Soil microbial biomass: The eco-physiological approach

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Abstract

In the 1980s ecosystem research projects were implemented world-wide since there was a pressing need to quantify the impacts of anthropogenic pollutants. Soil ecosystem analyses concentrated first on the quantification of the element and energy transfer between pools. Since mineralization of organic substrates and the release of nutrients and elements are due to the heterotrophic activity of the microbial decomposer compartment, this subsystem of terrestrial ecosystems gained importance. Direct microscopic observation methods were inadequate for the quantification of environmental impacts on the microflora. We adopted the maintenance requirement concept for the quantification of environmental impacts or stress effects on the soil microbial community. The paper gives a brief insight into the concept of maintenance from autecological studies and describes the underlying points which lead to our experimental approach of its application at the synecological level (i.e., microbial biomass as a single ecological entity) — a process which rested on long-term continuous research.

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1. Introduction

In the 1980s ecosystem research projects were implemented world-wide. Global pollution, which had led to forest decline, dying lakes and thinning of the ozone layer was a pressing environmental issue. These concerns continue to this day alongside those prompted by elevated CO₂ concentrations in the atmosphere and global warming. Thirty years ago there was a lack of empirical knowledge about how soil ecosystems function (Jarvis, 1987) yet a pressing need to quantify the impacts of anthropogenic pollutants. The realisation of the scale of our ignorance led to a great increase in funding and initiated a boom in ecosystem research. A number of these studies were follow-ups of projects of the International Biological Program (IBP) of the sixties and seventies, which in many ways, represented the first steps to ecosystem analysis. One important branch of the IBP research in Europe was the Solling Project in Lower Saxony (Germany). This was based at the Georg-August-University of Göttingen (Ellenberg, 1986) with follow-up projects such as “Ecosystems on Limestone Rocks” (SFB 135, 1981–1982), “Conditions of Stability of Forest Ecosystems” (1989–1993) and “Dynamics of Change of Forest Ecosystems” (1994–1998), all implemented at the Forest Ecosystem Research Center of the University of Göttingen and funded by the former Federal Ministry of Research and Technology (BMFT). The three much cited SBB papers which form the basis of this article (Anderson and Domsch, 1989, 1990, 1993) arose from our participation in the various follow-up projects. Of course the articles were based on a number of antecedent papers. Some of these are also discussed below, and were products of long-term, continuous research: research which was seen as a great challenge since the study of microbial eco-physiology in situ was in its infancy.

Almost thirty years ago ecosystem analyses concentrated on input/output analysis of fluxes. It was necessary to quantify the element (e.g., C, N, P) and energy transfer between pools and to us this seemed to be the most promising approach to understanding the function of an ecosystem. At first, the major emphasis was element dynamics and since mineralization of organic substrates and the release of nutrients and elements are due to the heterotrophic activity of the microbial decomposer compartment, this subsystem of terrestrial ecosystems gained importance. For the soil biologist it became obvious that direct microscopic observation methods, even after staining and plating techniques, were inadequate for the quantification of the total soil microflora as the factors used to convert microbial biovolume to microbial biomass or biomass-C rested on unproven assumptions (Jenkinson and Ladd, 1981). It was, therefore, a fortunate circumstance that the Rothamsted chloroform-fumigation incubation technique (CFIM) for the quantification of the total microbial biomass (bacteria plus fungi)
was published at this time (Jenkinson and Powson, 1976). The early comprehensive ecosystem models which were generated, i.e., the carbon and nitrogen cycle (e.g., Jenkinson and Rayner, 1977; Van Veen and Paul, 1981; Parton et al., 1987), took advantage of this technique. [N.B. A description of the land mark CFIM paper appears in Citation Classic I (Jenkinson et al., 2004)]. In addition, it was ground-breaking and offered many new approaches to studies of soil microbial ecology. Our colleague, John Anderson, who at that time was screening a great number of soils to determine the fungal to bacterial respiratory response (Anderson and Domsch, 1975) came across Jenkinson and Powson’s publication whilst doing a literature search. The excitement was great and discussions followed if the CFIM method could be used in combination with the induced respiration technique (SIR), a method which relies on calibration with the fumigation technique. It is the method which we used further on in our laboratory.

