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ESTIMATIONS OF MICROBIAL BIOMASS BY DIRECT AND INDIRECT METHODS WITH SPECIAL RESPECT TO MONITORING PROGRAMS

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Abstract: This review focuses on microbial biomass determinations in Antarctic terrestrial ecosystems. Such estimates are strongly related to individual methodological approaches. Standardizations of methods are needed with respect to monitoring programs in order to obtain directly comparable data from different environments. This discussion is also relevant to the problem of whether data of actual or potential measures of microbial activity should be used for long term studies in remote ecosystems.

1. Introduction

It is well known that microbes play an important role in the cycling of organic matter. Microbes are of central importance for soil fertility, processes of decomposition of organic matter, remineralization and immobilization of inorganic matter, and decomposition of xenobiotic substances. Investigations of this part of the food web are primary steps in analysis of microbial ecosystems. With regard to terrestrial Antarctic ecosystems, microbes (bacteria, fungi, cyanobacteria, algae) are of special interest because of the lack of other decomposing organisms, mainly meiofaunal components. The original food web of the soil ecosystem in Antarctic environments is shortened to direct interactions between producers and microbial decomposers; microbes represent the main link between heterotrophic and autotrophic processes (Fig. 1).

Microbial biomass in soils is defined as part of the organic matter. Microbial biomass is the living part of this material, *i.e.* the mass of living cells and organic matter derived therefrom. It should fullfill some basic assumptions which have been proposed by JENKINSON and LADO (1981):

—"Microbial biomass" is restricted to living cells and can be recycled after their death,

—"Microbial biomass" should be easy to extract from living cells and its concentration in individual cells should be constant,

—Reliable determinations of "microbial biomass" should be available.

Methods for determination of microbial biomass are well known and described extensively in various textbooks (*e.g.* PAUL and CLARK, 1989; ALEF, 1991). However, the large variety of methods has also produced a large variety of possible results. This

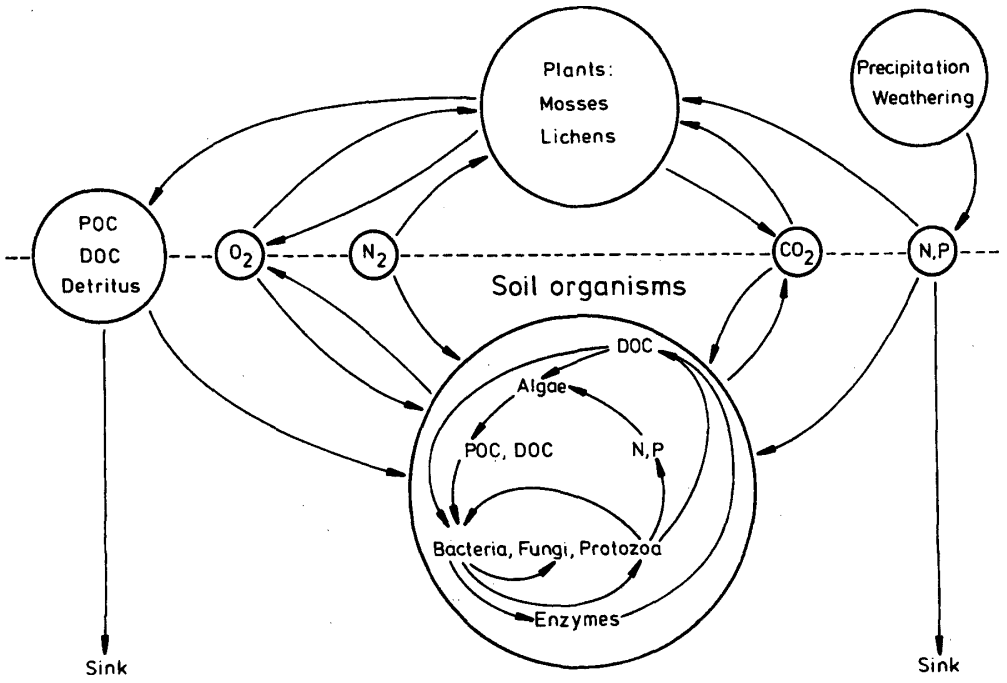

Fig. 1. Outline of interactions in terrestrial Antarctic ecosystems.

leads to different descriptive levels of the ecosystems in terms of biomass, and, further, to various types of calculations, recalculations and transformations of individual data sets.

