populations that are susceptible to the herbicide.

Substrate-Induced Respiration

Substrate-induced respiration (SIR) was significantly influenced by each herbicide during at least part of the first 9 days (Table 30). The 3-way ANOVA results also indicated that biomass values changed over time and the significant effects of the herbicide x time interaction for glyphosate and 2,4-D indicated the transitory nature of the difference between the 200 ppm herbicide and control treatments. In the case of 2,4-D and picloram SIR was reduced by 200 ppm herbicide during the first nine days, relative to the control and/or lower herbicide concentrations. If SIR can be presumed to be proportionally related to soil microbial biomass

Table 30. Substrate-induced respiration (in $\mu gCO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) under various herbicide regimes. For each herbicide, values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Herbicide Concentration (ppm)	Time after application (days) 1/3	1	3	9	27
a. 2,4-D					
0	21.9 a	21.6 a	21.8 a	21.9 a	20.2 a
2	22.5 a	22.0 a	22.2 a	21.8 a	22.0 a
20	22.2 a	21.7 a	21.7 a	22.4 a	21.6 a
200	17.1 b	16.6 b	16.7 b	23.8 a	20.1 a
b. Picloram					
0	15.4 a	15.5 a	14.4 a	15.9 a	15.6 a
2	16.3 a	16.3 a	15.2 a	17.0 a	16.1 a
20	15.7 a	16.0 a	15.0 ab	17.1 a	15.6 a
200	11.7 b	11.7 b	11.6 b	16.1 a	14.5 a
c. Glyphosate					
0	14.7 a	14.5 a	13.9 a	12.5 a	12.6 a
2	15.3 a	14.4 a	14.4.a	12.6 a	12.9 a
20	14.4 a	14.3 a	14.8 a	12.9 a	12.6 a
200	15.1 a	14.7 a	17.6 b	12.9 a	12.1 a

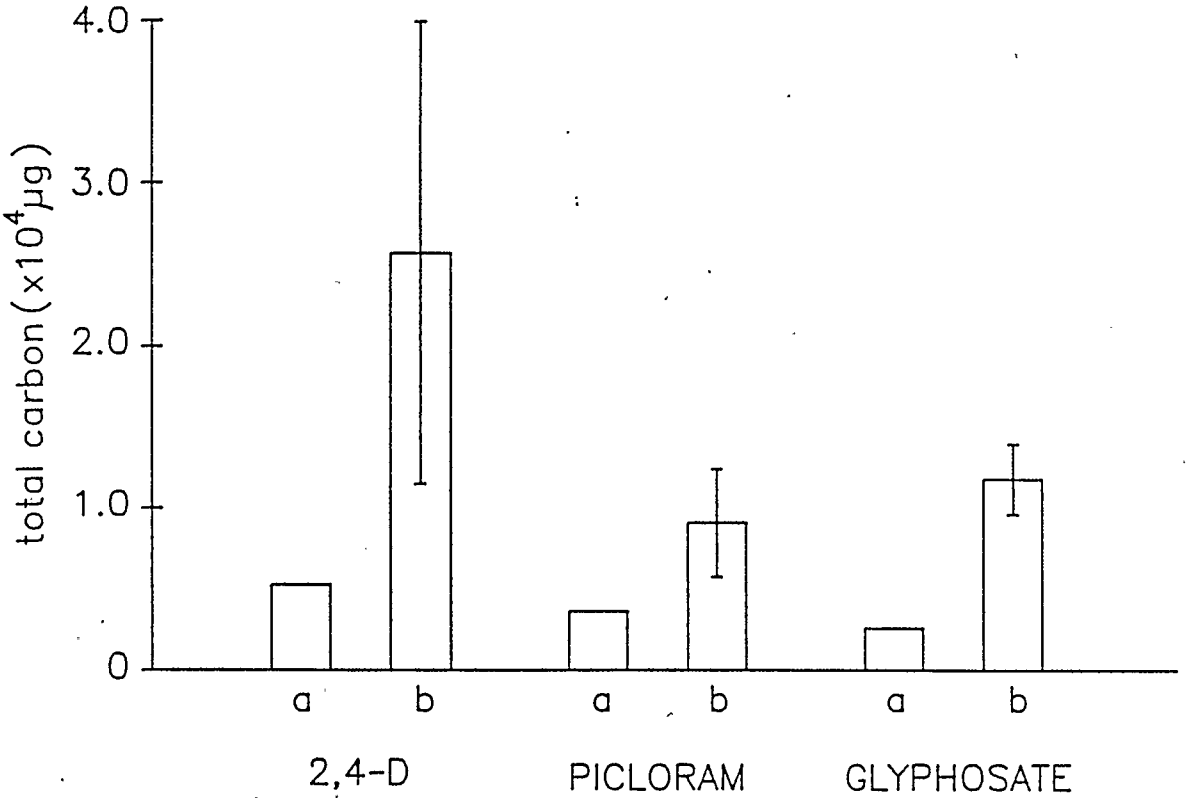
(a) 2,4-D — Day 0 SIR = 22.5 $\mu gCO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
ANOVA results: Herbicide (A): F = 19.0***; Time after application (B): f = 4.91**; Blocking: F = 5.06**; A x B interaction: F = 4.09***.

(b) Picloram — Day 0 SIR = 15.3 $\mu gCO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
ANOVA results: Herbicide (A): F = 8.38***; Time after application (B): F = 3.05*; Blocking: F = 5.03**; A x B interaction: F = 0.66 n.s.

(c) Glyphosate — Day 0 SIR = 144 $\mu gCO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
ANOVA results: Herbicide (A): F = 5.37**; Time after application (B): F = 63.9***; Blocking: F = 74.7***; A x B interaction: F = 7.13***.

Figure 16. Extent of respiration loss of C from soil caused by three herbicides.

a. Amount of carbon added per 60 g soil (d.w.) in 200 ppm herbicide.
b. Enhancement of CO_2-C loss through respiration due to addition of 200 ppm herbicide (i.e. CO_2-C loss in 200 ppm treatment - CO_2-C loss in 0 ppm control) within nine days of herbicide treatment, in 60 g soil (d.w.). Bars indicate mean ± 95% confidence interval, using students t-test.


4.0
3.0
2.0
1.0
0
total carbon (x10⁴µg)
a
b
a
b
a
b
2,4-D
PICLORAM
GLYPHOSATE

(Anderson and Domsch 1978b) then it can be inferred that a portion of the soil microflora was killed by 2,4-D and picloram. This could help explain the large enhancement of microbial respiration, through the rapid degradation of susceptible (killed) populations of microbes by non-susceptible microorganisms (Soulas et al 1984). This suggests that high levels of 2,4-D and picloram may enhance turnover rates of components of the microbial biomass, thus assisting in the release of CO_2 and immobilized nutrients (see Anderson and Domsch 1980; Jenkinson and Ladd 1981).

Glyphosate caused a signficant increase in SIR, on day 3 only, and only in the 200 ppm treatment. This suggests that the herbicide might have acted as a resource, or assisted in acting as a co-metabolite for the release of other resources, to increase biomass. This increase in biomass was transitory and presumably could not be maintained even until day 9.

The two other studies which have investigated herbicide influence on the soil microbial biomass have found relatively little effect. Soulas et al. (1984) observed that microbial biomass remained largely unchanged after additions of 3 - 4 ppm 2,4-D to soil. Duah-Yentumi and Johnson (1986) demonstrated, in a field experiment, that MCPA and simazine had little influence on the soil microbial biomass, but fungal biomass was suppressed by repeated paraquat application.

Basal Respiration: Substrate Induced Respiration Ratio

As outlined in Section 5.3, the ratio of basal respiration to S.I.R. is potentially useful for providing a relative measure of the respiration:biomass ratio as proposed by Odum (1969; 1985) for indicating the degree of ecosystem disturbance.

For each herbicide, 200 ppm applications enhanced this ratio relative to the controls or lower herbicide applications; this enhancement lasted for 9 days for 2,4-D and picloram, and 3 days for glyphosate (Table 31). Upon addition of high herbicide concentrations, (especially with 2,4-D and picloram), the microbial community was presumably in a considerable state of flux with susceptible microbes being killed and others therefore having a readily available source of carbon. Much of this available carbon would have been respired rather than incorporated into new microbial biomass. Even with glyphosate, it is possible that there could have been a shift in the microbial community structure (as indicated in a parallel study reported in Section 8.4) with significant microbial turnover but no great change in the microbial biomass, and a resultant increase in the respiration:biomass ratio. When the "stability" (= persistence: see Richards 1987) of the 200 ppm treated soils approached that of the 0 ppm controls towards the end of the experiment (i.e. 27 days), the basal respiration: SIR ratios of the treatment and control soils were not significantly different. The results of the 3-way ANOVA which determined significant time x herbicide interaction effects for all 3 herbicides indicated that enhancement of the ratio by the 200 ppm treatment was temporary.

Table 31. Basal respiration: Substrate-induced respiration ratio under various herbicide regimes. For each herbicide, values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Herbicide Concentration (ppm)	Time after application (days) 1/3		1		3		9		27	
a. 2,4-D										
0	.108	a	.095	a	.090	a	.080	a	.087	a
2	.122	a	.118	a	.101	a	.102	a	.099	a
20	.124	a	.110	a	.099	a	.075	a	.083	a
200	.231	b	.215	b	.195	b	.141	b	.112	a
b. Picloram										
0	.102	a	.082	a	.083	a	.074	a	.057	a
2	.112	a	.102	a	.085	a	.074	a	.060	a
20	.121	a	.111	a	.094	a	.074	a	.058	a
200	.181	b	.171	b	.145	b	.112	b	.056	a
c. Glyphosate										
0	.114	a	.107	a	.095	a	.085	a	.062	a
2	.119	a	.113	a	.093	a	.090	a	.063	a
20	.130	a	.118	a	.090	a	.084	a	.066	a
200	.163	b	.161	b	.152	b	.100	a	.066	a

(a) 2,4-D — Day 0 ratio = .106
ANOVA results (following $\log_e$ x transformation): Herbicide (A): F = 73.2***; Time after application (B): F = 16.6***; Blocking: F = 5.13**; A x B interaction: F = 2.70**

(b) Picloram — Day 0 ratio = .105
ANOVA results (following $\log_e$ x transformation): Herbicide (A): F = 67.8***; Time after application (B): F = 102.0***; Blocking: F = 5.84**; A x B interaction: F = 5.73***.

