

## MEASUREMENT OF MICROBIAL BIOMASS IN ARCTIC TUNDRA SOILS USING FUMIGATION-EXTRACTION AND SUBSTRATE-INDUCED RESPIRATION PROCEDURES

WEIXIN CHENG\* and ROSS A. VIRGINIA†

Systems Ecology Research Group and Department of Biology, San Diego State University,  
San Diego, CA 92182, U.S.A.

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**Summary**—The microbial biomass of seven arctic tundra soils was measured using both the chloroform fumigation-extraction procedure (biomass-C and -N) and the substrate-induced respiration method (biomass-C only) to test the suitability of these two methods for organic and mineral arctic soils. Results indicate that in general the two methods gave consistent microbial biomass-C measurements. Microbial biomass-C, estimated by the fumigation-extraction procedure (using  $K_{oc} = 0.35$ ) was highly correlated ( $r = 0.831$ ,  $P < 0.001$ ) with the microbial biomass-C values measured by the substrate-induced respiration technique across all soil types. Microbial biomass-N of the seven arctic soils measured by the fumigation-extraction procedure showed a higher linear correlation with the biomass-C values produced by the substrate-induced respiration procedure ( $r = 0.88$ ) than those produced by the fumigation-extraction procedure ( $r = 0.69$ ). This result seems to suggest that the fumigation-extraction procedure works better for microbial biomass-N measurements than for biomass-C in these arctic soil conditions. The fumigation-extraction and substrate-induced respiration methods, developed to study mostly temperate agricultural soils, can be successfully applied to arctic tundra soils.

### INTRODUCTION

It is well known that microbial biomass is the main acting agent for most soil biogeochemical processes in aquatic and terrestrial ecosystems (Paul and Voroney, 1980). Microbial biomass interacts with ecosystem productivity by regulating nutrient availability, determines soil C storage, and contributes to atmospheric CO<sub>2</sub> from respiration. A substantial amount of the stored terrestrial carbon (14% by Post *et al.*, 1982) is found as peat and dead organic matter in arctic tundra and other cold ecosystems. As a consequence, there is increasing interest in the importance of arctic ecosystems in the global balance of carbon. The role of these systems as a source or sink for CO<sub>2</sub> under varying climatic conditions is not clear (Billings, 1987). Under any climatic regime the soil microbial biomass will be a major contributor to C exchange with the atmosphere either directly from microbial respiration or through regulating soil organic matter via decomposition.

Information on the amount of soil microbial biomass and its activity in temperate and tropical ecosystems has increased greatly in the past decade (Parkinson and Coleman, 1991). In contrast, measurements of microbial biomass in arctic ecosystems have been scarce except for a few IBP studies

during the 1970s (e.g. Holding *et al.*, 1974; Miller and Laursen, 1974). This is probably due to inaccessibility and methodological problems specific to arctic soil conditions. Arctic tundra soils are usually at, or near, water saturation, have very high organic-C content, and usually contain large amounts of fresh plant materials.

Several methods are available to estimate microbial activity or biomass (Jenkinson and Ladd, 1981). The widely used fumigation-incubation method (Jenkinson and Powlson, 1976) has methodological problems with soils at or near saturation (Ross, 1987) or those that have received inputs of fresh organic matter (Shen *et al.*, 1987), conditions common to arctic tundra soils. Alternatives such as the fumigation-extraction (direct) method (Vance *et al.*, 1987; Sparling and West, 1988, 1989; Sparling *et al.*, 1990; Tate *et al.*, 1988; Ocio and Brookes, 1990) and the substrate-induced respiration method (Anderson and Domsch, 1978; West and Sparling, 1986; Cheng and Coleman, 1989) may overcome these difficulties and might be adapted to the study of arctic soils. For example, the fumigation-extraction method holds promise for anaerobic waterlogged soils (Inubushi *et al.*, 1991).

Our objective was to evaluate the potential of the fumigation-extraction and substrate-induced respiration procedures for determining microbial biomass in arctic tundra soils. This effort is part of a larger study to examine controls on soil respiration and microbial biomass distribution in arctic tundra systems.

