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MICROCALORIMETRY AND CO_2-EVOLUTION OF SOILS AND LICHENS FROM ANTARCTICA

Manfred BÖLTER

Institute for Polar Ecology, University of Kiel, Wischhofstrasse 1–3, Geb. 12, D-24148 Kiel, Germany

Abstract: Data on microcalorimetry and CO_2-evolution of soil samples and lichens from maritime and continental Antarctic habitats were compared. Values from soil samples showed a strong relationship to the organic matter content. Microbial biomass estimates determined from conversion factors were compared with biomass estimates from epifluorescence microscopy. There are basic differences in the microbial biomass data among individual samples. They can be attributed to different physiological states of the microbial communities and their original habitats. Heat production correlated well with CO_2-evolution. Data were obtained in ranges between 1 and 220 μWg^{-1} d.wt for soils and 240 and 2400 μWg^{-1} d.wt for lichens. These values are discussed with regard to microcalorimetric data from literature.

1. Introduction

Microbial biomass is part of a dynamic ecosystem with a continuous interchange between individual populations and their different energy sources, which influence community structure and its activity. In terrestrial Antarctic ecosystems, microbial activity is generally restricted by low levels of available nutrients (SMITH, 1985). Thus, very sensitive methods are needed in order to detect metabolic processes in this environment.

Direct microcalorimetry has been proposed as an indicator for overall microbial activity (LJUNGHOLM *et al.*, 1979a; SPARLING, 1983). It reveals the sum of the enthalpy changes of all physico-chemical processes and thus allows an appropriate measure for the energy flow in soil ecosystems. As an overall method it is suitable for quantitative comparisons of total budgets (BELAICH, 1980; PAMATMAT *et al.*, 1981). Additionally, total CO_2-evolution describes metabolic processes at an overall level. Both methods can be used as estimates of metabolic rates from natural samples and both methods are well established in soil microbiology (LJUNGHOLM *et al.*, 1979b; SPARLING, 1981a, b). No microcalorimetry data have been published yet from Antarctic soil environments and plants. During this study, a comparison between respirometry and microcalorimetry was carried out with samples from soils and lichens from two contrasting coastal terrestrial Antarctic environments in order to show their suitability as analytical methods in soil and plant ecological studies.

2. Materials and Methods

2.1. *Samples*

The investigation was carried out with soil and plant samples from Arctowski Station, King George Island, Maritime Antarctic (62°09′S, 58°28′W) and Casey Station, Wilkes Land, East Antarctica (66°17′S, 110°32′W) (Fig. 1), during the austral summers of 1986/87 and 1989/90, respectively. Soil samples were taken with special respect to the top level which was often formed by cushions of moss-lichen communities, algae and cyanobacteria-except those places in the vicinity of Arctowski Station which were covered with mats of Deschampsia antarctica and *Colobanthus quitensis*. Deeper layers were mainly formed by mineral soils. Samples were stored in a freezer (−25°C) until analysis in the laboratory in Kiel.

2.2. *Soil properties*

Dry matter was measured after exposure of subsamples to 105°C for 24 hours. Loss on ignition (LOI) was performed after combustion at 550°C for 6 hours. For the experiments conducted during this study, the samples were rewetted. Data of the water content at sampling inclusive data on organic and inorganic nutrients and other microbiological factors have been published in earlier reports (BÖLTER, 1991, 1992a, b, 1993).

2.3. *Microcalorimetry*

The microcalorimeters were double-twin heat flow calorimeters (PAMATMAT *et al.*, 1981; GRAF and BENGTSSON, 1984). Continuous voltages (120 mVK^{-1}) could be monitored by nanovoltmeters (DMM181 and DMM190, Keithley, FRG). Calibration constants for calorimeters A and B were 8.0 and 7.4 $mWmV^{-1}$, respectively. Incubation temperature was 20°C for the Casey samples and 15°C for the Arctowski samples. This difference was due to the different experimental apparatus. All samples (≈10–20 g w.wt) were preincubated for 4 h at 20°C or

Fig. 1. Locations of Stations Casey and Arctowski in the Antarctic (for details see text).

15°C, respectively. Reequilibration of the chambers with the heat sink occurred within 2–3 h. Although no direct data are available for the change of activity between these temperatures for calorimetry, data of CO_2-evolution generally show maxima at these temperatures (BÖLTER, 1991).