(c) Glyphosate — Day 0 ratio = .113
ANOVA results: Herbicide (A): F = 60.4***; Time after application (B): F = 130.5***; Blocking: F = 1.19 n.s.; A x B interaction: F = 7.06***.

Use of selective inhibitors

The extent of inhibition of SIR caused by streptomycin is shown in Table 32. This indicates that enhancement of the bacterial activity was caused by 200 ppm 2,4-D at day 9 and 200 ppm picloram during days 3 and 9. These increases occurred after the microbial biomass had been reduced. This suggests that the increases in microbial biomass in the 200 ppm treatments between day 3 and 9 for both herbicides was due at least in part to a proliferation in bacterial growth. Glyphosate had no significant effect on the extent of the inhibition of SIR by streptomycin.

The magnitude of inhibition of SIR caused by actidione is shown in Table 33. Enhancement of the inhibition was caused by 200 ppm 2,4-D on day 9 and 200 ppm glyphosate on day 3. For 2,4-D this suggests a proliferation of fungal growth coincident with the proliferation of bacterial growth. With glyphosate, the increase in fungal growth was coincident with the transitory enhancement of the microbial biomass. Picloram had no detectable effect on inhibition of SIR by actidione.

The use of selective inhibitors as indicators of microbial activity has demonstrated that where the degree of inhibition caused by streptomycin and/or actidione had been enhanced by herbicide addition, this occurred only when a significant increase in substrate-induced respiration (and therefore microbial biomass) was observed. In the case of 2,4-D and picloram, this occurred only when the SIR shifted from a depressed level, in the 200 ppm treatment, to that of the control soil. For glyphosate, this occurred

Table 32. Inhibition of substrate-induced respiration caused by streptomycin under various herbicide regimes (μgCO_2-C•h^{-1}• g $soil^{-1}$). For each herbicide, values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Herbicide Concentration (ppm)	Time after application (days) 1/3	1	3	9	27
a. 2,4-D					
0	2.93 a	2.87 a	3.25 a	2.69 a	2.09 a
2	2.92 a	2.87 a	2.42 a	2.47 a	2.17 a
20	3.15 a	2.80 a	2.80 a	2.95 a	2.76 a
200	3.03 a	1.70 a	2.54 a	5.07 b	1.86 a
b. Picloram					
0	1.94 a	2.57 a	2.16 a	1.89 a	1.44 a
2	2.17 a	2.46 a	2.01 a	2.01 a	1.71 a
20	1.78 a	2.01 a	1.97 a	2.24 a	1.94 a
200	2.31 a	2.34 a	3.15 b	3.63 b	1.33 a
c. Glyphosate					
0	1.48 a	1.26 a	1.18 a	1.21 a	0.80 a
2	1.67 a	1.44 a	1.48 a	1.21 a	1.03 a
20	1.44 a	1.41 a	1.52 a	1.21 a	0.88 a
200	1.36 a	1.21 a	1.71 a	1.10 a	0.90 a

(a) 2,4-D — Day 0 value = 3.15 μgCO_2-C•h^{-1}• g $soil^{-1}$
ANOVA results (following $\sqrt{x}$ transformation): Herbicide (A): F = 2.80*; Time after application (B): F = 5.73***; Blocking: F = 0.59 n.s.; A x B interaction: F = 3.05**.

(b) Picloram — Day 0 value = 1.94 μgCO_2-C•h^{-1}• g $soil^{-1}$
ANOVA results (following $\sqrt{x}$ transformation): Herbicide (A): F = 4.74**; Time after application (B): F = 9.07***: Blocking: F = 3.25*; A x B interaction: F = 3.38***.

(c) Glyphosate — Day 0 value = 1.63 μgCO_2-C•h^{-1}• g $soil^{-1}$
ANOVA results (following $\sqrt{x}$ transformation): Herbicide (A): F = 1.65 n.s.; Time after application (B): F = 5.73***; Blocking: F = 0.69 n.s.; A x B interaction: F = 3.05**.

Table 33. Inhibition of substrate-induced respiration caused by actidione under various herbicide regimes (μgCO_2-C•h^{-1}• g $soil^{-1}$). For each herbicide values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Herbicide Concentration (ppm)	Time after application (days) 1/3	1	3	9	27
a. 2,4-D					
0	6.05 a	6.28 a	6.73 a	6.71 a	6.63 a
2	6.94 a	6.12 a	7.01 a	6.81 a	6.78 a
20	7.61 a	6.58 a	7.84 a	6.78 a	7.16 a
200	6.54 a	7.34 a	7.16 a	11.40 b	6.02 a
b. Picloram					
0	5.38 a	5.82 a	5.15 a	5.07 a	5.57 a
2	6.12 a	6.36 a	5.50 a	5.73 a	5.80 a
20	5.90 a	6.13 a	5.82 a	6.05 a	5.57 a
200	5.42 a	5.50 a	5.74 a	5.80 a	5.50 a
c. Glyphosate					
0	3.68 a	4.39 a	4.51 a	3.60 a	4.09 a
2	4.66 a	5.14 a	4.74 a	3.86 a	4.54 a
20	3.60 a	3.90 a	4.09 a	3.56 a	3.90 a
200	4.62 a	4.13 a	6.96 b	3.93 a	3.94 a

(a) 2,4-D — Day 0 value = 6.89 μgCO_2-C•h^{-1}• g $soil^{-1}$
ANOVA results: Herbicide (A): F = 2.76*; Time after application (B): F = 3.22*; Blocking: F = 3.40*; A x B interaction: F = 3.86***.

(b) Picloram — Day 0 value = 5.83 μgCO_2-C•h^{-1}• g $soil^{-1}$
ANOVA results: Herbicide (A): F = 1.34 n.s.; Time after application (B): F = 0.42 n.s.: Blocking: F = 1.34 n.s.; A x B interaction: F = 2.36*.

(c) Glyphosate — Day 0 value = 4.70 μgCO_2-C•h^{-1}• g $soil^{-1}$
ANOVA results (following $\log_e x$ transformation): Herbicide (A): F = 6.41***; Time after application (B): F = 5.84***; Blocking: F = 6.75***; A x B interaction: F = 2.14*.

when SIR was enhanced temporarily above the control soil by 200 ppm herbicide during day 3. These results suggest that when SIR has been found to increase, it can be determined whether fungi or bacteria are responsible for this observed increase in microbial biomass.

This is further demonstrated when bacterial:fungal ratios are calculated from these data. This ratio was affected differently by each herbicide (Table 34). The herbicide 2,4-D had no effect on the ratio, suggesting that 2,4-D reduced bacterial and fungal communities equally. Picloram caused a transitory increase of the ratio, but only at 200 ppm on day 3 and 9. This suggests that the reduction in total microbial biomass treated with this herbicide may be due to a reduction in the fungal rather than bacterial component. Glyphosate caused a transitory decrease of the ratio at 200 ppm (relative to the 20 ppm treatment) and only at day 3 as a result of an increase in the fungal component. However, as outlined in Section 4.4.4, even when both actidione and streptomycin are added to soil samples, a considerable portion of SIR is not inhibited, and this is because selective inhibitors probably only apply to those organisms undergoing rapid protein synthesis at the time of respiration measurement.

6.4 CONCLUSIONS

Six microbial parameters were utilized to investigate whether three commonly used post-emergence agricultural herbicides exerted any detectable effects on the soil biota. For each

Table 34. Bacterial: fungal ratio under various herbicide regimes. For each herbicide, values in the same column followed by the same letter are not significantly different ($p < 0.05$).

Herbicide Concentration (ppm)	Time after application (days) 1/3	1	3	9	27
a. 2,4-D					
0	29:71 a	28:72 a	33:67 a	29:71 a	24:76 a
2	27:73 a	26:74 a	26:74 a	27:73 a	24:76 a
20	26:74 a	27:73 a	30:70 a	30:70 a	28:72 a
200	28:72 a	29:71 a	26:74 a	31:69 a	24:76 a
b. Picloram					
0	26:74 a	31:69 a	30:70 ab	27:73 a	20:80 a
2	26:74 a	28:72 a	27:73 a	26:74 a	23:77 a
20	23:77 a	25:75 a	25:75 a	27:73 a	26:74 a
200	30:70 a	30:70 a	35:65 b	39:61 b	20:80 a
c. Glyphosate					
0	29:71 a	22:78 a	21:79 ab	25:75 a	16:84 a
2	26:74 a	22:78 a	24:76 ab	24:76 a	19:81 a
20	28:72 a	27:73 a	27:73 a	25:75 a	18:82 a
200	23:77 a	23:77 a	20:80 b	22:78 a	19:81 a

(a) 2,4-D — Day 0 ratio = 31:69
ANOVA results: Herbicide (A): F = 2.36 n.s.; Time after application (B): F = 3.58*; Blocking: F = 35.5*; A x B interaction: F = 1.60n.s.

(b) Picloram — Day 0 ratio = 25:75
ANOVA results: Herbicide (A): F = 7.02***; Time after application (B): F = 10.2***; Blocking: F = 2.79*; A x B interaction: F = 2.84**.

(c) Glyphosate — Day 0 ratio = 26:74
ANOVA results: Herbicide (A): F = 4.05*; Time after application (B): F = 8.32***; Blocking: F = 2.61 n.s.; A x B interaction: F = 0.62n.s.

parameter, when significant effects occurred, they were transient, and only detected for the 200 ppm treatment. Assuming that the herbicide is mixed evenly to a depth of 5 cm, it is unlikely that the herbicide concentration following field applications exceeds 4 - 5 ppm (Anon. 1987), and even allowing that herbicide molecules may accumulate in specific microhabitats, it is unlikely that concentrations ever reach levels as high as 200 ppm. This indicates that effects of the three herbicides in the agricultural system are probably of relatively low importance with regard to the microbial parameters tested in this study.

