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An evaluation of the substrate-induced respiration method

Q. Lin, P.C. Brookes*

Soil Science Department, IACR-Rothamsted, Harpenden, Herts., AL5 2JQ, UK

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Abstract

Two ways of measuring substrate-induced respiration (SIR) following addition of glucose to soil, viz. solid glucose or glucose in solution, were tested on 13 soils sampled from arable, grassland and woodland sites ranging from pH 3.2 to 7.5. Generally similar patterns of CO_2 evolution were found between soils following addition of glucose as liquid or solid ($r = 0.93$) for unamended, ryegrass-amended and fumigated soils. Glucose added in solution to adjust the soils to 1.2-fold WHC was therefore preferred for analytical convenience. The optimum time of CO_2 measurement was between 0.5 and 2.5 h for routine use. It was found to be unnecessary to make any correction for CO_2 dissolved in the soil solution for soils below pH 6.5. Maximum inhibition of bacterial respiration was obtained at 4 to 8 mg added streptomycin and of fungal respiration at 8–12 mg added cyclohexamide g^{-1} soil. In a grassland soil (24% clay) the bacteria comprised $19 \pm 4.2\%$ and the fungi $82 \pm 4.0\%$ of the total biomass and in a grass ley (8% clay) the proportions were $25 \pm 1.2\%$ for bacteria and $76 \pm 4.5\%$ for fungal biomass.

Keywords: Substrate-induced respiration; SIR; Cyclohexamide; Streptomycin

1. Introduction

Most microorganisms in the soil are dormant (Jenkinson and Ladd, 1981), so their rate of respiration is low. However, their respiration can be stimulated by adding an easily decomposable substrate. Respiration may then rapidly increase to a maximum and remains at a constant rate for more than 4 h (Drobnik, 1960). Glucose is commonly used as a substrate because most soil microorganisms can readily utilize it as a carbon source (Stotzky and Norman, 1961). Anderson and Domsch (1978) suggested that the initial maximal respiration rate induced by glucose was proportional to the size of the original soil microbial biomass. The quantity of glucose added to achieve a maximal initial respiration rate varies greatly, depending on soil physical and chemical properties. Thus, Anderson and Domsch (1978) suggested that the quantity of glucose required to elicit the maximal respiration should be determined for each soil.

The substrate-induced respiration (SIR) rate is strongly influenced by soil water content (Wardle and Parkinson, 1990a). To avoid the influence of water content on the SIR measurement, West and Sparling (1986) added glucose (2 ml g^{-1} soil) rather than glucose powder to ensure excess water in all cases.

For reliable SIR measurement it is most important that the glucose should be distributed evenly throughout the soil. Adding glucose solution appears not only to give the best distribution of glucose in soil, but is also analytically very convenient. However, it is not certain that both approaches measure the same parameters under such different conditions. For example, West and Sparling (1986) pointed out that a large amount of CO_2 may be dissolved in soil solution when the soil pH is greater than 6.0. Similarly, Sparling and West (1990) reported that, above about pH 6.5, CO_2 measured by GC may be underestimated because CO_2 is retained in solution, whereas retention is small above this pH.

One of the perceived advantages of SIR is the

* Corresponding author. Tel.: +44-1582-763-133; fax: +44-1582-760-981.

E-mail address: philip.brookes@bbsrc.ac.uk (P.C. Brookes)

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Table 1
Soils

Soil No.	Field experiment	Sample depth (cm)	Organic C (%)	Total N (%)	pH	Management	Texture
1	Broadbalk Nil plot 32	0–23	0.88	0.095	7.5	winter wheat	clay loam
2	Broadbalk FYM plot 022	0–23	2.94	0.273	7.2	clay loam	clay loam
3	Broadbalk N_4PK plot 09	0–23	1.02	0.116	7.3	clay loam	clay loam
4	Broadbalk wilderness	0–10	4.42	0.401	6.7	deciduous forest	clay loam
5	Highfield grassland	0–10	4.08	0.364	4.8	grass	clay loam
6	Geescroft wilderness	0–10	6.65	0.389	3.2	deciduous forest	clay loam
7	Geescroft fallow	0–23	2.17	0.183	4.9	fallow	clay loam
8	Woburn long-term fallow	0–10	0.69	0.045	5.1	fallow	clay loam
9	Woburn ley-arable rotation: Plot 32	0–10	1.21	0.120	6.4	grass ley[a]	sandy
10	Woburn ley-arable rotation: Plot 72	0–10	2.00	0.147	6.1	grass ley[b]	sandy
11	Woburn ley-arable rotation: Plot 75	0–10	1.75	0.144	6.2	clover–grass ley[c]	sandy
12	Woburn ley-arable rotation: Plot 67	0–10	1.14	0.109	6.0	winter bean[d]	sandy
13	Woburn ley-arable rotation: Plot 60	0–10	1.42	0.127	6.4	clover–grass ley[e]	sandy

[a] From yr 1 of grass ley in a winter wheat; summer barley; 3 yr all grass ley rotation.
[b] From yr 8 of grass ley in a winter wheat; spring barley; 8 yr grass ley rotation.
[c] From yr 8 of clover–grass ley in a winter wheat; spring barley; 8 yr clover–grass ley rotation.
[d] From alternate winter wheat; spring barley; barley; winter bean.
[e] From second year of clover–grass ley in a winter wheat; spring barley; 3 yr clover–grass ley rotation.

measurement of the contribution of bacterial and fungal biomass to substrate-induced CO_2 respiration through coupling with antibiotics. Apparently successful measurements have been reported with this method in arable (Anderson and Domsch, 1973a,b, 1975), grassland (West, 1986; Wardle and Parkinson, 1990b) and rhizosphere–rhizoplane soils (Nakas and Klein, 1980) and in plant residues (Beare et al., 1990). However, when Ross et al. (1981) measured O_2 uptake following glucose addition they found no selective inhibition of O_2 uptake by streptomycin and cycloheximide. Unsuccessful application of this method has also been reported by West (1986) in an arable soil of low biomass content, and by Bewley and Parkinson (1985) in an organic soil.

Our aims were: (1) to optimise soil moisture content for SIR measurements, (2) to compare SIR responses to glucose in solid and aqueous forms, (3) to investigate relations between SIR response and soil physical and chemical conditions, e.g. organic C, clay content and pH and (4) to optimise conditions for selective inhibition measurements. The different glucose amendments were tested in unamended soils, ryegrass-amended soils and soils following 10 or 20 d incubation after 24 h $CHCl_3$ fumigation and its subsequent removal. The different treatments were selected to produce biomasses which were likely to have different community structures and activities.

This account is the first of three linked papers (Lin and Brookes, 1999a,b) in which we test different approaches to evaluate both the total soil microbial biomass and its microbial community structure (specifically the proportions of bacterial and fungal populations in soils of different types and managements given a number of treatments, viz. incubation unamended, amended with substrates e.g. ryegrass and glucose, and fumigated soils). In the second paper we reexamine the arginine ammonification method of Alef and Kleiner (1987) in conjunction with the use of selective inhibitors of bacterial and fungal activity (Lin and Brookes, 1999a) to see if this approach could be used to partition the biomass into its fungal and bacterial components. In the third paper (1998b) we compare SIR and selective inhibition with direct microscopic measurements of the proportions of fungi and bacteria in the microbial biomass (Lin and Brookes, 1999b).

2. Materials and methods

2.1. Soils

Thirteen soils were sampled at 0–10 cm or 0–23 cm with a 5 cm dutch auger. These soils were sieved moist at <2 mm and then incubated at 25°C for 7 d following adjustment of soil moisture to 40% of water-holding capacity. The conditioned (preincubated) soils were then stored at 5°C for up to 2 months prior to analysis. The detailed characteristics of these soils are given in Table 1.