\*Current address for correspondence: Institute of Ecology, University of Georgia, Athens, GA 30602, U.S.A.

†Current address: Environmental Studies Program, Dartmouth College, Hanover, NH 03755, U.S.A.

## MATERIALS AND METHODS

This study was conducted at the U.S. Department of Energy R4D study site in the Imnavait Creek watershed along the Dalton Highway in the northern foothills of Philip Smith Mountains in arctic Alaska (68°38' N, 149°25' W). Sampling was carried out on a southwest exposed slope in six different tundra habitats forming a drainage sequence. Three types (lichen heath, stone stripe and non-tussock-sedge dwarf-shrub tundra) were located on the top of the ridge, two types (tussock tundra and water track) were distributed in the mid-slope, and one type (riparian) was located at the base of the slope along Imnavait Creek. Plant communities, topography and soils at the R4D site have been described in detail by Walker *et al.* (1989) and are summarized in Table 1.

Six or seven plots (2 × 10 m) were randomly located in each habitat type. Within each plot a sample of the 0–5 cm layer below the green moss (or soil surface where no green moss layer exists) was taken with a 8 cm dia soil corer (in the three ridge-top habitats) or by cutting a 10 × 10 cm block of the organic soil material with a serrated knife. Samples were placed in sealed plastic bags, transported to the laboratory at Toolik Lake camp (ca 15 km away) and immediately stored in a refrigerator. All subsequent microbial assays were done within 2 weeks of sampling. Recognizable live plant materials (roots and shoots) in each sample were removed by hand picking. Coarse soil organic materials were cut into smaller pieces (< 1 cm). Each sample was homogenized by hand-mixing prior to subsampling for further analysis.

Substrate-induced respiration was measured using a soil respiration measuring system with continuous gas flows. The soil respiration system consisted of a LI-COR CO<sub>2</sub> analyzer (LI-6251), a water bath for controlling the incubation temperature and an air flow controlling and measuring unit. The configuration of this system is illustrated in Fig. 1. Substrate-induced (glucose) CO<sub>2</sub> evolution rates from each sample were measured at 22°C on a continuous

air flow-through system described by Cheng and Coleman (1989) with some modifications as shown in Fig. 1. Briefly, a 15 g fresh, homogenized subsample was placed in a 125 ml Erlenmeyer flask and glucose solution (60 g l<sup>-1</sup>) was added to the flask to bring the soil water content near to its holding capacity (no free standing solution) using a syringe. The flask was connected to the respiration measuring system and after about 40 min at an air flow rate of 180 ml min<sup>-1</sup>, the rate of CO<sub>2</sub> evolution from the soil sample became constant. At this point the CO<sub>2</sub> evolution rate was recorded as the substrate-induced respiration rate of that sample. The substrate-induced respiration rate of each sample was converted to microbial biomass-C using the equation of Anderson and Domsch (1978).

Soil microbial biomass-C and -N of all habitats was analyzed following the fumigation-extraction procedure (Tate *et al.*, 1988) with some minor modifications. Briefly, 10 g of fresh, homogenized subsamples were weighed into individual 100 ml glass beakers and fumigated with purified CHCl<sub>3</sub> for 24 h. After removal of residual CHCl<sub>3</sub> the fumigated soils were transferred into 250 ml plastic bottles, 50 ml of 0.5 M K<sub>2</sub>SO<sub>4</sub> solution added, and then shaken for 1 h on an orbital shaker at a speed of 110 revolutions min<sup>-1</sup>. The shaken mixture was then filtered through Whatman No. 40 filter paper and each extract was put in a 50 ml plastic bottle and immediately frozen for later analysis at San Diego State University. The concentration of total organic-C of each extract was analyzed on a total organic-C (TOC) Analyzer (Shimadzu 500). Total N in each extract was analyzed by using a micro-Kjeldahl digestion procedure followed by N determination on a Technicon II autoanalyzer. For calculation of microbial biomass-C, a K<sub>ec</sub>-factor of 0.35 was assumed (Sparling *et al.*, 1990). For calculation of microbial biomass-N, a K<sub>cn</sub> factor of 0.54 (Brooks *et al.*, 1985) was used.

