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Minireview

The Soil Microbial Biomass: Concept, Measurement and Applications in Soil Ecosystem Research

PHILIP BROOKES[1]*

[1] *Agriculture and Environment Division, IACR-Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, UK*

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For many purposes the soil micro-organisms can be considered as a single pool of living soil organic matter (the soil microbial biomass). Current methods to measure the microbial biomass are described and their merits and demerits discussed. The concept of the microbial biomass as a living soil organic matter pool is illustrated by reference to it as a labile reservoir of potentially plant-available nutrients. An estimate of the turnover times of biomass carbon (C) of 0.94 years and of biomass phosphorus (P) of 0.39 years shows that the turnover rates of nutrients within the biomass may be quite different. An understanding of the dynamics of biomass P is important. The lack of P availability in many tropical agricultural soils has been described as 'the bottle-neck of world hunger'. Even if P is supplied it may be rapidly and irreversibly fixed in these, usually, strongly P-fixing soils. By adding small rates of animal manures with the fertilizer, more biomass P is formed. During the process of biomass turnover, this P may be released slowly and taken up by the crop more efficiently. Thus, in a Kenyan P-fixing soil, crop yields were much larger when both manure and fertilizer P were given than when either were applied singly.

Key words: Microbial biomass measurements, microbial biomass P and C

The soil microbial biomass comprises all soil organisms with a volume of less than about 5×10^3 μm^3, other than living plant tissue, and can thus be considered as the living part of soil organic matter. Jenkinson[15] eloquently described it as *"the eye of the needle through which all the organic materials must pass"* as they are broken down to simple inorganic components including water, carbon dioxide, nitrate, phosphate and sulphate, that plants can use again.

Because it is living, the microbial biomass responds much more quickly to changing soil conditions, particularly decreases or increases in plant or animal residues, than does soil organic matter as a whole. Measurable changes in microbial biomass may thus reflect changes in soil fertility, due, for example, to changing soil management, long before such changes are reflected in changes in the total pool of soil organic matter.

The biomass, although comprising only about 1 to 4% of total soil organic matter, is an important labile reservoir of essential plant nutrients, e.g. nitrogen (N), phosphate (P) and sulphate (S). In arable Northern European soils it can easily contain 100 kg N ha^{-1} and up to 2 or 3 times more in grassland or woodland soils. It is now widely accepted that the fertility of both natural and agricultural ecosystems frequently depends upon the nutrients being very efficiently cycled within the organic pools of plants, microbes and organic matter. The biomass is thus both a sink and source of nutrients, which become available during the process of biomass turnover.

The microbial biomass concept

There have been many studies of individual soil microorganisms grown either in vitro or in soil under axeinic conditions. However, in terms of studying soil nutrient dynam-

* Corresponding author; E-mail: philip.brookes@bbsrc.ac.uk, Tel: +44–15827–63133, Fax: +44–15827–60981

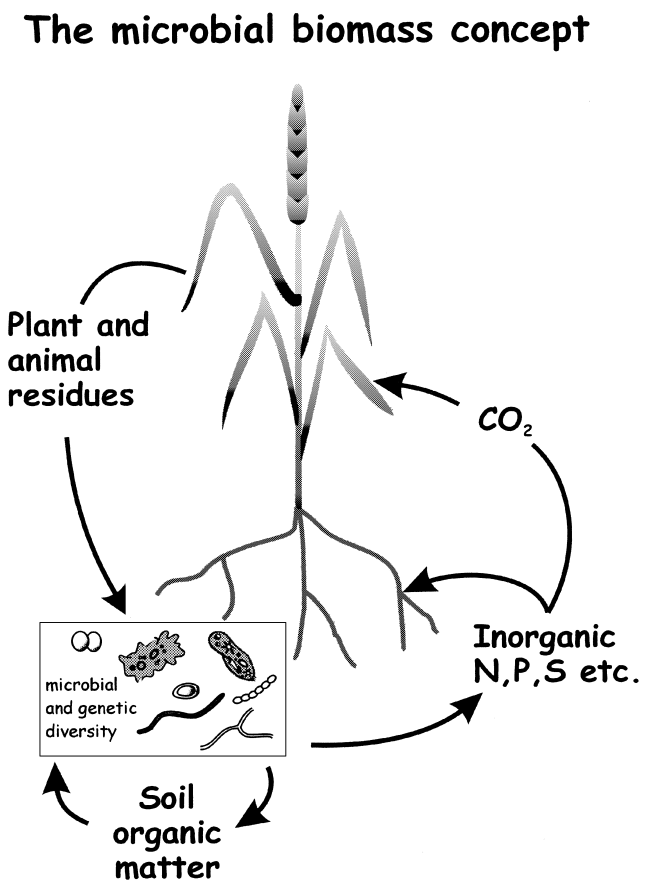

Fig. 1. The role of the microbial biomass in the cycling of plant nutrients.

ics, work with single species, or even a cluster of species of micro-organisms has not been generally useful. Exceptions include mycorrhizae and *Rhizobium* which have very specialised functions. Part of the problem is that so few soil micro-organisms have yet been identified and many cannot be cultured. Another problem is that soil microbial activity resulting, for example, in carbon dioxide (CO_2) evolution, N mineralization or immobilization, is the net result of complex interactions between the many thousands of microbial species and the many thousands of organic compounds, which, together, comprise soil organic matter.

