ADENOSINE TRIPHOSPHATE AND MICROBIAL BIOMASS IN SOIL

D. S. JENKINSON, S. A. DAVIDSON and D. S. POWLSON
Rothamsted Experimental Station, Harpenden, Herts AL5 2JQ, U.K.

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Summary—Two methods for measuring adenosine 5'-triphosphate (ATP) in soil were compared, one based on extraction with $NaHCO_3$-$CHCl_3$ and the other on extraction by a trichloracetic acid–phosphate–paraquat reagent. Recoveries of added ATP were greater with the $NaHCO_3$–$CHCl_3$ reagent but the extraction of "native" soil ATP by $NaHCO_3$–$CHCl_3$ was only about a third of that by TCA–phosphate–paraquat.

Microbial biomass C and ATP were measured in 8 contrasting English soils, using the fumigation method to measure biomass C and the TCA–phosphate–paraquat method to measure ATP. Except in one acid woodland soil, the ratio (ATP content of the soil)/(biomass C content of the soil) was relatively constant, with a mean of 7.3 mg ATP g^{-1} biomass C for the different soils. This value is very similar to that obtained earlier in a range of 11 grassland and arable soils from Australia. Taking the English and Australian grassland and arable soils together, there is a close ($r = 0.975$) linear relationship between ATP and microbial biomass C that holds over a wide range of soils and climates. From this relationship, the soil biomass contains 7.25 mg ATP g^{-1} biomass C, equivalent to an ATP-to-C ratio of 138, or to 6.04 μmoles ATP g^{-1} dry biomass.

The acid woodland soil (pH 3.9) contained much less biomass C, as measured by the fumigation method, than would have been expected from this relationship. This, and other evidence, suggests that the fumigation method for measuring microbial biomass C breaks down in strongly acid soils.

The ATP content of the biomass did not depend on the P status of the soil, as indicated by $NaHCO_3$-extractable P.

INTRODUCTION

Oades and Jenkinson (1979) showed a close correlation between microbial biomass and adenosine 5'-triphosphate (ATP) in a range of Australian soils. In these soils microbial biomass C could be estimated from the relationship

Biomass C in soil = 120 (ATP content of soil).

Our main aim in the present work was to see if this relationship also held for a range of soils from a cool temperate region. A subsidiary aim was to compare $NaHCO_3$ as extractant for soil ATP (Paul and Johnson, 1977) with the TCA–phosphate–paraquat extractant introduced by Jenkinson and Oades (1979).

MATERIALS AND METHODS

Soils

Six of the 8 soils used (Table 1) were sampled in July 1978 to a depth of 15 cm; soil 4 was sampled in March 1978 to 19 cm and soil 5 in April 1978 to 20 cm. All soils were sieved (<3.25 mm) and, if wetter than 50% w.h.c., spread out and allowed to dry at 25°C until the water content was just under 50% w.h.c. During drying the soils were constantly turned so that no part of the soil ever became air-dry. After sieving the soils were adjusted to 50% w.h.c. and stored under aerobic conditions for 6 days at 25°C in large closed steel drums containing soda-lime.

Analytical methods

These were as described by Jenkinson and Oades (1979) except that organic C was determined by a titrimetric method (Tinsley III method; Kalembasa and Jenkinson, 1973). Salt's method (1968) was used to measure 0.5 M $NaHCO_3$ extractable P. Soil NO_3^--N and NH_4^+-N were determined by a distillation procedure (Bremner, 1965). Soil ATP was measured using the TCA–phosphate–paraquat extractant as described by Jenkinson and Oades (1979), except that the soil was ultrasonified for 2 min during extraction with a M.S.E. 150 W ultrasonic disintegrator operating at full power. During extraction the disintegrator took 2 min to raise the temperature of the extractant-soil suspension from 0 to 55°C; in the original method, using a Branson B12 150 W sonifer, 1 min was sufficient. A 1-min treatment with the disintegrator was insufficient to disperse aggregates in a clay soil (soil 4); no aggregates remained after 2 min.