Soil total N was determined on a Technicon II Autoanalyzer following micro-Kjeldahl digestion. Soil total organic-C was determined by following the modified dichromate digestion procedure (Yeomans

Table 1. Summary of soil types (0–5 cm depth below green moss layer, numbers are means of 6 or 7 replicates, values in parentheses are standard errors). BD: bulk density

Name	Plant community	Typical site	N (%)	C (%)	C:N	BD (g/cc)
Lichen heath	<i>Dryas octopetala</i>	Windblown south-facing slopes on sandstone	0.930	16.84	19.36	0.379
	<i>Selaginella sibirica</i>		(0.171)	(3.42)	(2.59)	(0.031)
Stone stripe	<i>Cassiope tetragona</i>	Non-sorted stone stripes	0.504	11.53	25.55	0.310
	<i>Calamagrostis inexpansa</i>		(0.225)	(4.23)	(5.76)	(0.049)
Dwarf-shrub tundra	<i>Carex bigelowii</i>	Mesic sites on steeper slopes, some solifluction or cryoturbation	1.659	35.05	22.03	0.113
	<i>Sphagnum</i> spp		(0.139)	(1.10)	(2.23)	(0.011)
Water track	<i>Salix planifolia</i> spp	Water tracks, wet, mid-slopes	1.433	34.61	26.71	0.089
	<i>Sphagnum</i> spp		(0.166)	(2.10)	(4.70)	(0.003)
	<i>Eriophorum angustifolium</i>					
Tussock tundra (on tussock)	<i>E. vaginatum</i>	Stable mesic sites on flat or gentle slopes	0.481	43.09	91.52	0.106
Inter-tussock tundra	<i>Sphagnum</i> spp	As above, but in between tussocks	0.689	39.00	60.70	0.059
			(0.079)	(0.78)	(6.21)	(0.006)
Riparian	<i>Carex aquatilis</i>	Stream channels with a deep peat layer and moss covers	1.057	29.82	31.06	0.078
	<i>Sphagnum</i> spp		(0.111)	(2.84)	(5.65)	(0.005)

