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Modifications to the substrate-induced respiration method to permit measurement of microbial biomass in soils of differing water contents

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Summary

An improvement to the substrate-induced respiration (SIR) method of Anderson and Domsch (Soil Biol. Biochem. 10, 215–221) is described. In the original procedure endogenous CO_2 respiration of soil micro-organisms was stimulated by addition of glucose which removed the substrate limitation to soil microbial respiration. However, the water limitation to soil microbial respiration was not removed. The modifications proposed here are (1) to dissolve glucose in water prior to addition to soil and (2) to add glucose to soil on a concentration (weight of glucose/volume of water) basis to achieve a ratio of 2 ml glucose solution to 1 gram dry weight of soil. Thus, both substrate and water limitations to respiration are alleviated. Data are presented which assess these modifications including shape and size of the vessel used to contain soil, optimal CO_2 equilibration between liquid and gaseous phases, effect of substrate concentration, sampling frequency and interference from viable root fragments. The improved method permits estimation of induced microbial respiration (using gas chromatography) in a rapid and simple manner on soils of any water content. Calibration with biovolume-derived biomass C estimates on three soils of varying water contents produced a linear relationship between $\log_{10}$ respiration rate and the biomass C;

Biomass (μg C g^{-1} soil)	$= 433 \times \log_{10}$ respiration rate + 59.2 (μl CO_2 g^{-1} soil h^{-1})	r^2 = 71% at 25 °C

Biomass C determined by microscopy using non-vital stains probably included non-viable organisms which did not respire. From the above relationship, this non-respiring portion of the (biovolume-derived) biomass was calculated to be 59 μg C g^{-1} soil.

Key words: *Microbial biomass – Modification – Soil – Substrate-induced respiration*

Introduction

A variety of methods exist to estimate the size of the microbial biomass in soil. Of these methods, the most simple and rapid is substrate (glucose)-induced respiration (SIR), which stimulates a maximal respiratory response from the soil

biomass, measured conductimetrically as CO_2 evolution, and relates this response to biomass C [1].

Unfortunately, application of the SIR method is restricted to moist soils, as a basic requirement is a soil water content sufficient to dissolve the added substrate. A lack of water may also reduce microbial respiration in some soil samples [2].

In this study we propose an improvement to the SIR method which alleviates both these problems: glucose is dissolved in water prior to addition to soil and added at a ratio of 2 ml glucose solution to 1 g equivalent oven-dry weight of soil [3]. Data are presented which assess modifications to the original method, including use of sealed vessels to contain soil suspensions and use of gas chromatography to measure CO_2 respiration, and interference from viable root fragments in the assay. The improved SIR method is recalibrated with biovolume-derived biomass carbon estimates of grassland and arable soils, previously incubated at a wide range of water contents.

Materials and Methods

Soils

The soils used for method development were: (a) lowland, grazed pastures: Waikanae silt loam (Typic Udifluvent, 3.7% C, 0.4% N, pH 6.0) fertilized with approx. 30 kg superphosphate ha^{-1} $year^{-1}$ for 30 years; Pomare silt loam (Typic Dystrochrept, 4.6% C, 0.4% N, pH 5.2) unfertilized for 30 years; and Kaitoke silt loam (Typic Dystrochrept, 6.3% C, 0.4% N, pH 5.8); or (b) arable: Greytown fine sandy loam (Typic Udifluvent 1.3% C, 0.1% N, pH 6.3). Soils were sampled by taking randomly-selected cores when required (25 mm dia. × 75 mm depth; between January-December 1984), bulked, sieved ≤ 2 mm and incubated at 25 °C for ≤ 1 month prior to use. If necessary, soils were (1) air-dried by incubation, at 25 °C (most samples) or 37 °C with frequent turning and/or (2) rewetted by gradual addition of water using a syringe with frequent mixing between additions.

