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MICROBIAL ACTIVITY IN SOILS FROM ANTARCTICA (CASEY STATION, WILKES LAND)

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Abstract: During austral summer 1985/86 a comprehensive study on Antarctic soils was carried out at Casey Station, Wilkes Land. Samples of different locations were analyzed for inorganic nutrients and several organic constituents as well as for related microbiological properties. The data set of this presentation comprises data from 12 top layers of soils covered by different organic material: lichens, mosses and algae.

Microbial properties are described by total CO_2-evolution, ^{14}C-glucose uptake and thereof derived parameters such as turnover times of organic matter and their relationships to microbial biomass, estimated from ATP levels, and bacterial biomass and numbers, estimated by epifluorescence microscopy.

Results indicate high metabolic rates in these environments. Albeit ratios between gross metabolism (CO_2-evolution) and bacterial activity (^{14}C-turnover rates) show that a high contribution of the total metabolism must be due to other organisms than bacteria. This meets the results of fairly high amounts of ATP on the one hand and low bacterial biomass on the other hand.

1. Introduction

The description of Antarctic soils by chemical, biochemical and microbiological characteristics is rather scarce. Most authors focus on physical and geological descriptors, only few show interest in inorganic or organic nutrients and viable counts of bacteria or yeasts (*e.g.*, CAMERON *et al.*, 1970a, b; BAILEY and WYNN-WILLIAMS, 1982; VISHNIAC, 1984). Investigations on the terrestrial environment as ecosystems acting at the microbial level have been conducted mainly on Subantarctic Islands, such as Signy Island and Marion Island (*e.g.*, WYNN-WILLIAMS, 1985a, b; SMITH, 1987), whereas continental Antarctic has been widely excluded—except the region of the Ross Desert, which, however, represents a special kind of desert biology.

On the continent, however, we find the largest regions of ice free areas in the Antarctic, covered with various types of mosses, lichens and other cryptogams as producers in quite different assembladges and, hence, forming a great variety of ecological systems. The interactions within these systems as well as their trophic relationships are only poorly understood.

Although a great variety of methods for the analysis of production and remineralization processes at the microbial level have been established recently (*e.g.* measurements of enzymatic activities, *cf.*, HOPPE, 1983), determinations of different parts of microbial biomass or microbial activity (*e.g.*, MEYER-REIL, 1987), those methods are used only

seldom in terrestrial microbiology in Antarctic environments.

The present study is an attempt to combine those parameters of microbial activity with conservative ones such as concentrations of organic and inorganic nutrients. This data set is analyzed in a holistic view on the various interrelationships and in balancing some of the individual characteristics in order to establish the acting level of these ecosystems.

2. Meterials and Methods

Sampling was carried out during austral summer 1985/86 (Nov.–Jan.) at a hill near Casey Station, Wilkes Land, Antarctica. The study is part of a comprehensive study on the biology on lichens, mosses and soils at Kiel University which is carried out in continental and maritime Antarctic. Results of these investigations have been published in previous reports, *e.g.*, KAPPEN *et al.* (1986, 1987).

The soil samples were analyzed by a broad spectrum of characteristics (see Table 1) in order to get an idea of the processes and the standing stock of the microbial system. Further, many characters describing physical and chemical characteristics of the soil samples were analyzed. Most of these properties have been analyzed in the laboratory at Kiel University. For this purpose, the samples were deep frozen immediately after sampling and tansported deep frozen to Kiel. Only measurements on ATP, water content, and gas exchange were carried out in the field laboratory at Casey Station; these experiments were repeated in Kiel in order to estimate changes during the transport. With regard to the properties mentioned, changes were negligible and thus transport and storage were considered to be of no significant influence also for other parameters. This should be true at least for inter-sample variations and, hence, for purposes of balances for this set of samples, although changes in the samples themselves can never be excluded completely. However, studies on botanical samples did

Table 1. Brief account of the methods used during the soil microbial/chemical studies and references.

Descriptor	Procedure	Reference
Water content	Drying at 105°C	
Organic matter	Combustion at 550°C	
Inorganic nutrients/anions and cations in the soil solution	MERCK "Spectroquant" reagent kits	
Protein	SIGMA "Protein assay kit"	
Particulate organic carbon and nitrogen	HEREAUS CHN-Analyser	
Bactrial number and biomass, size classes	Epifluorescence microscopy	ZIMMERMANN *et al.*, 1978
ATP	Tris-buffer extraction	ERNST, 1970
Free amino acids	Ortho-phthalaldehyd reaction	DAWSON and LIEBEZEIT, 1983
Carbohydrates	Class reaction	DAWSON and LIEBEZEIT, 1983
^{14}C-Glucose uptake/respiration	Non-kinetic approach	HARRISON *et al.*, 1971, MEYER-REIL, 1978
Exoenzymatic activity	Methylumbelliferyl-substrates	HOPPE, 1983
Community respiration	Gas exchange chambers	KAPPEN *et al.*, 1986, 1987

not show any drastic effect caused by storage (L. KAPPEN, pers. commun.). NAKATSUBO and INO (1987) showed this also for studies on nitrogen fixation. Further analysis on the samples analyzed here also did not show evident changes in their microbiological descriptors.