CHAPTER 7

7. INFLUENCE OF FIELD APPLICATIONS OF GLYPHOSATE AND 2,4-D ON THE MICROBIAL BIOMASS

7.1 GENERAL INTRODUCTION

Although the majority of studies concerned with investigating herbicide side-effects of the soil microflora and soil processes have used laboratory incubation techniques (see Simon-Sylvestre and Fournier 1979; Greaves and Malkames 1980), there are certain difficulties in attempting to extrapolate such information to the field. Determination of the appropriate concentration of herbicide to mix into soil samples in such studies is problematic, and in many studies unrealistically high and inappropriate concentrations of herbicide have been applied (Domsch et al. 1983). Also it is difficult to relate the importance of detected herbicide side-effects on a given biotic variable relative to the background of spatial and temporal biotic variability which occurs in the field (Greaves et al. 1980; Cook and Greaves 1987).

Domsch et al. (1983) concluded that most detected side-effects of pesticide applications on the soil microflora are relatively unimportant because depressions of over 90% of many soil processes often occur due to variability in natural factors. Comparisons of herbicide effects with the influences exerted by other factors are most effectively made using field studies. Cook and Greaves (1987) indicated 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.

When a herbicide is applied in a field situation, it may affect the soil biota either through direct or indirect mechanisms. Direct mechanisms involve the chemical exerting an influence on the physiology of various soil organisms, either by acting as a toxin or a resource. Indirect effects involve the habitat of the soil biota being affected by the herbicide, e.g. as a result of the reduction of plant cover (Eijsackers and van der Drift 1976; Purvis and Curry 1984). Most laboratory incubation studies only involve assessment of the direct effects of the chemical on soil biota, in terms of whether a given herbicide application inhibits the activity and/or abundance of soil organisms. In field experiments, where herbicides are applied to replicated field plots (along with adequate controls) soil organisms may also respond to indirect effects of the chemical, e.g. reductions of weed biomass killed by the herbicide. Some studies have attempted to remove the indirect pesticide effects in field plots by handweeding (e.g. Fratello et al. 1985).

The experiments outlined in this chapter are divided into two sections with the following objectives:

(i) To determine whether field-level application of 2,4-D and glyphosate exert appreciable effects on soil microbial biomass dynamics and activity, in relation to natural variability (1987 data: section 7.2).

(ii) To investigate whether microbial biomass and activity is affected differently by herbicides in the absence vs. presence of handweeding treatments and crop plants. By simultaneously measuring changes in microbial variables and plant biomass across different herbicide treatments, and in using different combinations of weeds and crop plants, attempts were made to determine whether any detectable effects of the herbicide could be separated into direct or indirect effects of the chemicals on the soil microflora (1988/89 data: section 7.3).

7.2 RELATIVE IMPORTANCE OF EFFECTS OF HERBICIDES AND ENVIRONMENTAL VARIABLES ON THE MICROBIAL BIOMASS

7.2.1 METHODS AND MATERIALS

Plot details

A portion of the 80 X 80 m study site described in Chapter 2 was cultivated to depth of 15 cm in late May 1987. Sixteen plots (4 treatments x 4 replicates) were subsequently set up in a randomized block design; each plot being 2.5 x 2.5 m. A 1.0 m wide buffer zone was used to separate adjacent plots. The following treatments were used:

(a) Glyphosate was applied at the rate of 4 kg ha^{-1} active ingredient, in the form of Roundup (Monsanto®)

(b) 2,4-D was applied at 3 kg ha^{-1} active ingredient in the form of 2,4-D-ester (Dow Chemicals)

(c) Handweeded plot treatment, without herbicide addition. These plots were handweeded every 3 - 5 days.

(d) Non-handweeded plot treatment without herbicide addition.

Appropriately dilute herbicide solutions were applied to the plots by hand watering. Control plots received an equivalent volume of water. The concentrations used represented the uppermost levels recommended for use in Alberta (Ali 1988). Herbicides were applied on May 31, 1987.

A non-weeded and weeded control treatment were both used (i.e (c) and (d) - see Purvis and Curry 1984) because it was considered that the herbicide additions may interfere with plant biomass in the treatment plots, which might in turn indirectly influence the abundance of soil organisms. This may obscure the direct effects of the chemical on the organism (see Eijsackers and van der Drift 1976). By maintaining control plots both with and without weeding this effect could be examined, and if found to be important, plant biomass could be used as a covariate when the effects of the chemical were being analyzed.

Data collection

At each of the 4 sampling days (i.e. 1, 5, 15, and 45 days after herbicide application) two positions were randomly selected in

each plot. At each position a 15 x 15 x 15 cm soil sample was collected, and the following determinations made.

1. Microbial biomass data. Each sample was passed through a 4 mm sieve and the following measurements taken:

(a) basal respiration, determined exactly as in Section 5.2

(b) substrate induced respiration (S.I.R.) using 30 g (d.w.) soil, exactly as determined in Section 4.3.2

(c) basal respiration:substrate induced respiration ratio, i.e. ratio of (a) to (b)

(d) inhibition of S.I.R. by 10 000ppm streptomycin sulphate, exactly as outlined in Section 4.4.2

(e) inhibition of S.I.R. by 15 000ppm actidione exactly as outlined in Section 4.4.2

(f) bacterial: fungal ratio using the ratio of (d) to (e)

All CO_2 measurements for (a) - (f) were made using an infrared gas analyzer at a continuous flow rate of 178 ml min^{-1}.

2. Environmental variables. Data on subsamples of each sample were collected with the intention of using them as covariates to help explain variation on the microbial data. For each sample the following determinations were made, using the methodologies outlined in Section 2.2:

(a) Gravimetric moisture content (%)

(b) Percent organic carbon

(c) Soil pH

(d) NH_4^+, NO_3^-, and PO_4^{3-} concentrations

3. Vegetation cover. At day 45, two 30 x 30 cm quadrats were located within each plot. These were placed in exactly the same position as that used for soil sample collection on that day. Within each quadrat, all vegetation was clipped at ground level, and sorted into the monocotyledonous and dicotyledonous components. The dry weight of each component was then determined after oven drying at 80°C for 24 hours. Determinations of plant biomass were not made earlier because during the other 3 sampling times plant biomass in all plots was negligible; a possible consequence of dry warm conditions through May and June 1987.

Statistical analysis

Data for each of the six microbial variables was analyzed as follows:

1. 3-way analysis of variance or covariance, testing for the following effects: herbicide treatment, time of sampling, herbicide x time interactions, and blocking. Covariates involved any of the environmental or vegetation variables which could statistically significantly reduce the error (within) sums of squares in this analysis. Whenever herbicide effects were significant, the data for each

sampling time was analyzed using two-way ANOVA (herbicide treatment and blocking), and multiple comparison analysis was performed across herbicide treatments using Tukey's h.s.d.

2. For each biotic variable, the fraction of variability in the data set explained by presence/absence of herbicide was compared with that explained by the various environmental variables. This analysis was performed for each sampling date. This involved determinations of r^2 between the biotic variable and each herbicide or environmental variable. For the herbicide variables r^2 determinations were made after applying dummy variables to each herbicide treatment, i.e. 1 = herbicide applied; 0 = no herbicide applied (Draper and Smith 1982).

7.2.2 RESULTS AND DISCUSSION

Basal respiration

Soil basal respiration was unaffected by glyphosate but was significantly enhanced by 2,4-D at sample day 1 when both soil carbon and soil moisture were extracted as covariates (Table 35). This enhancement by 2,4-D is consistent with the trends observed in the laboratory-incubation results presented in Chapter 6, where respiration of incubated soil samples was enhanced by 200 ppm 2,4-D. However, unlike that investigation, the concentration used in this study was probably closer to 1 - 4 ppm 2,4-D, assuming equal mixing of the chemical through the cultivated portion of the soil

Table 35. Relationship between soil basal respiration and application of herbicides.

	Time (Days)			
	1	5	15	45
(a) <u>treatment</u>:				
control (non-weeded)	2.33a	2.10a	1.98a	1.64a
control (weeded)	2.26a	2.65a	2.13a	1.82a
2,4-D	3.39b	2.77a	2.21a	1.84a
glyphosate	2.07a	2.08a	1.83a	1.68a
(b) <u>% explained</u>				
2,4-D	46.0**	13.6*	2.5 ns	0.9ns
glyphosate	(-)11.8ns	(-)5.4ns	(-)4.1ns	(-)0.8ns
% H_2O	13.0*	36.8**	3.2ns	5.2ns
% carbon	21.2**	37.0**	23.0**	2.2ns
pH	0.0ns	6.6ns	(-)1.0ns	1.7ns
NH_4^+	28.7**	20.6**	19.5ns	(-)0.0ns
NO_3^-	2.7ns	1.8ns	2.8ns	(-)4.8ns
PO_4^{3-}	11.8ns	3.0ns	25.1**	4.8ns
dicot. biomass	-	-	-	(-)1.2ns
monocot. biomass	-	-	-	0.0ns

(a) • Basal respiration ($\mu g\ CO_2\text{-}C.g\ soil^{-1}.hr^{-1}$) in plots of different treatments.
- Day 0 value = 2.28 $\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
- Within the same column, numbers followed by the same letter are not significantly different at p = 0.05
- Covariates: soil carbon: t = 2.78**, soil moisture: t = 2.19*

(b) • Percentage of variation (r^2) in data set for basal respiration explained by presence vs. absence of 2 herbicides, and environmental variables.
- *: $p(r^2 = 0) < 0.05$; **: $p(r^2 = 0) < 0.01$; ns: $p(r^2 = 0) > 0.05$
- (-) indicates correlation coefficient, r, was below 0

profile (Domsch et al. 1983; Anon 1987). However, it is likely that the concentration of 2,4-D near the surface was higher than this because the chemical may not have had time to disperse through the profile within the first 24 hours following application. While only patterns of stimulation caused by 2,4-D were noted in this investigation, other studies have typically found patterns of inhibition of CO_2 production (e.g. Malunchuk and Joyce 1983; Schinner in Curry 1986).

The presence (vs. absence) of 2,4-D explained 46% of variation in basal respiration for the day 1 data; this is higher than that attributable to spatial variation in any of the soil chemical or environmental variables measured. The effect is, however, transient, and by day 5 variations of soil C, H_2O and NH_4^+ explain a much higher percentage of variability in the data than does 2,4-D. Although significant relationships occur between basal respiration and these three soil environmental factors, little can be inferred about the effects of these factors on the respiration rate. This is especially true of NH_4^+ concentration, which may be related to basal respiration because higher microbial activity results in higher mineralization of soil organic matter (including nitrogen) or because extractable NH_4^+ and soil respiration are jointly influenced by other factors.