Modified substrate-induced respiration

Fresh soil was weighed into a vessel (see Experimental Variables) and sufficient water added to attain a soil suspension with a ratio of 2 ml water to 1 g equivalent oven-dry weight of soil, i.e., the volume of water already present in the soil was accounted for in the calculation of the additional volume of water required. Glucose was dissolved in the water prior to its addition to the soil. The amount of glucose dissolved in the water was calculated on the basis of achieving the desired glucose concentration in the final ratio of water to soil.

Vessels (3–5 replicates/treatment) containing the soil and glucose solution were left open to the atmosphere (25 °C) for 0.5 h prior to sealing with a vaccutainer stopper and then incubated for up to 5 h at 25 °C. Concentrations of CO_2 were estimated by sampling 1 ml of headspace gas and analysing on a Carle GC8500 gas chromatograph (GC) [2, 3]. Total rate of CO_2 evolution was expressed as μl CO_2 g^{-1} soil h^{-1}, assuming a nominal volume of 0.5 ml for each g equivalent oven-dry weight of soil and that a negligible proportion of CO_2 was in the liquid phase (soil-glucose solution). As in the original method [1], no allowance for indigenous

respiration is required and we have expressed respiration in units of volume rather than mass. Use of volume units with no correction for barometric pressure can result in small errors (< 5%) which are not considered significant.

Experimental variables

A series of experiments was undertaken to optimize the equilibration of CO_2 between liquid and gaseous phases. The six separate or combined variables studied were:

(1) vessel volume; McCartney bottle (28.5 ml) containing 1 g soil (2 ml glucose solution, nominal headspace volume 26 ml); or conical flask (59.3 ml) containing 3 g soil (6 ml glucose solution, nominal headspace volume 52.8 ml);
(2) surface area of soil suspension; a vertical or horizontal bottle;
(3) static or shaken incubation of vessels throughout the assay;
(4) with or without (±) swirling bottle on a vortex immediately prior to sampling;
(5) ± sonication (20 μW for 30 s) of soil suspension prior to commencing the assay; and
(6) assessing hourly changes in respiration rate.

Root respiration

Kaitoke soil was used to assess interference from viable root fragments in the modified SIR method. This soil is not subjected to summer drought and therefore is likely to contain a high proportion of viable roots and was thus suitable for use in laboratory air-drying experiments.

Respiration rates of freshly sampled whole cores (hand crumbled and contained in 600 ml capacity preserving jars), sieved soil (≤ 2 mm), sieve-extracted roots (>2 mm), freshly-grown, excised maize roots, and root-amended soil, were measured (Table 2). Maize (*Zea mays* var. Fantastic hybrid) was germinated on damp sand (25 °C), roots excised after 72 h growth, washed, cut into 1 cm lengths and weighed. Fresh roots were used less than 30 min after excision.

Biovolume-biomass C

Soil mycelial organisms and bacteria were enumerated on membrane filters by epifluorescent microscopy. Mycelia were stained with fluorescent brightner [3] and bacteria with acridine orange [4]. Further details on the subsamples, dilution, filter and microscope field replication are given by West [3]. Biovolume:biomass C conversion factors (CF) were calculated assuming buoyant densities of 1.09 g/ml and dry matter contents (wt./wt.) of 21 and 30% for fungi and bacteria, respectively [5], with 50% of cell dry weight as carbon. Final CF values (μl to μg C) were 115 for mycelial organisms and 164 for bacteria.

Calibration of modified substrate-induced respiration with biovolume-biomass C

Prior to calibration analyses, 100 g portions of previously air-dried (25 °C) Waikanae, Pomare and Greytown soils were left dry or wetted up to a range of pressure potentials (see Fig. 5) and incubated for 7 days at 25 °C. The objective was to produce a range of biomass levels in each soil positively correlated with water content.