Many of the procedures of analyzing nutrients and metabolic activities are taken from marine science and needed some changes for the use on soil samples. Since these procedures (*e.g.* measurements of organic and inorganic nutrients), however, were carried out in the soil solution, the procedures could follow close to the methods described in the literature (*cf.* Table 1).

The complete matrix for statistical and multivariate analysis comprises 12 sites (samples) and 67 descriptors. This matrix is analyzed by rank correlation and subsequently by cluster analysis. The algorithms are complete and average linkage. Details of this statistical and mathematical analysis can be taken from the program scheme of BÖLTER *et al.* (1980) and additional considerations by BÖLTER and MEYER (1986). All procedures were run on the PDP-10 (DEC-system) at the computer center of Kiel University.

3. Results

Statistical analysis: The statistical properties of the parameters show very high variability. Only a few of them, some size classes of bacteria, the ratio of bacterial biomass/bacterial number and C/N, show a coefficient of variability $<50\%$. This points to a strong heterogeneity of the individual samples, *i.e.* refers to a strong patchiness of the sampling area, even on a small scale: the total sampling area did not cover more than 500 m^2. The distance between some samples was less than 1 m.

The correlation analysis showed many interrelationships which, however, cannot be presented here in detail. The structuring of this matrix by cluser analysis gives some insight into the prevailing interrelationships.

As such one large group established by this procedure contains the following characters:

Water content, total organic material, ammonia, nitrate, protein, sum of inorganic N, particulate organic carbon (POC) and nitrogen (PON), C/N, ATP, polycarbohydrates, bacterial biomass/ATP, most exoenzymatic activities.

These parameters represent nearly a complete graph, *i.e.* each of these descriptors is connected with another.

A second group combines:

Biomasses of cocci $<0.5\,\mu$m and rods 1–1.5 μm, and numbers of cocci $<0.5\,\mu$m and rods 0.5–1 μm, 1–1.5 μm.

This group indicates concomitant variations of the mentioned bacterial size classes.

Further groups contain:

—Calcium content, disintegrated phosphate, nitrite, chloride, iron, polyphosphate.

—Bacterial biomass/number, biomasses and numbers of rods 1.5–2.5 μm, 2.5–4 μm.

—Free amino acids (FAA), monocarbohydrates (MCHO), FAA/MCHO, polycarbohydrates (PCHO)/MCHO.

Without going into detail into all these interrelationships, we should focus on some evident relations as examples for the illustration of this environment.

Bacterial biomass: The mean value of bacterial biomass as estimated by epifluorescence microscopy is about 0.305 μg C g^{-1} w.wt. (median: 0.269 μg C). The corresponding bacterial number from this method has a mean value of 31.16×10^6 g^{-1} w.wt. The size distribution of the bacterial population illustrates this figure (Table 2). This analysis shows that the bacterial biomass is dominated by small rods (0.5–1.5 μm: 66.16%; 0.5–2.5 μm: 83%). The number, however, is governed by the smallest fractions of rods and cocci (80.5%).

Organic nutrients: The amounts of particulate organic carbon (POC) and nitrogen (PON) were estimated by CHN-analysis and showed a mean value of 4.74% C, the C/N ratio is in mean 12.7. Labile compounds, as measured for free amino acids (FAA), monocarbohydrates (MCHO) and polycarbohydrates (PCHO) show values of 136 μg g^{-1}, 32.4 μg g^{-1}, and 272.2 μg g^{-1}, respectively, *i.e.* these constituents represent 0.3%, 0.07%, and 0.57%, respectively, of the total organic matter as estimated by combustion. These amounts, at least FAA and MCHO, however, can be regarded as "available material" to microorganisms because of the mild extraction procedures used here (extraction by cold water).