As with organic matter itself, using a 'black box' approach—measuring the microbial biomass as a single, undifferentiated unit[19]—has proved surprisingly useful in studying soil organic matter dynamics. I hope to illustrate these concepts further in this paper. In doing this, I am mindful of the development of powerful techniques in molecular biology which will enable us to study the survival and biology of single microbial species in whole soil, with its full suite of organisms intact[12]. This approach will almost certainly increase our understanding of microbial survival in soil, and of the factors controlling specific processes. However, for ecosystem studies and investigations into carbon or nutrient flows that result from large consortia of microbes processing a wide range of substrates, the 'black box' approach, in which the microbial population is treated as an undifferentiated whole, still has much to offer.

Here I outline some of the methods that we have developed to measure the soil microbial biomass. I also attempt to show what these methods have revealed about some of the characteristics of the biomass and about its role in the maintenance of soil fertility.

Measuring the soil microbial biomass

Direct microscopic counting

Microscopic counting is still the most direct method of estimating the amount of microbial biomass in soil, but is technically difficult and completely unsuitable for routine use. Thin films are prepared from an agar-soil suspension, mounted on microscope slides and then treated with an appropriate stain. Phenolic aniline blue is often used as it stains protein and is thus considered to give an estimate of the entire population. The numbers and sizes of spherical organisms and the lengths and diameters of fungal hyphae are measured and converted to total biomass by using conversion factors for specific gravity, percentage carbon content and percentage dry matter, obtained from micro-organisms grown in vitro. Other stains, especially fluorescent ones like fluoroscein isothiocyanate or acridine orange, are much easier to count with but do not stain the full range of organisms. This method is discussed in more detail by Jenkinson *et al.*[20] and Jenkinson and Ladd[16].

Fumigation-Incubation method

Jenkinson and Powlson[19] showed that more CO_2 was evolved from a soil fumigated with chloroform, following fumigant removal and aerobic incubation, than from a similar non-fumigated soil. They subsequently showed that this extra CO_2 (the CO_2 flush) came from the microbial cells, killed by $CHCl_3$, as they were decomposed by the subsequent recolonizing population. They suggested that measurement of the CO_2 flush could provide an estimate of the amount of biomass in soil.

The standard Fumigation-Incubation (FI) method uses soil, first incubated at 40–50% water-holding capacity (WHC) for 7–10 days at 25°C, then given a 24 hour fumigation with *ethanol-free* $CHCl_3$, followed by fumigant removal and a 10 day aerobic incubation of the soil following readjustment to 50% WHC. Biomass C (B_c) is then calculated

from: $B_c=F_c/k_c$ where F_c=[(CO_2-C evolved from the fumigated soil)] minus [(CO_2-C evolved from the non-fumigated soil)], both over the 10 day period. The constant k_c is taken to be 0.45 under these conditions, on the basis that approximately 45% of the carbon in micro-organisms *added* to soils, followed by fumigation and incubation as described above, is evolved as CO_2 in 10 days. This method has been widely used since its introduction and, provided the soils are first incubated as above, results are in quite good agreement with measurements obtained by direct microscopy[20,39]. Biomass C measurements can be converted to total biomass by assuming that the biomass contains 45% C on a dry weight basis.

The FI method cannot be used in soils that have recently been air-dried. Air-drying both kills some of the biomass and renders some non-biomass C decomposable[36]. In addition, FI measurements are unreliable in soils which contain much free $CaCO_3$, soils which have recently received fresh substrates[23], waterlogged soils[14] or soils with a pH below about 4.5[39].

Biomass N can also be estimated similarly by measurement and appropriate calibration of the flush of inorganic N which also occurs during FI.

Fumigation-Extraction method

Vance *et al.*[40] first showed a close linear relationship between biomass C measured by FI and E_c, where E_c=[(organic C extracted by 0.5 *M* K_2SO_4, from a 24 h fumigated soil) minus (organic C extracted from a similar, non-fumigated soil)]. They proposed that biomass C can be estimated from the relationship: Biomass C=2.64 E_c.

The fumigation-extraction method (FE) does suffer from the disadvantage that comparatively small amounts of C have to be measured in 0.5 *M* K_2SO_4. Vance *et al.*[40] used a dichromate digestion method. However, the C can be more conveniently determined by an automated system using persulphate and U.V. oxidation, which gives essentially the same results but more rapidly and easily[41].

Chloroform fumigation also increases the amount of total N extractable with 0.5 *M* K_2SO_4. Brookes *et al.*[7] showed that this extra N also comes from the microbial biomass and proposed that biomass N could be estimated from the relationship: Biomass N=2.22 E_N, where E_N is analogous to E_c.