Results and Discussion

Effect of soil water content on substrate-induced respiration

Peak (i.e., optimal) respiratory response (μl CO_2 g^{-1} h^{-1}) of soil at 25% water content occurred with glucose amendments in the range of 2.5–10 mg glucose g^{-1} equivalent oven dry weight of Waikanae soil (Fig. 1). Amendments < 2.5 mg or > 10 mg g^{-1} soil resulted in significantly ($P < 0.05$) reduced responses.

Expressed as mg glucose ml^{-1} soil water, optimal levels of amendment were 10–40 mg ml^{-1}. The original SIR method [1] refers to amendment on a weight of glucose:dry weight of soil basis. This creates a potential problem as no standardized water content (gravimetric or pressure potential) was specified and measurements could conceivably be attempted with air-dried through to saturated soil. In these extreme circumstances concentrations of glucose in the soil water (assuming full dissolution of glucose, unlikely in dry soils) can be 6–160 mg ml^{-1} which will produce respiratory responses below optimal (Fig. 1). At < 10 mg glucose ml^{-1} there is probably insufficient substrate to saturate microbial respiratory metabolism whilst > 60 mg glucose ml^{-1} probably raises soil water osmotic potential to levels which inhibit respiration in this soil [6]. However, if glucose is added on a constant concentration basis (weight of glucose:volume of soil water) this problem can be avoided.

Glucose addition can remove respiratory limitations induced by low substrate availability, but does not remove the possibility of water availability limiting respiration [2, 7].

Respiration rates determined immediately after rewetting a previously air-dried soil showed significant variation, differing by up to a factor of 2 dependent on the soil water content (11 and 23 μl CO_2 g^{-1} soil h^{-1} at 13 and 43% water content, respectively; experiment A, Table 1). Substrate availability may also have been a factor limiting respiration in the drier soils, due to an incomplete dissolution and a

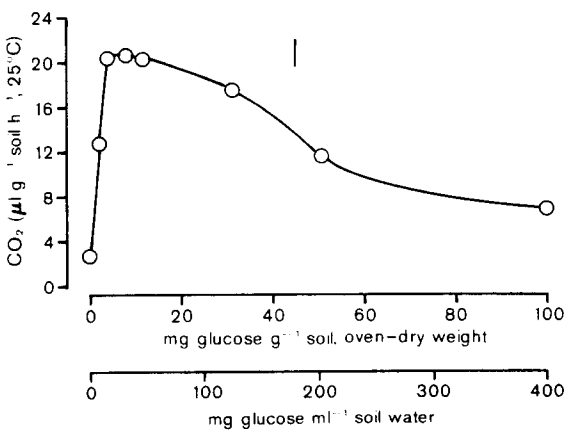

Fig. 1. Effect of glucose on the respiration rate of field moist (25% water content, −92 kPa) Waikanae soil (25 °C). Glucose expressed on a weight or concentration basis. Bar represents 95% uncertainty interval.

TABLE 1

EFFECT OF WATER CONTENT ON RESPIRATION OF WAIKANAE SOIL (25 °C) AMENDED WITH GLUCOSE ON A CONSTANT WEIGHT OR CONCENTRATION BASIS

Soil water content[a] (% w/w)	Respiration (μl CO_2 g^{-1} soil h^{-1})[b]	
	Glucose addition	
Experiment A	10 mg g^{-1} soil	60 mg ml^{-1} soil water
13	11.2[a]	11.6[a]
25	19.0[b]	17.8[b]
43	23.3[c]	23.5[c]
100	18.5[b]	17.2[b]
200	17.2[b]	18.8[b]
Experiment B	no glucose	10 mg g^{-1} soil
10	1.7[a]	10.2[b] (0.17)[c]
23	6.7[c]	19.0 (0.35)
41	8.9[b]	24.1 (0.35)

[a] Soil air-dried, rewetted to required water content and assayed immediately using 1 g soil equivalent dry weight in McCartney bottles.

[b] Values with different suffixes significantly different ($P \leq 0.05$) within experiments.