Probably temporal changes in the soil moisture content also exert a stronger effect than 2,4-D on soil respiration, even at day 1. The results presented in Chapter 5 indicate that in adjacent plots, a change of soil moisture of 20% in June - July 1987 could

cause an enhancement of basal respiration of up to 100%. In the present investigation enhancement of basal respiration by 2,4-D was only 47%, relative to the controls.

Substrate-induced respiration (S.I.R.)

Substrated-induced respiration was found to be temporarily enhanced by 2,4-D at sampling days 1 and 5, after soil carbon, moisture, and pH were extracted as covariates (Table 36). No significant glyphosate effects were detected. This is in contrast to the results of the study presented in Chapter 6, where only patterns of neutrality or inhibition of S.I.R. were caused by 2,4-D. This indicates that the soil microbial biomass responds differently to 2,4-D in different conditions, and this may be attributable to the the spectrum of microorganisms present in each system. In the previous study (Chapter 6), soils were incubated at 55% moisture, while in the present experiment the soil moisture levels at days 1 and 5 ranged between 21.8% and 51.3%. Perhaps these different field moisture levels encourage microbial communities with different relationships with 2,4-D from those observed in the laboratory, to exist. Therefore, different soil conditions can cause the relative microbial biomass to demonstrate different responses to the same chemical. If S.I.R. can be presumed to provide a relative measure of the size of the soil microbial biomass (Anderson and Domsch 1978b), then it can be concluded that the microbial biomass was enhanced by 2,4-D, either through 2,4-D acting as a resource or as a cometabolite for attacking other resources. This effect was

Table 36. Relationship between substrate-induced respiration and application of herbicides.

	Time (Days)			
	1	5	15	45
(a) <u>treatment</u>:				
control (non-weeded)	17.1ab	16.7a	13.0a	17.5a
control (weeded)	16.1a	17.4a	13.4a	18.8a
2,4-D	19.9b	21.6b	15.8a	18.8a
glyphosate	15.9a	17.3a	11.8a	17.5a
(b) <u>% explained</u>				
2,4-D	18.8*	13.8*	6.2ns	1.3ns
glyphosate	(-)4.2ns	(-)1.4ns	(-)3.6ns	(-) 1.4ns
% H_2O	42.3**	58.5**	50.8**	18.2*
% carbon	48.7**	60.5**	52.1**	20.3**
pH	5.0ns	24.2**	21.2**	21.3**
NH_4^+	21.3**	10.1ns	4.9ns	0.9ns
NO_3^-	1.0ns	(-)1.0ns	(-)21.0**	(-)7.6ns
PO_4^{3-}	0.3ns	0.3ns	(-)0.3ns	2.0ns
dicot. biomass	-	-	-	0.2ns
monocot. biomass	-	-	-	(-)2.5ns

(a) • Substrate-induced respiration (μg CO_2-C.g soil^{-1}.hr^{-1}) in plots of different treatments.
• Day 0 value = 16.3 μg CO_2-C•h^{-1}•g soil^{-1}
• Covariates: soil carbon: t = 3.29**, soil moisture: t = 2.64*; soil pH: t = 2.25*

(b) • Percentage of variation (r^2) in data set for substrate-induced respiration explained by presence vs. absence of 2 herbicides, and environmental variables.

Legend as for Table 35

maintained for the first five days after which time the effect of the herbicide was no longer detectable.

Although S.I.R. was significantly affected by 2,4-D during the first sampling periods of the study, this effect was relatively small compared with spatial variation of some of the environmental variables. Presence vs. absence of 2,4-D explained less than half of the variation explained by soil moisture or soil carbon and sometimes less than that explained by NH_4^+ or pH.

Using the results presented in Chapter 5, it can be determined that a 20% increase in soil moisture can cause an increase of 80% in S.I.R.; S.I.R. was enhanced only 20 - 22% by 2,4-D. The results of the present study indicate that even when significant herbicide effects can be detected they may be relatively unimportant compared with natural spatial and temporal variation in the soil microbial biomass caused by natural environmental factors.

Basal respiration:substrate-induced respiration ratio

The value of this ratio was unaffected by glyphosate, and was only influenced by 2,4-D during day 1 when soil moisture was used as a covariate (Table 37). If this ratio can be presumed to provide relative values of the basal respiration:microbial biomass ratio (Insam and Domsch 1988) then this ratio is increased, and this indicates that the soil system is temporarily in a state of disturbance following 2,4-D application. This would involve a reduction of efficiency of the microbial biomass, in that a higher percentage of substrate was respired as CO_2 than incorporated in production

Table 37. Relationship between the basal respiration:substrate-induced respiration ratio and application of herbicides.

	Time (Days)			
	1	5	15	45
(a) treatment:				
control (non-weeded)	.136a	.126a	.152a	.094a
control (weeded)	.140a	.152a	.160a	.047a
2,4-D	.170b	.128a	.140a	.098a
glyphosate	.130a	.120a	.155a	.096a
(b) % Explained				
2,4-D	27.1**	0.2ns	(-)2.3ns	(-)0.2ns
glyphosate	(-)16.3*	(-)4.0ns	(-)0.2ns	(-)0.2ns
% H_2O	17.0*	(-)11.0ns	(-)46.8**	(-)8.0ns
% carbon	(-)8.1ns	(-)13.3*	(-)16.1*	(-)18.8*
pH	(-)17.7*	(-)32.6**	(-)43.1**	(-)73.8**
NH_4^+	7.4ns	8.7ns	4.9ns	(-)0.0ns
NO_3^-	5.9ns	36.4**	42.3**	14.1*
PO_4^{3-}	37.4**	8.6ns	33.9**	4.8ns
dicot. biomass	-	-	-	(-)5.6ns
monocot. biomass	-	-	-	7.8ns

(a) • B.R.:S.I.R. ratio in plots of different treatments.
• Day 0 value = 0.140
• Covariates: soil carbon: t = 2.35*

(b) • Percentage of variation (r^2) in data set for B.R.: S.I.R. ratio explained by presence vs. absence of 2 herbicides, and environmental variables.

Legend as for Table 35

or maintenance of microbial tissue (Insam and Domsch 1988). Consistent with Odum's theory of ecosystem succession (Odum 1969, 1985), the B.R.:S.I.R. ratio of the 2,4-D treatment became closer to that of the controls when the herbicide effect had ended. This is consistent with the results presented in Chapter 6 which observed patterns of stimulation of the ratio following 2,4-D application.

Although there was a statistically significant relationship between the B.R.:S.I.R. ratio and the presence vs. absence of 2,4-D in day 1, this explained only 27% of the variation in the data set, while variations in soil moisture, pH, and PO_4^{3-} each also explained comparable fractions of the total variation. Although PO_4^{3-} explained a high percentage of variation in the B.R.:S.I.R. ratio, it is unclear whether the ratio is directly influenced by phosphate levels or whether both variables are jointly influenced by other factors.

Inhibition of S.I.R. caused by streptomycin and actidione

The magnitude of reduction of S.I.R. caused by either streptomycin or actidione was found to be unaffected by glyphosate, but enhanced by 2,4-D, for both days 1 and 5 following 2,4-D application (Tables 38, 39). In both cases soil moisture was used as a covariate. According to the discussion in Section 4.4.4, this inhibition may be used as an indicator of the activity of the bacterial and fungal components. The results presented here suggest that the activity of both bacteria and fungi are temporarily enhanced by 2,4-D. This enhanced activity may be attributable to the herbicide

Table 38. The extent of inhibition of S.I.R. by streptomycin, in plots of different herbicide treatment.

	Time (Days)			
	1	5	15	45
(a) <u>treatment</u>:				
control (non-weeded)	1.63a	1.00a	1.22a	1.47a
control (weeded)	1.58ab	1.53ab	1.13a	1.31a
2,4-D	3.02b	2.33b	1.18a	1.42a
glyphosate	1.63a	1.00a	1.24a	1.19a
(b) <u>% explained</u>				
2,4-D	21.9**	37.0**	0.2ns	0.0ns
glyphosate	(-)4.9ns	(-)15.9**	0.2ns	(-)1.9ns
% H_2O	32.4**	2.6ns	7.6ns	0.0ns
% carbon	30.8**	9.7ns	10.2ns	0.0ns
pH	1.2ns	0.1ns	0.3ns	2.1ns
NH_4^+	21.3**	0.2ns	5.3	(-)2.1ns
NO_3^-	3.5ns	0.0ns	(-)0.0	(-)6.5ns
PO_4^{3-}	6.3ns	10.1ns	11.5ns	(-)0.0ns
dicot. biomass	-	-	-	1.6ns
monocot. biomass	-	-	-	(-)0.0ns

(a) • Inhibition of S.I.R. by streptomycin ($\mu g\ CO_2\text{-}C.g\ soil^{-1}.hr^{-1}$) in plots of different treatments.
• Day 0 value = 1.66 $\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$
• Covariates: soil moisture: t = 1.82 (p = 0.068)

(b) • Percentage of variation (r^2) in data set for inhibition of S.I.R. by streptomycin explained by presence vs. absence of 2 herbicides, and environmental variables.

Legend as for Table 35

Table 39. The extent of inhibition of S.I.R. by actidione, in plots of different treatmens.

	Time (Days)			
	1	5	15	45
(a) <u>treatment</u>:				
control (non-weeded)	5.22ab	5.72a	3.89a	5.87a
control (weeded)	4.22a	6.16a	3.88a	6.46a
2,4-D	7.63b	8.13b	5.29a	6.73a
glyphosate	5.55a	5.69a	3.60a	5.83a
(b) <u>% explained</u>				
2,4-D	17.8*	17.7*	12.6*	2.5ns
glyphosate	(-)0.2ns	(-)16.2*	(-)3.2ns	(-)0.6ns
% H_2O	41.5**	49.6**	54.6**	18.3*
% carbon	30.6**	51.0**	45.4**	12.3*
pH	7.2ns	24.2**	18.5*	13.8*
NH_4^+	18.7*	2.0ns	15.4*	(-)0.0ns
NO_3^-	0.0ns	(-) 4.8ns	(-)7.6	(-)1.8ns
PO_4^{3-}	0.0ns	0.9ns	15.4*	13.8*
dicot. biomass	-	-	-	(-)0.8ns
monocot. biomass	-	-	-	0.0ns

(a) • Inhibition of S.I.R. by actidione ($\mu g\ CO_2$-C.g soil^{-1}.hr^{-1}) in plots of different treatments.
• Day 0 value = 5.05 $\mu g\ CO_2$-C•h^{-1}•g soil^{-1}
• Covariates: soil moisture: t = 3.55***

(b) • Percentage of variation (r^2) in data set for inhibition of S.I.R. by actidione explained by presence vs. absence of 2 herbicides and environmental variables.