The relationship between dead primary products (litter), soil organic matter (Corg), and soil microbial biomass (Cmic) became an important issue in soil microbial ecology with respect to soil ecosystem analyses in the eighties. The key question was how do these carbon sources affect microbial productivity? Physiological terms used in ‘autecological’ studies (i.e., eco-physiological studies at the species level) such as the maintenance carbon requirements, growth rate, yield or turnover time were adopted and discussed with respect to the soil microbial community (e.g., Babiuk and Paul, 1970; Gray and Williams, 1971; Parkinson et al., 1978; McGill et al., 1981). In particular we were attracted to the concept of ‘maintenance’ which was originally tested with axenic cultures (McGrew and Mallette, 1962; Marr et al., 1963; Pirt, 1965). The adoption of the maintenance requirement concept and the study of its application at the synecological level (i.e., microbial biomass as a single ecological entity) was the basis for our three SBB papers.

2. Maintenance energy demand and soil microbial biomass

The close relationship between ecology and microbial physiology is encapsulated in the term eco-physiology. In the early days (during the 1950s and 1960s) the study of ecological parameters (e.g., temperature, pH, oxygen supply, nutrients) and their influence on microbial physiology were conducted under in vitro conditions using either batch culture or continuous culture techniques and concentrating at the response of one species at a time. To simulate more natural milieu and substrate competition between microorganisms, the pure culture experiments were followed by mixed culture studies. The real pioneers here were Hans Veldkamp and Holger Jannasch (Veldkamp and Jannasch, 1972; Jannasch and Mateles, 1974; Veldkamp, 1976). Although it is nowadays taken for granted that properties of microorganisms measured under in vitro conditions do have relevance to events in the natural environment, when we embarked upon our ecological studies, it was a highly controversial issue in the literature. The informative reviews by Gray and Williams (1971), Tempest (1978), Tempest and Neijssel (1978), Lynch and Poole (1979) and Tempest et al. (1983) were key publications back then and helped us to become acquainted with this subject matter.

When the chemostat culture technique was applied to understand the relationship between growth and utilization of a carbon source in bacteria, the term “yield”, Y, was introduced (Herbert, 1958): $Y = -\frac{dx}{ds}$ where dx is the increase in biomass and ds is the amount of carbon substrate used. Herbert (1958) showed experimentally that not all the carbon supplied was used for growth because a portion of it was used for maintaining the cell integrity and viability of the existing population. He called this “endogenous metabolism” since even at zero growth rate a carbon substrate demand remained; endogenous metabolism was later termed “the specific maintenance rate” $\alpha$ (Marr et al., 1963). Pirt (1965) building on the work of Marr et al. (1963) and also Schulze and Lipe (1964) introduced the terms maintenance energy demand or “maintenance coefficient” (m, g substrate g$^{-1}$ biomass h$^{-1}$). The portion of a maintenance energy demand is expressed as substrate (s) consumed per unit cell mass per unit time: thus, $\left(\frac{ds}{dt}\right)_{M} = -n\alpha$. Pirt (1982) refined this maintenance concept of microorganisms further by introducing a constant maintenance energy requirement independent of growth, the energy needed for maintaining cell integrity (synonymous with the endogenous metabolism of Herbert, 1958) and a growth rate dependent maintenance requirement which could be the production of energy storage products. Since the concept of maintenance demand was to be transferred to soil organisms growing under extremely carbon-limited conditions (Lynch, 1979) and with very long generation times (Nedwell and Gray, 1987) it became obvious that endogenous respiration would be the most important portion of the total maintenance or “transfer” term. For practical purposes the term m in our studies included the amount of additional carbon needed for energy purposes and which are either catalysed immediately and/or are laid down as reserve material for energy utilization at a later stage. The maintenance energy debate continues and a recent publication by Van Bodegom (2007) appeared where the former concept of maintenance is questioned, particular with respect to the definition of maintenance and generated equations. It is beyond the scope of this paper to take this debate since its application to soil microbial biomass will necessitate some thought and interpretation. Former extrapolated specific maintenance rates for soil microbes were in the range 0.003–0.04 g substrate g$^{-1}$ biomass h$^{-1}$ (Babiuk and Paul, 1970; Shields et al., 1973; Behera and Wagner, 1974; Barber and Lynch, 1977; Paul and Voroney, 1980). However, these values frequently exceeded the carbon input data to a soil calculated on a yearly basis and it became clear that the total maintenance demand of growing cells without carbon limitations must be different from organisms in a carbon-limited natural environment.