2.4. *CO_2-Evolution*

All experiments for determination of CO_2-evolution (samples about 20 g w.wt) were carried out in temperature controlled chambers with continuous gas flow (0.5 l min^{-1}). The gas was analyzed by an infrared gas analyzer (Leybold Heraeus, FRG, *cf.* KAPPEN *et al.*, 1989). CO_2-evolution of the Casey samples was performed with their actual water content. Experiments with the Arctowski samples were run after rewetting (final water content: 50% water of dry weight). Experiments were run at temperature steps of 5°C between 0 and 30°C. Data used here refer to microcalorimetry.

3. Results

Data for the CO_2-evolution of the soil samples and further information about samples are presented in Table 1. Surface samples showed significantly more activity than the deep-layer samples, especially those samples which contain high amounts of organic matter, as expressed by the loss on ignition (LOI).

The data of Casey can be pooled into three categories based on amounts of LOI and CO_2-evolution: a) Soil samples 1A, 2A and 7A; b) soil samples 1B, 2B, 7B, 8A, 8B, 9A, 9B; and c) lichen samples C3 (*Umbilicaria decussata*), C4 (*Pseudephebe minuscula*), C5 (*Usnea sphacelata*), C6 (*Usnea antarctica*). Similarly, the samples from Arctowski were arranged in this way:

—group A: surface samples with high organic matter content,
—group B: surface samples with low amounts of organic matter,
—group C: deep layer samples with generally low amounts of organic matter.

Wide ranges of heat output data were found in the soil and lichen samples. The results indicate an influence of organic matter on microbial activity in soil samples and they also seem to be related to the water content. A relationship was assumed between LOI and CO_2-evolution or energy loss, respectively, although the data were insufficient to establish a regression function. Lichen thalli revealed much higher values of CO_2-output.

Thermograms of samples 1A and 1B and of sample C4 (*Pseudephebe minuscula*) are presented in Fig. 2. The steady decrease of heat output of samples 1A and 1B showed a negative exponential shape. This shape was obtained for all soil samples. The strongest decrease occurred during the first 12 h. It became more steady for another period of about 12 h and fell to nearly zero after that. Thermograms of the lichen samples showed somewhat different shapes compared to those with the soil samples, *i.e.* there was a longer steady state phase at a high level of heat production. Lichen samples kept high heat production even after 20 h (32.8–84.5%) with respect to the actual heat production after 4 h. Lower, but still significant values (19.7–30.9%) were found in soil

Table 1. Characteristics of the samples and results of the CO_2-evolution from Casey and Arctowski Station.

a) Samples from Casey Station.

Sample	NO.	Depth (cm)	LOI[1] (%)	H_2O[2] (%)	CO_2-evolution[3] (µg CO_2 g^{-1} h^{-1})
Soil	1A	0–1	13.8	83.5	56.4
	1B	1–3	2.1	34.2	3.7
	2A	0–1	14.0	88.7	55.5
	2B	1–3	6.3	44.8	8.3
	7A	0–1	14.4	84.6	31.6
	7B	1–3	3.1	39.0	3.5
	8A	0–1	2.4	24.9	2.0
	8B	1–3	2.3	23.5	0.9
	9A	0–1	3.4	47.7	12.8
	9B	1–3	1.8	33.3	4.2
Lichen					
C3 *U. decussata*			97.1	70.6	[4]
C4 *P. minuscula*			73.5	89.0	
C5 *U. sphacelata*			97.2	72.9	
C6 *U. antarctica*			98.0	52.9	

[1] LOI= loss on ignition (% of dry weight);
[2] H_2O= actual water content (% of sample wet weight) during incubation.
[3] Incubation at 20°C.
[4] For gas exchange data see Fig. 3.

b) Samples from Arctowski Station.

Sample	Depth (cm)	*n*		LOI (%)	CO_2-evolution[1] (µg CO_2 g^{-1} h^{-1})
Soil	A 0–1	8	min	16.8	11.7
			max	44.6	97.3
Soil	B 0–1	6	min	10.4	0
			max	16.9	5.8
Soil	C 1–6	14	min	10.0	0
			max	31.3	11.2
Lichens[2]		15	min	4.5	26.9
			max	76.5	144.8

[1] Incubation at 5°C.
[2] For individual gas exchange date of lichens see Fig. 3.

samples 1A, 2A, and 7A.