Legend as for Table 35

acting as a resource, or through killing certain components of the microbial biomass, causing other components to become more active through the flush of decomposition (Jenkinson and Powlson 1976) and resulting in an increase in microbial basal respiration and possibly S.I.R.. The study outlined in Chapter 6 also found enhancement of both bacterial and fungal activity by 2,4-D but this was only evident 9 days after herbicide application; nevertheless, the directions of the observed trends are similar in both cases.

With regard to bacterial activity, presence vs. absence of 2,4-D explained 29% of the variation in the data set for day 1, which was similar to that explained by spatial variation in soil moisture, carbon, and NH_4^+ (Table 38). However, on day 5, 2,4-D explained 37% of the variation in bacterial activity, considerably higher than any environmental variable.

Although the fungal activity was significantly enhanced by 2,4-D, the percentage of variation explained by 2,4-D was small compared with the effects of soil moisture and soil carbon (Table 39). However, in both day 1 and 5, the value of r^2 between soil moisture or carbon, and fungal activity, was significantly enhanced when presence vs. absence of 2,4-D was also considered.

If the results from Tables 38 and 39 are used to construct bacterial: fungal ratios using the approach of Anderson and Domsch (1973, 1975), then it can be seen that glyphosate had no significant effect on the ratio while 2,4-D did (see Table 40). This ratio was

Table 40. Relationship between the bacterial:fungal ratio, and application of herbicides.

	Time (Days)			
	1	5	15	45
(a) <u>treatment</u>:				
control (non-weeded)	24:76a	15:85a	24:76a	20:80a
control (weeded)	27:73a	20:80ab	23:77a	17:83a
2,4-D	28:72a	22:78b	18:82b	17:83a
glyphosate	23:77a	15:85a	26:74a	17:83a
(b) <u>% explained</u>				
2,4-D	23.2**	19.4*	(-)14.0*	(-)0.5ns
glyphosate	(-)17.3*	(-)16.9*	0.6ns	(-)0.5ns
% H_2O	1.0ns	(-)20.0*	(-)21.0*	(-)5.6ns
% carbon	(-)1.3ns	(-)8.3ns	(-)15.3ns	(-)3.4ns
pH	(-)3.5ns	(-)14.3*	(-)17.6*	(-)0.0ns
NH_4^+	11.0ns	(-)0.5ns	(-)0.2ns	(-)2.8ns
NO_3^-	10.9ns	2.8ns	12.1ns	(-)3.2
PO_4^{3-}	22.1**	11.6ns	18.7*	0.3ns
dicot. biomass	-	-	-	2.8ns
monocot. biomass	-	-	-	0.4ns

(a) • Bacterial:fungal ratio in plots of different treatments.
• Day 0 value = 25:75
• Covariates: none

(b) • Percentage of variation (r^2) in data set for bacterial:fungal ratio explained by presence vs. absence of 2 herbicides(-) and environmental variables.

Legend as for Table 35

significantly enhanced on day 5 and reduced at day 15, relative to the controls. This suggests that 2,4-D encouraged the bacterial activity more than the fungal activity on day 5, and this bacterial activity was reduced relative to the fungal activity on day 15. Although this ratio was significantly influenced by 2,4-D on day 15, neither of the components of this ratio were significantly influenced (Tables 38,39). However, the value for the fungal activity was almost significantly higher in the presence of 2,4-D relative to the controls during this sampling period (Table 39). The values for the ratio suggest that after an initial lag period following 2,4-D application, the activity of the bacteria was enhanced relative to the fungi, then later the fungal activity was enhanced relative to that of the bacteria. Perhaps the fungi are slower than the bacteria to respond to a 2,4-D application.

The bacterial:fungal ratio was affected by 2,4-D only slightly, with presence vs. absence of 2,4-D explaining 19% and 14% of the variation in the data set at day 5 and 15 respectively (Table 40). Other variables which explain equivalent amounts of variation at these times include soil moisture, carbon, pH, and PO_4^{3-}.

Importance of herbicide effects on the soil microflora

Glyphosate was not found to exert significant effects on any microbial parameter and will not be discussed further in this section.

2,4-D influenced each of the six microbial variables used in the present study. The results were consistent with variables

measuring activity and relative biomass all indicating enhancement of the microflora by 2,4-D. Nevertheless, the effects were transient, and with one exception, all variables were affected by 2,4-D only for the first five days following application of the herbicide.

The effect of 2,4-D on the microbial variables was often relatively low (even when significant) compared with spatial variation in environmental variables. The most important of these variables were soil moisture and soil carbon content. Soil moisture has previously been determined as a major force influencing soil organisms (Bottner 1985; West et al. 1987). Soil carbon reflects the available level of substrate for the system to function (Insam and Domsch 1988). Temporal changes in soil moisture content have been determined (Chapters 4 and 5), to cause large changes both in S.I.R. and basal respiration and these changes are much larger than the effects of applying 2,4-D at the recommended rate to the same soil. Spatial variability in soil pH, and PO_4^{3-}, NO_3^-, and NH_4^+ concentrations sporadically explained a significant fraction of variability in the six biotic variables tested, but no strong consistent trends were noted. However, whenever these relationships were significant, it is unclear as to what was the cause-effect relationship. While it is possible that the soil microflora is affected by these soil environmental factors, it is also likely that these factors are affected by microbial activity. Furthermore, it is possible that these biotic and environmental variables appear

correlated with each other merely because they are both jointly affected by other factors.

Because the effects of 2,4-D on the soil microflora were transient and often minor compared with spatial variation in environmental variables, the effects of 2,4-D can be considered to be of relatively low ecological consequence, even when they are statistically significant. The approach of Domsch et al. (1983) suggests that because natural factors often cause inhibition of microbial processes of over 90%, most reported pesticide effects are relatively unimportant, a conclusion supported by the present study. As noted by Cook and Greaves (1987), pesticide studies are most appropriately reported against a background of the natural dynamics of the variable under consideration. The approach used in this study, i.e., partitioning the total sums of squares observed for each biomass variable in order to assess their importance, allows for direct comparison of pesticide effects with other factors which influence the soil microflora.

With regard to plant biomass measurements, as made during day 45, it appears that the soil microflora was little affected by the presence of dicotyledoneous and monocotyledonous plants, because the correlation coefficients between any of the microbial variables and the total plant biomass was not significantly different from zero under any circumstance. This is despite the dicotyledonous weed flora being significantly reduced at day 45 by 2,4-D. (Glyphosate had no effect on subsequent above-ground flora.) Therefore, any effect of 2,4-D on the microflora is due to direct effects of

the chemical, and not due to indirect effects caused by the plant being killed by the chemical. Also, at days 1 and 5, when the effects of 2,4-D on the microbial biomass were most pronounced, plant biomass on any of the plots was negligible.

7.3 INFLUENCE OF HERBICIDES ON THE MICROBIAL BIOMASS AND ACTIVITY IN THE PRESENCE AND ABSENCE OF CROP PLANTS AND WEEDS

7.3.1 METHODS AND MATERIALS

Plot description

Forty-eight 3 x 3 m plots were set up in the field in the spring of 1988 (i.e. 12 treatments x 4 blocks). Adjacent plots were separated by a buffer zone of 1 m. Barley seeds (Klondyke variety) were sown (June 10) in six of the 12 plots per block, at a depth of 6 cm and a density of 180 kg ha^{-1}. Because of the severity of drought that summer (see Chapter 2) these seeds died shortly after germination and were replanted on June 17, 1988. Extreme dry and warm weather made it necessary to water all plots by hand daily, at the rate of 40 ℓ/plot until the drought ended about two weeks later.

Throughout the course of the experiment, half the barley and half the non-barley plots were handweeded every 3 to 6 days. Thus, for each block there were 3 plots for each combination of handweeding/non-handweeding vs. barley/non-barley. For each group

of three plots for each combination a different herbicide treatment was used, i.e. control, 2,4-D, and glyphosate.

The 2,4-D plots were amended at a rate equivalent to 4 kg ha^{-1} active ingredient, applied on July 23, 1988, when the barley plants were at the three-leaf stage. The concentration and time of application of the herbicide were those recommended in Alberta for control of broadleaf weeds (Ali 1988).

The glyphosate plots were amended at the rate equivalent to 5 kg ha^{-1} active ingredient. This was applied on September 4, 1988, when the barley plants had produced harvestable seed and many of the plots were heavily infested with broad leaf weeds and quackgrass (Agropyron repens). The application rate and post-harvest age are those recommended in Alberta for control of weeds in the autumn season (Ali 1988).

All plots not treated with herbicide on a given day were amended with an equivalent quantity of water, i.e. 8 ℓ.

Data collection (1988)

For each of the 2,4-D treated and control plots, one sample approximately 15 x 15 x 15 cm was collected on the following days after herbicide application: July 24, 1988 (day one after 2,4-D application), July 27, 1988 (day 4), August 3, 1988 (day 11), August 28, 1988 (day 36), September 12, 1988 (day 50), and May 10, 1989 (day 291). For each replicate "glyphosate" and "control" plot, one sample was collected on each of the following days: September 6, 1988 (day 2), September 12, 1988 (day 8), and May 10, 1989 (day 248).

Each sample was passed through a 4 mm sieve and all visible plant roots were removed. The following determinations were then made:

(a) basal respiration (B.R.), determined as in Section 5.2

(b) substrate-induced respiration (S.I.R.) determined as outlined in Section 4.3.2

(c) B.R.:S.I.R. ratio, i.e. the ratio of (a) to (b)

The following environmental variables were measured for each sample, using the methods outlined in Section 2.2.