About 16% of the *total* N released by $CHCl_3$ after 24 h and extracted by K_2SO_4 is in either ammonium-N or α-amino N. These forms react with ninhydrin giving a blue/purple colour and measurement of ninhydrin-N can be used to estimate the amount of biomass C[2].

Following $CHCl_3$-fumigation there is also an increase in inorganic P (P_i) made extractable to 0.5 *M* $NaHCO_3$. Brookes *et al.*[8] reported that biomass P could be estimated from measurement of this increase in P_i, with a correction made to account for incomplete extraction of P_i due to fixation on soil colloids etc. and assuming that about 40% of the P in the soil microbial biomass is extracted by 0.5 *M* $NaHCO_3$ following $CHCl_3$-fumigation.

Sulphur is also released from the biomass during $CHCl_3$ fumigation and its measurement after extraction can also be used to estimate biomass S[10,33].

The FE method offers some considerable advantages over FI. Biomass measurements can be made across the whole pH range[39], in soils containing actively decomposing substrates, e.g. cereal straw, both in the laboratory[24] and in the field[25] and in freshly sampled soils, all conditions where FI is unreliable. Reliable biomass measurements by FE have also been reported in paddy (i.e. waterlogged) soils[14].

As with FI, the FE method is suitable for use with isotope tracer studies. FE has the big advantage that the labelled biomass that develops as substrates decompose can be measured immediately after substrate addition[26]. This is impossible with FI. In most situations FE has now replaced FI as the routine method to measure microbial biomass. However, FI remains the standard method against which all others are calibrated.

Adenosine 5'-triphosphate

Adenosine 5'-triphosphate (ATP) is only found in living cells and has a very short exocellular existence (a few hours) in soils. It can be quantitatively extracted from the biomass by ultrasonification in the presence of a highly acidic reagent (e.g. trichloroacetic acid, TCA) to inhibit phosphatase activity. The reagent we use[17] is comprised of an aqueous solution of paraquat (0.25 *M*), sodium dihydrogen phosphate (0.5 *M*) and trichloroacetic acid (0.5 *M*). Following ultrasonics, the filtered soil extracts can be analysed immediately or stored frozen (−18°C) for weeks or months. A set of extractants 'spiked' with a known quantity of ATP (usually 25 pmol 50 μl^{-1}) are extracted simultaneously. The partial recoveries of the 'spike' are used to correct for incomplete extraction of native microbial ATP. The ATP is finally assayed by the fire-fly luciferin-luciferase enzyme system using a bioluminometer or scintillation counter set to count 'out of coincidence'.

Characteristics of the soil microbial biomass

Biomass size

Measurements of soil microbial biomass C usually show that it comprises about 1 to 4% of soil organic C, with the

largest proportions in grassland or woodland rather than arable soils. A more illuminating way of considering the biomass size is in terms of its fresh weight. The microbial biomass in the plough layer of the unfertilized plot of the Broadbalk Continuous Wheat Experiment at Rothamsted contains about 500 kg C ha^{-1}. If we assume that living microbial cells contain 50% C and 90% water then the total C immobilized in the cells of the biomass converts to 10 t ha^{-1} of living tissue. This is equivalent to approximately 100 sheep per hectare which gives some idea of the size of this population.

Only a very small proportion of the total species of micro-organisms in the biomass have been properly identified. It is almost certain that some of them (as yet unidentified) will have properties which are ultimately very useful (e.g. antibiotic production). For this, if no other reason, it is clearly in our best interest to conserve soil in a fully functioning state and avoid polluting it with heavy metals, or other toxic materials. While the expression "don't treat soil like dirt" is a much over-used cliché (and lecture title) in soil science, it does at least carry a useful message.

The biomass as a sink-source of plant nutrients

The soil microbial biomass can be considered as a labile pool of essential plant nutrients such as N, P and S, which are held in a form largely protected from loss due to leaching or fixation. Until the development of the fumigation-extraction method it was not possible to quantify the sizes of the microbial pools of these nutrients as they developed during the early decomposition of crop or animal residues. This newer methodology made this possible[24].

There is evidence that the biomass may utilize nutrients preferentially from plant residues rather than from the soil nutrient pool[26]. Thus, the composition and characteristics of plant residues will have a major influence on the availability of nutrients to crops and upon subsequent recycling. Factors such as substrate C/N ratio, percentage of readily decomposable and resistant plant tissue or lignin content will have important effects upon the uptake and subsequent mineralization of nutrients in crop residues[3].

Therefore the correct management of crop residues and annual manures, while important enough in the generally more productive agriculture in temperate regions, is vital in managing soil fertility in the tropics. It is also clear that the fertility of both natural and agricultural tropical ecosystems depends upon the nutrients being very efficiently cycled within the organic pools of plants, microbes and soil organic matter. In this way, losses of nutrients from the ecosystem are minimised.

Table 1. Nutrients immobilized in the soil microbial biomass.