This effect can be attributed to internal processes of oxygen and/or nutrient depletion or effects of disturbances. Hence, readings after 12 h incubation time were chosen for comparisons between samples. Readings for soil and lichen

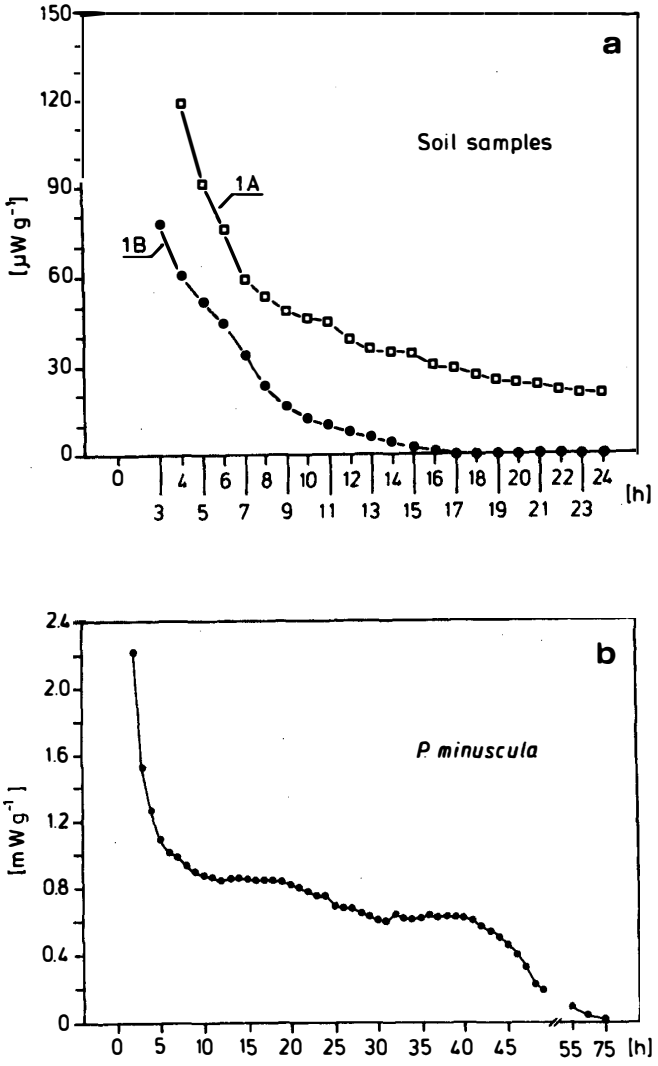

Fig. 2. a) Thermograms of soil samples (1A: surface layer, 0-1 cm; 1B: deep layer, 1-3 cm). b) Thermogram of the lichen Pseudephebe minuscula.

samples from Casey at different times are presented in Table 2 and in Fig. 3. Heat production of lichen thalli was significantly higher than that of most soil samples. Similar results were found in an experiment with the lichen *Caloplaca regalis* from Arctowski Station (expedition 1984/85) which showed decreasing heat production from 1.28 to 0.97 mWg^{-1} over the incubation period from 6–100 h (BÖLTER and GRAF, unpubl. results).

Basically, data of heat output are concomitant to those of CO_2-evolution: Soil samples with high amounts of organic matter showed significantly higher heat production than those from mineral soils. Lowest heat outputs were found in barren soils and soils of deeper layers. The grouping according to LOI and CO_2-evolution also showed evidence for a decrease in heat production during the course of the experiments.

A basic grouping of the Arctowski samples showed the following results according to their heat output: a) soil surfaces (0–2 cm) with high amounts of

Table 2. Date of microcalorimentry of soil and lichen samples from Casey and Arctowski Station, Antarctica.

a) Casey

Sample	Time 4 h	12 h	20 h
1A	117.4	37.7	23.2
1B	60.0	7.2	0
2A	221.0	123.3	68.3
2B	37.2	1.7	0
7A	208.6	131.4	57.1
7B	15.1	1.4	0
8A	15.8	2.2	0
8B	9.7	1.2	0
9A	130.0	5.0	0
9B	55.5	1.0	0
U. *decussata*	2383	1750	1526
P. *minuscula*	1282	846	794
U. *sphacelata*	740	459	242
U. *antarctica*	1808	1353	1527

Readings after 4, 12, and 20 h incubation in $\mu W\, g^{-1}$.

b) Arctowski

Summary of readings ($\mu W\, g^{-1}$) after 12 h.
For individual data see Fig. 4.