(a) gravimetric moisture content

(b) soil organic carbon content

Before the removal of each soil sample, a 50 x 50 cm sub-plot was set up and all above-ground vegetation was clipped at ground level. This was sorted into dicotyledonous weeds, monocotyledonous weeds, and barley plants. The dry weight of these was determined after oven drying at 80°C for 24 hours. In addition, for the May 10, 1989 samples (day 291 for 2,4-D; day 248 for glyphosate), the total surface dead material was collected from each plot, and dry weight determined as for the vegetation samples.

Data analysis

For each sampling time and herbicide, the data for basal respiration, substrate-induced respiration, and the B.R.:S.I.R. ratio were analysed using 4-way analysis of variance or covariance, testing for the following effects:

(a) effect of presence vs. absence of barley

(b) effect of presence vs. absence of weeds

(c) effect of presence vs. absence at herbicide application

(d) effect of blocking

(e) all the possible 2-way interactions of effects (a) - (c)

Covariates represented those environmental or vegetational variables which significantly reduced the total error sums of squares within the analysis. The data for each combination of weeding/non-weeding x barley/non-barley x time after application was also analysed using 2-way ANOVA, testing for the effects of the herbicide, and blocking.

7.3.2 RESULTS AND DISCUSSION

Basal respiration

Basal respiration was not found to be significantly influenced by 2,4-D, except at day 4 (barley-planted, weeded plots) and day 291 (non-barley, weeded plots) (Table 41). However, there were no consistent effects of any of the variables tested on basal respiration, including either a direct herbicide effect, or an

Table 41. Interactions between 2,4-D application and soil basal respiration.

		Days after 2,4-D application						
		0	1	4	11	36	50	291
(a) Treatment								
barley weeded	(herbicide)	.76	.77	.63	1.66	1.09	1.08	.94
	(control)	.76	.87	.72	1.88	.98	1.09	.67
barley non-weeded	(herbicide)	.79	.71	.65	1.71	1.15	1.08	.81
	(control)	.79	.86	1.27*	1.60	1.11	1.09	.85
no barley weeded	(herbicide)	.72	.98	.72	1.52	1.07	.95	.86
	(control)	.72	.69	.77	1.50	1.03	.98	.61*
no barley non-weeded	(herbicide)	.73	.87	.70	1.70	.87	1.13	.73
	(control)	.73	.81	.81	1.66	.98	1.06	.82
(b) ANOVA results	Barley (A)		ns	ns	ns	ns	ns	ns
	Handweeding (B)		ns	ns	ns	ns	ns	ns
	2,4-D (C)		ns	*	ns	ns	ns	ns
	Blocking (D)		ns	ns	ns	**	ns	*
	A x B		ns	ns	ns	ns	ns	ns
	A x C		*	ns	ns	ns	ns	ns
	B x C		ns	ns	ns	ns	ns	*

(a) Basal respiration ($\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) in plots of different plant and herbicide treatments
***, **, * indicate that differences between pairs of numbers subjected to the same barley x handweeding treatments are significantly different at p = 0.001, 0.01, and 0.05 respectively

(b) Effects of treatments on basal respiration as found by 4-way analysis of variance
***, **, * indicate significance of treatment effects at p = 0.001, 0.01, and 0.05 respectively; ns indicates effect is not significant at p = 0.05.

effect of herbicide x barley or herbicide x weeding interaction. It is likely that because of the large number of pairwise comparisons (Table 41), some of these may indicate erroneously significant treatment effects due to type I error. This may be true especially for those effects which are significant at $p = 0.05$, but not $p = 0.01$. Any effect of 2,4-D on basal respiration, direct or indirect, appears to be slight and negligible when the spatial and temporal variability of the data set as a whole is considered. The plant biomass, as influenced by handweeding or barley planting, had no significant effect on microbial respiration, and although significant reductions in dicotyledonous biomass were caused by 2,4-D on days 36 and 50, this did not translate into a detectable effect on basal respiration. Unlike the results obtained in 1987 (Section 7.2) neither soil moisture nor soil carbon content were found to serve as useful covariates, nor did they appear to exert any significant influence on the soil respiration rate.

The effects of glyphosate on basal respiration were similar to the effects noted for 2,4-D. No significant glyphosate effect was detected, except for a weak barley x glyphosate interaction at day 2 (Table 42). Other (non-herbicide-related) treatment effects on basal respiration were occasionally significant but no consistent trends were detected. Plant cover, soil carbon, and soil moisture did not individually exert any detectable effect on basal respiration, and therefore were not used as covariates. If any undetected herbicide effects did occur, these are probably negligible compared with spatial and temporal variation in basal respiration.

Table 42. Interactions between glyphosate application and soil basal respiration.

		Days after glyphosate application			
		0	2	8	248
(a) Treatment					
barley weeded	(herbicide)	1.70	1.70	1.04	.78
	(control)	1.70	1.66	1.05	.68
barley non-weeded	(herbicide)	1.62	1.44	.80	.97
	(control)	1.62	1.37	1.06	.85
no barley weeded	(herbicide)	1.71	1.30	.80	.75
	(control)	1.71	1.66	.95	.61
no barley non-weeded	(herbicide)	1.61	1.41	1.22	.81
	(control)	1.61	1.41	1.04	.82
(b) ANOVA results	Barley (A)		ns	ns	ns
	Handweeding (B)		**	ns	ns
	2,4-D (C)		ns	ns	ns
	Blocking (D)		ns	ns	**
	A x B		ns	***	ns
	A x C		*	ns	ns
	B x C		ns	ns	ns

(a) Basal respiration ($\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) in plots of different plant and herbicide treatments.

(b) Effects of treatments on basal respiration as found by 4-way analysis of variance

Legend as for Table 41

Substrate-induced respiration (S.I.R.)

2,4-D had no detectable effect on S.I.R. except at day 36 when there was a marginally significant herbicide influence (Table 43); however, this isolated result may be due to type I error. Other treatment effects were occasionally marginally significant but no consistent effects of any treatment on S.I.R. were found except for the influence of soil moisture content. Spatial variability in soil moisture content exerted considerable influence on variation in S.I.R. throughout the experimental period (except at day 50). This is consistent with the observations made in Chapters 4, 5, and 6. There appears to be no important consequence of 2,4-D application on S.I.R. (or therefore, presumably soil microbial biomass) regardless of whether barley plants or weeds were present or not.

Values for S.I.R. were not found to be significantly influenced by glyphosate (Table 44) except for a reduction in S.I.R. at day 8 in plots without either barley plants or handweeding. This result was significant at $p = 0.01$, suggesting that it may represent a real treatment effect rather than merely being attributable to type I error. However, it is unclear as to why this treatment effect should only occur when weeds are not removed, especially because there did not appear to be any relationship between the quantity of plant biomass (weeds or barley) and S.I.R. The only other variable which exerted a strong effect on S.I.R. was soil moisture, and this occurred only in the May 1989 (day 248) sampling.

Table 43. Interactions between 2,4-D and substrate-induced respiration (S.I.R.).

		Days after glyphosate application						
		0	1	4	11	36	50	291
(a) Treatment								
barley weeded	(herbicide)	12.1	13.0	12.0	17.7	11.3	13.2	10.4
	(control)	12.1	12.4	11.0	17.4	10.9	14.0	9.6
barley non-weeded	(herbicide)	12.5	12.3	12.0	18.3	11.3	13.0	11.7
	(control)	12.5	12.4	12.2	17.1	10.9	12.9	10.8
no barley weeded	(herbicide)	12.8	14.4	13.4	15.0	12.1	12.2	10.3
	(control)	12.8	12.9	13.0	16.2	11.2	12.0	9.0
no barley non-weeded	(herbicide)	13.5	12.7	11.9	16.5	10.3	12.7	10.7
	(control)	13.5	13.6	12.8	16.3	11.2	13.0	11.0
(b) ANOVA results	Barley (A)		ns	ns	ns	ns	ns	ns
	Handweeding (B)		ns	ns	ns	*	ns	ns
	2,4-D (C)		ns	ns	ns	ns	ns	*
	Blocking (D)		ns	ns	ns	***	ns	ns
	A x B		ns	ns	ns	ns	*	ns
	A x C		ns	ns	ns	ns	ns	ns
	B x C		*	*	ns	ns	ns	ns
	covariates:soil moisture		***	***	***	***	-	***

(a) S.I.R. ($\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) in plots of different plant and herbicide treatments.

(b) Effects of treatments on S.I.R. as found by 4-way analysis of variance

Legend as for Table 41

Table 44. Interactions between glyphosate addition and substrate-induced respiration (S.I.R.).

		Days after glyphosate application			
		0	2	8	248
(a) Treatment					
barley weeded	(herbicide)	12.0	12.6	13.4	10.4
	(control)	12.0	12.8	14.0	9.6
barley non-weeded	(herbicide)	12.9	12.0	12.1	11.3
	(control)	12.9	12.5	12.9	10.8
no barley weeded	(herbicide)	12.6	12.6	11.8	10.2
	(control)	12.6	11.1	12.0	9.0
no barley non-weeded	(herbicide)	11.4	11.7	10.6	9.8
	(control)	11.4	13.8	13.0*	11.0
(b) ANOVA results	Barley (A)		ns	ns	ns
	Handweeding (B)		ns	ns	ns
	2,4-D (C)		ns	*	ns
	Blocking (D)		ns	ns	*
	A x B		ns	ns	ns
	A x C		ns	ns	ns
	B x C		*	ns	ns
	covariates:soil moisture		-	-	***

(a) S.I.R. ($\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) in plots of different plant and herbicide treatments.

(b) Effects of treatments on S.I.R. as found by 4-way analysis of variance

Legend as for Table 41

Soil moisture content exerted a dominant effect on the S.I.R. data obtained in the 2,4-D study on July 24, July 17, August 3, and August 28, 1988 and in the 2,4-D and glyphosate studies on May 19, 1989. However, there was no significant effect of moisture on S.I.R. on September 6 and 13, 1988. This is because soil moisture levels were higher in September 1988 than during the other sampling times, and were not limiting to such an extent that spatial variability in S.I.R. was affected. At these September sampling times, even when soil moisture did not exert a significant influence, there were no consistent effects of other treatments on S.I.R. despite the background variability in S.I.R. being lower than in other sampling times during the study.