[c] Values in parentheses = ratio of non-amended respiration to amended respiration

heterogeneous distribution of glucose. However, the respiration rate of soil at 13% water content (−832 kPa) did not differ significantly when amended at the rates of 10 mg glucose g^{-1} or 7.5 mg g^{-1} (60 mg ml^{-1} soil water; experiment A, Table 1), which suggests that here substrate concentrations did not limit respiration.

Rewetting air-dried soil results in high concentrations of soil-derived, organic C and organic and inorganic N and P in soil solution [8, 9]. These may affect the modified substrate-induced respiration method if they are a source of microbial substrate which, when in combination with glucose, produces a larger respiratory response than glucose alone. However, it appears unlikely that these soil-derived compounds have this effect as the ratio of respiration from non-amended soil to respiration from amended soil *increases* with increasing soil water content (experiment B, Table 1). Moreover, although compounds solubilised on rewetting air-dried soils can stimulate microbial respiration [2, 10], use of casamino acids, yeast extract or glucose with supplementary soluble inorganic N and P does not produce respiratory responses markedly different from glucose alone [1, 11, unpublished data].

We therefore propose the following modifications to the original SIR method; that (1) glucose is dissolved in water prior to addition to soil, (2) the rate of amendment is calculated on a concentration basis and (3) glucose solution is added to soil at a ratio of 2 ml solution to 1 g equivalent oven-dry weight of soil. Essentially, soil is suspended in an aqueous growth medium for the duration of the assay.

Optimal equilibration between liquid and gaseous phases of substrate-induced, respired CO_2

The measured soil respiration rate was significantly lower in the modified (soil wa-

ter content 200% w/w) than in the original SIR method (Table 1) even though all the treatments should have had identical microbial biomass and similar rates of respiration.

In the modified SIR method, vessel volume was not a significant variable, but the surface area:volume ratio of the glucose solution significantly affected the rate of CO_2 equilibration, with a positive correlation between surface area and the concentration of CO_2 in the headspace (Fig. 2). Shaking throughout the assay also significantly increased CO_2 concentration (Fig. 2), but sonication prior to the assay did not. This infers that the difference in measured respiration rates between the modified and original SIR methods is a function of differences in CO_2 equilibration between gaseous and liquid phases, and not a problem related to soil dispersion and substrate penetration.

Swirling bottles on a vortex mixer immediately prior to sampling produced a CO_2 equilibration as effective as that achieved using continual shaking. Thus the recommended incubation procedure for CO_2 measurements by GC is to incubate McCartney bottles containing soil and glucose solution in a vertical position (at 25 °C) and to swirl them violently for 5 s on a vortex mixer immediately prior to sealing with a vaccutainer stopper and/or headspace sampling.

The pH of the soil solution can, theoretically, also influence the equilibration of gaseous and liquid phase CO_2. With a soil solution pH of ≤ 6.0 the theoretical distribution of CO_2 between these phases (using sealed vessels) favours the gaseous phase by a ratio of $\geq 10{:}1$ at equilibrium (H. J. Percival, personal communication). In more neutral soil solutions the theoretical distribution of CO_2 rapidly shifts in favour of the liquid phase (e.g., at pH 7.5 the ratio is 1:1). Thus, respiration rates estimated from headspace gas composition could be 50% or less of the real respiration rate in such soils. Thus, we currently recommend that the modified SIR method be limited to use on soils with a pH ≤ 6.5.