Seven of the soils came from Rothamsted Experimental Station, UK. They ranged from about 0.9 to 6.7% organic C and from about pH 3.2 to 7.5. Six others came from Woburn, UK, and ranged from about 0.7 to 2.0% organic C and from pH 5.1 to 6.4. Soils No. 5 and 9 (Lin and Brookes, 1996) and a clay soil from a non-experimental field at Woburn were

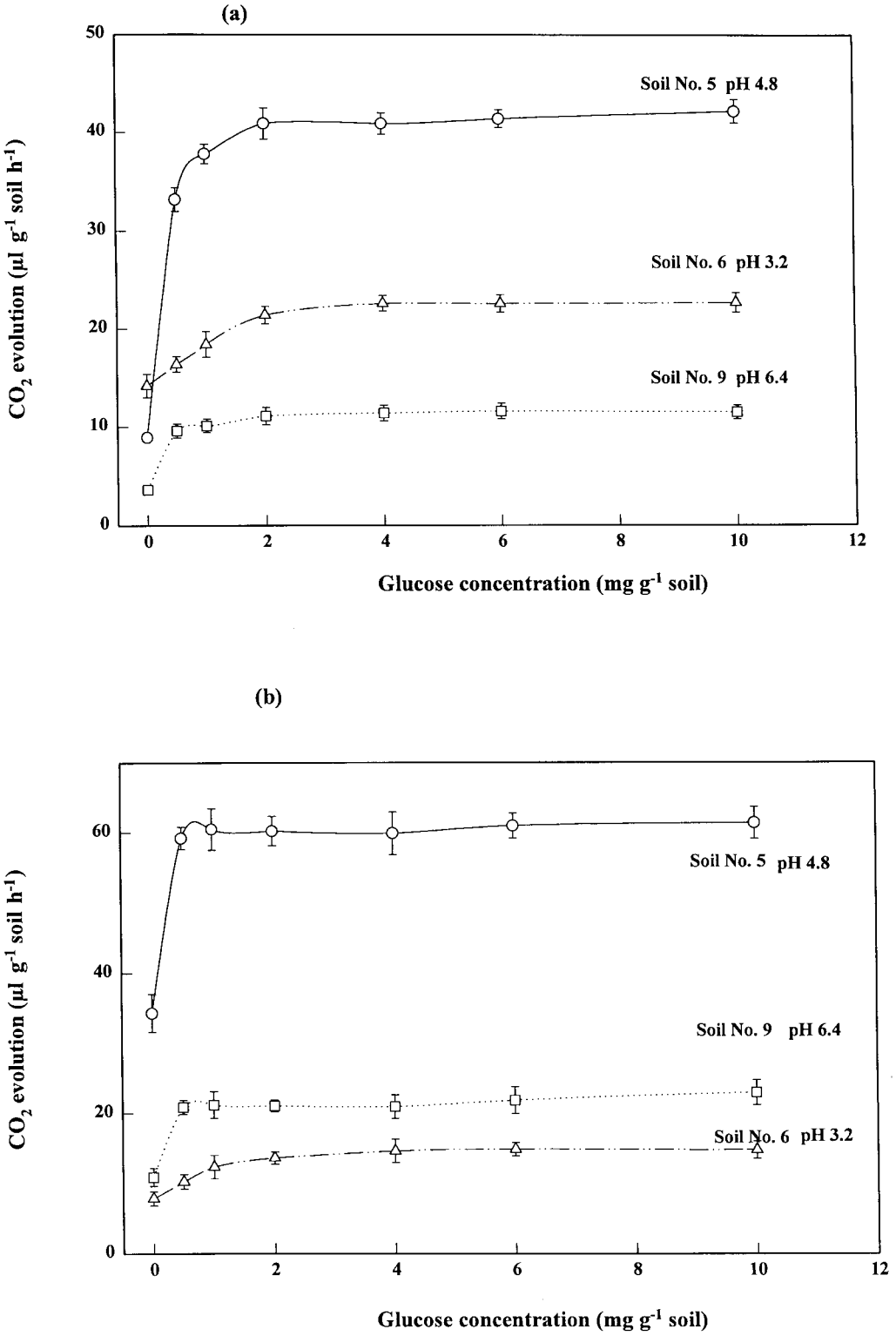

Fig. 1. The response of the soil microbial biomass to the addition of (a) solid and (b) aqueous glucose in the soils of different pH. Bar is standard deviation.

used for measuring the proportions of bacterial and fungal biomass. The conditioning treatment of the clay soil was the same as described above. This soil contained 56% clay (< 2 μm), 1.60% organic C, 0.13% total N and had a pH of 6.2.

2.2. Ryegrass amendment

Portions of moist soil No. 3–13 (approximately 1 kg) were amended with finely-ground ryegrass (2 wt%). Variable small amounts of distilled water were added to adjust soil moisture to 50% of water-holding capacity. The soils were then incubated in a container with water and soda-lime at 25°C for 10 d for soil No. 3, 5, 6, 8, 9 and 10 and for 20 d for the other soils.

2.3. Fumigation–incubation

Portions of unamended soils No. 3, 5, 6, 8, 9 and 10 (approximately 0.5 kg) were fumigated with alcohol-free $CHCl_3$ and then incubated as described above for 10 d following removal of $CHCl_3$. Portions of soil No. 4, 7, 11, 12 and 13 (approximately 0.5 kg) which had been incubated with ryegrass for 10 or 20 d (see above) were also fumigated. They were then incubated for 10 d after $CHCl_3$ removal as described above.

2.4. Measurement of carbon dioxide evolution following glucose addition

Three portions of moist soil, No. 5, 6 and 9, each containing 10–40 g oven-dry (o.d.) soil, were amended with a series of glucose concentrations (0.5, 1, 2, 4, 6 and 10 mg g^{-1} o.d. soil) either in solution (to adjust soil moisture to 120% WHC) or as a solid (ground with talcum into a fine powder). The soils were then incubated at 25°C (with shaking at 150 rev min^{-1} after adding liquid glucose), and CO_2 evolution was determined over 0.5–6.5 h after glucose addition (Fig. 1).

2.5. Measurement of carbon dioxide dissolved in soil solution

Moist soils were fumigated with alcohol-free $CHCl_3$ for 48 h at 25°C. After removal of $CHCl_3$, the same individual weights of the fumigated soil and talcum or distilled water (to adjust soil moisture to 120% WHC), as used in standard SIR measurements, were weighed into 250 ml Quickfit flasks. Pure CO_2 was added to provide a series of CO_2 concentrations. Carbon dioxide concentrations in the flasks were immediately determined after CO_2 addition (initial CO_2 concentrations). The flasks were then incubated at 25°C for 2 h as in SIR measurements. Carbon dioxide concentrations in the flasks were measured again (final CO_2 concentrations). Measurement of CO_2 concentration in the soil without pure CO_2 addition was also done (control CO_2 concentrations). The quantity of CO_2 dissolved in soil solution was calculated from:

$$(\text{initial } CO_2 \text{ concentration}) - [(\text{final } CO_2 \text{ concentration}) + (\text{control } CO_2 \text{ concentration})]$$

2.6. Measurement of selective inhibition of CO_2 evolution following glucose addition

Three portions of moist soil (10–30 g) were amended with (a) glucose (6 mg g^{-1} soil), (b) glucose + streptomycin, (c) glucose + cycloheximide, and (d) glucose + streptomycin + cycloheximide. Glucose powder was used here because adding glucose solution resulted in a somewhat poorer correlation between SIR rate and soil ATP content and biomass C measured by FE (Qimei Lin, unpublished Ph.D. thesis, University of Nottingham, 1994). The glucose and inhibitors were ground with talcum (1:4 glucose to talcum ratio) into a fine powder before use. The soil samples were then incubated at 25°C and CO_2 evolved was determined by GC

3. Results and discussion

3.1. The response of soil microorganisms to glucose addition

Carbon dioxide evolution following addition of solid glucose increased rapidly up to a maximum rate, at 2 mg glucose g^{-1} soil, of 40.9 for soil No. 5 and 11.2 μl CO_2 g^{-1} soil h^{-1} for soil No. 9. In both cases the maximum rate was obtained at an addition of 4 mg glucose g^{-1} soil (Fig. 1).