	C	N	P
		kg ha^{-1}	
Broadbalk[a]			
Unfertilized	180	26	7
NPK	200	26	6
Farmyard manure	310	46	27
Woodland	570	84	54
Highfield Grassland	890	130	65

[a] All 0–10 cm soil depth

Due to its large size, the amounts of nutrients, e.g. N and P immobilized in the cells of the microbial biomass are quite large (Table 1). They are also usually considerably larger in grassland than arable soils. This reflects the much larger annual inputs of C in grassland than in arable soils. In low-input soils, a large proportion of the plant-available nutrients, especially N, P and S, will be derived from the mineralization of nutrients immobilized within the cells of the microbial biomass.

Soil microbial biomass as an early warning of changing soil conditions

There is generally a reasonably close linear relationship between amounts of microbial biomass C and amounts of soil organic C in arable, grassland and woodland soils[4,6,13,16,23,29,31,35]. The soil microbial biomass increases or decreases in response to changes in soil management much more quickly than soil organic matter as a whole, where such changes can often take many years before being detectable by classical chemical analysis[1,6,16]. Ayanaba *et al.*[6] and Adams and Laughlin[1] reported that changing from forest or grassland to arable management caused much greater decreases in biomass C than total soil organic C.

Powlson *et al.*[28] showed that 18 years of straw incorporation in two Danish field experiments (Studsgaard and Rønhave) caused 40–50% increases in biomass C and N whereas total soil organic C and N only increased by 5%, a statistically insignificant increase (Figs. 2–3). Both CO_2-C evolution and N mineralization over the 0–60 day period were significantly greater in the soils which had received straw than in control soils where the straw had been burnt. At Rønhave the increase in mineralized N was 38% and at Studsgaard 50% (Fig. 4). This is direct evidence that an increased rate of return of crop residues to soil increases the labile pool of soil organic matter where mineralization-immobilization occurs, to a much greater extent than the soil organic matter as a whole. Some of this N that is mineral-

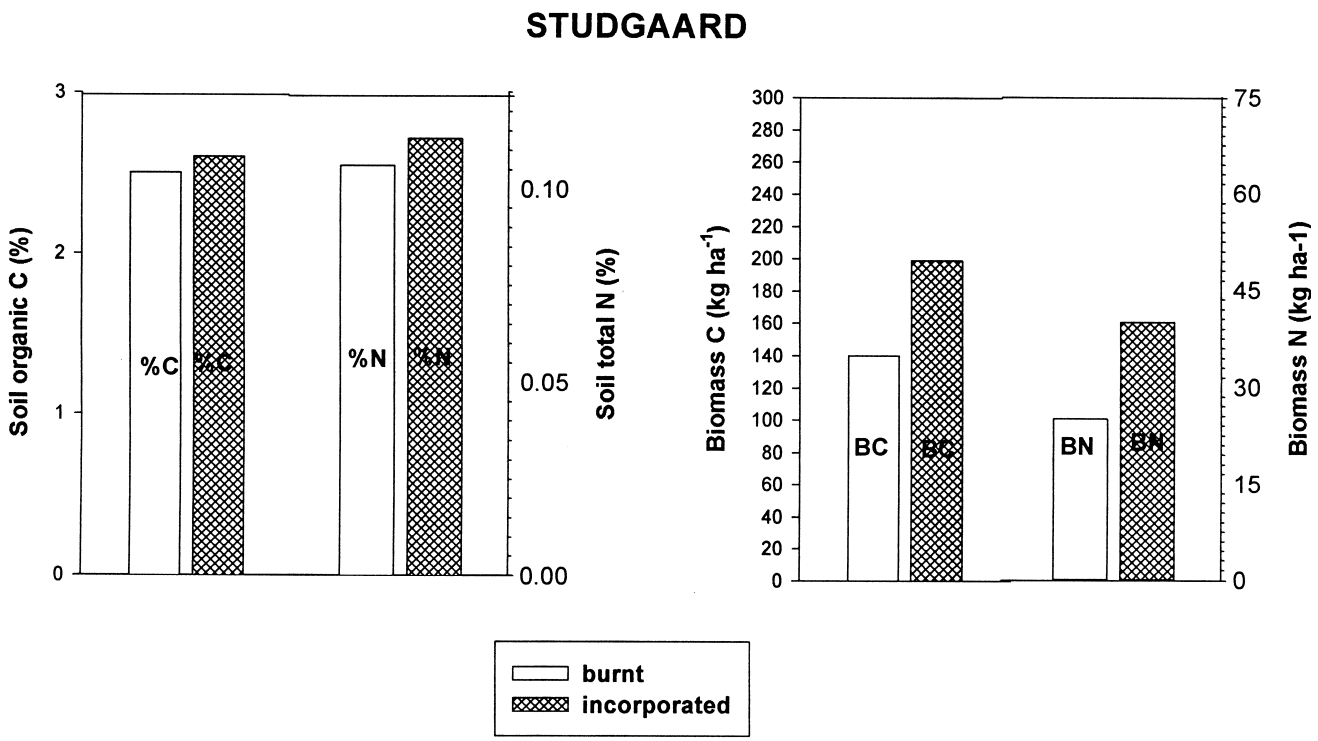

Fig. 2. Percentage soil organic C and N and biomass C and N in Studgaard field soils where straw was burnt or incorporated for 18 years.

ized will be derived from mineral N immobilized during straw decomposition and some from N originally present in the straw[26,30]. The additional mineralization of N in straw-treated soils during the 60 d laboratory incubation was equivalent to more than 20 kg N ha^{-1} at both sites. Increases of this magnitude in the field, if they occur, are of agronomic significance and would permit fertilizer N applications to be decreased to some degree.