Soils group	*n*	min	max
A	8	12	90
B	6	2	97
C	14	3	60

organic matter: 0.012–0.09 mWg^{-1}; b) soil surfaces (0–2 cm) with low amounts of organic matter: 0.002–0.097 mWg^{-1}; c) deep layers (2–4 and 4–6 cm): 0.003-0.06 mWg^{-1}; and d) lichen samples: 0.21–2.07 mWg^{-1}. There was no evident differentiation between macro and micro lichens.

The values of heat production of all Casey samples from deep layers and those from barren soils were very low or even below detection level (Table 2). This looks different for the soils from Arctowski (Fig. 4). There were several low values within samples of group A and high values within groups B and C (*cf.* Tables 1, 2). These discrepancies must be attributed to internal processes of the samples with relation to different active communities. Different patterns were obtained also from LOI, POC, monocarbohydrates and bacterial biomass (for references on organic nutrients and data on bacterial biomass see above).

Direct comparisons between the data from respirometry and microcalorimetry were difficult because of the different behavior of the samples during the experiments. While the CO_2-output did not show a decrease during the course of

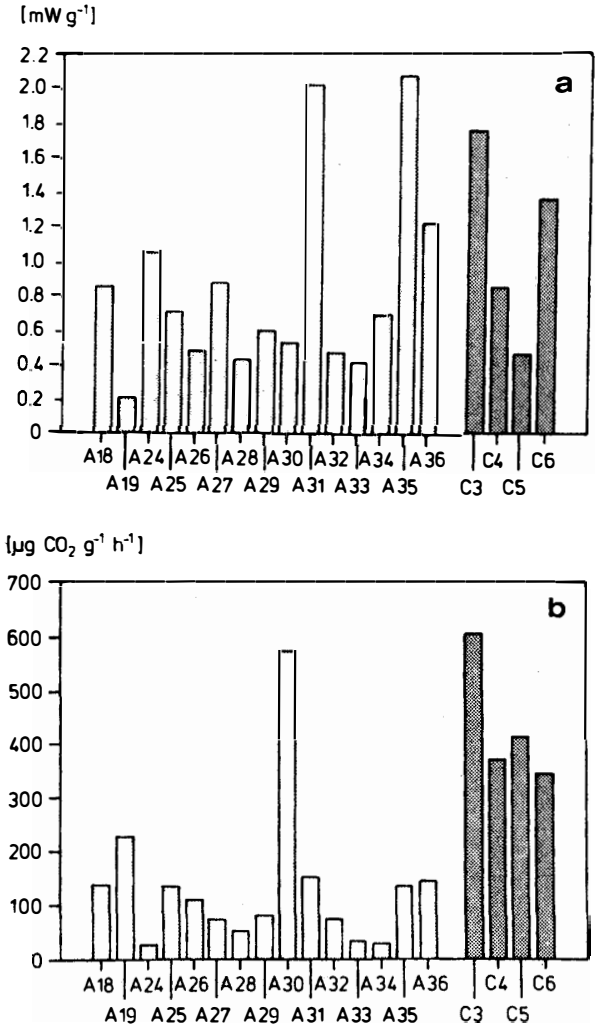

Fig. 3. *a) Heat output of individual lichen thalli from Casey and Arctowski.*
b) Gas exchange of individual lichens from Casey and Arctowski.

Sample	*Lichen species/community*
A18	Usnea antarctica
A19	Alectoria *sp.*
A24	U. aurantiaco-atra
A25	Leptogium puberulum
A26, A27	Ochrolechia frigida
A28	Placopsis contortuplicata
A29, A36	Stereocaulon alpinum
A30	Cornicularia *sp.*
A31	Alectoria *sp.*
A32	U. antarctica
A33	*moss community with* O. frigida
A34	*community of different microlichens*
A35	Parmelia saxatilis
C3	Umbilicaria decussata
C4	Pseudephebe minuscula
C5	U. sphacelata
C6	U. antarctica