For the quantification of carbon cycle interrelationships via microbial biomass and determinations of microbial turnover times in terrestrial systems, the maintenance carbon requirement of the total microbial biomass would have to be subtracted from an estimated total carbon pool. Since maintenance coefficients had not been established under in situ conditions we accepted the challenge and this was for us the first step towards an experimental soil microbial ecology.

3. Testing the applicability of eco-physiological parameters at the synecological level

As mentioned above knowledge about the physiological responses of organisms to environmental factors has been gained from autecological studies. We followed the assumptions made by Tempest et al. (1984), that principles and mechanisms of growth or survival under axenic conditions have relevance to cells of a total multi-species community when considered as a single ecological entity. However, the specific rate constants of cell activities (e.g. growth or maintenance) may not be transferable. When we began our studies on physiological parameters, the term “activity” was widely used in soil microbiology. For instance, the respiratory “activity” was at that time related to a weight or volume of soil and not to the actual quantity of microbial cells under study. In strict
terms, only the determinations of constants (performance per unit biomass and time under similar temperature and water conditions) would allow the direct comparison of soil microbial activities of different soils. The work described in our paper “Maintenance carbon requirements of actively-metabolizing microbial populations under in situ conditions” (Anderson and Domsch, 1985a) was the first attempt to relate the CO2 output of the microbial community to the existing microbial biomass. We adopted Pirt’s (1975) term metabolic quotient, q, for the CO2 output per unit biomass, (qCO2) and here we describe the experiments since the knowledge gained was the basis for much of our subsequent research. Maintenance and eco-physiological quotients such as growth rate (µ), yield (Y), turnover time (d) (Anderson and Domsch, 1986) and carbon uptake kinetics (Vmax, Km) (Anderson and Gray, 1990) all had a big influence on the work which appeared in the three papers.

The application of the substrate-induced respiration technique for total microbial biomass determination with the use of the “Ultragas 3 CO2-Analyzer (Anderson, 1982)” allowed us to record the hourly CO2 production rates from the soil samples which had been initially spiked with glucose. We learned, when testing different soils which had different levels of microbial biomass and by applying increasing amounts of glucose, that CO2 output kinetics were similar throughout the course of incubation: samples with an excess of glucose showed an increase in the rate of CO2 production (due to cell proliferation) after the initial response maxima, while samples with an insufficient glucose supply remained below this initial response maxima. However, one particular glucose supply induced a special type of CO2 response curve, where the CO2 efflux rate it became evident that values for qCO2 as could be shown previously for the optimal growth rate equalled zero. The experimentally determined maintenance coefficients of the actively metabolizing biomasses of three soils ranged from 0.012 h⁻¹ for two agricultural soils to 0.03 h⁻¹ for one forest soil and were in agreement with maintenance values from in vitro single cell systems (background literature is given in Anderson and Domsch, 1985a) or extrapolated m values as cited above. Since the characteristic state of the major part of a total microbial community is dormancy and not anabolic activity, the next step for us was the determination of the m value of the dormant community. We adopted the concept proposed by Sinclair and Topiwala (1970) where maintenance “represented” a constant death rate of a culture. Viewed in this way, maintenance in an eco-physiological sense could be reduced to the question: how much carbon is necessary to maintain a constant level of microbial biomass? We experimentally determined this in order to calculate m. Further, as stated above, the majority of the microbial community is dormant and as an approximation, the basal respiration (the CO2-C respired from the basal CO2 efflux rate it became evident that values for m (obtained from the carbon rations supplied) where not equal to values for qCO2 as could be shown previously for the optimal carbon supplied and active microbial biomasses. Instead the carbon released by CO2 (qCO2) was higher than the carbon needed to maintain the biomass (m) (Anderson and Domsch, 1985b). The conclusion drawn at that time was that in mainly dormant populations a large part of the “true m” must be met by endogenously derived carbon sources. However, m values were up to two orders of magnitudes and qCO2 values one order of magnitude below anabolically activated soil microbial communities; this verified a physiological difference between anabolic active and dormant communities with respect to their maintenance carbon demand. What emerged, in addition, was that both metabolic quotients responded with increases when the temperature was increased. This indicated a higher energy demand. It was the beginning of our systematic study of soil microbial communities and their response to suboptimal conditions or environmental stress — such as soil

![Figure 1](attachment:image.png)

**Fig. 1.** (a) Example of a CO2 response curve of an arable soil (Chernozem) after amendment with increasing concentrations of glucose. (b) Response of the microbial population of the same soil to additional glucose-C amendments added after decrease of the stable CO2-C response rate. Arrows indicate time of additional glucose application. Figures are adapted from Figs. 1 and 4, Anderson and Domsch (1985a).