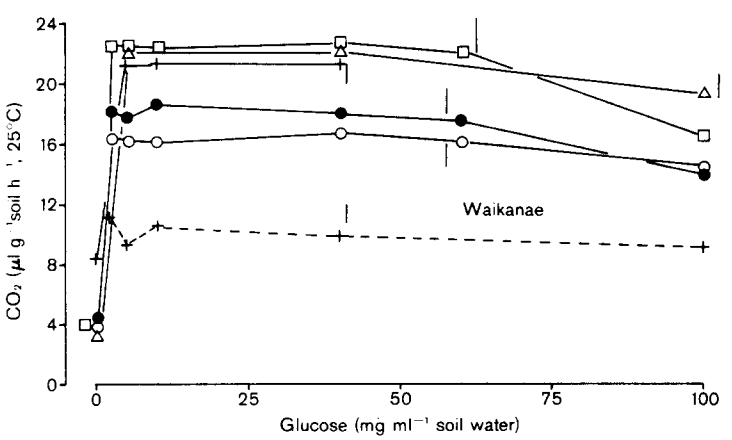

Fig. 2. Effect of shaking treatments on the respiration rate of Waikanae soil (25 °C) in glucose solutions of increasing concentration. ○, low surface area (SA, vertical bottle), unshaken; ● high SA (horizontal bottle), unshaken; △, high SA, shaken at 80 rpm; □, high SA, shaken at 100 rpm; +, high SA, shaken at 130 rpm; +(– – –), low SA, unshaken with vortex swirling and air-dried soil. Details as in Fig. 1.

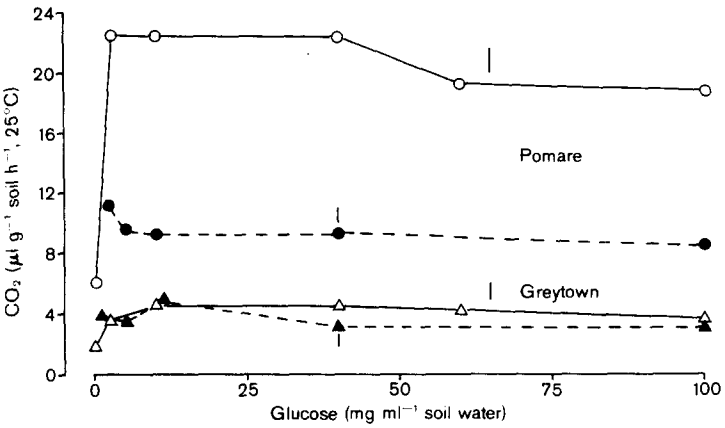

Fig. 3. Respiration rates of fresh and air-dried Pomare and Greytown soils (25 °C) in glucose solutions of increasing concentration: o (–), fresh Pomare soil; • (– – –), air-dried Pomare soil; ▵ (–), fresh Greytown soil; ▲ (– – –), air-dried Greytown soil. Modified substrate-induced respiration method using McCartney bottles, 2 h assay period and vortex swirling (see text). Details as in Fig. 1.

Effect of substrate concentration on substrate-induced respiration of soil

The range of optimal glucose concentrations was 2.5–60 mg ml^{-1} irrespective of assay treatment or soil (Figs. 2 and 3). Optimal concentrations were 2.5–60 mg and 2.5–40 mg glucose ml^{-1} for Waikanae and Pomare soils, respectively (Fig. 3), and 10–40 mg ml^{-1} for Greytown soil. Succeeding studies on very high biomass, effluent-treated soils determined a similar range of optimal concentrations, 5–60 mg glucose ml^{-1} (J.C. Cowling, personal communication).

Air-drying of the grassland soils significantly reduced their microbial biomass (Figs. 2 and 3), but did not greatly alter their range of optimal glucose concentrations: the difference between the respiration response of moist and air-dried soils was far greater than differences in optimal concentration ranges. Consequently, a range of optimal glucose concentrations that is common to both moist and air-dried soils can probably be assumed in seasonal studies of soil.

A glucose concentration of 30 mg ml^{-1} appears to produce an optimal respiration response in all the soils we have studied. Therefore we recommend that this substrate concentration be used whenever time constraints do not permit the determination of the optimal glucose concentrations (maximal respiratory responses) in soils. Exceptions are low bulk density, high organic matter soils such as peats or forest litters where optimal substrate concentrations will probably be >30 mg ml^{-1} and must be determined before experimentation.