When glucose was added in solution, the maximum rate of CO_2 evolution occurred at 0.5 mg g^{-1} soil for soil No. 5 and 9, and again 4 mg glucose g^{-1} soil was required for soil No. 6. The maximal rates with addition of glucose solution were 60.5, 15.7 and 21.3 μl CO_2 g^{-1} soil h^{-1} for soil No. 5, 6 and 9, respectively. This may suggest that addition of glucose solution gave a better distribution of glucose in soil, as suggested by West and Sparling (1986). The initial maximal CO_2 evolution rates were increased by 1.5-fold and 2.0-fold for soil No. 5 and 9, respectively, and by about 1.4-fold in the acidic soil No. 6. It is possible that the two different ways of glucose addition (as solid and aqueous) might stimulate a different section of the soil biomass. This will be discussed later.

Anderson and Domsch (1978) found a rate of 0.25 to 4 mg glucose g^{-1} soil to be optimal for their 12 soils depending upon the initial amount of biomass. West and Sparling (1986) found that 7.5 mg glucose g^{-1} soil, corresponding to 30 mg ml^{-1} soil water, was

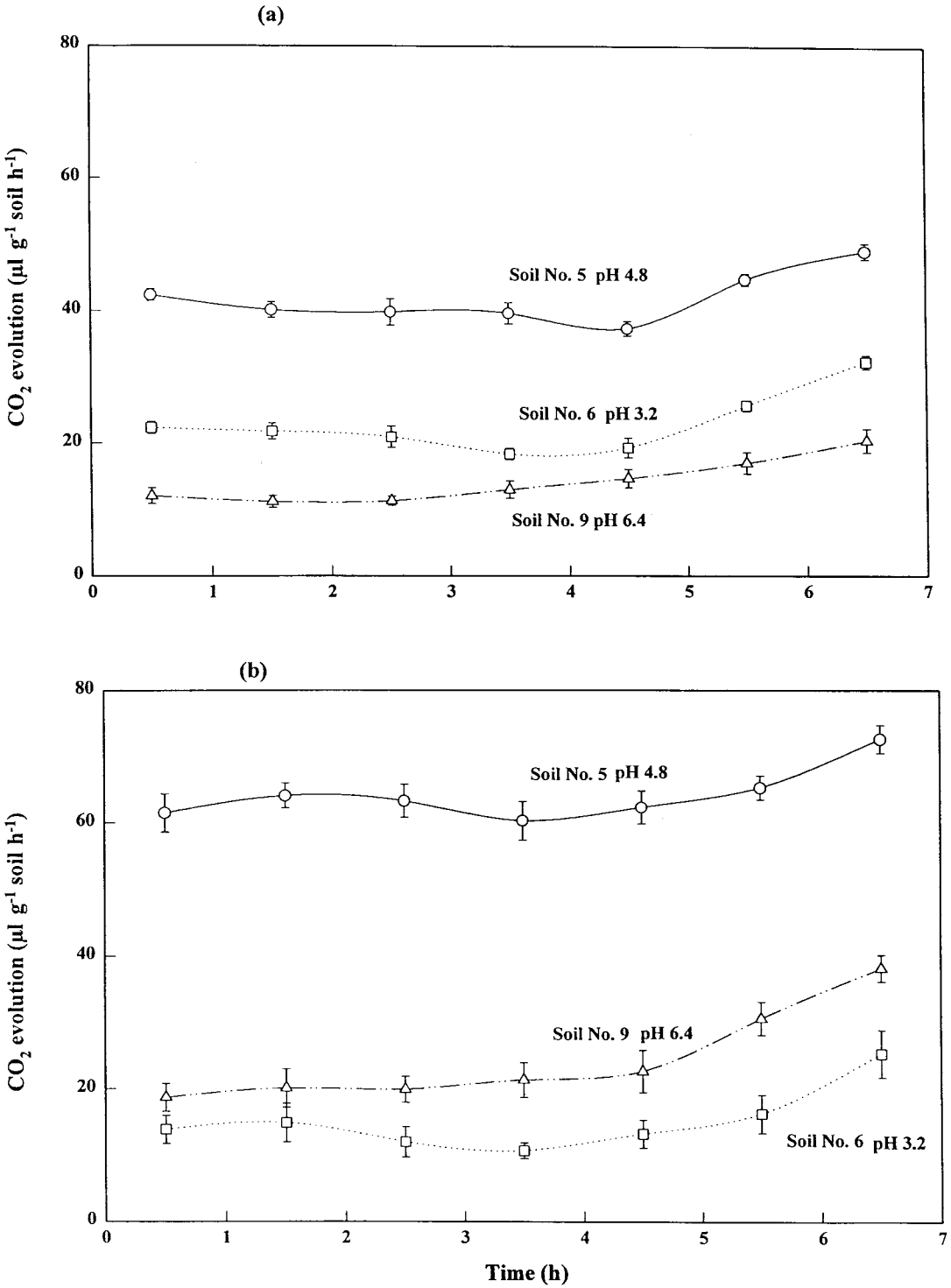

Fig. 2. CO_2 evolution following addition of (a) solid and (b) aqueous glucose during incubation. Bar is standard deviation.

optimal for their three soils. We found less difference in optimum glucose rates between soils. The initial maximal rate of CO_2 evolution was obtained for all of the soils with an addition at, or mainly below, 4 mg glucose g^{-1} soil.

Anderson and Domsch (1978) and West and Sparling (1986) found that more glucose was needed in soils with a high organic C content. In our work, soil No. 6 had a very high organic C content, 6.65%. More glucose (about 4 mg g^{-1} soil) was also required in this soil to achieve the initial maximal rate of CO_2 respiration. However, since soil No. 6 also had a very