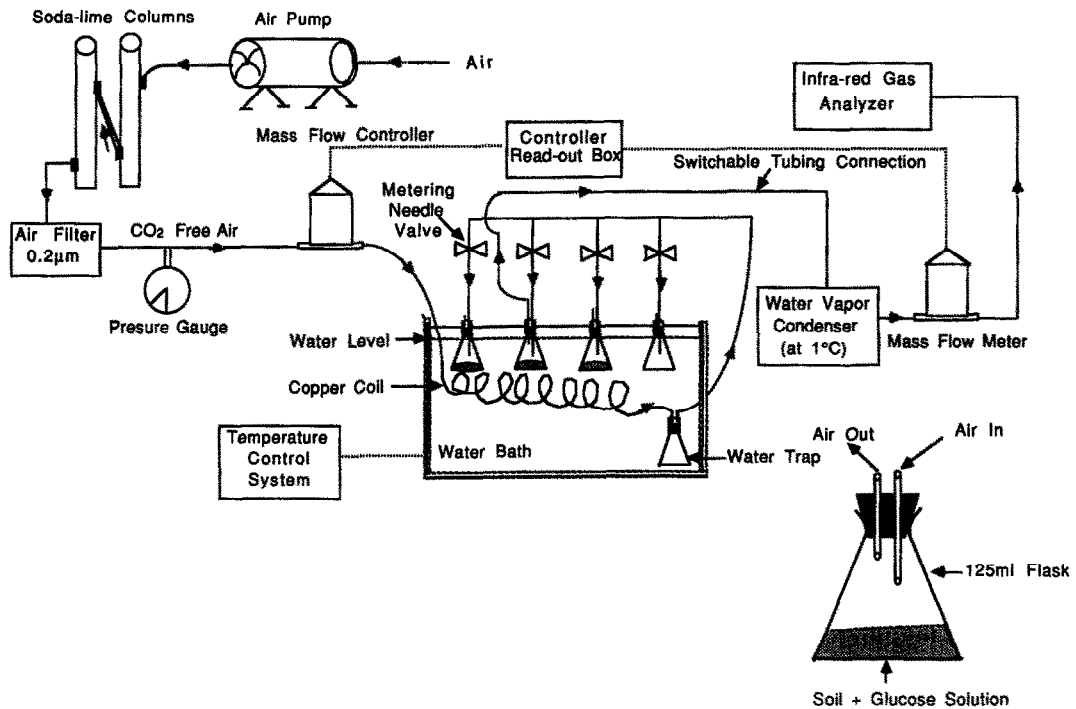


Fig. 1. Substrate-induced respiration measuring system configuration. — Air tubing (1/4 in O.D.); ..... electrical wire.

and Bremner, 1988). Soil bulk density was determined by oven-drying (60°C) of soil sample from a known volume for at least 72 h.

RESULTS AND DISCUSSION

The soils examined ranged considerably in their bulk densities, organic-C contents, and total N contents. Lichen heath and stone stripe soils from the upper-ridge contained more mineral materials and relatively more well-decomposed organic matter than the rest. The mid-slope tussock tundra and inter-tussock tundra soils are highly organic (43 and 39% C) but contain very little N (0.48 and 0.69%) (see Table 1), and therefore have high C:N ratios (91.5 and 60.7) relative to the other soils. The water track

and dwarf-shrub tundra soils are organic (35% C) but also contain higher amounts of N (1.4 and 1.7%), resulting in lower C:N ratios (26.7 and 22.0) than the tussock soils. The riparian soils are continuously saturated and mostly consist of dead moss material.

Results of microbial biomass-C in these arctic tundra soils measured by the fumigation-extraction and substrate-induced respiration procedures are shown in Table 2. Microbial biomass ranged from 2.99 mg C g<sup>-1</sup> soil to 13.9 for the fumigation-extraction procedure and from 2.33 to 12.8 for the substrate-induced respiration procedure. This large range of microbial biomass values can be attributed to the wide range of organic-C contents and bulk densities of the soils tested. In order to compare these results among themselves and with those in the literature, we expressed results as mg

Table 2. Microbial biomass carbon in arctic tundra soils measured by the fumigation-extraction method and the substrate-induced respiration method (mean and standard error in parentheses)

Biomass	Method <sup>1</sup>	Soils <sup>2</sup>						
		IT	L	DT	R	S	T	W
mg C g <sup>-1</sup> soil dry wt	FE	13.9 (1.50)	4.80 (1.09)	8.68 (1.63)	7.44 (1.01)	2.99 (1.03)	8.87 (0.62)	11.2 (1.17)
	SIR	10.3 (1.17)	4.71 (0.99)	7.13 (1.55)	9.34 (1.11)	2.33 (0.81)	6.43 (0.55)	12.8 (1.82)
	<i>t</i> -test <sup>3</sup>	*					**	
mg C g <sup>-1</sup> Soil C	FE	35.8 (4.2)	27.1 (3.0)	24.5 (4.1)	21.9 (2.7)	28.2 (4.9)	20.6 (1.5)	32.2 (2.3)
	SIR	26.8 (3.1)	26.9 (2.5)	20.1 (4.0)	27.6 (3.5)	20.6 (2.9)	14.9 (1.3)	36.4 (4.5)
	<i>t</i> -test	*					**	
C:N in MB	FE/FE	21.8 (1.76)	11.2 (0.57)	13.5 (0.49)	9.74 (1.68)	10.5 (1.11)	18.3 (0.64)	10.8 (1.13)
	SIR/FE	16.2 (1.48)	11.2 (0.56)	10.9 (0.64)	12.1 (1.63)	8.30 (1.07)	13.3 (0.94)	12.0 (1.47)
	<i>t</i> -test	**		***			****	

<sup>1</sup>FE: fumigation-extraction method; SIR: substrate-induced respiration method.