Effect of sampling frequency on the measured level of substrate-induced respiration of soil

The frequency of headspace sampling in an assay is dependent on the response of the soil to substrate addition [1]. In the soils we used there was no great variation in hourly CO_2 respiration rates within the duration of the assay (0.5–5.5 h after glucose amendment; see Fig. 4).

Consequently for our soils we were able to reduce the sampling to two times only, at the start and the finish of the incubation period. Incubation periods ranged between 2 and 5 h and depended on the previously determined respiratory response of the soil.

However, before this simplified sampling routine can be used, the respiratory response of the soils under investigation must be determined as specified in the original method [1]. With soils where the respiratory response is known to fluctuate substantially within the assay period, the sampling regime should be more frequent.

Contribution from roots to substrate-induced respiration of soil

In fresh soil, tested within 24 h of collection, roots were estimated to make a substantial contribution (31–38%) to the total SIR response (Table 2). However, when soil was either incubated for 7 days prior to analysis, or air-dried, the contribution of indigenous grassland roots to the total SIR response was greatly reduced, dropping to 18% in incubated soil and 8% in air-dried soil (Table 2). Sieving was also effective in reducing the contribution from roots. Root respiration was calculated to be the same irrespective of whether roots were assayed separately or mixed with soil. The contribution of fresh excised roots was greater than that from sieved-out indigenous roots, but the pattern of decline after preincubation and the additive effect on total soil respiration was similar.

We therefore recommend that soils be sieved before use in the SIR method, but if intact cores are used, that they be incubated for ≥ 7 days prior to analysis. This will substantially reduce the contribution of roots to the total SIR response. If intact cores of dry soil are assayed, the contribution of roots to the total SIR response will already be negligible and prior incubation will not be needed.

Thus, errors may be introduced if the modified SIR method is used with soils where living roots are present, but this limitation is also common to the chloroform fumigation [10] and ATP content [12] procedures to estimate soil biomass C [13].

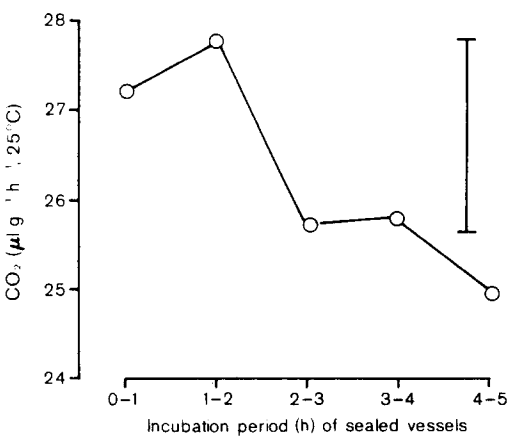

Fig. 4. Hourly respiration rates of Waikanae soil (25 °C) in glucose solution (2 ml of 30 mg glucose ml^{-1}:1 g oven dry equivalent weight of soil). Details as in Fig. 1.

TABLE 2

CONTRIBUTION FROM ROOTS TO THE RESPIRATION OF GLUCOSE-AMENDED KAITOKE SOIL (25 °C)

Treatment	Respiration rate (μl CO_2 h^{-1})			
Experiment A[a]	**Moist soil**			
	Fresh		*Incubated*[b]	
Soil from sieved cores	15.1		13.6	
Root from sieved cores	7.7		2.9	
Intact cores with root				
expected[c]	26.3	NSD[e]	16.5[d]	NSD[e]
observed	24.9		16.2	
Experiment B	**Moist soil**		**Air-dried soil**	
Soil from sieved cores	24.9		17.7[d]	
Root from sieved cores	14.4		1.5[d]	
Excised maize root	26.1		n.d.	
Sieved soil with sieve-extracted root				
expected[c]	39.3	NSD[e]	19.2[d]	NSD[e]
observed	38.3		18.6	
Sieved soil with excised maize root				
expected[c]	51.0	NSD[e]	n.d.	
observed	53.8		n.d.	