This last point leads to controversy about methods for determining microbial biomass and prevents direct comparisons between different data sets. It is also a crucial point in setting long term studies such as monitoring programs, especially when different methods are used by individual researchers. Thus, this point will be considered in more detail, keeping in mind the main background of this symposium.

Considering monitoring of microbial biomass and activity we have to be aware of the following constraints:

—the time scales of microbiological processes,
—local patchiness,
—methods of determination,
—mathematical and statistical handling of appropriate data sets.

2. Subunits of Microbial Biomass and Microbial Communities

As mentioned above, microbial biomass can be regarded as part of the organic matter in soil ecosystems. The great diversity of microorganisms and their different roles and niches in the ecosystem have produced different empirical subunits with regard to functional levels. This is also due to the fact that microbiologists are working in different parts of the microbial ecosystem. On the one hand, investigations of the species or community level are carried out, *i.e.* distinct organisms (*e.g.* nitrifiers, aerobes, fungi, etc.). On the other hand, reactions at the community level are investigated (*e.g.* total heat production, CO_2-evolution, enzymatic reactions etc.).

We have to distinguish between measurements of numbers and biomass of microorganisms and their activities. This means that different techniques of biomass estimations (direct or indirect) apply to inidividual properties of the microbial ecosystem: different organisms, different physiological states of the total population or of parts of it. Thus, comparisons between those results need to consider carefully the trophic structure of the ecosystem with regard to the role of its individual components. Any different trophic level needs its special sampling device and sampling strategy.

3. Methodological Considerations

Here I discuss the different methods, their interpretations and the comparisons of individual results. Methodological considerations are especially important when comparisons are carried out between earlier studies and more recent studies in the Antarctic environments.

3.1. Microbial (bacterial) number and biomass: Colony forming units and microscopic approaches

Early studies carried out in the Antarctic deal mainly with colony forming bacteria (cfu), yeasts or fungi on different media (*e.g.* SIEBURTH, 1965; CAMERON *et al.*, 1970; CAMERON, 1972). Those studies have been extended partially with modified methods (*e.g.* HIRSCH *et al.*, 1988).

The use of microscopic counting techniques has given quite different data sets with regard to bacterial numbers and thereof calculated microbial biomass (*e.g.* RAMSAY, 1983; WYNN-WILLIAMS, 1985; BÖLTER, 1989, 1991, 1992; ROSER *et al.*, 1993a) depending on the individual methodological approach. Studies which use image analysis provide more detailed information about changing size structures of the bacterial community (WYNN-WILLIAMS, 1988; BÖLTER *et al.*, 1993).

3.2. Microbial and bacterial biomass: Indirect methods

Concomitant to studies on bacterial counting and thereof derived bacterial biomass, indirect methods have shown many applications in soil studies. As such, ATP has been regarded generally as a marker for soil microbial biomass (*cf.* ANDERSON and DOMSCH, 1978; PAUL and CLARK, 1989; ALEF, 1991)—also in Antarctic ecosystems (FRIEDMANN *et al.*, 1980; VESTAL, 1988; BÖLTER, 1989, 1990a; ROSER *et al.*, 1993a, b). More recently, lipid phosphate has been used as an equivalent for bacterial biomass (VESTAL, 1988; BÖLTER, unpubl.).

3.3. Microbial biomass: Ecophysiological methods

The introduction of ecophysiological methods into microbiological investigations of Antarctic ecosystems has yielded a great quantity of data from modern techniques, but also a great diversity of results. Their interpretation depends strongly on methodological effects. For example, data are available on total respiration of microbiological communties, *i.e.* gross community respiration (WYNN-WILLIAMS, 1984; BÖLTER, 1989, 1991; ROSER *et al.*, 1993a, b), activity with regard to specific

substrates, mainly glucose (RAMSAY and STANNARD, 1986; BÖLTER, 1990b), or determinations of the activity of specific enzymes (BÖLTER, 1989; 1992; ROSER *et al.*, 1993a, b). Results of these methods are used for conversions of metabolic rates into terms of microbial biomass (ROSER *et al.*, 1993b; BÖLTER, 1994).