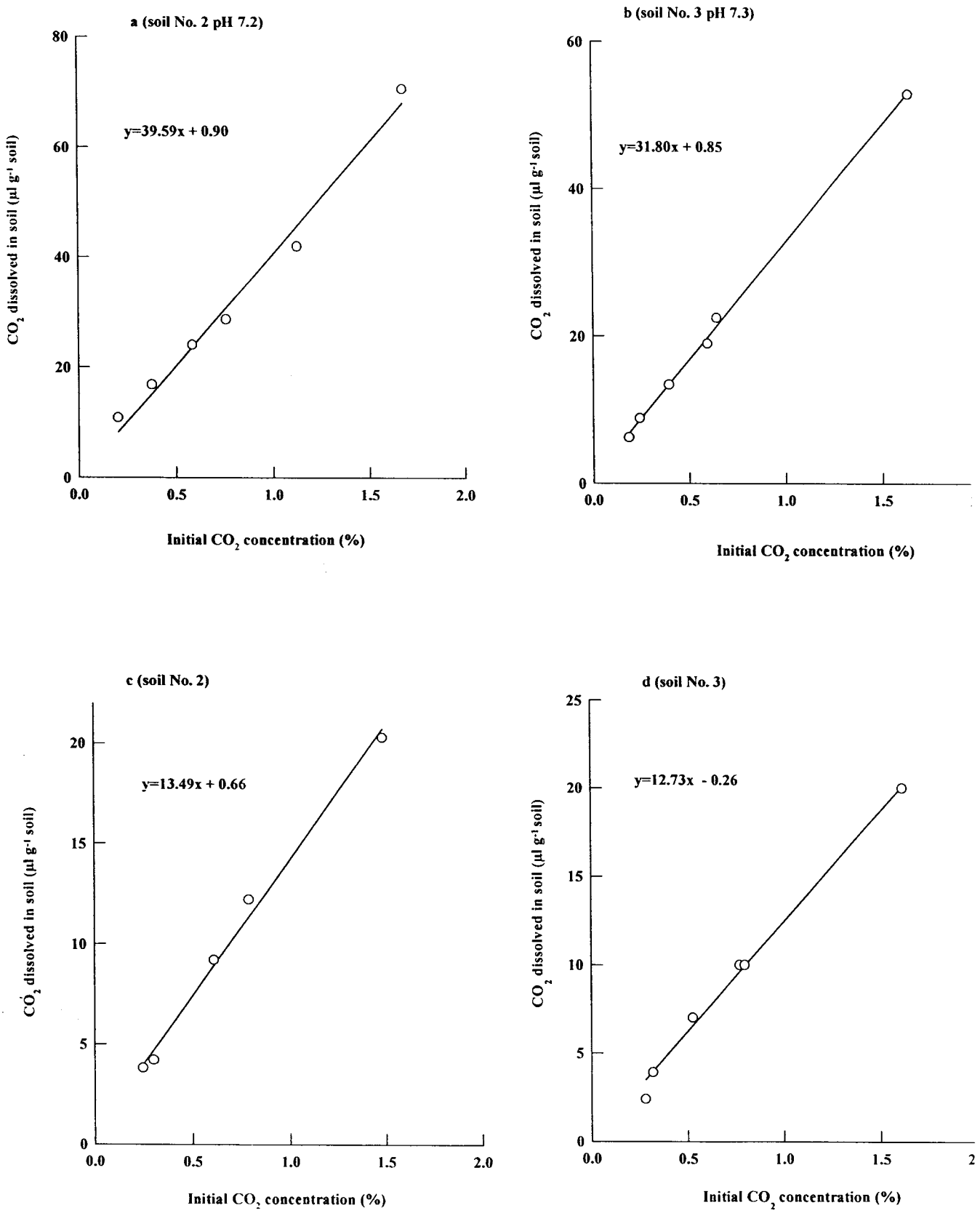

Fig. 3. Relationship between the quantity of CO_2 dissolved in soil solution and gaseous CO_2 concentration above soils (a and b: soil moisture was adjusted to 1.2 WHC by adding distilled water; c and d: soils amended with talcum, 0.4 WHC).

low pH (3.2) this could also have been the explanation.

West and Sparling (1986) reported that CO_2 evolution was significantly decreased when glucose addition exceeded 60 mg ml^{-1} soil water, presumably due to osmotic effects. In our experiment glucose concentrations ranged from 0.42 to 71 mg ml^{-1} soil water. No inhibition of microbial respiration was observed at any concentration. Thus, in routine work, 6 mg g^{-1} soil was used for all soils in this study.

3.2. Carbon dioxide evolution during incubation following glucose addition

Moist soil No. 5, 6 and 9 were amended with solid or aqueous glucose (to adjust soil moisture to 1.2-fold WHC) at 6 mg glucose g^{-1} soil, and incubated at 25°C (with shaking at 150 rev min^{-1} after addition of glucose solution). Carbon dioxide evolution was determined at 0.5, 1.5, 2.5, 3.5, 4.5, 5.5 and 6.5 h after glucose addition. After each sampling the flasks were flushed with CO_2-free air for 1 min and then equilibrated at room temperature for 2 min. This procedure had been tested (results not given) and found to be adequate to eliminate gaseous CO_2. During flushing the soils were manually shaken to remove any CO_2 trapped in the soils. The results are shown in Fig. 2.

Carbon dioxide evolution following addition of solid glucose increased slowly over the 6.5 h incubation for soil No. 9. In contrast, for soil No. 5 and 6, CO_2 evolution decreased slowly during the initial 4.5 h, and then increased rapidly over the remaining period of incubation. Similar patterns of CO_2 evolution were found when aqueous glucose was added.

Drobnik (1960) divided glucose mineralization into two main parts: primary and secondary oxidation. In primary oxidation CO_2 evolution increased very slowly during the initial 4 h, then increased rapidly to a maximum over the next 12 h. The slow mineralization rate of glucose in the initial 4 h was considered to be due to the enzymes originally present in the soil microbial biomass and, possibly, excellular enzymes. The logarithmic increase in CO_2 evolution was considered to be due to the synthesis of glucose-induced enzymes.

To determine microbial biomass by SIR it is self-evident that it is most important to select the glucose mineralization rate most closely proportional to the size of the original soil microbial biomass. Anderson and Domsch (1978) found three patterns of CO_2 evolution following glucose addition to soil. For pattern 1 the measurement was of CO_2 evolved when the slope became zero. For pattern 2 and 3, CO_2 evolution during the initial 1 h after glucose addition was used as an indicator of the amount of original microbial biomass. West and Sparling (1986) found no great variation in hourly CO_2 respiration rates throughout the duration of the assay (0.5–5.5 h) after glucose addition. In our soils, there were similar patterns of CO_2 evolution after glucose addition to those described by Anderson and Domsch (1978). This might suggest that the microorganisms did not change their physiological status during the short term incubation (at least 4 h) after glucose addition. Thus, for routine use, CO_2 evolution at 0.5–2.5 h after glucose addition was chosen as the best estimator of the SIR rate.

3.3. The effects of carbon dioxide dissolved in soil solution on SIR measurements

Fumigated moist soils received increasing concentrations of pure CO_2 and were then incubated under the same conditions as for SIR measurement, except that glucose was not added. The initial and final CO_2 concentrations in the flask headspace were determined with or without addition of pure CO_2. The results are shown in Fig. 3.

Carbon dioxide can react with alkaline solution as follows:

$$CO_2 + 2[OH^-] \Leftrightarrow [HCO_3^-] + [OH^-] \Leftrightarrow [CO_3^{2-}] + H_2O$$

Alkaline soils contain large amounts of Ca, Mg and Fe, mainly as carbonates. Depending on the partial pressure of CO_2 in soil air, an equilibrium exists between the sparingly soluble carbonate salts of Ca, Mg and Fe and the corresponding soluble hydrogen carbonate. For example:

$$CaCO_3 + CO_2 + H_2O \Leftrightarrow Ca(HCO_3)_2$$

With higher CO_2 concentration in soil air, more CO_2 will be trapped as metallic hydrogen carbonates. Although these salts are more soluble in water than carbonates, the 10 min of flushing in Anderson and Domsch's system is insufficient to remove all of the CO_2 dissolved in the soil solution (Martens, 1987). Carbon-14 labelling experiments also showed a large amount of CO_2 dissolved in alkaline soil solution. Martens therefore suggested that a continuous air-flowing system should be used in SIR measurement. West and Sparling (1986) modified the original method by adding glucose solution, equivalent to 2 ml g^{-1} soil. Although the modified method was convenient and suitable for soils of different water contents, there was an inherent problem caused by CO_2 dissolving in soil solution. Cheng and Coleman (1989) developed a simple continuous air-flowing system, but the accuracy was decreased (Van Cleve et al., 1979). Certainly, if routine measurements are to be made, investment in a reliable automated system, e.g. Heinemeyer et al. (1989), would be recommended.

In our work, soils No. 1–3 were above pH 7.0, soil No. 4 had a pH of 6.7, and soil No. 9–13 had pHs

Table 2
CO_2 dissolved in fumigated soil No. 9 (pH 6.36) (CO_2 in flask headspace)

Glucose powder addition		Glucose solution addition	
Initial CO_2 (%)	Final CO_2 (%)	Initial CO_2 (%)	Final CO_2 (%)
0.058	0.076	0.066	0.086
0.147	0.178	0.256	0.286
0.304	0.357	0.368	0.375
0.443	0.476	0.465	0.423
0.694	0.688	0.685	0.613
0.927	0.875	0.894	0.802
1.411	1.135	1.321	0.856

from 6.0 to 6.4. The quantity of CO_2 dissolved in soil solution increased linearly with increasing CO_2 concentrations in soil No. 1–4 (Fig. 3). The correlations were highly significant ($P < 0.001$). Three times more CO_2 was dissolved in the soils at 1.2-fold WHC than at 40% WHC. However, for soil No. 9–13, a significant quantity of CO_2 dissolved in the soil solution was observed only at very high CO_2 concentrations, $>0.4\%$ and 0.7% at soil moisture of at 1.4-fold and 1.2-fold WHC, respectively (Table 2).

We therefore propose that, for routine use, it is not necessary to correct for the CO_2 dissolved in soil solution below pH 6.5 when a small quantity of soil (<30 g per 250 ml flask) is used. Under these circumstances the CO_2 concentration in the flask remains lower than the critical level which results in a significant amount of CO_2 dissolving in soil (see also Sparling and West (1990) for a discussion of this).

3.4. The effects of water content on SIR rate

Moist soils No. 5 and 9 were amended with different volumes of glucose solution to provide different water contents (0.4, 0.6, 0.8, 1.0 (=100%), 1.2, 1.4, 1.6 and 2.0-fold WHC), at a constant glucose concentration of 6 mg glucose g^{-1} soil. The amended soils were then incubated at 25°C with or without shaking at 150 rev min^{-1}. Carbon dioxide evolution was measured between 0.5 to 2.5 h after glucose addition (Fig. 4).