[a] Experiments analysed separately using *t* tests.

[b] Incubated for 7 days at 25 °C, soil at 30% water content.

[c] Expected, total respiration expected by summation of respiration from separate soil and root components. Observed, measured total respiration from intact soil/root system.

[d] Significant difference between columns within rows ($P \leq 0.05$).

[e] NSD, not significantly different within columns ($P > 0.05$).

n.d., not determined.

Calibration of modified substrate-induced respiration method with biovolume-biomass C

The optimal calibration regression equation, accounting for 71% of the variance, was an exponential function relating a log-transformed respiration rate to biovolume-biomass C with an intercept of 59 μg non-responding biomass C g^{-1} soil at 25 °C (Fig. 5).

In the original method [1] the calibration was between SIR and the chloroform fumigation procedure [10] on 12 soils of unreported pressure potential values and was represented by a linear equation accounting for 92% of the variance and with an error term of 3.7 μg biomass C g^{-1} soil at 22 °C. However, of the 12 soils calibrated, the two soils with a high organic C content and microbial biomass were very influential in producing this linear equation. The relationship between SIR and chloroform fumigation derived from the remaining ten soils, with biomass levels $\leq$ 1100 C g^{-1} soil, appears far from linear. Subsequent calibration between the original SIR method and biomass C estimated by the biovolume procedure in decomposing leaf litter found a failure to correlate significantly, this failure being

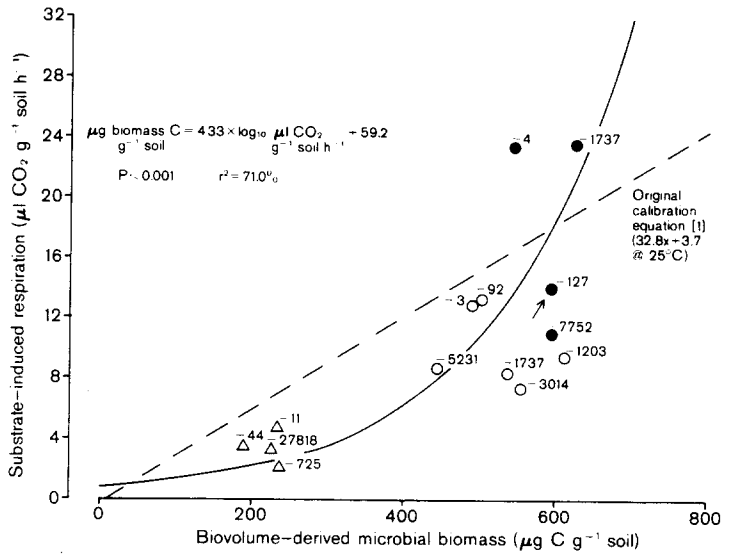

Fig. 5. Correlation between respiration rates (using the modified substrate-induced respiration method) with microbial biomass C (from biovolume measurements) in Greytown (△), Pomare (●) and Waikanae (○) soils. Numbers represent the pressure potential (kPa) of the soils at the time of sampling. Arrow denotes a repeat measurement undertaken at later date on extensively air-dried, then rewetted soil. Original calibration equation adjusted to account for use at 25 °C.

attributed to the biovolume including non-viable 'biomass' which would be incapable of a respiratory response to substrate addition [14]. Such staining of inactive or dead biomass in the biovolume procedure is a commonly recognised phenomenon [4, 10, 15–18] and we believe that this also occurred in our calibration. Such an error was probably responsible for the clustering of data (Fig. 5) and for the lack of any significant correlations ($r < 0.10$) between gravimetric water content or pressure potential (kPa) and biovolume-biomass C in any of the three soils observed.