4. Comparison of Methods-Conversion Factors

The use of conversion factors is common and very critical in ecological studies. It is well known that cell constituents and parameters of activities vary with environmental factors or physiological conditions, *e.g.* temperature, moisture, nutrient availability and others. The lack of direct relationships between colony forming units and microscopic countings of bacteria is generally well accepted. Nutrients and incubation techniques are regarded as reasons for those discrepancies. So, why should we find definite (linear) relations between any other (arbitrary) parameter of microbial number, biomass and activity? Normally, the basic assumptions for such relationships (linearity and homogeneity) cannot be verified in natural ecosystems. Nevertheless, such relationships have been documented between microbial biomass and ATP, phospholipids, chlorophyll, lipopolysaccharides, muramic acid, ergosterol and teichoic acid (*e.g.* FRY, 1988; ALEF, 1991). Some of these relationships show wide variability within the conversion factors (Table 1).

This broad variability of conversion factors is related to two facts:
a) The variabilty in the original data, *i.e.* the data from which those conversion factors are taken show wide ranges;
b) The procedures by which the data are produced, *e.g.* the different methods for extractions of ATP from cells or the laboratory conditions under which measurements of microbial activity were performed.

Table 1. Conversion factors from different units of microbial standing stock or activity to microbial biomass carbon in terrestial ecological studies.

From	Factor	Reference
ATP	250 µg C/µg ATP	*VESTAL (1988)
	40–1000 µg C/µg ATP	PAUL and CLARK (1989)
	100–200 µg C/µg ATP	*ROSER *et al.* (in press)
Direct count	130–650 fg C/µm	FRY (1988)
SIR	25 µl CO2 h^{-1}/mg C	ANDERSON and DOMSCH (1978)
		*ROSER *et al.* (1993b)
FDA-hydrolysis	12 abs. units ml^{-1} h^{-1}/205 µgC	*ROSER *et al.* (1993b)
Lipid-PO_4	1 µmol Phospholipid/480 µgC	*VESTAL (1988)
	1 nmol PO_4-P/20 µg Biomass	*BÖLTER (unpubl.)

*Studies performed in the Antractic.

5. Sources of Error

Without good knowledge about the methods of individual investigations, *i.e.* the constraints on the basis of which individual relationships were established, it is impossible to compare the results directly, weighing them or even calculating any trend from such data sets. An excellent example of such sources of error has been presented recently during an intercalibration of the bacterial direct count method by NAGATA *et al.* (1989), who found variations of the bacterial number in marine water samples of orders of magnitude when analyzed in different laboratories. Other examples of such systematic errors produced by individual investigators may be found in the literature for different environments (*e.g.* DOMSCH *et al.*, 1979; INGHAM *et al.*, 1991). Besides those methodological comparisons we also have to bear in mind:
—strong patchiness of the ecosystem itself, especially of Antarctic soils,
—natural (but hidden) time scales of the ecological processes, which produce random errors (see Fig. 2).

Systematic errors can be solved, random errors might be used for descriptions —if there is a sufficient data base and statistical methods have shown its original nature. But decisions between these classes of errors are not easy. The recognition of such problems has led to many articles about parameter estimation and modeling problems (*e.g.* LOELHE, 1987).

Ecological investigations of Antarctic terrestrial ecosystems are best suited for studies at low levels of nutrient concentrations and concomitant microbial activity. This is due to low levels of energy input and plant production (SMITH, 1985). Many ecophysiological studies result in extreme low levels of activity or even in naughts of individual properties of the microbial ecosystem. However, high activities can be monitored at places with high amounts of organic matter and proper environmental conditions (*e.g.* BÖLTER, 1989, 1992). This fact demonstrates problems of combining microbial data sets from different Antarctic sites without detailed knowledge about their environmental conditions and which give individual fingerprints to all other data.

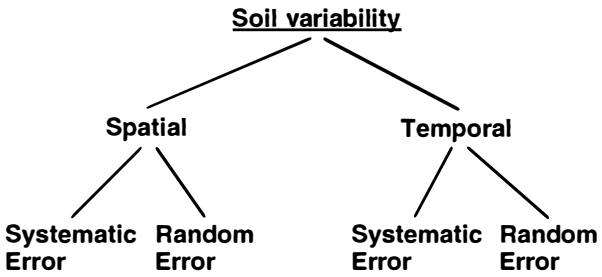

Fig. 2. Outline of errors during ecological studies.