Carbon dioxide evolution following addition of either glucose solution or glucose powder remained constant between 0.4 and 0.8 WHC either with or without shaking. However, at 1.2-fold WHC without shaking during incubation, it decreased rapidly from 40.2 to 22.8 for soil 5 and from 14.6 to 6.8 μl CO_2 g^{-1} h^{-1} soil for soil No. 9. This was probably due to O_2 limitation which inhibited microbial respiration at the higher soil water contents.

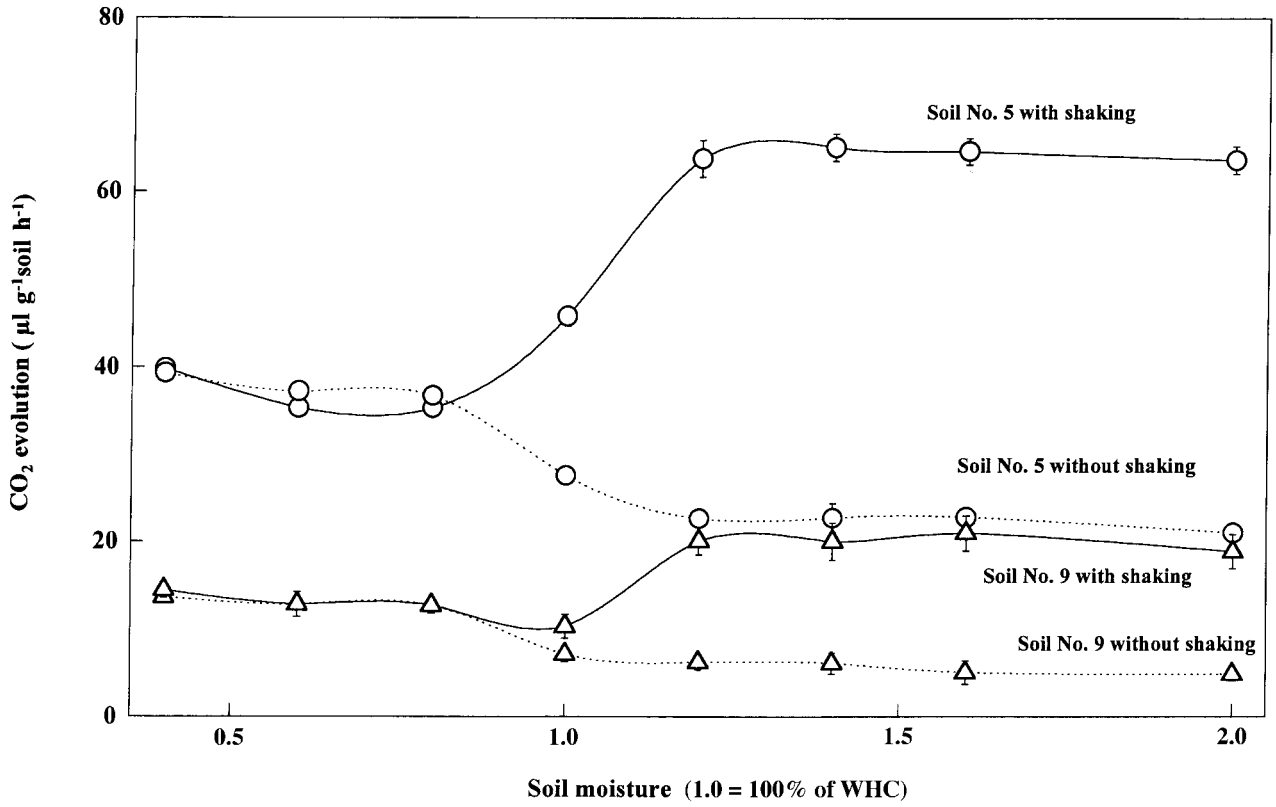

Fig. 4. The effects of soil moisture on the SIR measurements. Bar is standard deviation.

When the soils were shaken during incubation more O_2 would be supplied. Shaking resulted in a rapid increase in CO_2 evolution from 0.8 WHC to a maximum of 1.2 WHC. Carbon dioxide evolution increased by more than 50%, from 40.2 to 65.4 and 14.6 to 20.7 μl CO_2 g^{-1} h^{-1} soil for soils No. 5 and 9, respectively. This is discussed in the next section.

3.5. Carbon dioxide evolution following glucose addition

Moist soils were amended with solid glucose or aqueous glucose at 6 mg g^{-1} soil, and incubated at 25°C (with shaking at 150 rev min^{-1} after aqueous glucose addition). The evolution of CO_2 was estimated between 0.5 to 2.5 h after glucose addition. Carbon dioxide evolution was also determined in the soils without glucose addition, but the same amount of talcum or distilled water was added as used in SIR measurement. The quantity of CO_2 dissolved in soils No. 1–4 was corrected by the method described above. The results are shown in Table 2.

When moist soils (0.4 WHC) were amended with talcum or soil moisture adjusted to 1.2 WHC with distilled water, an appreciable amount of CO_2, termed basal CO_2 respiration in this study, was evolved and could be measured in the unamended soils over the 2 h incubation. The basal CO_2 respiration ranged from 2.1–14.3 (at 0.4 WHC) and 1.5–50.4 μl g^{-1} h^{-1} soil (at 1.2 WHC). For sandy loam soils, a much higher basal CO_2 respiration rate was obtained at 1.2 WHC than at 0.4 WHC. This could be due to increased substrate availability in the soils with high water contents (1.2 WHC) than with low water contents (0.4 WHC). In contrast, because of lower rates of diffusion of O_2 in the soils of high clay content and poor physical structure, e.g. soils No. 1, 3 and 7 (Williams, 1977), lower basal CO_2 respiration was measured at 1.2 WHC than at 0.4 WHC. The basal CO_2 respiration was on average 35% of the SIR rate measured following addition

Table 3
The correlation coefficients between the basal CO_2 respiration and total C, total N and K_2SO_4-extractable C (n = 13)

Soil moisture	Total N	Total C	K_2SO_4-extractable C
0.4 WHC	0.88	0.94	0.81
1.2 WHC	0.47	0.42	0.31