Basal respiration:substrate-induced respiration ratio

The basal respiration:substrate-induced respiration (B.R.:S.I.R.) ratio was not consistently influenced by either 2,4-D or glyphosate during the study period (Tables 45,46). There was no consistent effect of handweeding, barley planting, or spatial variability in soil moisture content, although spatial variability in soil carbon content inexplicably had a strong influence on the ratio during one isolated sampling time, i.e. August 3, 1988 (Table 45: day 11). As outlined earlier (Section 6.3), the B.R.:S.I.R. ratio can be used as an indicator of the level of ecosystem disturbance caused by herbicide application (Odum 1969, 1985). Therefore in the present study, neither 2,4-D nor glyphosate exerted a detectable disturbance effect. The samplings at September 4 and 6, 1988

Table 45. Interactions between 2,4-D and the basal respiration: substrate-induced respiration (B.R.:S.I.R.) ratio.

		Days after 2,4-D application						
		0	1	4	11	36	50	291
(a) Treatment								
barley weeded	(herbicide)	.063	.059	.053	.094	.096	.082	.090
	(control)	.063	.070	.065	.108	.089	.078	.070
barley non-weeded	(herbicide)	.063	.058	.054	.093	.102	.083	.069
	(control)	.063	.069	.104	.094	.102	.084	.079
no barley weeded	(herbicide)	.056	.068	.054	.101	.088	.078	.083
	(control)	.056	.053	.059	.098	.092	.082	.068
no barley non-weeded	(herbicide)	.054	.069	.059	.102	.084	.089	.068
	(control)	.054	.060	.063	.103	.088	.082	.075
(b) ANOVA results	Barley (A)		ns	ns	ns	ns	ns	ns
	Handweeding (B)		ns	ns	ns	ns	ns	ns
	2,4-D (C)		ns	*	ns	ns	ns	ns
	Blocking (D)		ns	ns	ns	*	ns	*
	A x B ns		ns	ns	ns	ns	ns	
	A x C *		ns	*	ns	ns	ns	
	B x C ns		ns	ns	ns	ns	ns	
	covariates:soil moisture		-	-	***	-	-	-

(a) B.R.:S.I.R. ratio in plots of different plant and herbicide treatments.

(b) Effects of treatments on the B.R.:S.I.R. ratio as found by 4-way analysis of variance or covariance

Legend as for Table 41

Table 46. Interactions between glyphosate addition and the basal respiration:substrate-induced respiration (B.R.:S.I.R.) ratio.

		Days after glyphosate application			
		0	2	8	248
(a) Treatment					
barley weeded	(herbicide)	.142	.135	.078	.075
	(control)	.142	.130	.075	.070
barley non-weeded	(herbicide)	.126	.120	.066	.086
	(control)	.126	.110	.084	.079
no barley weeded	(herbicide)	.136	.103*	.068	.074
	(control)	.136	.150	.082	.068
no barley non-weeded	(herbicide)	.141	.121	.115	.083
	(control)	.141	.102	.084	.075
(b) ANOVA results	Barley (A)		ns	ns	ns
	Handweeding (B)		*	ns	ns
	2,4-D (C)		ns	ns	ns
	Blocking (D)		ns	ns	*
	A x B		ns	*	ns
	A x C		ns	ns	ns
	B x C		ns	ns	ns
			*	ns	ns

(a) B.R.:S.I.R. ratio in plots of different plant and herbicide treatments.

(b) Effects of treatments on B.R.:S.I.R. ratio as found by 4-way analysis of variance

Legend as for Table 41

(Table 46: days 0 and 2) indicated much higher ratio values than for the other sampling periods, presumably because these measurements followed a recent rainfall event, which could have acted as a disturbance event.

Importance of herbicide effects on the soil microbial biomass

While the results based on 1987 data, outlined in the previous section (Section 7.2), indicate that 2,4-D significantly enhanced B.R., S.I.R. and the B.R.:S.I.R. ratio the same trend was not observed in the experiments outlined in this section. There was no sign of a consistent direct or indirect effect of 2,4-D on the microbial variables regardless of whether weeds and/or barley plants were present or not. Although 2,4-D significantly reduced dicotyledonous and total weed biomass, this did not translate into a net microbial response. In the previous (1987) study it was concluded that effects of 2,4-D were statistically significant but relatively unimportant compared with effects caused by spatial and temporal variation in certain environmental variables. In the present study, 2,4-D effects were not even consistently significant. This is possibly because the area was subjected to a near record drought prior to setting up the experiment, and subsequent extreme changes in soil moisture status throughout the experimental period could have contributed to obscuring effects of 2,4-D which might otherwise have been detected. The relatively strong effects of time after herbicide application on each microbial parameter probably reflect

changes in the soil moisture status. The mean soil moisture content rose to 46.0% from 34.7% between days 4 and 11, and fell to 33.5% by day 36. This strongly paralleled shifts in B.R. and S.I.R. The evidence in the present study reinforces that of the previous one, where soil moisture status exerted a dominant effect on the soil microflora, and herbicide applications did not.

Similarly, glyphosate did not influence any of the microbial variables in a consistent manner. This agrees with the results in Section 7.2, and suggests that at field-level applications of the herbicide, there is no detectable effect on the soil biomass or its activity.

In no instance did plant biomass consistently influence any of the soil biotic variables investigated, and reduction of weed cover by the herbicide had no detectable short-term effects on these variables. Although there was a marginally significant effect of herbicide application in reducing the surface litter on the plots in the following spring (i.e. May 10, 1989), this did not result in differences between treated and untreated plots with regard to the size and activity of the soil microbial biomass. Therefore, the present investigation suggests that in the short term there is neither a direct effect of 2,4-D nor glyphosate on the soil microbial biomass and its activity (in plots with all plants removed), nor an indirect effect due to the reduction of plant cover caused by herbicide addition.

CHAPTER 8

8. GENERAL DISCUSSION

8.1 METHODS FOR MICROBIAL BIOMASS MEASUREMENTS

The soil microbial biomass plays an important role in carbon and nutrient cycling, and acts as a labile sink of available nutrients (Anderson and Domsch 1980). Therefore the turnover of the microbial biomass regulates the supply of plant nutrients, and this influences ecosystem productivity (McGill et al. 1986). Quantification of the total microbial biomass is therefore of interest because it acts as an indicator of soil fertility and nutrient immobilization in microbial tissue. The total microbial biomass is comprised of active and inactive components; the active component contributes directly to short-term soil processes while the inactive component does not.

Quantification of the total microbial biomass has been assisted by the development of physiological techniques for such studies, the first of these being the fumigation-incubation method (Jenkinson and Powlson 1976). However, determination of the total microbial biomass using this method may be problematic because of difficulties associated with selecting an appropriate non-fumigated "control" treatment (Jenkinson 1988), incomplete fumigation (Ingham and Horton 1987), and variability in the parameter k_c (Ross 1987; Jenkinson 1988).

The investigations outlined in chapters 3 and 4 indicated that $\underline{k}_c$ may differ amongst soil types, and soil samples preincubated under different soil moisture conditions. Whether or not these variations in $\underline{k}_c$ determination may be important in a given study depends upon the objectives of that study. If an approximate value of the amount of organic C immobilized in the microbial pool is required, or if different treatments or soil types with vastly differing biomass values are being compared, then variations in $\underline{k}_c$ may not be highly important and the use of a constant $\underline{k}_c$ factor may be adequate. However, when accurate estimates of biomass are required, or when treatment effects on the microbial biomass may be small (<20%) but still significant (and of interest), then difficulties in interpreting results based on the use of a constant $\underline{k}_c$ factor may occur. When a constant $\underline{k}_c$ factor is used, a value of 0.41 (Anderson and Domsch 1978a) would probably be suitable, as it is well within the range of values determined in Chapter 4.2 (i.e. 0.25 - 0.47). The use of the fumigation-incubation method may also be problematic in forest soils where $\underline{k}_c$ values have not been effectively determined. Furthermore, although variations in bacterial:fungal ratios were not found to influence $\underline{k}_c$-factor estimation significantly in the present study, this may exert an influence if this ratio is found to vary substantially in response to treatment effects. Therefore, it can be recommended that when the fumigation-incubation method (or related techniques) is used to determine microbial biomass values for soils of different treatments,

preliminary data should first be obtained to determine whether $\underline{k}_c$ values are likely to remain independent of treatment effects.

The three physiological techniques proposed for predicting microbial biomass C (i.e. fumigation-incubation, oxygen uptake, and S.I.R.) predicted different responses of the microbial biomass to soil moisture, and did not correlate well with each other across the 120 soil subsamples used. Statistical re-evaluation of previously determined calibrations (Chapter 3) also indicated that correlation coefficients between fumigation-based methods and those involving glucose-stimulation may actually be quite low. This may be in part because of the problems associated with fumigation-based methods outlined above, including variability of the $\underline{k}_c$, $\underline{k}_{EC}$, and $\underline{f}_{5d}$ factors. However, the S.I.R. method also relies on a fundamental assumption for which little evidence exists, i.e. that all components of the microbial biomass respond equivalently immediately upon glucose addition. It is likely that inactive microorganisms do not respond immediately to glucose (Van de Werf and Verstraete 1987a,b), yet these microorganisms constitute most of the total soil microbial biomass (Sparling 1985; Van de Werf and Verstraete 1987b). Therefore, it is likely that fumigation-based methods apply to active and inactive microbes, while S.I.R. and related techniques apply only to the active components. Uncertain relationships between fumigation-incubation and S.I.R. may occur if the fraction of the total biomass which is active varies between soils or between treatments. This may be especially true across a soil moisture gradient where microbial biomass may be inactivated by soil moisture limitation but not

necessarily killed. Therefore it is recommended that fumigation-based methods be applied for assessing the total microbial biomass, while S.I.R. be applied with the recognition that the active microbial biomass may be the principal contributor to respiration following glucose addition.

Application of S.I.R. for absolute quantification of the active microbial biomass may be problematic, especially because it is usually calibrated against fumigation-incubation. There may, however, be some potential for calibrating S.I.R. against microscopic methods for quantifying active microbial biomass (see Söderström 1979; MacDonald 1980) both within and bbetween soil types. However, S.I.R. can provide useful information on relative values on the active portion of the total microbial biomass, such as has been done in Chapters 5, 6, and 7.