6. Consequences for a Monitoring Program

The above-mentioned topics should have consequences in constructing monitoring programs of microbial properties of terrestrial Antarctic ecosystems. When (If)

setting up such a program at the level of the microbial food chain, three main questions should be answered:
—What shall be analyzed?
—When shall it be analyzed?
—Where shall it be analyzed?

6.1. What shall be analyzed?

Due to the many ecological methods (Fig. 3) which may show us parts of the different secenarios of the "ecological theatre" (HUTCHINSON, 1965), some of them have to be selected. As monitoring programs should last for at least a decade, emphasis should be placed on the most robust individual methods rather than the most recent methodological advances, because monitoring has to look into two directions, past and future. The incorporation of old data should not be neglected in order to set up baselines and trends of ecological studies.

There is nearly no microbiological method which has been used in an Antarctic environment for more than two consecutive seasons. Some basic data are available about some colony counts, some descriptions of isolated bacterial (or fungal) strains and their physiological properties. More recently, studies about communities have been conducted on ecophysiological methods.

So, it is today's dilemma to select adequate parameters of the microbial community for monitoring purposes. Such selection should be made by including the following ideas:
—Robustness of parameters, *i.e.* parameters will be accepted for a long time;

Microbiological parameter		
	may describe	by method
Standing stock of biomass	indirect	e.g. ATP, lipid-PO_4
	direct	e.g. cfu, microscopy
Activity	potential	e.g. SIR, BOD, V_{max}, enzymes
	actual	e.g. actual uptake of substrates (e.g. glucose, leucine)
	specific	e.g. enzymes (MUF-substrates), carbohydrate degradation
	overall	e.g. FDA-hydrolysis, CO_2-evolution, heat production

Fig. 3. Outline of different microbiologcial parameters.

—Reproducibility of methods, *i.e.* different laboratories should come out with comparable results;
—Feasibility of methods, *i.e.* methods can be performed at different places (stations), and incorporated into new programs;
—Acceptance of parameters and methods, *i.e.* many nations (or researchers) should agree in such a program that it can provide data not only from one place of a large continent.

Further, monitoring programs have to be coordinated with other projects which have been established on a global base, *e.g.* the global change programs.

6.2. *When (how often) shall analysis be performed?*

The above mentioned temporal variability sets another frame to a monitoring program. Although seasonal effects are described for Antarctic habitats, short term fluctuation with regard to scales of days or even hours, *e.g.* during short warmings of surface layers, seems to be of great importance. This includes effects of repeated freeze-thaw cycles and strong diurnal gradients as shown for rock faces or soil beds (*e.g.* WALTON, 1982; SMITH, 1986; BÖLTER *et al.*, 1989; BÖLTER, 1992). Concomitant to temperature gradients, shifts of moisture can be observed.

It seems obvious that such strong short term environmental impacts override seasonal signals, a fact which can be found in observations of microbial activity (BÖLTER, 1992) as well as in microbial populations' structures (DAVEY, 1991; LING, personal comm.).

Since sampling, however, is often performed more under expeditionary than under controlled experimental conditions, an incidental mixture of microbial properties will be the result–when considering parameters describing actual activities or actual standing stocks.

Having this in mind, it is worth considering descriptors of potential activity which might be more relevant as indicators of long term changes. The signal of a potential activity might be "preserved" in an environment for a longer time than that of an actual event which cannot be more than a snapshot (see Fig. 4). Nevertheless, short term variability can show features of the flexibility of metabolic processes. However, studies based on potential activities may resume more shifts on a long time basis than those of measures of actual activities, *e.g.* processes of adaptations to higher temperatures, different nutrition, new species or genes and others.

6.3. *Where should sampling be carried out?*

Monitoring programs should be carried out at well defined places. Different strategies for the search for research sites have been described in detail by SMITH (1992). Keeping in mind the strong spatial variability of Antarctic soils and that –*sensu stricto*–no place can be sampled twice, we have to look for areas of similar environmental patterns. Such places can be found, but they have to be checked carefully for effects of human activity. Monitoring programs also should take into account the basic different habitats of Antarctic ecosystems, such as those large areas of desert pavements, those of lichen heaths in continental Antarctica or *Deschampsia antarctica* meadows and moss beds in the maritime Antarctic.