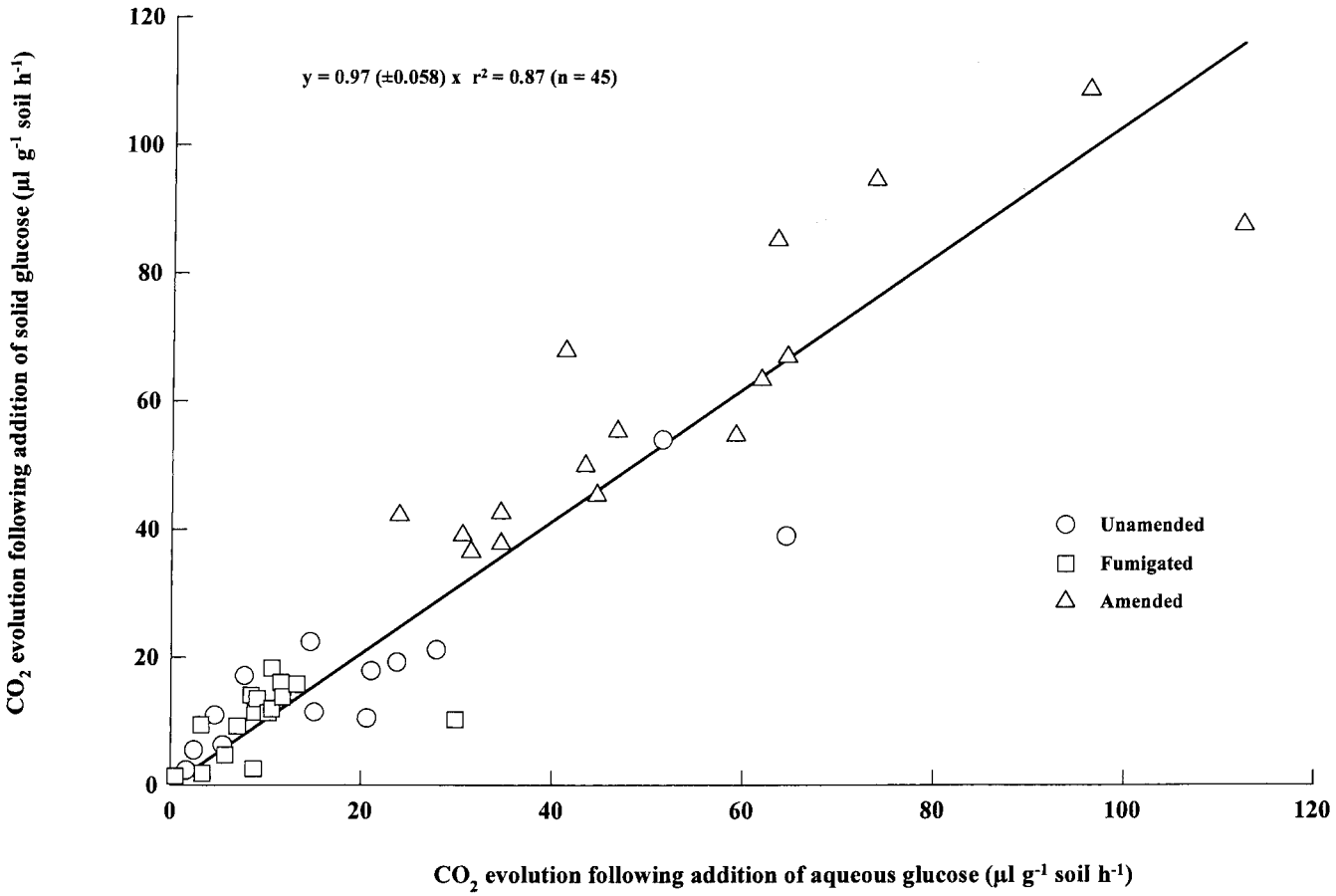

Fig. 5. Relationship between CO_2 evolution following the addition of solid and aqueous glucose.

Table 4
The correlations between SIR rate and soil organic C and total N content in the unamended soils ($n = 13$)

	SIR-s[a]		Net SIR-s		SIR-a[b]		Net SIR-a	
Organic C	0.68[c]	0.91[d]	0.51[c]	0.89[d]	0.54[c]	0.90[d]	0.44[c]	0.79[d]
Total N	0.84[c]	0.91[d]	0.71[c]	0.89[d]	0.73[c]	0.88[d]	0.61[c]	0.77[d]

[a] SIR rate following addition of solid glucose.
[b] SIR rate following addition of aqueous glucose.
[c] All 13 soils were included in statistical analysis.
[d] Soil No. 6 was excluded from statistical analysis.

of solid glucose (SIR-s) and 48% of that following addition of aqueous glucose (SIR-a).

Generally, a greater proportion of SIR was attributed to the basal respiration in soils of low biomass content (soils No. 1 and 8) than in soils of larger biomass content (soils No. 4 and 5). Thus, the smaller biomasses had higher specific respiration rates. More than 68% of SIR-a was attributable to the basal respiration for soils No. 5 and 9 at 1.2 WHC. No explanation can be offered. It was not, for example, attributable to different microbial C-to-soil organic C ratios (Q. Lin, loc. cit.).

There were statistically significant correlations between the basal CO_2 respiration with addition of talcum (0.4 WHC) and soil organic C content, K_2SO_4-extractable C and total N content. However, very weak correlations were found between basal respiration following addition of water (1.2 WHC) and the above variables (Table 3). The reason is not clear.

Carbon dioxide evolution following addition of solid glucose ranged from 2.4 to 53.9 and aqueous glucose from 1.7 to 59.2 μl g^{-1} h^{-1} soil in the unamended soils. There was a highly significant correlation between SIR-s and SIR-a, $r = 0.93$ ($P < 0.001$) (Fig. 5). Generally, adding glucose solution produced more CO_2 evolved than did addition of solid glucose, but not always. For example, for soils No. 6 and 7, addition of solid glucose gave twice as high SIR rates as glucose solution. However, for soils No. 5 and 9, SIR rates following solid glucose were only 50% of those due to adding aqueous glucose. When the basal CO_2 respiration was deducted from the SIR rate, i.e. net SIR rate, the correlation was not improved ($r = 0.92$, $P < 0.001$) (Table 4).

There was a very poor relationship between CO_2 evolution following addition of solid or aqueous glucose and soil organic C when all the soils were included in the analysis (Table 4). Using net SIR values did not improve the relationship. In contrast to organic C, the relationship between SIR rate and total N was strong. Analogous to the correlation of arginine mineralization with soil organic C and total N (Lin and Brookes, 1999a), when the acid soil, soil No. 6, was omitted, the correlations were markedly improved.

Table 5
The correlation between CO_2 evolution following addition of solid glucose (SIR-s) (y) and liquid glucose (SIR-a) (x) in the unamended, amended and fumigated soils (ND means not determined)

Soil treatment	SIR		Net SIR	
	Regression	Coefficient	Regression	Coefficient
Unamended ($n = 13$)	$y = 0.64\ x$	0.93	$y = 0.93\ x$	0.92
Amended ($n = 16$)	$y = 0.81\ x$	0.87	$y = 1.30\ x$	0.98[a]
Fumigated ($n = 16$)	$y = 0.34\ x$	0.42	ND	ND

[a] $n = 10$.

Sparling (1981) also reported weak correlations between SIR biomass, soil organic C and total N. In contrast, Speir et al. (1984) found high correlations between them.

3.6. Ryegrass amendment

When soils were amended with ryegrass their SIR rates increased from 2- to 150-fold (Fig. 5). A greater increase in SIR rates was found in the soils which had lower original SIR rates when the soils were amended with ryegrass. The SIR rates did not much change between 10 and 20 d incubation. This suggested that the microbial biomass content and its activity were constant between 10 to 20 d after ryegrass amendment, as shown by Wu et al. (1993). The basal CO_2 respiration ranged from 5.8 to 18 and 7.6 to 21.8 μl CO_2 g^{-1} h^{-1} soil following addition of talcum (0.4 WHC) and water (1.2 WHC), respectively. Generally, higher values were obtained with addition of water than with addition of talcum. The basal CO_2 respiration was around 30% of the SIR rates, lower than that in unamended soils, but less variable across the soils.

There was a highly significant correlation between CO_2 evolved following addition of solid (SIR-s) and aqueous glucose (SIR-a) to ryegrass-amended soils: (SIR-s) = 0.81 (SIR-a); $r = 0.87$ ($P < 0.001$) (Table 5). Generally, adding glucose solution produced higher rates of CO_2 evolution than addition of solid glucose. In contrast, the net SIR rate following addition of glucose solution was lower than that following addition of solid glucose. This implied that the respiration of the microbial biomass in the amended soil was limited at 1.2 WHC. As found in the unamended soils, the values of SIR-s and SIR-a were significantly different for some soils (e.g. soils No. 5, 6 and 12).