<sup>2</sup>IT: inter-tussock; L: lichen heath; DT: dwarf-shrub tundra; R: riparian; S: stone stripe; T: tossuck tundra; W: water track.

<sup>3</sup>Comparison of means between FE and SIR, \**P* < 0.1; \*\**P* < 0.05; \*\*\**P* < 0.01; \*\*\*\**P* < 0.001.

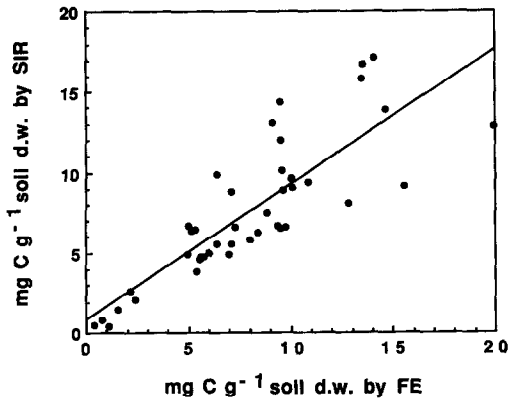


Fig. 2. Relationship between the microbial biomass carbon values ( $\text{mg C g}^{-1}$  soil dry wt) measured by the fumigation-extraction (FE) procedure and those measured by the substrate-induced respiration (SIR) method for seven arctic tundra soils. Regression function:  $Y(\text{SIR}) = 0.812 + 0.842X(\text{FE})$  ( $r = 0.831$ ,  $P < 0.001$ ).

microbial biomass-C  $\text{g}^{-1}$  soil organic-C using soil organic-C data in Table 1 and the  $\text{g}^{-1}$  dry soil based microbial biomass data in Table 2. We found that  $\text{mg}$  microbial biomass-C  $\text{g}^{-1}$  soil organic-C of these soils follows the order of inter-tussock > water track > stone stripe > lichen heath > dwarf-shrub > riparian > tussock using the fumigation-extraction procedure, and the order of water track > riparian > lichen heath > inter-tussock > stone stripe > dwarf-shrub > tussock using the substrate-induced respiration procedure. These microbial biomass results are comparable to and well within the range of values reported in the literature (i.e. Insam and Domsch, 1988; Insam *et al.*, 1989), mostly from temperate regions. The range of C:N ratios in the microbial biomass of these arctic tundra soils are similar to those found for agricultural, grassland and temperate forest soils (Voroney and Paul, 1984; Shen *et al.*, 1987; Sparling and West, 1989) except for a few high values measured for inter-tussock and tussock soils using the fumigation-extraction procedure.

Microbial biomass-C, estimated by the fumigation-extraction procedure (using  $K_{\text{ec}} = 0.35$ ) was highly correlated ( $r = 0.831$ ,  $P < 0.001$ ) with the microbial biomass-C values measured by the substrate-induced respiration technique across all soil types (Fig. 2). It is unlikely that this significant correlation was obtained solely as a consequence of the wide ranging of the biomass-C values among the soils we tested. This problem has been discussed by Wardle and Parkinson (1990). The correlation between the two sets of measurements is much higher if only the three soil types on the ridge top are included, which is a subset of the total and has a narrower range in biomass-C values (Fig. 3). Furthermore, microbial biomass-C as percentage of total soil-C measured by the fumigation-extraction procedure was also significantly ( $P < 0.001$ ) correlated to that measured by the substrate-induced respiration

procedure even though the range of values was small (1–5%) (Fig. 4). These results agree well with the finding of Sparling *et al.* (1990) for grassland soils and indicates that each method gave consistent results for these arctic soils.

