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Microbial biomass and mineralization-immobilization of nitrogen in some agricultural soils

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Summary. The chloroform fumigation-incubation method (CFIM) was used to measure the microbial biomass of 17 agricultural soils from Punjab Pakistan which represented different agricultural soil series. The biomass C was used to calculate biomass N and the changes occurring in NH_4^+-N and NO_3^--N content of soils were studied during the turnover of microbial biomass or added C source. Mineral N released in fumigated-incubated soils and biomass N calculated from biomass C was correlated with some N availability indexes.

The soils contained 427–1240 kg C as biomass which represented 1.2%–6.9% of the total organic C in the soils studied. Calculations based on biomass C showed that the soils contained 64–186 kg N ha^{-1} as microbial biomass. Immobilization of NO_3^--N was observed in different soils during the turnover of microbial biomass and any net increase in mineral N content of fumigated incubated soils was attributed entirely to NH_4^+-N.

Biomass N calculated from biomass C showed non-significant correlation with different N availability indexes whereas mineral N accumulated in fumigated-incubated soils showed highly significant correlations with other indexes including N uptake by plants.

Key words: Available N – Fumigation method – N immobilization-remineralization – Microbial biomass

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Heterotrophic microorganisms control the flow of carbon and the cycling of nutrient elements in terrestrial ecosystems and the microbial biomass implicates it as a major nutrient sink during C immobilization and a source during its mineralization. Although soil microorganisms constituting the biomass do not represent a major fraction of the organic and inorganic nutrient pools in most ecosystems (Paul and Voroney 1980), they are now recognised as a source/sink for the major nutrient elements such as N, P, S and C (Paul and van Veen 1978; Anderson and Domsch 1980; Paul and Voroney 1980).

During the past few years many studies have been published on the microbial biomass and its role in plant nutrition (Jenkinson and Ladd 1981) and a variety of methods have been developed to measure the size of microbial biomass and its nutrient content (Jenkinson and Powlson 1976; Anderson and Domsch 1979; Domsch et al. 1979). However, the fumigation technique of Jenkinson and Powlson (1976) has been the most widely used method for estimating biomass carbon. Ayanaba et al. (1976) have used the biomass C to calculate biomass N while Anderson and Domsch (1980) used C content of pure microbial material as a measure for calculating biomass N. Some other workers have used NH_4^+-N released in fumigated incubated soils to estimate biomass N (Paul and Juma 1981; Azam et al. 1985). The difficulty in the latter approach is that denitrification and immobilization of mineral N in fumigated incubated soils may give erroneous results for biomass N. This difficulty has apparently been overcome by Voroney and Paul (1984), who calculated the biomass N from k_N estimated by the in situ development and degradation of microbial biomass using

^{14}C and ^{15}N in the presence of excess of C and N supply as ^{14}C glucose and ^{15}N KNO_3, respectively. They used this k_N value to calculate the biomass N. Compared with these methods, Anderson and Domsch (1980) used C content of pure microbial cultures to calculate biomass N.

The present investigation was carried out to study (a) the biomass of some agricultural soils, (b) immobilization-remineralization of mineral N during the turnover of microbial biomass or exogenous C supply and (c) the role of microbial biomass in N nutrition of plants using some well-known methods of studying soil N availability for comparison.

Materials and methods

Experiment 1

Surface soils from 17 different agricultural soil series were collected from the Lahore, Sheikhupura and Gujranwala districts of the Punjab province in Pakistan. Some of the properties of the soils are presented in Table 1. Percentage base saturation, electrical conductivity (EC) and pH (H_20) were determined by the methods given in the USDA Handbook No. 60 (USDA 1954). Using the method of Watanabe and Olson (1965) $NaHCO_3$-extractable P was obtained and textural analysis was carried out by the Boyoucos method (1962). For organic C, the wet combustion method described by Malik et al. (1979) was followed. Mineral and total N was estimated by the methods of Bremner and Keeney (1965) and Bremner (1965), respectively.

The soils used in this study differed widely in their characteristics (Table 1). Carbon content ranged between 0.5% and 1.48% and N content between 0.05% and 0.13%. Other characteristics also varied over a wide range. Air-dried and sieved (2-mm) soil samples were used for the studies.