3.7. Fumigation

When soils were fumigated and then incubated for 10 d following fumigant removal, the SIR rate was about 20% of the original. There was a very poor relationship between CO_2 evolution following addition

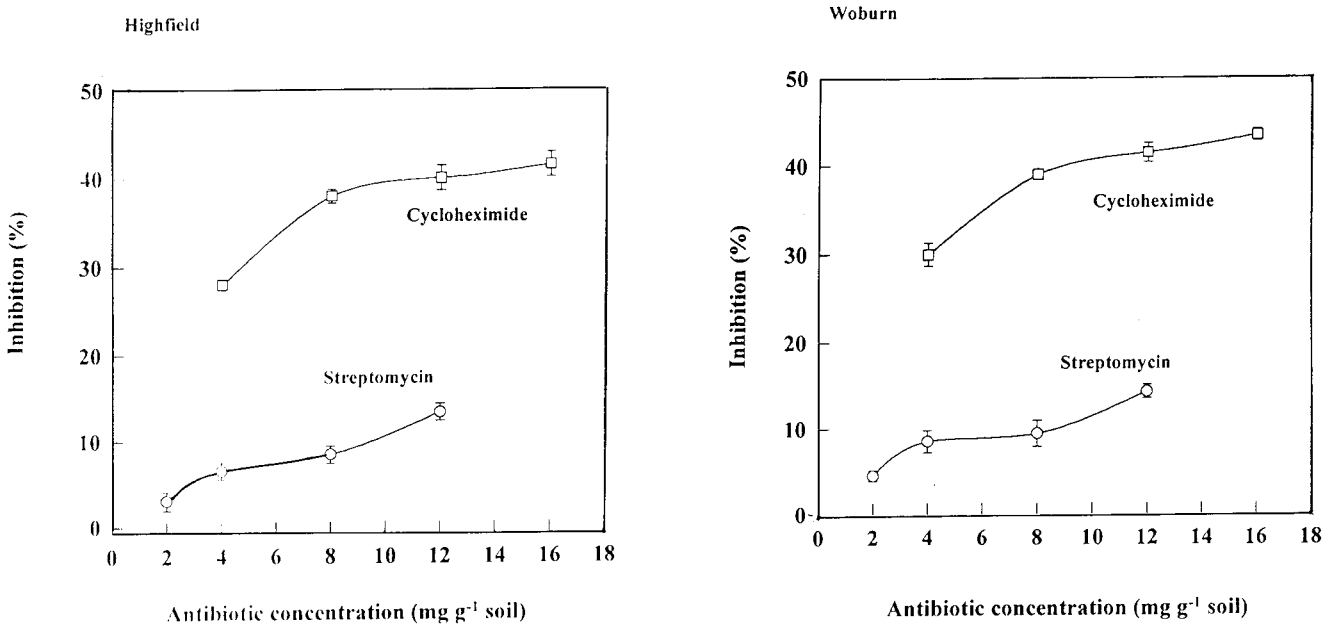

Fig. 6. Relationship between inhibition of substrate-induced respiration and antibiotic concentrations. Bar is standard deviation.

of solid (SIR-s) and aqueous (SIR-a) glucose ($r = 0.42$, $P < 0.1$) (Table 5). However, when soil No. 5 was excluded from the analysis, the relationship was improved ($r = 0.79$, $P < 0.001$). The most likely reason for the generally weak relationship between SIR-a and SIR-w measurements is the analytical problem of measuring relatively small amounts of, and small changes in, CO_2 evolution against a large and possibly somewhat variable basal respiration rate in soils which have already undergone considerable metabolic changes due to substrate addition and fumigation.

3.8. Overall relations between SIR measurements following addition of aqueous or solid glucose

Combining all of the data, SIR-s = 0.97 (±0.058) SIR-a. The regression accounted for 87% of the variance in the data. The intercept was not significantly different from zero (Fig. 5). Generally, similar amounts of CO_2 were evolved following addition of either solid or aqueous glucose in the unamended, amended and fumigated-incubated soils. However our data shows that this is not always the case (see above). Unfortunately it is not yet possible to predict with certainty which soil physical, chemical or biological factors (or their interactions) cause the differences. We have not yet resolved this problem and it has to be bourne in mind. However, since it is more convenient to add the glucose in solution rather than in solid form, this was to be the preferred procedure in future work.

3.9. Optimal rates of antibiotics for selective inhibition of substrate-induced respiration

Three portions of moist soil were amended with (a) glucose solution (6 mg g^{-1} moist soil), (b) glucose solution + streptomycin (2, 4, 8 or 12 mg streptomycin g^{-1} moist soil) and (c) glucose solution + cycloheximide (4, 8, 12 or 16 mg cycloheximide g^{-1} moist soil). The soil samples were then incubated at 25°C, and the SIR rates were determined at 0.5 to 2.5 h after the amendments (Fig. 6).

Table 6
Inhibition of SIR rate by antibiotics in Woburn clay soil

	Control[a]	Streptomycin (mg g^{-1} soil)					Cycloheximide (mg g^{-1} soil)			
		0.4	1.6	3.2	8	16	4	8	12	16
SIR rate (CO_2 μl g^{-1} soil h^{-1})	20 ± 0.4[b]	20 ± 0.7	19 ± 0.3	20 ± 0.5	23 ± 1.5	25 ± 0.8	17 ± 0.3	16 ± 0.6	16 ± 0.5	15 ± 0.3

[a] Glucose addition only.
[b] Standard error of the difference of the mean.

Table 7
The measurement of the ratio of bacteria-to-fungi in soil No. 5 (Highfield grassland soil) (nc—not calculated)

	Paired inhibitor concentration (mg g^{-1} soil)			
Cyclohexamide	8		12	
Streptomycin	4	6	4	6
Time interval 0.5–2.5				
Synergistic effect	1.03	1.02	1.00	1.00
Bacteria:Fungi	18:85	23:79	17:78	22:78
Total combined inhibition (%)	43.4	46.5	45.1	47.7
Time interval 2.5–4.5				
Synergistic effect	0.98	1.04	0.93	1.01
Bacteria:Fungi	12:86	24:80	nc	22:79
Total combined inhibition (%)	45.0	48.3	49.6	51.5
Time interval 4.5–6.5				
Synergistic effect	1.07	1.18	1.04	1.18
Bacteria:Fungi	nc	nc	16:88	nc
Total combined inhibition (%)	50.7	54.0	53.3	57.4

Generally, inhibition of SIR by streptomycin showed an inverse-sigmoidal dose–response curve in the two soils. At addition rates below 4 mg g^{-1} moist soil, the inhibition decreased rapidly because the amount of the added inhibitor was not enough to inhibit bacterial respiration. The inhibition increased again at addition rates above 8 mg streptomycin g^{-1} moist soil. This might be due to adding too much inhibitor so as to lose selectivity to bacteria in the inhibition of the SIR rate. We therefore considered that true maximum inhibition was obtained at 4–8 mg streptomycin g^{-1} moist soil. The inhibition of SIR at these rates was about 10% for these soils. Exceptionally, SIR was stimulated by adding streptomycin in the Woburn clay soil (Table 6). Bewley and Parkinson (1985) and West (1986) also found that streptomycin stimulated microbial respiration in both an arable and an organic soil. The explanation has not been established.

The inhibition of SIR by cycloheximide approached a maximum between the addition rates of 8–16 mg g^{-1} moist soil. The maximum inhibition of SIR was about 45%. The optimal rates of streptomycin were thus in the range of 4–8 mg streptomycin and 8–12 mg cycloheximide g^{-1} moist soil.

Anderson and Domsch (1975) added 0.5–3 mg streptomycin and 0.5–4 mg cycloheximide g^{-1} soil to their five soils, much lower rates than those used here. However, Bewley and Parkinson (1985) applied 1–16 mg streptomycin and 1–32 mg cycloheximide g^{-1} soil to the three organic soils and one mineral soil. Wardle and Parkinson (1990b) added 10 mg streptomycin and 15 mg cycloheximide g^{-1} soil. The different rec-

Table 8
The measurement of the ratio of bacteria-to-fungi in soil No. 9 (8th year ley of Woburn ley-arable rotation) (nc—not calculated)

	Paired inhibitor concentration (mg g^{-1} soil)			
Cyclohexamide	8		12	
Streptomycin	4	6	4	6
Time interval 0.5–2.5				
Synergistic effect	1.0	0.95	1.05	0.98
Bacteria:Fungi	26:80	24:71	26:80	24:74
Total combined inhibition (%)	42.2	47.3	43.1	46.5
Time interval 2.5–4.5				
Synergistic effect	1.22	1.24	1.11	1.23
Bacteria:Fungi	nc	nc	nc	nc
Total combined inhibition (%)	44.0	48.2	48.2	48.2
Time interval 4.5–6.5				
Synergistic effect	1.22	1.36	1.15	1.19
Bacteria:Fungi	nc	nc	nc	nc
Total combined inhibition (%)	48.2	51.5	50.9	52.5

ommended rates are not, therefore, generally widely disparate from each other. In contrast, West (1986) required rates of streptomycin equivalent to 260 mg g^{-1} soil and cycloheximide equivalent to 64 kg g^{-1} soil to 'significantly inhibit' respiration in a New Zealand pasture soil. In another New Zealand soil, respiration was even stimulated. Based on our work we suggest 4–8 mg streptomycin and 8–12 mg cyclohexamide g^{-1} soil as optimum rates.