Location-specific analyses showed that the correlation between the two sets of measurements (substrate-induced respiration and fumigation-extraction) was significant for stone stripe soil ( $P < 0.001$ ), dwarf-shrub tundra soil ( $P < 0.001$ ), lichen heath soil ( $P < 0.01$ ), water track soil ( $P < 0.05$ ) and tussock tundra soil ( $P < 0.05$ ), but was not significant for inter-tussock soil and riparian soil (Table 3). A possible cause of the poor correlations between the fumigation-extraction values and the substrate-induced respiration values of inter-tussock soil and riparian soil was that they contained large amounts of recently dead moss materials. Only 4 replicates were tested for the riparian soil which may also have contributed to the low correlation mentioned above.

Across all the soils microbial biomass-N, estimated by the fumigation-extraction procedure (using  $K_{\text{en}} = 0.54$ ) was significantly correlated with the microbial biomass-C value measured by the substrate-induced respiration technique ( $r = 0.88$ ,  $n = 42$ ,  $P < 0.001$ ) and by the fumigation-extraction procedure (using  $K_{\text{ec}} = 0.35$ ) ( $r = 0.69$ ,  $n = 42$ ,  $P < 0.001$ ) [Fig. 5(A) and (B)]. If we assume that both the fumigation-extraction and the substrate-induced respiration methods provide equally good estimates of microbial biomass-C, then a similar linear correlation should exist between C and N estimates from both methods. However, the correlation between microbial biomass-N and microbial biomass-C from the fumigation-extraction procedure was much lower than that between microbial biomass-N estimated from fumigation-extraction procedure and microbial

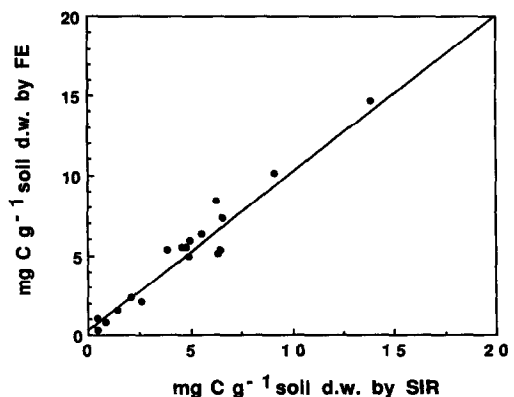


Fig. 3. Relationship between the microbial biomass carbon values ( $\text{mg C g}^{-1}$  soil dry wt) measured by the fumigation-extraction (FE) procedure and those measured by the substrate-induced respiration (SIR) method for three arctic tundra soils (lichen heath, stone stripe and dwarf-shrub tundra). Regression function:  $Y(\text{FE}) = 0.218 + 1.050X(\text{SIR})$  ( $r = 0.974$ ,  $P < 0.001$ ).

Table 3. Regression functions of microbial biomass C (unit:  $\text{mg g}^{-1}$  of oven-dried soil) measured by fumigation-extraction method ( $Y$ ) vs microbial biomass C measured by substrate-induced respiration method ( $X$ ) for seven arctic soils

Soil type	Function	Coefficient
Lichen heath	$Y = -0.080 + 0.979 X$	$r = 0.94, n = 6, P < 0.01$
Stone stripe	$Y = 0.048 + 1.188 X$	$r = 0.99, n = 6, P < 0.001$
Dwarf-shrub tundra	$Y = 1.237 + 0.974 X$	$r = 0.98, n = 6, P < 0.001$
Water track	$Y = 3.944 + 0.521 X$	$r = 0.86, n = 6, P < 0.05$
Tussock tundra (on tussock)	$Y = 2.911 + 0.848 X$	$r = 0.80, n = 7, P < 0.05$
Inter-tussock	$Y = 7.094 + 0.581 X$	$r = 0.48, n = 7, \text{NS}^*$
Riparian	$Y = -0.403 + 0.794 X$	$r = 0.93, n = 4, P < 0.1$

\*NS = not significant.