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>I*</th>
<th>II</th>
<th>III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass-C (µg.g⁻¹ soil)</td>
<td>147 ± 6.1b</td>
<td>996 ± 121</td>
<td>1982 ± 32</td>
</tr>
<tr>
<td>Mean CO2-C rate (µg CO2-g⁻¹ soil h⁻¹)</td>
<td>1.83 ± 0.004</td>
<td>12.7 ± 0.4</td>
<td>25.2 ± 0.12</td>
</tr>
<tr>
<td>Mean qCO2 (µg CO2-C g⁻¹ Cmic h⁻¹)</td>
<td>0.0124</td>
<td>0.0127</td>
<td>0.0127</td>
</tr>
<tr>
<td>Glucose-C maintenance ration (µg CO2-C g⁻¹ soil h⁻¹)</td>
<td>1.82</td>
<td>12.7</td>
<td>61.8</td>
</tr>
<tr>
<td>Mean CO2-C rate (µg C-glucose g⁻¹ soil h⁻¹)</td>
<td>1.85 ± 0.007</td>
<td>12.9 ± 0.10</td>
<td>25.0 ± 0.13</td>
</tr>
<tr>
<td>Maintenance coefficient (m) (µg C-glucose g⁻¹ Cmic h⁻¹)</td>
<td>0.0124</td>
<td>0.0127</td>
<td>0.031</td>
</tr>
</tbody>
</table>

*Soils I, II — agricultural soils (Cambisol and Chernozem); soil III — forest soil (Rendzina).

b Standard deviation (SD); n = 15.

* Mean CO2-C rates after application of maintenance rations (as shown in Fig. 1b).
acidification. To quantify stress, we used the q\textsubscript{CO2} of basal respiration as an approximation of the true maintenance demand.

4. Determining soil microbial community differences using eco-physiological parameters

The basis of two of our papers (Anderson and Domsch, 1989, 1990) rested on Odum’s (1969) theory on bioenergetics of ecosystem development. This says that the development of diversity (plants, animals and microbes) in ecosystems coincides with an increase of an efficient use of energy during the progress from a developmental stage to maturity and quasi-equilibrium and at this point there is a low community respiration per unit biomass. When equilibrium is attained, input to any particular C compartment is equal to output, and there is no further accumulation or reduction of organic matter in that compartment. Extrapolated to soils and the microbial biomass compartment, this would mean that at maturity there should be a low microbial community respiration per unit microbial biomass (q\textsubscript{CO2}) and a high microbial biomass supported per unit energy source (C-source), which would be reflected in a high ratio of biomass-C-to-total organic C (the C\textsubscript{mic}-C\textsubscript{org} ratio). Since Odum’s theory also encompasses the diversity of substrate litter inputs from the primary producer to the soil system, we assumed that agricultural long-term permanent monoculture soils (M) could be considered as less diverse in litter input in comparison to long-term continuous crop rotation plots (CR). After analysis of over 100 plots with a long-term management history (at least 15 years to approximate a quasi-equilibrium state) from 26 sites at different locations in Europe, we were able to show that these two management systems differed: mean % C\textsubscript{mic} in C\textsubscript{org} for M soils was to 2.3 and for CR soil 2.9: this coincided with almost twice the value for q\textsubscript{CO2} for M plots as compared to CR plots. Since these differences were not related to soil texture or pH, the assumption made was that the different management practices (monoculture as opposed to crop rotation), must have determined the different energy demands of the microbial pools. It became clear to us that we should repeat this analysis by investigating natural soil systems. As we were involved in forest ecosystem research projects at the University of Göttingen at that time, we had the opportunity to study forest stands with different litter inputs, comparing simple systems of beech monocultures with mixed beech-oak stands over a long-term period. The paper which is the third in the sequence (Anderson and Domsch, 1993) reports on the effects of soil pH on the microbial biomass which emerged after the study of over 100 forest stands. The pH effect was so pronounced in showing an increased q\textsubscript{CO2} and a decreased C\textsubscript{mic}-to-C\textsubscript{org} ratio under acidic conditions in comparison to neutral stands, that we assumed that we had found a microbial community stress indicator in the sense of Odum (1985) who commented “repairing damage by disturbances requires diverting energy from growth and production to maintenance”. To pursue our original goal: the comparison of pure beech stands to mixed beech-oak stands, we had to search for stands with comparable pH and age. This screening went on for years and was finally completed and the data analysed in 1998 after studying of over 1000 stands. The findings of this extensive investigation were published recently in a collaborative book project (Bramme and Khanna, 2009). We could, indeed, show that the more complex beech-oak stands had a higher percent C\textsubscript{mic} in C\textsubscript{org} than that of pure beech stands which suggested a more efficient use of the available carbon by the beech-oak microflora. For mature beech-oak stands on neutral soils the mean percent C\textsubscript{mic} in C\textsubscript{org} was 2.7 and for the pure beech stands 2.3 which, coincidentally, are similar values to those found for agricultural soil systems (see above). On the other hand, the unit respiration released per unit microbial biomass, the q\textsubscript{CO2}, was significantly lower in the more complex beech-oak system than in pure beech stands (Anderson, 2009). We believe it confirms our assumption of a development of a more efficient microbial community in terrestrial systems with increasing diversity of organic matter input. Whether the observed differences of efficient carbon use are attributed to greater species richness within the microbial community remains an open question. This is a challenge to which we expect the molecular biologists to rise.