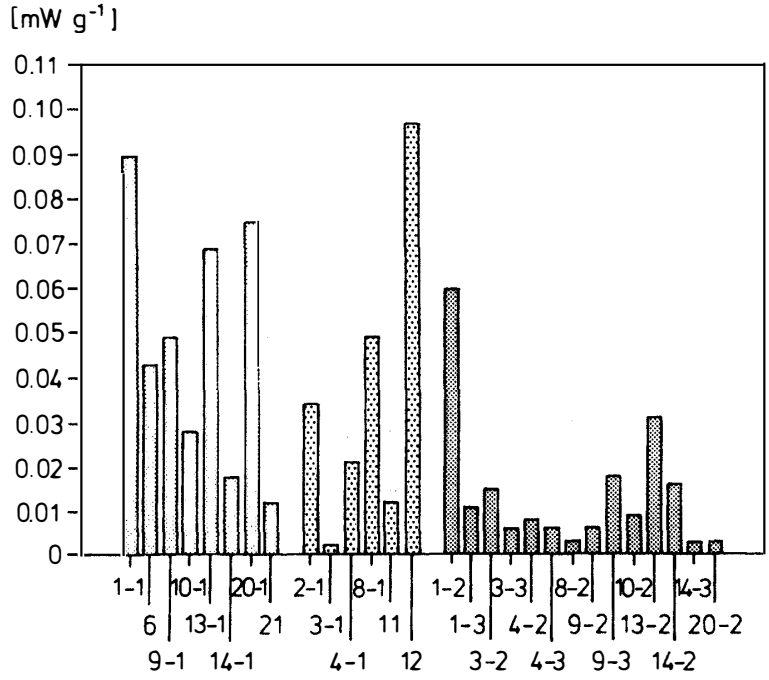

Fig. 4. Heat output of soil samples from Arctowski.

the experiments, the heat production decreased non-linearly. Analysis of the data set from Arctowski showed the same tendencies as the soil samples from Casey.

4. Discussion

The ranges of these results of the heat output gave some hints for further comparisons with data from the literature (Table 3). Comparable results to the values of heat production of the top layers (samples 1A, 2A) have also been reported from temperate environments (SPARLING, 1983; ZELLES *et al.*, 1987a, 1990; ALEF *et al.*, 1988). This points to a high metabolic potential of Antarctic soils, *i.e.* their microbial community. The low values found here are comparable to soils from Sweden (LJUNGHOLM *et al.*, 1979a, b) and from Scotland (SPARLING, 1981a, b), although different methodological approaches in microcalorimetry (incubation temperature, preincubation, substrate additions) prevent direct comparisons with those data from the literature.

It is obvious that these data cannot show more than potential activity due to the (artificial) laboratory conditions. This holds true for temperature and moisture regimes, although there are no other important disturbances (*e.g.* sieving, addition of nutrients). Comparably high temperatures and moisture contents have been monitored in the original soil and plant environment (BÖLTER, 1989, 1992a). Therefore, these conditions are not out of the range of actual ecophysiological constraints.

Ecologically, such high potential activities are not desirable effects in the original environment because they would lead to quick depletion of both internal

Table 3. Date from literature of heat production from different soils.

Ref.	Soil types	Condition	Heat production ($\mu w\ g^{-1}$)
1	spruce forest	30°C	20
	clayish field	addition of	5
	beech/oak forest	glucose	18
2	cultivated soil, 15 cm depth	25°C	4–8
3	Scottish soils	22°C	1.7–35
4	Scottish soils	22°C	1.9–30.8
5	Scottish soils	22°C	1.7–171.8
	–fresh organic	120% H_2O	167
	–fresh mineral	20–25% H_2O	11.4
6	Forest soils:	25°C,	
	Of_1	addition of	2500–4000
	Of_1	glucose	400–1680
	Oh		90–225
	Ah		10–80
7	Forest soils 0–20 cm	30°C, addition of glucose	32–330
8	Forest podzol soils:		
	Of_1		1024
	Of_2		291
	Oh		121
	Aeh		26
	Ah 1		6

References: 1: LJUNGHOLM *et al.* (1979a), 2: LJUNGHOLM *et al.* (1979b), 3: SPARLING (1981a), 4: SPARLING (1981b), 5: SPARLING (1983), 6: ZELLES *et al.* (1987b), 7: ALEF *et al.* (1988), 8: ZELLES *et al.* (1990).

and external substrates of the organisms. But it has to be borne in mind that such conditions last for only short times (BÖLTER *et al.*, 1989) and, hence, extrapolations to longer time spans are not useful. This also holds true for the lichen samples. In most cases high temperature input is concomitant to a quick decrease in moisture to low levels that prevent metabolic activity (BÖLTER, 1992a).