3.10. Selective inhibition of substrate-induced respiration following addition of inhibitors

The fungal and bacterial inhibitors of respiration (cycloheximide and streptomycin, respectively), in combination, were added to moist soil and then incubated at 25°C. The SIR rate was determined at 0.5–2.5, 2.5–4.5 and 4.5–6.5 h after the amendments. The relative proportions of bacterial and fungal biomass were calculated from:

bacterial respiration $(A-B)$
fungal respiration $(A-C)$
bacterial proportion 100 $[(A-B)/(A-D)]$
fungal proportion 100 $[(A-C)/(A-D)]$

The term A is CO_2 evolved following addition of glucose only; B is CO_2 evolved following addition of glucose + streptomycin; C is CO_2 evolved following addition of glucose + cycloheximide and D is CO_2 evolved following addition of glucose + streptomycin + cycloheximide (Tables 7 and 8).

If there were no synergistic effects then the ratio $[(A-B)]/[(A-D)]$ should be 1.0. In fact, the ratio ranged from 0.93 to 1.36, generally increasing with assay time. Addition of both antibiotics as a mixture to the soils did not result in complete inhibition of the SIR rate. The total combined inhibition of the SIR rate ranged from 36 to 60% and increased with assay time. Beare et al. (1990) also found that the synergistic effect increased, but the total combined inhibition decreased with assay time. The total SIR inhibition obtained here was comparable to that reported by Anderson and Domsch (1975) and West (1986) but lower than that reported by Beare et al. (1990). Substrate induced respiration analysis for paired concentrations of antibiotics which meet the criteria for maximum selective inhibition can be used to calculate fungal and bacterial contributions to the total SIR response. Optimum joint concentrations of antibiotics are indicated when the ratio $[(A-B)+(A-C)]/(A-D)$ most nearly approaches 1, and total combined inhibition $((A-D)/A \times 100)$ is maximal. According to Anderson and Domsch's (1975) equation:

$$A - [(A - B) + (A - C)] = D \pm 5\%\ D$$

or

$$(A - B) + (A - C) = A - D \pm 5\%\ D$$

$$[(A - B) + (A - C)]/(A - D) = 1 \pm 5\%\ (D/(A - D)$$

since

$(A - D)/A = \alpha$ (Total combined inhibition of SIR)

then

$(A - D) = \alpha A$

and

$D = A(1 - \alpha)$

so

$$[(A - B) + (A - C)]/(A - D) = 1 \pm 5\%\ [(1 - \alpha)/\alpha]$$

This suggests that the data are considered valid only as long as the synergistic effect is between the range $1 \pm [(1-\alpha)/\alpha]$. According to this equation, in the total microbial biomass of the Highfield Grassland soil, the bacteria comprised 12–24% and the fungi 78–88%, with means of $19 \pm 4.2\%$ for the bacteria and $82 \pm 4.0\%$ for the fungi (Table 7). The ratio of bacterial-to-fungal biomass in the Woburn ley-arable rotation experiment plot 32 was 25 (±1.2)-to-76 (±4.5) (Table 8). These results are comparable with those of Anderson and Domsch (1975) and Wardle and Parkinson (1990b). They therefore support the hypothesis that the fungal biomass usually dominates both the microbial biomass, and microbial metabolism in agricultural and forest soils based on the results of using this method.

3.11. Limitations in the use of the selective inhibition method

The main limitations are (1) that the antibiotics used are often insufficiently specific, (2) they are often easily inactivated by soil or degraded by the surviving soil microorganisms and (3) the separate use of selective inhibitors of fungal and bacterial biomass must, by definition, suppress the metabolic activities of these two populations. This may result in additional energy becoming available to the non-suppressed organisms for metabolism.

The specificity of streptomycin and cycloheximide has been tested by Martin (1950), Corke and Chase (1956), Johnson (1957), Williams and Davies (1965), Anderson and Domsch (1975) and Beare et al. (1990) and others. Cycloheximide is a glutarimide antibiotic that inhibits protein biosynthesis in organisms that have 80s ribosomes. The inhibition affects both peptide initiation and extension by an effect on the donor ribosome site (Obrig et al., 1971). Streptomycin blocks synthesis of protein on the 70s ribosomes (Dubin et al., 1963). Eucaryotic cells have both 80s and 70s ribosomes in their cells, while the prokaryotic cells have only 70s ribosomes (Stanier et al., 1977). Thus, cycloheximide depresses not only fungal respiration but also algae, protozoa and other eucaryotes (Siesler and Siegel, 1967). However, the latter populations form

Table 9
The ratio of bacterial to fungal respiration in soil No. 5 (Highfield grassland soil) (S is streptomycin and C cycloheximide)

Paired inhibitor concentration (mg g^{-1} soil)	Time intervals (h)		
	0.5–2.5	2.5–4.5	4.5–6.5
S_4C_8	0.22 ± 0.02	0.13 ± 0.04	0.20 ± 0.01
S_4C_{12}	0.22 ± 0.02	0.13 ± 0.03	0.18 ± 0.01
S_6C_8	0.29 ± 0.01	0.29 ± 0.04	0.40 ± 0.02
S_6C_{12}	0.28 ± 0.01	0.28 ± 0.04	0.37 ± 0.01
S_8C_8	0.31 ± 0.01	0.22 ± 0.01	0.36 ± 0.08
S_8C_{12}	0.31 ± 0.02	0.21 ± 0.01	0.33 ± 0.07

only a small proportion of the total biomass in most soils. It was not considered in this study. Streptomycin seems also to inhibit fungal respiration. However, experiments both in situ and in vitro suggest that streptomycin does not inhibit fungal growth (e.g. Anderson and Domsch, 1975; Beare et al., 1990).

Cycloheximide is a neutral antibiotic which is weakly bound to soil and retains a relatively high degree of activity in soil. However, streptomycin is a basic antibiotic which can be strongly bound and inactivated by soil. The antibiotics can also act as substrates to soil microorganisms (Ivarson and Sowden, 1959; Stamatiadis et al., 1990). Addition of excess inhibitors and a shorter assay time could counteract the inactivation. Therefore, the highest possible addition rate of antibiotics and the shortest time of incubation which give maximal selective inhibition, according to the criteria given in Section 3.10, should be determined for each soil.

Ivarson and Sowden (1959) found that bacterial populations increased greatly in mixed bacterial and fungal cultures receiving cycloheximide, while fungal growth was stimulated when streptomycin was present. Anderson and Domsch (1975) warned that removal of competitors by adding selective inhibitors could result in faster growth of surviving microorganisms. If this happens, the ratio of bacterial and fungal respiration $((A-B)/(A-C))$ would change proportionally. This did not occur in our work during the assay, which suggests that the ratios of bacterial and fungal respiration did not greatly change during the assay (Table 9).

3.12. Conclusions of the use of the selective inhibition method

There appear to be some merits in using the selective inhibition method to estimate the relative proportions of bacterial and fungal biomass in soil. However, it should be noted that both inhibitors inhibit, in total, about half the respiration of the total microbial biomass, which we then divide into 'fungal' and 'bacterial' biomass. The structure of the remaining part is currently not known. If this unknown biomass consists of fungi and bacteria in similar proportions to those in that part of the biomass which has been inhibited, then the selective inhibition method is valid. If it does not, i.e. the community structure is different between the inhibited and non-inhibited parts, the method is in error. Thus, Bewley and Parkinson (1985) suggested that this method should be used and tested in conjunction with other independent methods such as direct microscopy or specific biomarkers, e.g. ergosterol or phospholipid analyses etc. While some work has been done (see for example West 1986) for various reasons the results were difficult to interpret. We consider that a rigorous test of this type is long overdue as SIR coupled with Selective Inhibition is almost the 'standard' method for measuring the proportions of bacterial and fungi in soil. We therefore conducted such a comparison. The results are given by Lin and Brookes (1999b).

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