ATP was also measured in soil using the extraction technique introduced by Paul and Johnson (1977). They extracted soil for 2 min in a Waring blender with $CHCl_3$ and 0.5 M $NaHCO_3$ (adjusted to pH 8.5 with NaOH), centrifuged the suspension and removed any $CHCl_3$ dissolved in the clear aqueous extract by evacuation. The extract was then diluted and ATP determined by the luciferin–luciferase method in a photometer. We used the Paul and Johnson procedure to obtain a $CHCl_3$-free $NaHCO_3$ extract but then measured ATP in a 50 μl portion of the *undiluted* extract by the procedure used in the TCA–phosphate–paraquat method (Jenkinson and Oades, 1979), using a liquid scintillation spectrometer. The calibration curve (made in the presence of the same amount of 0.5 M $NaHCO_3$ as present in the soil extracts) was linear over the 1–25 pmole range, using the same plot as with the TCA–phosphate–paraquat method.

Table 1. Description and analyses of soils

Soil	Location	Site	Cropping or vegetational cover	Clay (%)	pH	Organic C (%)	Carbonate C (%)	Total N (%)	$NaHCO_3$ extractable P ($\mu g\ g^{-1}$ oven-dry soil)
1	Rothamsted[a]	Broadbalk Continuous Wheat Experiment, farmyard manure plot (Plot 22, sect. 5)	Wheat[d]	23	7.9	2.66	0.12	0.262	149
2	Rothamsted[a]	Broadbalk Continuous Wheat Experiment, unmanured plot (Plot 03, sect. 5)	Wheat[d]	21	8.3	0.79	0.24	0.097	10
3	Rothamsted[a]	Broadbalk Wilderness (wooded section)	Deciduous woodland	26	7.7	3.78	0.11	0.337	14
4	Boxworth[b]	Cultivations for Cereals Experiment, direct drilled plot receiving NP (Plot 3)	Wheat	43	8.0	2.45	1.12	0.287	31
5	Headley Hall[c]	Cultivations for Spring Barley Experiment, direct drilled plot receiving NPK (Block 3)	Barley	26	7.3	1.51	0.02	0.167	17
6	Rothamsted[a]	Geescroft Wilderness	Deciduous woodland	28	3.9	2.98	0	0.232	43
7	Rothamsted[a]	Park Grass Continuous Hay Experiment, unmanured unlimed plot (Plot 3d)	Permanent grassland	21	5.3	3.85	0	0.339	4
8	Rothamsted[a]	Park Grass Continuous Hay Experiment, limed plot receiving PKNaMg (Plot 7a)	Permanent grassland	20	6.9	3.91	0.03	0.391	151

[a] From Rothamsted Farm (Batcombe Series).
[b] From Boxworth Experimental Husbandry Farm (Hanslope Series).
[c] From the University of Leeds Farm (Wothersome Series).
[d] Fallowed in the year samples were taken.

Counts per pmole ATP were slightly greater with $NaHCO_3$ extracts than with TCA–phosphate–paraquat extracts. In order to correct for recovery of added ATP, soils were extracted with $CHCl_3$ and either 0.5 M $NaHCO_3$ or a 0.5 M $NaHCO_3$ solution 0.5 μM with respect to ATP. Recovery of added ATP was calculated from the difference between the ATP contents of the spiked and unspiked extracts, just as in the TCA–phosphate–paraquat method.

Biomass C was measured by a slight modification of the fumigation method (Jenkinson and Powlson, 1976), using less soil (100 g moist weight) and incubating under drier conditions (50% w.h.c.). Fumigation was done at 25°C in the presence of soda-lime. While fumigation was in progress, the unfumigated control soils were also kept at 25°C over soda-lime. ATP was extracted at the same time as the $CHCl_3$ fumigations were begun—at the end of the 6-day pre-incubation. ATP measurements (both by $NaHCO_3$ extraction and by TCA–phosphate–paraquat extraction) were in quadruplicate, biomass C measurements in triplicate and all other measurements in duplicate.