Metabolic activity: The data of the gas exchange experiments gave a mean figure of 6.35 mg CO_2 h^{-1} g^{-1} w.wt. at 6°C. This temperature serves as a level for the description of often prevailing environmental conditions. Hence, these data on overall metabolic activity may account for a general feature of actual processes. This would be a carbohydrate consumption of 4.17 mg h^{-1} g^{-1} w.wt., and encompasses 3.8% of the total organic matter (mean value: 110 mg g^{-1}), or 8.8% of the POC (mean value: 47.4 mg g^{-1}). The sum of the above given data of the available organic material is in the order of 0.44 mg g^{-1}, *i.e.* 0.93% of the total organic matter as estimated by CHN-analysis. This amount of material is available for heterotrophic organisms and their biomass production. It should, however, be noted that higher temperatures may increase the

Table 2. Size distribution of the bacterial population analyzed from Antarctic surface soils. The values are given in percent (%) of the total population. "Other" forms mentioned in this table are e.g. larger filamentous rods.

Size class (μm)	Biomass (%)	Number (%)
Cocci –0.5	9.25	47.54
Cocci 0.5–1	1.33	1.92
Rods 0.5–1	33.71	32.99
Rods 1–1.5	32.45	13.29
Rods 1.5–2.5	16.86	3.80
Rods 2.5–4	2.97	0.40
Other	3.43	0.06
	100.00	100.00

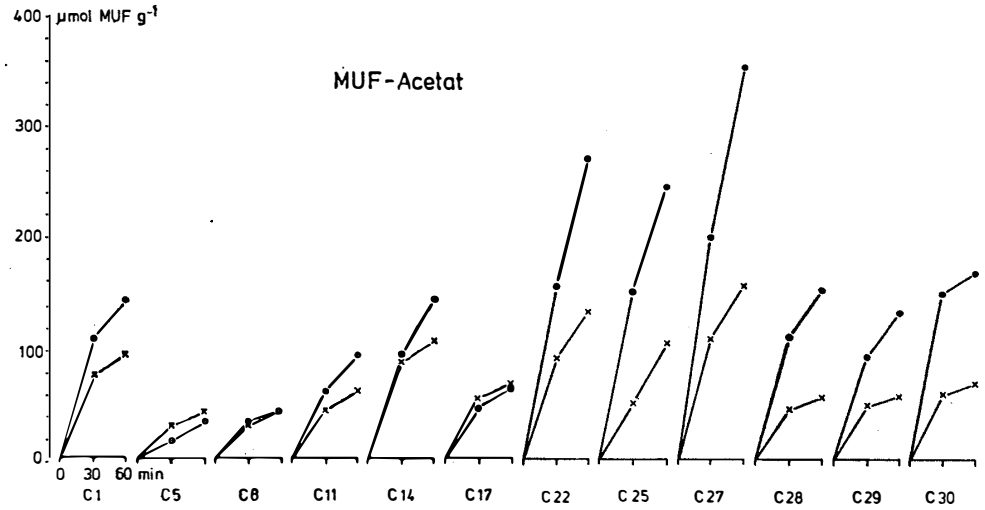

Fig. 1. Release of free MUF from MUF-acetate after incubation of soil samples after 30 and 60 min at 2°C (crosses) and 18°C (dots). The values are calculated from the calibration curve of free MUF (concentration of MUF vs. relative fluorescence).

metabolic rates drastically. For instance, sample 1 (a crust of dead moss with some on growing crustose lichens) did not reach a maximum of respiratory activity at 22°C. This points to the fact that temperature has to be considered as an important controlling factor for the metabolic processes at this level.

Concomitant analysis of the uptake and respiration of ^{14}C-labelled glucose, however, showed very short turnover times of this substrate in the range of 10–15 h or even less in samples with high concentrations of organic matter. Incubations were carried out at 5, 15 and 25°C. This indicates that the bacterial population (which can be considered to be an active part on the degradation of the labile compounds) has a high affinity to these organic compounds. Further, it is notable that the portion of respired glucose is generally more than 50% and may reach levels up to 90%. This points to the fact that the maintenance of the metabolism takes a lot of energy. High temperatures (15, 25°C) are generally favorable and produce higher figures of the mentioned metabolic rates. Highest values of uptake rates were recorded at 15°C, a further increase in temperature shows only slight effects on metabolic rates. These results are going along with those of the gas exchange experiments and support the results presented above.

Enzymatic activities: The activity measurements of exoenzymes acting on MUF-β-D-Glucoside, MUF-β-D-Galactoside, MUF-Acetate, MUF-Butyrate, and MUF-Phosphate (related hydrolases and phosphatase) showed also rather high figures. Incubations were performed at 2 and 18°C. The influence of the temperature could also be shown to be positive. The activity rates was highest in samples with high levels of organic material. Figure 1 illustrates the data gained from MUF-Acetate. Values of activity are recalculated from free MUF which served as a standard of the fluorescence.