Similar results were also reported by Saffigna *et al.*[32] for Australian soils. This, and much other similar work, supports the original idea of Powlson and Jenkinson[29] that the biomass is a much more sensitive indicator of changing soil conditions than is total soil organic matter content so that the biomass can serve as an "early warning" of such changes long before they may be determined by classical chemical analyses.

Biomass as a sink or source of plant nutrients in low-input agriculture

Soil nutrient availability in low-input farming systems mainly depends upon the mineralization of crop residues, animal manures and of native soil organic matter. Many farmers outside of the developed world are too poor to afford much inorganic fertilizer so that there is usually a net removal of nutrients from the soil in the harvested crop. This will often be coupled with a decline in soil organic matter with time as the inputs of organic C in the crop residues or animal manures are seldom equal to the annual losses of organic C and N caused by microbial mineralization and soil erosion. Many farmers are therefore faced with declining soil fertility, with a resulting decrease in crop yield.

It is clear therefore that the correct management of crop residues and animal manures, while important enough in the generally more productive agriculture in temperate regions, is an essential part of the agricultural economics in developing countries, especially in tropical climates. If these organic inputs could be better managed this would have the direct result of improving crop yield, by increasing soil nutrient availability, decreasing erosion, improving soil structure and increasing soil water holding capacity.

The rate and efficiency of mineralization of the nutrients held in crop or animal residues, mediated by the soil microbial biomass, is a key factor in determining the availability of nutrients to crops. It is also becoming widely accepted that the fertility of both natural and agricultural tropical ecosystems depends upon the nutrients being very efficiently cycled within the organic pools of plants, microbes and soil organic matter. In this way, losses of nutrients from the ecosystem are minimized. For example, many tropical soils have exceedingly high P-fixation capacities so that P is

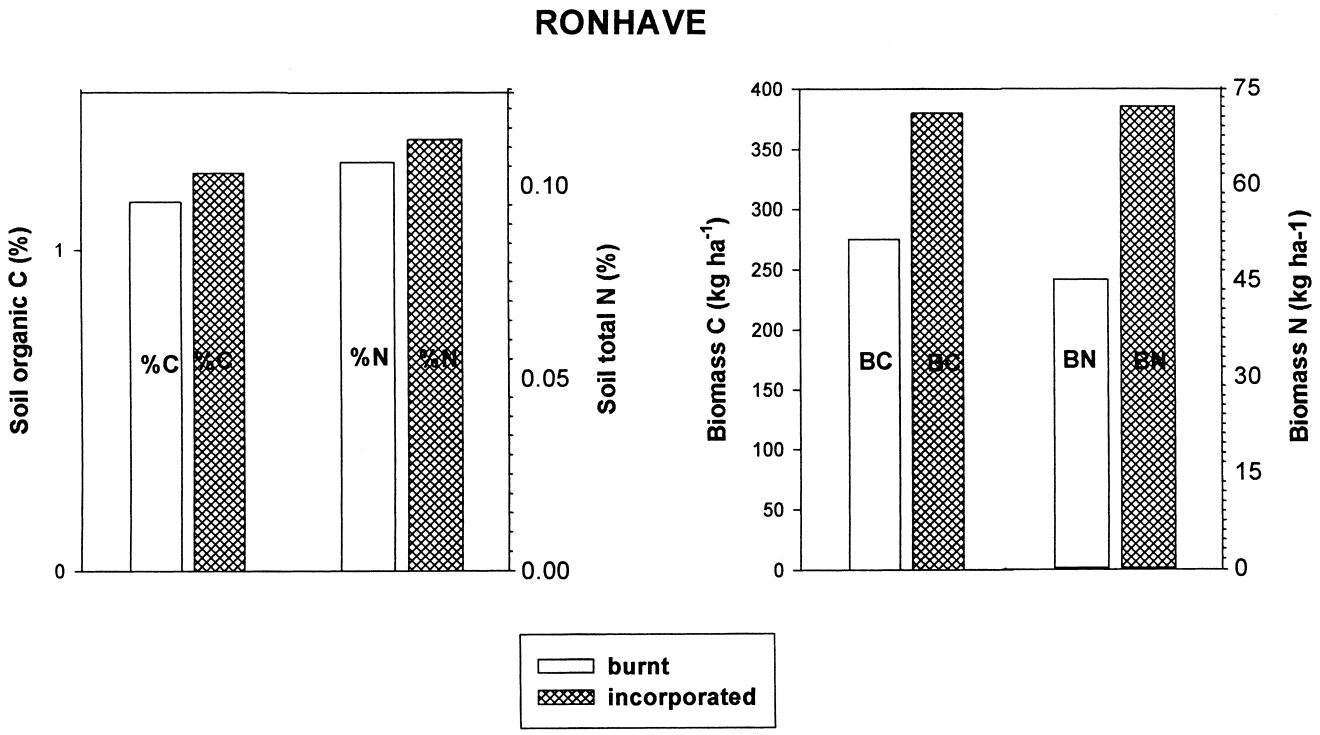

Fig. 3. Percentage soil organic C and N and biomass C and N in Rønhave field soils where straw was burnt or incorporated for 18 years.