RESULTS AND DISCUSSION

Extraction of ATP from soil by $NaHCO_3$ and TCA–phosphate–paraquat

The recovery of added ATP was consistently greater with $NaHCO_3$ than with TCA–phosphate–paraquat for an extractant-to-soil ratio of 10 (Table 2). In contrast, the overall extraction of "native" soil ATP by $NaHCO_3$ was only about a third of that by TCA–phosphate–paraquat, even though there was a close ($r = 0.96$) correlation between the amounts extracted from the different soils by the two methods. Under the conditions tested, the TCA–phosphate–paraquat method cannot therefore be replaced by the $NaHCO_3$–$CHCl_3$ procedure, even though the latter has the advantage of using much less toxic and unpleasant reagents. Soil 1 (2.66% organic C) and soil 2 (0.79% organic C) are from adjacent plots on the Broadbalk Continuous Wheat Experiment and contain similar amounts of clay (Table 1). With both extractants, the recovery of added ATP was less with soil 1 than with soil 2, indicating that ATP is sorbed on soil organic matter during extraction.

ATP content of the soils

ATP contents ranged from 1.22 to 8.74 μg ATP g^{-1} oven dry soil, the grassland and forest soils containing most and the arable soils least. For the 8 soils there was a fairly close ($r = 0.92$) correlation between total N and ATP content and also between organic C and ATP content ($r = 0.91$). The 6 Rothamsted soils have been under the same management for many years, are all located on the soil series and have developed under the same climatic conditions. In such a group, the largest amounts of organic matter will be found in soils with the largest annual inputs of organic matter (Jenkinson and Rayner, 1977); these are also the soils with most ATP (Table 2).

The ATP content of the soil microbial biomass

In calculating the ATP content of the soil biomass by dividing the measured ATP content of the soil by its measured biomass C content, it is assumed that all the ATP is in the soil biomass. Reasons for thinking that this is so have already been set out (Jenkinson and Oades, 1979). With one exception, the ATP content of the soil biomass was relatively constant in the different soils (Table 3), with a mean of 7.3 ± 1.6 mg ATP g^{-1} biomass C (excluding soil 6). These results are similar to those previously obtained with Australian soils (Oades and Jenkinson, 1979) and Fig. 1 shows the combined results for 6 English and 11 Australian soils, excluding two Australian forest soils and two English woodland soils. There was a close ($r = 0.975$) linear relationship between ATP and biomass for the 17 soils, the regression equation being

$$y = (116.3 \pm 6.8)x + (43.8 \pm 30.0) \quad (1)$$

where x is the ATP content of the soil in μg g^{-1} oven-dry soil and y is the biomass C content, also in μg g^{-1}. The corresponding regression through the origin (Fig. 1) for the 17 soils is

$$y = (124.3 \pm 4.2)x \quad (2)$$

again excluding the 4 forest and woodland soils. This regression accounts for 98.1% of the variance. Considerable confidence can now be placed on this relationship, holding, as it does over a wide range of cultivated and uncultivated soils under very different climatic conditions.

Table 2. Comparison of TCA–phosphate–paraquat and $NaHCO_3$ as extractants for ATP in soil

Soil	Recovery of added ATP from soil (%)		ATP content of soil[a] (μg ATP g^{-1} oven-dry soil)	
	TCA–phosphate–paraquat	$NaHCO_3$	TCA–phosphate–paraquat	$NaHCO_3$
1	73.1[b]	87.1[b]	3.14	1.20
2	80.0[b]	97.1[b]	1.22	0.55
3	80.9[c]	91.0[b]	7.53	2.00
4	84.8[c]	84.2[b]	3.91	1.30
5	78.5[b]	94.8[b]	2.49	0.92
6	72.4[b]	94.2[b]	3.38	1.29
7	81.3[c]	96.2[b]	8.40	2.81
8	84.2[c]	91.0[b]	8.74	3.33
Coefficient of variation, %[d]	5.8	4.5	10.6	12.2

[a] Corrected for recovery of added ATP in this and subsequent Tables.
[b] Extractant-to-soil ratio 10 ml g^{-1} soil.
[c] Extractant-to-soil ratio 20 ml g^{-1} soil.
[d] Within-soil sampling error.