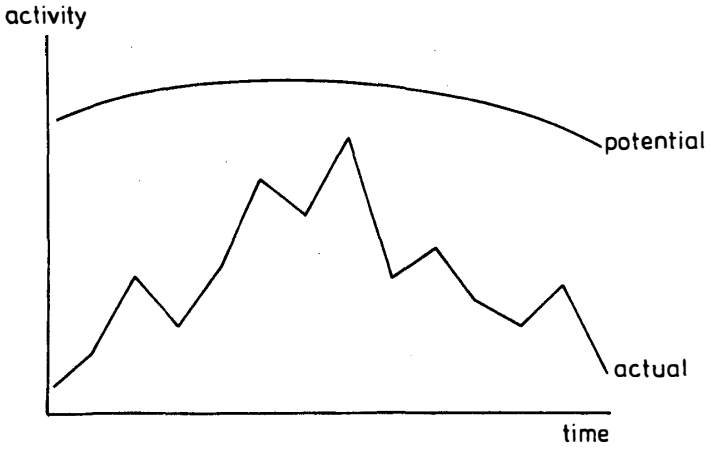

Fig. 4. Sketch for the differentiation between actual and potential activity in an ecosystem.

Those places should be visited at regular intervals and they should be accessable without strong logistic support. In a recent paper by BOCKHEIM *et al.* (1993) a transect is proposed along the line from South America to Australia, basically with respect to different climatological, pedological and biological zonations for monitoring sites: 1) Tierra del Fuego (subantarctic forest), 2) Macquarie Island (subantarctic low tundra), 3) Signy Island (subantarctic high tundra), 4) King George Island (Antarctic subpolar desert), 5) Wilkes Land (Antarctic polar desert), 6) Taylor Valley (subxerous cold desert), 7) Lake Vanda (xerous cold desert), 8) Beardmore Glacier (ultraxerous cold desert). It would be worth including SSSI sites because we have already general knowledge about their soil cover and other important descriptions.

Those places should be sampled also for other than microbiological properties, mainly pollution, in order to correlate changes in the biology with other factors. Further, such data should be used as baselines for comparisons with comparable studies which will be performed in other areas, *e.g.* the Arctic or temperate environments.

7. Consequences of Monitoring Programs

Such a program needs strong cooperation not only at the level of institutions (or countries) but also of their participants. It cannot be a place to ride one's own horse, especially with regard to the places, times, and parameters.

A broad agreement should be reached so that everyone will be speaking the same language when interpretating the data. This holds especially true for ecological data, which are often regarded as "hard facts" (especially when mathematics or statistics is involved) but should be regarded as soft data. FAGERSTRÖM (1987) stated that "there are no 'hard data' in ecology, it is only our hard skulls that shelter the belief in such data". We have to consider carefully that most microbiological data should be regarded in the first respect as being more qualitatively than quantitatively, *i.e.* such a data base is primarily descriptive. A good description, however, may be worth more than a bad analysis. And we should be aware that the analysis of such qualitative data

is as difficult as a quantitative approach (FRYER, 1987). Such analysis also would need the introduction of new mathematical tools, such as fuzzy logic or expert systems.

This means that we have to be careful in interpretating such data, especially as long as we have no hard criteria to judge levels or trends. Monitoring cannot be more than a registration of individual parameters.

8. Conclusion

It is obvious that microbiologists should cooperate closely with other disciplines. Much more information is needed about the various constituents and the availability of organic matter—basic parameters for calculations of microbial activity. Much effort has to be put into sampling strategies, *i.e.* sampling with minimum stress on the organisms and the ecosystem, in order to avoid artifacts of basic methodologies.

Setting up a monitoring program for microbiological properties seems to be a great challenge. It needs much more effort than *e.g.* registration of climatological parameters. We approach the "ecological theatre" without removing the curtain and with only little knowlegde about the acting artists. But as we have some tools available to observe standing stock and activity, we should start as soon as possible, before we come to the decision that it is too late to see the basic features of fairly undisturbed ecosystems.

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