Glucose-stimulation techniques such as S.I.R. can be used to provide useful information on the active microbial biomass other than its relative magnitude. When glucose is added to soil samples, the respiratory response of the biomass can be assessed over time if an effective system for regular measurement of CO_2 release or O_2 uptake is available. Visser et al. (1984) and Visser and Parkinson (1979) utilized such an approach to determine the response of the microbial biomass to topsoil storage and chemical perturbations respectively, and concluded that the microbial biomass in systems under stress responded more slowly to added glucose than in conditions of relative stability. This respiratory delay indicated that in unfavourable conditions the microbial biomass is physiologically

stressed, and this inhibits its responsiveness to a rapid influx of substrate.

When S.I.R. is used in combination with bacterial and fungal protein synthesis inhibitors, additional information can be obtained on the dynamics of the soil microbial biomass. Addition of glucose to soil samples initiates temporary microbial growth, which is accompanied by protein synthesis. Simultaneous addition of glucose and bacterial or fungal protein synthesis inhibitors can therefore be used to determined whether this protein synthesis is primarily of bacterial or fungal origin. As indicated in Chapter 6, such information can be used to determine whether increases in S.I.R. may be attributed primarily to increases in bacterial or fungal protein synthesis. Selective inhibition can also be used for determining bacterial:fungal ratios of this active component (see Anderson and Domsch 1973, 1975), but this first requires two main assumptions which are difficult to fulfil, i.e. (1) that the 40-60% of S.I.R. which is not inhibited by either inhibitor has the same bacterial:fungal ratio as that component which is inhibitor-sensitive; and, (2) bacteria and fungi respire at the same rate per unit biomass upon glucose addition.

When S.I.R. is measured, useful additional information can be simultaneously obtained by measuring the activity of the microbial biomass, in terms of basal respiration. This approach was used in Section 4.4 to demonstrate that inactive micro-organisms can be immediately reactivated when dry soils are rewetted. Basal respiration data can also indicate the availability of resources in

the soil system, and this can be effectively demonstrated when the soil is recovering from disturbance and approaching a condition of relative stability (= persistence: Richards 1987). In such a situation the basal respiration increases then gradually decreases. This is most likely because a component of the microbial biomass is killed by the disturbance, and is then decomposed, resulting in a temporary "flush of decomposition" (Jenkinson and Powlson 1976).

The ratio of basal respiration:microbial biomass or B.R.: S.I.R. can provide additional information on the response of the microbial biomass to disturbance. This ratio is based on Odum's theory of ecosystem succession (Odum 1969, 1985), part of which predicts the ratio of total respiration to total biomass in an ecosystem decreases over time during succession, and increases during periods of stress, because ecosystems become more efficient in conserving resources as ecosystem stability (= persistence: Richards 1987) increases. This ratio was applied to soil ecosystems by Insam and Domsch (1988) who concluded that since most of the net primary productivity (NPP) of above-ground systems passes through the soil system, it was possible to simplify Odum's model by replacing total respiration and total biomass with basal respiration and microbial biomass. In the present investigation, the ratio of B.R.: S.I.R. was used as a measure of the respiration:active biomass ratio, and it provided a useful indication of the response of the microbial biomass to disturbance, either through herbicide application or wetting/drying cycles, where the ratio increased then decreased again during periods of relative stability. This

indicates that during periods of disturbance and instability the microbial biomass becomes inefficient in immobilizing carbon reserves for growth and maintenance, and respires a higher proportion of incoming C as CO_2, than in periods of stability. Therefore, this ratio enables assessment as to how the efficiency of the microbial biomass (in terms of carbon-utilization) may vary across a disturbance gradient.

8.2 EFFECTS OF SOIL MOISTURE ON MICROBIAL BIOMASS

While S.I.R. may provide a useful measure of how the active microbial biomass responds to variations in soil moisture content, different results were obtained depending upon whether or not soils were remoistened simultaneous to glucose addition. When soils were remoistened S.I.R. was largely independent of initial soil moisture content, while without remoistening S.I.R. was very strongly related to soil moisture status. This leads to a possible conclusion that there are in fact two separate and distinct pools of inactive microbial biomass:

(i) Those microorganisms which are inactive because of soil moisture stress, and therefore cannot respond immediately to glucose addition. These are immediately activated upon remoistening.

(ii) Those microorganisms which are inactive over a longer time frame, and cannot be activated by glucose addition, even in the presence of optimum soil moisture conditions.

(n.b. These distinctions are contingent upon the definition of the active microbial biomass as proposed by Van de Werf and Verstraete 1987a,b, i.e. that microbial biomass which can respond immediately to glucose addition).

If these two pools can be presumed to be distinct, then S.I.R. with remoistening in dry soils measures all these microorganisms in pool (i), while S.I.R. without remoistening excludes all those microorganisms in both pools (i) and (ii). It should be emphasized that these S.I.R. values can provide only a relative measure of the active microbial biomass, because there is no known effective way of calibrating the method. Therefore, in the present investigations all S.I.R. measurements have been kept in units of respiration rate (i.e. $\mu g\ CO_2\text{-}C \cdot h^{-1} \cdot g\ soil^{-1}$) rather than converted to biomass figures (i.e. μg biomass C g $soil^{-1}$). However, even as a relative measure of active microbial biomass S.I.R. is still problematic because the response of the microbial biomass to added glucose may vary under different circumstances. It is possible that a microbial community with a high ecological efficiency will respire a lower fraction of the added glucose as CO_2 than one which is less efficient.

Determination of the response of the total microbial biomass (inactive plus active) to soil moisture gradients is problematic, especially because the two fumigation-based techniques used (i.e. based on CO_2 release and O_2 uptake) yielded very different results on the same samples. However, when the results for the CO_2-C released and O_2 taken up by control (non-fumigated) samples is

considered, similar patterns were found. In both cases respiration was much higher in samples that were dried and remoistened compared with those held at a constant high moisture level. It is reasonable to assume that this respiration enhancement was attributable to microorganisms killed by drying. Why this was not detected in the use of the fumigation-incubation assay (even with k_c values determined for each sample and treatment) remains unclear. Previous studies have used related approaches to assess drying effects on the soil microbial biomass. These also demonstrate that the microbial biomass is influenced by drying-rewetting cycles, although this is difficult to quantify. For example, Shields *et al*. (1974) and Van Veen *et al*. (1987) have used microbial biomass labelled with ^{14}C *in situ* to demonstrate that the flush of respiration (in the form of $^{14}CO_2$) following rewetting of dry samples is of microbial origin. Nevertheless, the fraction of microbial biomass killed by drying and the turnover of microbial biomass attributable to drying/rewetting cycles is difficult to determine.

8.3 EFFECTS OF HERBICIDES ON MICROBIAL BIOMASS

The investigations outlined in Chapters 6 and 7 were concerned with determining whether the application of commonly used herbicides exerted any real influence on variables associated with the soil microbial biomass, i.e. basal respiration, S.I.R., the basal respiration:S.I.R. ratio, and extent of inhibition by selective inhibitors. S.I.R. was selected for use in this study because it provides an indication of the response of the active microbial

biomass to the herbicide, and because the technique allows simultaneous rapid processing of a large number of samples. The results of the laboratory incubations (Chapter 6) indicated that all three herbicides tested exerted some effect on the microbial biomass (at least in terms of its activity) but only at concentrations of 200 ppm herbicide. Even the effects of 200 ppm herbicide on the variables studied were relatively slight when compared with the effects of natural temporal and spatial variability (see Chapter 5). Furthermore, herbicide concentrations of 200 ppm are well in excess of the concentrations of herbicide that may be experienced following field level applications, possibly by as much as two orders of magnitude (Domsch *et al*. 1983; Anon 1987).

The temporary enhancement of soil S.I.R., and especially the fungal component, by 200 ppm glyphosate, can be complemented by other information which was collected in a parallel study but not reported in detail here. In that study soil was preincubated for 0, 1, 3, 9, and 27 days under glyphosate regimes of 0, 2, 20, and 200 ppm. Assessments of the principle fungal species were made on both organic and mineral particles using the procedure of fungal isolation described in section 2.3. Significant temporary enhancement of three of the four principal fungal species by 200 ppm glyphosate was noted on organic fragments, i.e. *M. alpina*, *T. harzianum*, and *A. sphaerospermum* (the fourth species, i.e. *M. hiemalis*, was unaffected). *Trichoderma harzianum* was also significantly enhanced by 200 ppm glyphosate on mineral particles. These shifts in fungal species composition were temporary and most obvious at day 3; this

coincided with enhancement of S.I.R. and the fungal contribution to S.I.R. by 200 ppm glyphosate. Therefore, the evidence presented in Chapter 6 which suggests that the fungal biomass is temporarily enhanced by glyphosate is reinforced by data suggesting that some of the fungal species are also temporarily enhanced, with a resulting shift in fungal community structure. The influences of 200 ppm glyphosate on the fungal community structure were also determined to be relatively unimportant compared with other sources of variability in the data set, and this again complements the biomass data given in Chapter 6. This was demonstrated using the ordination technique "Detrended Canonical Correspondence Analysis" (Ter Braak 1987; Ter Braak and Prentice 1988) which detected statistically significant herbicide effects on the fungal community data compared with natural variability in the data set.

The field studies (Chapter 7) did not detect any consistent herbicide effect on the microbial variables measured, except for significant enhancements of microbial biomass and activity by 2,4-D in the 1987 experiment. This enhancement by 2,4-D at field-level applications is in total contrast to the results obtained by adding 2,4-D to laboratory-incubated samples where only patterns of inhibition were noted, and only at 200 ppm herbicide. There is no obvious reason for this discrepancy, although it is possibly attributable in part to the soil moisture levels of the laboratory-incubated samples being higher than for those in the field study, with possible differences in microbial community structure, and therefore in the microbial response and susceptibility to 2,4-D.

Despite the differences observed in the microbial response between laboratory and field experiments, both studies indicated that responses of the microbial biomass to herbicide additions, when detectable, are slight when compared to the more extreme responses of this biomass to environmental variables, especially soil moisture content fluctuations. The data collected in this investigation indicate that while it is difficult to predict how the microbial biomass may respond to herbicide addition in most field conditions, it is most likely that the herbicides applied in the experiments outlined here exert minimal influence on the soil microbial biomass, especially when variations in other factors responsible for regulating the soil microbial biomass are considered.

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