rapidly and irreversibly fixed and becomes unavailable to plants. However, if cycled within the organic pools, as described above, such losses from plant-available forms can be minimised[34].

There is evidence that the microbial biomass constitutes an organic matter pool of potentially available, but protected, plant nutrients in tropical ecosystems. Thus, Singh *et al.*[37] reported that the microbial biomass is an important source of plant-available N in tropical soils. The biomass declined in size as N mineralization increased following the rewetting of such soils, precisely during the period when plant growth was most rapid. They therefore considered that the microbial biomass acted both as a sink and a source of nutrients in these nutrient-poor systems. It thus functioned by accumulating and conserving nutrients in a biologically active form during the dry period (large biomass—slow turnover) when the ability of plants to extract nutrients from soil was low. It then released nutrients rapidly once the soils became wet, so stimulating plant productivity (small biomass—fast turnover). Until recently it was not possible to quantify the sizes of the microbial pools of plant nutrients, e.g. N, P, S as they formed during the early decomposition of crop or animal residues. Neither was it possible to monitor the fluxes of nutrients under these conditions as they passed through the biomass, and thence into mineralizable forms. Recent breakthroughs in methodology now make this possible[24,26,43], using both unlabelled and isotopically-labelled plant material and other substrates.

Improving phosphorus fertilizer use efficiency in P-fixing soils in Africa

We are testing the concept of the microbial biomass as a pool of potentially available plant nutrients experimentally in Africa[5]. Phosphorus is the limiting nutrient in many African soils. This is partly because more is removed in the crop than is replaced by additions of manures or (even more rarely) inorganic fertilizer. Another reason is that many soils chemically fix P on soil surfaces, where it is then removed from the plant-available pool. One way of possibly overcoming this problem is to apply inorganic fertilizer P together with an organic fertilizer, e.g. farmyard manure. The organic matter may decrease P fixation by masking sites which would otherwise fix P. The microbial biomass which decomposes the manure will also have a large demand for P as it grows. Thus P will thus be immobilized within the microbial cells and so protected from fixation by the soil colloids. As the biomass declines, following exhaustion of the manure, the microbial P will be mineralized to inorganic P, which plants can use again.

Preliminary results were most encouraging. In both 1997

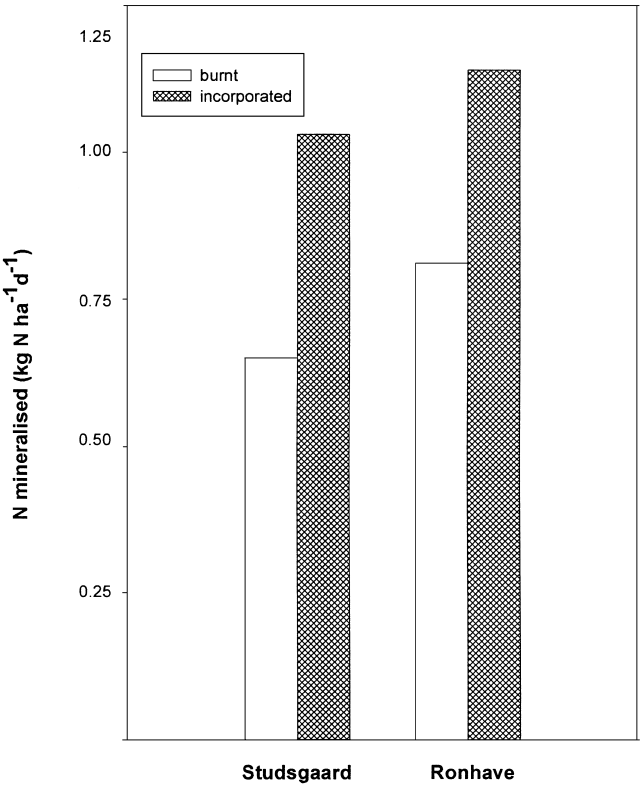

Fig. 4. N mineralised during 60 days in the laboratory from Danish field soils where straw was burnt or incorporated for 18 years.

and 1998 the (Farmyard Manure+P) treatment gave significantly larger yields than when FYM or P were applied singly to a strongly P-fixing soil at Malava (Fig. 6). Differences were much less when the treatments were applied to a non-P fixing soil at Mau Summit. The improvements in yield were certainly not due to the additional inorganic nutrients in the manures. Less than 2 kg P ha^{-1} was supplied in this way.