Comparisons between heat production and calorimetry are possible under the assumption that the liberated energy per mol glucose during oxidation yields a constant amount of heat per consumed amount of O_2 or produced CO_2 and that

the respiration quotient RQ=1 (IVLEV, 1934; BELAICH, 1980). Direct heat production and the CO_2-evolution data were within comparable ranges. Reasons for inconsistencies can be attributed mainly to different experimental designs: The microcalorimetric experiments were run under static conditions, whereas respirometry was a dynamic approach with a steady flow of air which prevented accumulation of gaseous products that might negatively affect further microbial activity.

On the other hand, it has been shown that the measure of gas exchange tends to overestimate metabolic rates (DE JONG *et al.*, 1979; SAKAMOTO and YOSHIDA, 1988). The reason for this has been discussed with respect to out flow of air from the soil microniches which contain higher amounts of CO_2 than the flowing gas stream. Furthermore, the steady exchange of gas may lead to a change in the populations and may stimulate specific organisms which are less active under natural conditions.

The microcalorimetry and respirometry data refer to an active microbial community. Bacterial biomass (as estimated from epifluorescence microscopy) in the soils from Casey was found to be in the range of 5.0 to 99.2 μgCg^{-1} d.wt in the soil samples and between 1.3 and 86.7 μgCg^{-1} d.wt in the lichen samples of Casey (BÖLTER, 1992a). Bacterial biomass of soil samples from Arctowski ranged between 0.05 and 11.0 μgCg^{-1} d.wt, and that of lichen samples showed values between 0.69 and 15.94 μgCg^{-1} d.wt. Assuming a conversion factor from heat production to microbial biomass of 12 to 20 mWg^{-1} biomass C (SPARLING, 1983), the following ranges of biomass were estimated:

a) soils
   - –Casey: 0.05–10.95 mg biomass C g^{-1} d.wt
   - –Arctowski: 0.10–8.08 mg biomass C g^{-1} d.wt.

b) lichens
   - –Casey: 22.90–145.80 mg biomass C g^{-1} d.wt;
   - –Arctowski: 10.00–172.50 mg biomass C g^{-1} d.wt.

ANDERSON AND DOMSCH (1978) proposed a conversion factor from CO_2-output to microbial biomass (1 *ml* CO_2 h^{-1} =40 mg microbial biomass C) for substrate induced respiration (SIR). Thus the data gained from the experiments of this study may be lower. Different conversion factors to those from ANDERSON and DOMSCH (1978) for Antarctic ornthogenic soils have been established by ROSER *et al.* (1993b). However, using this above mentioned factor for the gas exchange data of the soil samples, the following biomass ranges were estimated:

Casey: 0.07–4.12 mg C g^{-1} d.wt.

Arctowski: 0–10.58 mg C g^{-1} d.wt.

These data were much higher than those obtained from direct analyses of the bacterial community with regard to total bacterial number and biomass by epifluorescence microscopy (*cf.* BÖLTER, 1992a; BÖLTER *et al.*, 1993). This may be due to the fact that only a small part of the bacterial population is removed from the sand grains or thalli. It also holds true for those soil samples which contain microlichens or mosses. These data confirmed the high amounts of ATP and enzymatic activity in the Casey samples. Hence, bacterial biomass covered only a

small part of the total (active) microbial biomass (BÖLTER, 1992a; ROSER, 1993a).

The wide ranges of heat output and CO_2-evolution point to a very diverse ecosystem. This can be attributed to the different types of microbial communities. Algae, cyanobacteria, microlichens and other organisms have considerable influence on the state of the ecosystem with regard to such overall estimates of metabolic parameters. Their contribution to the total microbial community has to be analyzed very carefully and to be taken into consideration separately when energetic balances are carried out.

The data showed that individual methods could not give an appropriate picture of these ecosystems since individual properties (LOI, bacterial biomass, heat output, respiration) seemed to be uncoupled, at least for the soils from Casey. STOTZKY (1972) stated that all methods for ecological research had their advantages and restrictions. There is no single method which can provide a characterization of all processes in complex environments. This leads to the conclusion that only a variety of different methods can reveal a realistic picture of the interrelationships in those ecosystems such as Antarctic soils.

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