4. Discussion

These preliminary results of a comprehensive study on terrestrial Antarctic ecosystems showed high overall rates in total microbial activity, *e.g.* CO_2-evolution,

exoenzymatic activity, and in total microbial biomass, *e.g.* ATP values.

As stated above, the total bacterial biomass figured out 0.305 μg C g^{-1} w.wt. This amount seems to be very low in comparison to other estimates of organic material in the samples. For instance, the total organic material (measured by combustion) is about 110.4 mg g^{-1} w.wt., the amount of particulate organic carbon (POC) is 47.4 mg g^{-1} w.wt. These two figures match very well if we assume that about 50% of the total organic matter can be attributed to POC (MEYER-REIL, 1987). The amount of ATP has been estimated at 18.3 μg g^{-1} w.wt. Assuming that 2% of the total organic matter in soils (SPARLING, 1985) may be regarded as total microbial biomass (*i.e.* bacteria + fungi + algae + microfauna), then 2.2 mg C should encompass this estimate. This figure is based on a conversion factor of 120 from ATP to microbial biomass (OADES and JENKINSON, 1979), JENKINSON *et al.* (1979) report a factor of 138. It has, however, to be borne in mind that these factors may vary largely with envionmental conditions and the population's state of growth (NANNIPIERI *et al.*, 1978). According to these calculations, however, the bacterial biomass (0.305 μg C) would then represent only 0.014% of the total microbial biomass. Obviously, these calculations are rather rough, albeit they indicate the order of magnitude in which we have to go forward and that we have to look more carefully other constituents of the microbial population in future. Microscopic inspection of fresh samples (R.I.L. SMITH, pers. commun.) and later analyses in the laboratory did not show significant abundances of nematodes or other animals. However, these samples should have a high contribution of fungi and algae as also indicated by their high respiratory activity which will be considered below.

The bacterial biomass contributes only to a small part of the total microbial standing stock, and figures of bacterial biomass found here seem to be lower than other estimates, *e.g.*, WYNN-WILLIAMS (1985c) finds 10^8 to 10^9 bacteria g^{-1} w.wt. in soils of Signy Island, although a direct comparison between these figures is difficult. The ratio between bacterial biomass and number, however, is fairly in the range of that described in the literature, although no data are available from Antarctic environments. For instance, MEYER-REIL (1987) finds in marine sediments from temperate environments values between 11×10^{-9} and 20×10^{-9} μg cell, my data show a figure of 9.8×10^{-9} μg $cell^{-1}$. The size class distribution fits into the general distribution pattern of sediment bacteria (MEYER-REIL, 1987), but it points to a larger part of the smallest size classes.

The measured figures of respiratory and exoenzymatic activity but also the high affinity to ^{14}C-labelled glucose point to a healthy and active microbial (bacterial) population. The possible stimulation of the metabolic rates by high temperatures, up to 30°C, indicates a mesophilic metabolism rather than an adaptation to a cold environment. This encompasses results of R.I.L. SMITH (1986) and own measurements which showed soil and rock temperatures at this location to be up to 40°C, although only for short times. More temperate conditions (10–20°C), however, can last for longer times (hours) in the soils. Hence, the microbial population can make use of these conditions very well and act rather effectively on the prevailing nutrient status. Although the data on bacterial activity presented here may be regarded as relatively high, the overall nutrient state (C : N ratio, concentations of monocarbohydrates and amino acids) refer to high metabolic rates. The C : N ratio (mean value: 12.7) indicates a situation of net mineralization (STEVENSON, 1986) and thus points to the so-called "active soils".

Results from the statistical analysis showed that the environment is rather patchy. Sampling sites which are located very close to each other (in the scale of some dm) showed very large differences in their nutrient content, microbial standing stock and metabolic activity. This is expressed by the high variability of the individual descriptors, as mentioned above. Thus, it becomes rather difficult to give significant mean values for this environment, *i.e.* accurate balances of nutrients and metabolic rates as well as extrapolations for larger areas.

This state of patchiness may relate to a very "young" system where soil-forming processes are in an early stage and that different processes are not so well linked as in other environments. This may also be due to the fact that the Antarctic soil system is lacking in organisms which might act on bioturbation, *i.e.* active transport of soil particles underneath the surface or within different layers. Hence, only cryoclastic processes or melt water and related diffusion can be regarded as mediators of nutrients, effects which are limited to short periods.

The resutls of this study have to be confirmed by more data of this investigation and further studies which have been carried out in other areas. Nevertheless, they have shown us some margins of the system and gave us some indications of the high metabolic activity, but we have to look carefully into the different parts of this system in order to increase our knowledge of this environment.

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