Turnover of the soil microbial biomass

The methods available to measure the 'standing crop' of soil microbial biomass, while having their limitations, have given estimates of pool sizes of C, N and P in the biomass which generally fit with perceived reality. They certainly allow us to work towards an understanding of soil nutrient dynamics which would be impossible if we could only study micro-organisms as single species or families in soil[27]. Linked with this is the concept of biomass turnover, leading to estimates of the flux of nutrients through the biomass. It is really by these processes that soil nutrients are made available to plants by microbial activity. Estimates of biomass turnover times, defined for example for P as: [(Biomass P content, kg P ha^{-1})/(Annual input of P into the biomass, kg P ha^{-1} yr^{-1})] and flux of P through the biomass, as [(Biomass P content, kg P ha^{-1})/(Biomass P turnover time, yr^{-1})] can provide estimates of soil nutrient fluxes in

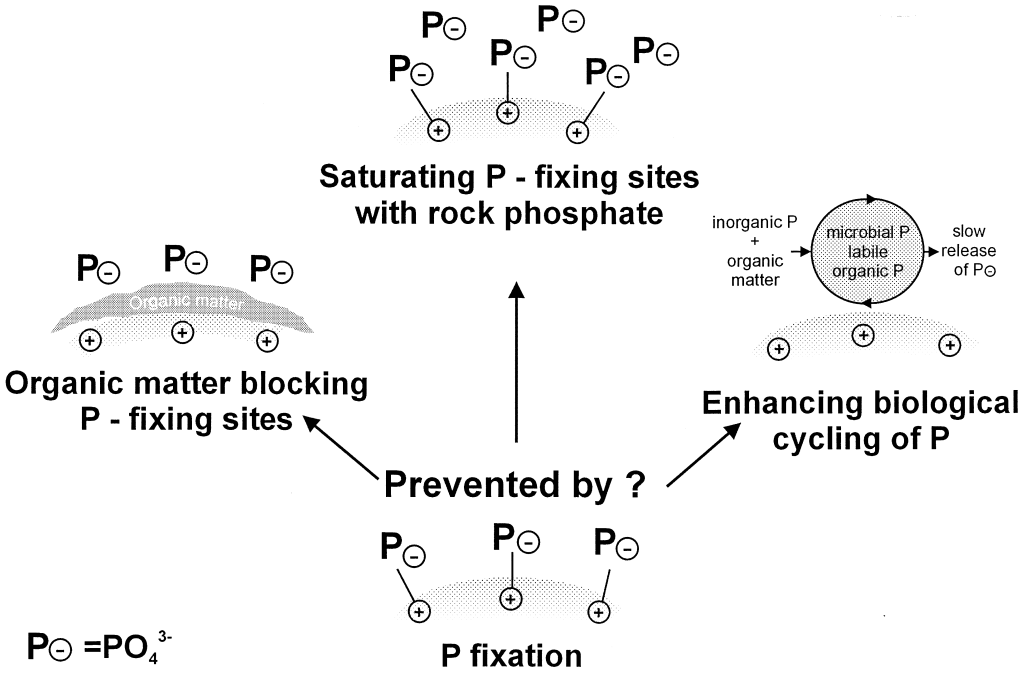

Fig. 5. Possible strategies to overcome the effects of P fixation in soils.

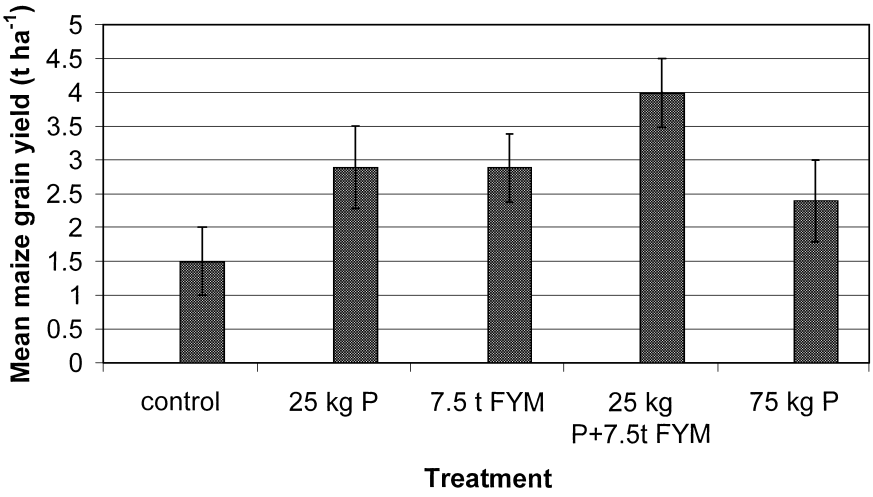

Fig. 6. Effect of applied fertilizer P with or without FYM on maize grain yield at Malava.

agricultural or natural ecosystems.