biomass-C measured by the substrate-induced respiration technique. From Fig. 5(B), there is a clear indication that the fumigation-extraction procedure produced reasonable microbial biomass values for soil samples which showed lower microbial biomass-C and -N contents, but not as well for the rest which had much higher microbial biomass-C and -N values. The fumigation-extraction method (if assumed  $K_{ec} = 0.35$ ,  $K_{en} = 0.54$ ) gave unreasonably and significantly higher C:N ratios of microbial biomass for tussock ( $P < 0.01$ ), inter-tussock ( $P < 0.05$ ) and dwarf-shrub tundra ( $P < 0.05$ ), and significantly higher microbial biomass values both in terms of  $\text{mg microbial-C g}^{-1}$  oven-dried soil and  $\text{g}^{-1}$  total soil organic-C than the substrate-induced respiration method (Table 3). These results indicate that the fumigation-extraction procedure ( $K_{ec} = 0.35$ ) overestimated microbial biomass-C for soils of higher C:N ratio.

Various  $K_{ec}$  values can be obtained if the biomass-C numbers from substrate-induced respiration are used as the reference (i.e. are assumed to be the correct estimate). The  $K_{ec}$  values produced in this way ranged from 0.22 to 0.6 among these 40 arctic samples (data not shown). This wide range of  $K_{ec}$  values may indicate problems in either one of the two methods or both.

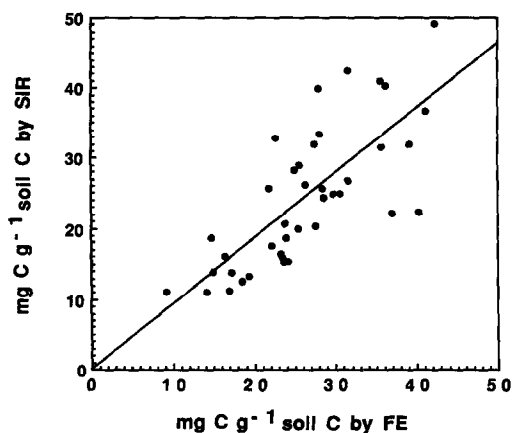


Fig. 4. Relationship between the  $\text{g}^{-1}$  soil C based microbial biomass C values ( $\text{mg C g}^{-1}$  soil organic C) measured by the fumigation-extraction (FE) procedure and those measured by the substrate-induced respiration (SIR) method for seven arctic tundra soils. Regression function:  $Y(\text{SIR}) = 0.036 + 0.923X(\text{FE})$  ( $r = 0.751, P < 0.001$ ).

The  $K_{ec}$  values calculated using the biomass-C numbers from substrate-induced respiration as the reference were significantly ( $P < 0.001$ ) correlated (positive) with the C:N ratios of the fumigation flush ( $\text{K}_2\text{SO}_4$  extracts after chloroform fumigation) for the arctic samples and Sparling and West (1988) found similar results from some New Zealand grassland soils (Fig. 6). This suggests that the relative extractabilities of microbial biomass-C and -N after