Table 3. Respiration rate, biomass content and ATP content of soils

Soil	CO_2 evolved in 10 days (μg C g^{-1} oven-dry soil) Unfumigated	Fumigated	Biomass C (μg C g^{-1} oven-dry soil)	ATP content of soil[a] (μg ATP g^{-1} oven-dry soil)	ATP content of biomass (mg ATP g^{-1} biomass C)
1	113	371	517	3.14	6.1
2	64	169	210	1.22	5.8
3	389	773	768	7.53	9.8
4	127	428	603	3.91	6.5
5	43	241	396	2.49	6.3
6	158	180	43	3.38	78.6
7	368	821	905	8.40	9.2
8	333	899	1133	8.74	7.7

[a] Using TCA–phosphate–paraquat as extractant.

From the slope of the regression through the origin, microbial biomass contains 8.05 mg ATP g^{-1} biomass C. This is probably a little large, being based on a value of 0.5 for the k constant in the fumigation method of measuring biomass. Taking k at 25°C as 0.45, as suggested by Anderson and Domsch's (1978) results (see Oades and Jenkinson, 1979, for a discussion of this), the ATP content of the soil biomass in the 17 soils becomes 7.25 mg ATP g^{-1} biomass C. This corresponds to an ATP-to-C ratio of 138 or to a value of 6.04 μmoles ATP g^{-1} dry biomass, assuming that dry microbial biomass contains 46% C (the mean C content of all 41 organisms examined by Jenkinson, 1976, and Anderson and Domsch, 1978). Although these values are very similar to those proposed by Oades and Jenkinson (1979) they are to be preferred as they are based on a wider range of soils. However, in applying them to other conditions, it must be remembered that our value for the ATP content of the soil biomass only applies to aerobic soils that have not recently received large additions of fresh substrate and that have not been air-dried, frozen or otherwise maltreated prior to measurement. It should also be remembered that it applies to soils that have had a pre-incubation at 25°C: soils pre-incubated at other temperatures may not give the same result.

The two Australian forest soils and the two English woodland soils excluded from Fig. 1 all contain more ATP per g biomass C than the value calculated from the slope of regression (2). Whether or not this holds

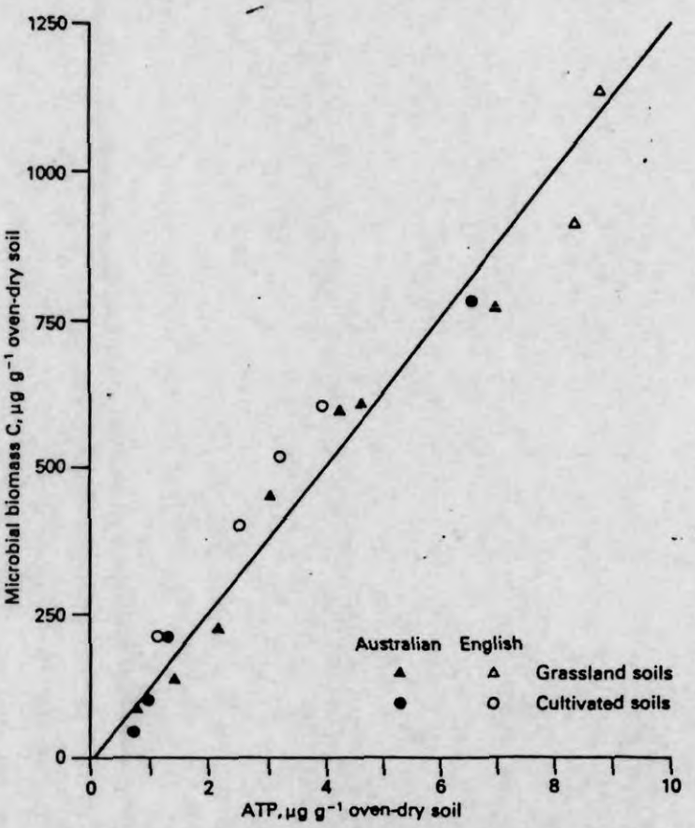

Fig. 1. ATP and microbial biomass C in 11 Australian and 6 English soils.