The metabolic quotient for CO\textsubscript{2} (q\textsubscript{CO2}) has found world-wide application in soil microbial ecology. It started with our former colleague Heribert Insam (now at the University of Innsbruck) who applied Odum’s theory on “bioenergetic economisation with successive age of an ecosystem” by studying soils of reeding glaciers (Insam and Haselwandter, 1989) and our former colleagues Andreas Fließbach, Rainer Martens, Hans Reber, using the q\textsubscript{CO2} as an stress indicator of soils contaminated with heavy metals (Fließbach et al., 1994). Further, the q\textsubscript{CO2} and the C\textsubscript{mic}-to-C\textsubscript{org} ratio were implemented as soil microbial parameters in the continuous observation programme of 90 soil sites in Lower Saxony, Germany, 1991 (Höper and Kleefisch, 2001).

5. Some concluding remarks

We look back with some pride on the large number of publications where metabolic quotients, particular the specific respiration, q\textsubscript{CO2}, have been applied for the quantification of environmental effects on the microbial community in soils. And we still believe that metabolic quotients have a great and as yet unrealised potential for improving our understanding of the development of microbial communities in the ecosystem that they inhabit. Unfortunately, now and then, the relative ease of their determination leads to misapplication. For example, a high q\textsubscript{CO2} is sometimes explained with a high microbial activity and is interpreted as a positive property. In ecological terms, however, a high q\textsubscript{CO2} reflects a high maintenance carbon demand, and if the soil system cannot replenish the carbon which is lost through respiration, microbial biomass must decline. Also, care must be taken how and when basal respiration is determined. In our publications on metabolic quotients we have discussed the fact that we are dealing with physiological quotients (Anderson, 1994). With respect to the microbial biomass in soil, we know that it is not static over the year. It undergoes many changes, including those in its physiological status. The specific respiration, q\textsubscript{CO2}, will be particularly affected, since the CO\textsubscript{2} efflux for its calculation rests on the ratio of the growing to dormant portion of cells. Here, although self-evident, it is of paramount importance that the time (e.g., season) of sampling is comparable if physiological comparisons are to be made.

The holistic approach which we pursued was necessary at that time to understand the relationship between soil microbial biomass its energy transfer and carbon demand. To us, the detection of an energy economy at the microbial biomass level, together with an increase in the diversity of litter input, was really exciting. What the physiological quotients could not provide was direct proof of a greater species diversity in such soil systems. For a decade now, the possible linkage between above-ground biodiversity as a controlling factor of below-ground species diversity has become a research issue (e.g., Wall and Moore, 1999; Hooper et al., 2000; Wardle et al., 2004). At least for the time being the emphasis lies on descriptive techniques such as Biolog\textsuperscript{®}, phospholipid fatty acid (PLFA) or DNA analyses for determining the microbial community structure. We believe a combination of a holistic approach (e.g., metabolic quotients) and specific molecular techniques for testing whether microbial diversity is related to a microbial communities’ energy economy could open a new
chapter in microbial ecology especially given the continuity of research such studies would need.

References
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