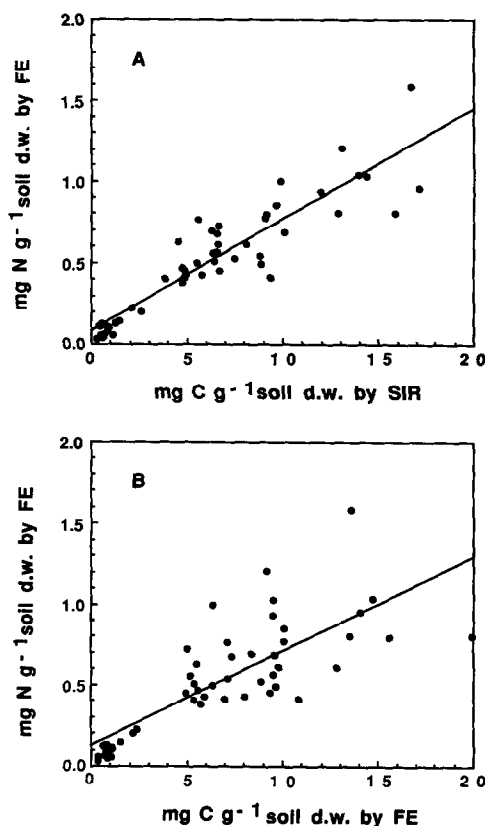


Fig. 5. Relationship between microbial biomass C ( $\text{mg C g}^{-1}$  soil dry wt) measured by the substrate-induced respiration procedure and microbial biomass N ( $\text{mg N g}^{-1}$  soil dry wt) measured by the fumigation-extraction procedure. (A) Regression function:  $Y_A = 0.08 + 0.068X_A$ ,  $r = 0.917, P < 0.001$  and relationship between microbial biomass C ( $\text{mg C g}^{-1}$  soil dry wt) and microbial biomass N ( $\text{mg N g}^{-1}$  soil dry wt) both measured by the fumigation-extraction procedure. (B) Regression function:  $Y_B = 0.12 + 0.059X_B$ ,  $r = 0.803, P < 0.001$ .

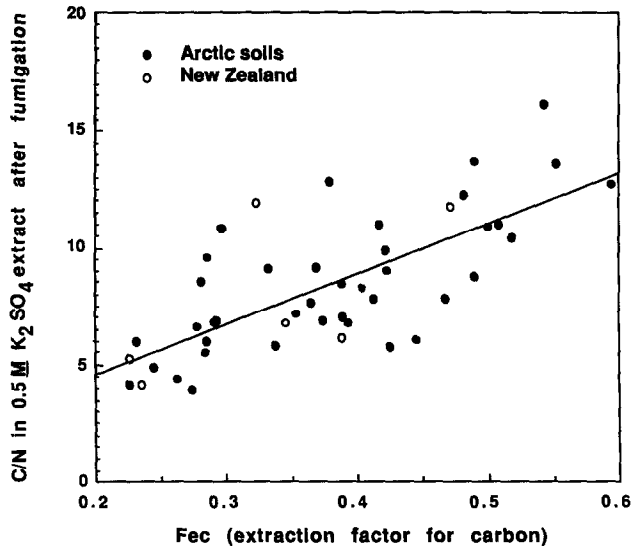


Fig. 6. Correlation between extraction factor for carbon,  $F_{ec}$ , and C:N ratio in the extract ( $K_2SO_4$ ) after fumigation. The solid circles represent arctic soils and the open circles represent soil from New Zealand grassland (Sparling and West, 1988). The regression function:  $Y(C:N) = 0.3 + 21.55X(F_{ec})$  ( $r = 0.72$ ,  $P < 0.001$ ).

chloroform fumigation vary significantly as a function of soil type if the reliability of the substrate-induced respiration measurements is assumed.

Our results signal some further questions concerning the fumigation-extraction method. In term of consistency and reliability of the  $K_2SO_4$  extractable microbial-C vs microbial-N after fumigation, is C or N more suitable to use as the basis for the biomass calculation? Jenkinson (1988) noted that the soluble organic matter released by fumigation may partition differently between soil and extract in different soils. Our results seem to support this proposition. Amato and Ladd (1988) demonstrated that microbial biomass-C measured by the fumigation-incubation procedure was highly correlated with the ninhydrin-reactive N extract by 2 M  $K_2SO_4$  from chloroform-fumigated soils. Their results indicated that the ninhydrin-reactive N extracted by 2 M  $K_2SO_4$  from chloroform-fumigated soils was mainly organic and of microbial origin, so it can be used to measure microbial biomass-C. They also noted that anthrone-reactive C (carbohydrate) was released from soil fumigation, but there was no correlation between the amount of anthrone-reactive C extracted after chloroform fumigation and the amount of microbial biomass-C estimated by the fumigation-incubation procedure.

We found that the fumigation-extraction procedure can provide reliable information of microbial biomass-N as well as biomass-C across the wide range of soil condition found in arctic systems. The substrate-induced respiration and fumigation-extraction procedures produced fairly consistent results. The role of arctic tundra systems in the global carbon cycle will require increasing effort to understand microbial biomass distribution and activity.

Our results indicate that the fumigation-extraction and the substrate-induced respiration methods, developed to study temperate mostly agricultural soils, can be successfully applied to arctic tundra.

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