Implicit in our assumption that soil water content and biomass would be positively correlated was that a 7 day incubation, post rewetting and prior to calibration analysis, would provide sufficient time for extensive decomposition of the biomass killed by the air-drying process [10]. However, amount of water and microbial activity are positively correlated in soil [2] and in the drier soil treatments, where the rate of microbial activity is limited by the low water content (or high pressure potential), a larger proportion of non-viable 'biomass' would probably be included in the total biovolume-biomass estimate. Such an error bias toward the drier soil treatments could account for the necessity of an exponential calibration equation.

Alternatively, the proportion of glucose-C respired as CO_2-C may have been positively correlated with the water content at which the soil was incubated for 7 days prior to analysis. This appears unlikely, based on previous data in this paper. Data from biomass analysis using the modified SIR method on a wider range of 18 grassland soils [13] also demonstrated that the various gravimetric water contents (or

pressure potential) at which these soils were incubated prior to analysis showed no correlation with the subsequent respiratory response to substrate amendment. Further, when air-dried Pomare soil was rewetted to a range of water contents and incubated for 7 days, biomass size was positively correlated with water content as determined by the chloroform fumigation procedure (unpublished data).

Studies of air-drying the range of 18 grassland soils have provided further, convincing evidence that the modified SIR method produces a respiratory response which correlates strongly with the soil microbial biomass. After air drying, the increase in $NaHCO_3$-extractable inorganic P (P_i) from the soils could be predicted from the percent decline in the SIR-estimated respiration rates [13]. The correlation coefficient (r) between respiratory decline and P_i increase was 0.97 using the original, linear calibration equation [1] and 0.96 using the exponential calibration equation derived here.

Thus, the respiration rates derived by the modified SIR method reflect relative changes or differences, within or between soils, and are thus of value per se. However, the extent of such differences, when converted to biomass C, could vary substantially dependent on the use of either a linear or an exponential calibration equation. Based on the available data, we cannot firmly recommend either calibration equation to obtain biomass C values from the measured respiration rates. This is because the alternative methods to determine biomass C, such as the biovolume or chloroform fumigation procedures appear unreliable when applied to drier soils [10]. Using the modified SIR method, estimates of biomass C can be obtained with the original, linear calibration equation [1] recalibrated for use at 25 °C, if it is assumed that there was no water limitation to induced-respiration with the original calibration and that the relationship between induced-respiration and biomass C extends to drier soils. In soils at field water status with no water limitation to respiration, such biomass C estimates will be lower (approx. 20%) when obtained with the modified than the original SIR method. This is because the measured respiration rate is significantly lower in the modified method. Alternatively, the exponential calibration equation (Fig. 5) produced using the modified method is also unsatisfactory as it too is derived from clustered data. In attempting a calibration over a wide range of soil water contents (or pressure potentials) rather than a wide range of soils at a nominal, high field water content, we believe that a major deficiency in the biovolume procedure, using non-vital stains, has been highlighted. To produce a reliable calibration equation between the modified SIR method and biomass C on soils of varying water contents, biovolume procedures based on vital stains or autoradiographic methods are probably necessary.

At present we consider that by relieving both the substrate and water limitations to soil microbial respiration, the proposed modifications to the original SIR method offer real improvements and substantial benefits for use in a variety of soil studies. These improvements include measurement of respiration rates with a low coefficient of variability in a rapid (minimum 2.5 h) and simple manner, use with soils of any initial water content, no requirement for prior incubation of soils so respiration (biomass) can be estimated at the time of sampling, and use with the modified selective respiratory inhibition method to provide estimates of the eukaryote and prokaryote components of soil [3]. However, we recommend that caution be used in

data interpretation if respiration rates are converted to biomass C using either the original or new calibration equations. We also recommend such caution if the method is applied to soils with a large amount of live roots present or a pH > 6.5, and that the method is not used on soils with microbial populations which are extensively anaerobic as these can metabolize glucose-C to CH_4-C as well as CO_2-C [19].

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