ally for forest and woodland soils remains to en. Unfortunately, the two Australian forest soils been stored under conditions different from those for all the others, so that undue weight cannot ut on these two measurements (see Oades and inson, 1979). One of the two English woodland (soil 3) contained only marginally more ATP s biomass (9.8 mg ATP g^{-1} biomass C) than the ilated value of 8.05 mg, but the other, soil 6, rrently contained 10 times as much. This soil will onsidered separately.

biomass content of the acid woodland soil (soil 6)

wo results suggest that the biomass content of 6, as given in Table 3, is too small. The ATP tent of the biomass is an order of magnitude ter than in any of the other soils in Table 3, and ed of microorganisms in general (Knowles, 1977). other result was obtained in earlier work with 6 (Jenkinson *et al.*, 1976): biomass as measured by fumigation method was 46 μg C g^{-1} soil, much than that measured by direct microscopic obser- ion of stained microbial cells (330 μg C g^{-1} soil). two different methods of measuring biomass gave ncordant results in all the other soils studied. The crepancy was explained by postulating (Jenkinson *al.*, 1976) that the strongly acid soil 6 (pH 3.9) did leed contain the very small biomass indicated by fumigation method and that in this soil an abnor- lly large proportion of the organisms sized and unted under the microscope were dead. This planation must now be abandoned.

The particular sample of soil 6 used in the present ork came from the same part of Geescroft Wilder- ss as that used 9 yr earlier. Measurements on the 69 and 1978 samples gave similar results: thus the ganic C contents were 2.95 and 2.98% respectively; H 3.9 and 3.9; CO_2-C evolution from $CHCl_3$-treated il 152 and 180 μg g^{-1} and N mineralization from HCl_3-treated soil 23 and 24 μg g^{-1} soil. It is there- re possible to relate observations made on the 1969 mple (Jenkinson *et al.*, 1976) to the 1978 sample ith a fair degree of confidence.

Assuming that the biovolumes in the 1969 and 1978 mples were the same and that biovolume measure- ents give a correct measure of biomass C, then soil contains 10.2 mg ATP g^{-1} biomass C, little greater an the other values in Table 3, which have a mean f 7.3. The ATP results in Table 3 thus suggest that iomass as determined by the fumigation technique s only about a tenth of what it should be in this articular soil. It is clear that extreme soil acidity epresses the flush of decomposition in fumigated soil. lthough we do not yet know how.

Microbial biomass and mineralization of N

The usual increase in mineralization of NH_4^+-N occurred when the fumigated soils were incu- bated (Table 4). As observed before (see for example, Powlson and Jenkinson, 1976) the ratio (CO_2-C evolved)/(N mineralized) fell sharply on fumigation. There was no nitrification in fumigated soils and in- deed fumigated incubated soils contained slightly less NO_3^--N than the unfumigated unincubated controls, presumably because of denitrification during or after fumigation.

One soil (soil 7, from unmanured unlimed grass- land) contained no NO_3^--N at the beginning of the experiments and did not mineralize N when incu- bated *un*fumigated, although it evolved more CO_2-C during incubation than the companion soil 8 (from limed fertilized grassland). Presumably mineral N was being immobilized in soil 7 before and during incuba- tion. However when this soil was fumigated and incu- bated, much more NH_4^+-N was mineralized than could be immobilized.