Jenkinson and Rayner[21] proposed a turnover time of 2.5 yr for biomass C measured in the Broadbalk Continuous Wheat Experiment under UK field conditions and a turnover time for N of 1.52 y was proposed for the biomass in soils of the same experiment, again measured under field conditions[18]. The measurements of turnover of biomass C were based upon measurements of inputs and declines of ^{14}C in soil as a result of the atomic bomb tests in the 1960s. At this time a pulse of ^{14}C-labelled C entered the global soil organic matter pool giving, hopefully, a unique chance to undertake these measurements. The turnover time for biomass N of 1.52 yr[18], also obtained under field conditions, was done by adding ^{15}N-labelled inorganic N fertilizer to the soil in both cases to obtain these values. Full experimental and theoretical details of these and other field measurements of biomass turnover are given by others[11,18,21]. While it is clear that these and similar measurements are best made under field conditions, the cost, expertise and time required (often several years) often makes this an impossibility. The need to use radioactive isotopes in many cases causes further restrictions, although the increasing use of the non-radioactive isotope ^{13}C may accelerate research into C dynamics under field conditions. Certainly, any proposal to measure biomass P dynamics, using ^{32}P or ^{33}P in the field would now face a plethora of restrictions and regulations which would daunt all but the strongest hearted.

Recently we developed a procedure (based on that proposed by Wu[42]) to measure the turnover times of biomass P and biomass C simultaneously in the same soil, under the same conditions in the laboratory[22]. The method involves addition of ^{14}C-labelled substrates (in this case glucose) to soil containing $KH_2{}^{32}PO_4$ which has been allowed to equilibrate for 5 d with unlabelled native soil inorganic P prior to glucose addition. The apparent turnover times of biomass C and P were estimated by applying first-order kinetics rate equations to the declines in ^{32}P- and ^{14}C-labelled biomasses at 25°C and 40% water holding capacity (WHC). Assuming that turnover times of biomass under field conditions in a temperate climate are about 4 times slower than under the above laboratory conditions, Chaussod *et al.*[11], this gives mean field turnover rates for both native and recently synthesised biomass P of about 0.4 yr and for biomass C of about 1.0 yr, measured in a Rothamsted soil of about 23% clay. Using these values, the mean biomass P flux through 6 UK arable soils was about 40 kg P ha^{-1} yr^{-1} and about 140 kg P ha^{-1} yr^{-1} for UK grasslands.

The faster turnover time for P than C seems reasonable as the P will be almost entirely within the cell membranes and cytoplasm of the micro-organisms, while the C will also be an important constituent of the cell wall. Microbial cell walls are known to be much more stable in soil than the intracellular components[16]. The results strongly indicate that the microbial biomass is far from being a static component of the total soil organic matter pool and that the flux of nutrients through it can be surprisingly large. Phosphorus coming from biomass turnover will help replenish soil inorganic P pools.

It must be emphasised that our ideas about the measurement of biomass turnover times and the quantification of fluxes of nutrients through the biomass are still evolving. In particular the fluxes of P through the biomass as measured by ^{32}P seem large, although there are few, if any, similar measurements to serve as comparisons. Calculated fluxes of

Table 2. Biomass turnover times and C and P fluxes.

Soils	Turnover times		Flux through biomass	
	Biomass C	Biomass P	C	P
	Years		kg ha^{-1} y^{-1}	
Arable	0.94	0.39	300	44
Grassland	0.94	0.39	927	146

P through the biomass, usually based on 'standing crop' measurements of biomass P and an assumed turnover time of 1.5–2.5 years[9,38] are generally around 10 kg P ha^{-1} yr^{-1} for temperate arable soils and 25 kg P ha^{-1} yr^{-1} for grassland or forest. More work is needed to evaluate these discrepancies. We also do not know the proportions of nutrients that are made available to plants during biomass turnover. Some will be sorbed by the soil, some will be utilized directly by the soil microbial biomass, and some may be lost by leaching or erosion.

Conclusions

The microbial biomass concept was introduced as a viable, working method in soil science nearly thirty years ago[19]. It has therefore stood the test of time. It is a 'black box' or holistic approach, measuring the entire population, and with suitable modifications, the nutrient fluxes through it. Therefore, the approach makes no attempt to take any account of microbial diversity and its implications in the functioning of the soil microorganisms or their interactions. In some ways this is a clear weakness, in others it is most certainly a strength.

The newer approaches of measuring microbial diversity have developed rapidly. Initially, only crude microscopic measurements were available. However, the newer molecular approaches, such as microbial fatty acid and nucleic acid based measurements opened up the biomass 'black box' in ways which were, hitherto, impossible.

In some rather obvious cases, such as single processes like biological N_2-fixation, or the introduction and monitoring of a single introduced organism, the benefits of diversity over biomass measurements may be quite obvious. However, in other more complex situations, such as organic matter mineralization, where suites of organisms or genes may be involved the 'black box' approach is still a very valuable one. Certainly, the newer methodologies are going to play a key role in the future. However, I am equally sure that soil will continue to throw us more questions than answers, whichever methodologies we may ultimately be capable of developing.

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