Ayanaba *et al.* (1976) suggested that soil microbial biomass could be roughly estimated from the rela- tionship $B = 8F_n$ where B is the biomass C content of the soil in μg g^{-1} oven-dry soil and F_n the flush of mineral N caused by fumigation, also in μg g^{-1}. A similar relationship holds for the results in Table 4: the regression (through the origin) being $B = 7.73F_n$. Soil 6 was excluded from this regression.

The ratio (ATP content of soil)/(flush of mineral N) was remarkably constant in the different soils, ranging from 0.051 to 0.076, again with the exception of the strongly acid soil 6. This is further evidence in sup- port of the hypothesis that the extra N released when soils are fumigated and incubated comes from the killed biomass. As before, the large ratio in soil 6 suggests that the flush of decomposition is repressed in this soil.

The effect of P on the ATP content of the soil biomass

As a result of long-continued manurial treatments (see Table 1) soils 1 and 2 (from the dunged and un- manured plots of the Broadbalk Continuous Wheat Experiment, respectively) differ widely in the amounts of P they contain (Johnston, 1969), as do soils 7 and 8 (from unfertilized and fertilized grassland; Warren and Johnston, 1964). There are also large differences in 0.5 M $NaHCO_3$ extractable P (Table 1). Soil 1, receiving dung annually since 1843, contains 15 times more $NaHCO_3$ extractable P than soil 1, unmanured since 1839. Soil 8, receiving P and K annually since 1856, contained 38 times more $NaHCO_3$ extractable P than soil 7, unmanured since 1856.

However, these differences in P status were not reflected in the ATP content of the biomass, which was only slightly greater in soil 1 than in the corre- sponding unmanured soil 2 (Table 3). In the pair of grassland soils this was reversed; the ATP content of the biomass in the high-P soil 8 being somewhat less than that in the unmanured soil 7. It follows that the P status of a soil, as measured by $NaHCO_3$ extract- able P, can vary over a very wide range without affecting the ATP content of the soil microbial bio- mass.

In contrast, Nannipieri *et al.* (1978) showed that the biomass formed when glucose decomposed in soil in the presence of an adequate P and N supply con- tained more ATP per unit biomass than that formed from glucose and N alone. This discrepancy probably arises because our measurements were made on the native population of the soil at a time when little of it was in active growth; theirs on fresh, rapidly- changing populations, some grown under deliber- ately-created conditions of extreme P deficiency. Clearly, our value for the ATP content of the soil biomass should not be used under such conditions.

Table 4. Mineralization of nitrogen in soils

Soil	Mineral N in soil						N mineralized during *incubation*		Flush of *mineral* N[b]	Ratios			
	Initial		After 10-day incubation										
			Unfumigated		Fumigated		Unfumigated	Fumigated[a]		CO_2-C evolved/N mineralized			ATP content of soil/ flush of mineral N
	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	(NH_4^+ + NO_3^-)N			Unfumigated	Fumigated	Flush[c]	
	(μg mineral N g^{-1} oven-dry soil)												
1	1	28	1	32	56	26	5	55	50	23	6.7	5.2	0.063
2	1	12	1	14	20	11	2	18	16	32	9.4	6.6	0.076
3	5	20	4	30	122	19	8	117	109	49	6.6	3.5	0.069
4	0	25	2	31	84	23	7	84	77	18	5.1	3.9	0.051
5	2	22	1	24	42	19	1	40	39	43	6.0	5.1	0.064
6	8	32	3	39	32	29	2	24	22	79	7.5	1.0	0.154
7	6	0	5	1	116	2	−1	112	113	—	7.3	4.0	0.074
8	7	13	6	28	173	11	13	166	153	26	5.4	3.7	0.057

[a] Corrected for NO_3^--N lost during fumigation: 3 μg NO_3^--N g^{-1} oven-dry soil or less in all cases.
[b] N mineralized by fumigated soil less N mineralized by unfumigated soil over the same period.
[c] i.e. ratio of *extra* CO_2-C evolved by fumigated soil to *extra* N mineralized by fumigated soil.

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