Estimation of microbial biomass

Portions (100 g) of soils in 250-ml Erlenmeyer flasks were brought to 60% of their respective water-holding capacity (WHC) and incubated for 5 days at 30 ± 1°C. Preincubation for 5 days was considered appropriate since our unpublished results and those published by other workers (van Veen et al. 1985) show an initial immobilization of N during the first 3–7 days (indicating a maximum build-up of microbial population) followed by remineralization (degradation of microbial biomass) as estimated by the method of Jenkinson and Powlson (1976). One hundred-gram portions of each soil in triplicate were taken in Erlenmeyer flasks and fumigated with chloroform. Fumigated soils were inoculated with 1 g of the respective untreated soil after removing chloroform vapour through repeated evacuations. Triplicate soil samples were left untreated. Flasks containing untreated and fumigated soils were sealed with rubber bungs having 5-ml-capacity glass cups attached to the base. The cups contained 4 ml 10% NaOH solution to trap CO_2. Flasks were incubated at 30 ± 1°C for 10 days and CO_2 absorbed in the alkali was estimated twice during this incubation period using a titrimetric method (Stotzky 1965). Biomass C was estimated by using the formula: $B = F/K$ (Jenkinson and Powlson 1967). The value of K was taken as 0.45 (Oades and Jenkinson 1979). Mineral N (NH_4^+ and NO_3^-, separately and together) was also estimated before and after incubating fumigated and untreated soils. Biomass N was calculated from biomass C values using the relationship N:C = 0.15 (Anderson and Domsch 1980).

N mineralization

One hundred-gram portions of different soils were filled in flat-bottomed plastic containers, brought to 60% of their respective water-holding capacity and incubated at 30 ± 1°C for 8 weeks. Moisture was maintained at 60% WHC throughout the incubation period. After incubation the soils were analysed for total mineral (NH_4^+ and NO_3^-)-N.

Extraction procedure

The extractant was 0.1N $KMnO_4$ in 1N H_2SO_4 prepared on the day of extraction. For extraction, 2-g portions in triplicate from each

Table 1. Properties of soils representing some agricultural soil series (classification based on the seventh approximation of the USDA comprehensive soil classification system)

Location of agricultural soils	%C	%N	C/N	SP	EC mmhos cm^{-1}	pH	$NaHCO_3^-$ extractable P (ppm)	% clay	Cropping history
Kamoke	0.97	0.11	8.8	63.4	0.9	7.8	7	50	Clover-fallow
Gujranwala LL	1.05	0.08	13.1	43.3	0.9	8.0	6	31	Wheat-fallow
Bhalwal	0.84	0.05	16.8	41.0	2.2	7.9	14	31	Under rice
Miranpur	0.82	0.10	8.2	41.0	0.9	7.8	4	27	Wheat-fallow
Hafizabad	0.52	0.06	8.7	37.2	1.2	7.7	5	18	Clover-fallow
Bhalike	0.69	0.05	13.8	41.5	1.4	7.9	8	26	Wheat-fallow
Gajiana	0.64	0.12	5.3	43.0	0.9	7.8	9	27	Wheat-fallow
Satghra	0.74	0.10	7.4	54.4	4.8	7.9	27	27	Wheat-fallow
Gujranwala UL	0.95	0.13	7.3	49.6	1.0	7.7	15	27	Clover-fallow
Pindorian D	0.75	0.06	12.5	35.4	0.9	8.0	11	17	Spiked millet-fallow
Faisalabad	0.98	0.09	10.9	35.9	1.7	7.8	5	18	Vegetable crop
Shahpur	1.13	0.08	14.6	44.7	3.2	7.9	15	27	Wheat-fallow
Shahdra	0.86	0.08	10.8	46.5	2.7	7.8	8	17	Cropped
Rasulpur	0.92	0.07	13.1	26.7	1.6	4.2	6	7	Wheat-fallow
Kotli	0.90	0.10	9.0	57.5	1.0	7.2	12	49	Wheat-fallow
Pindorian	1.38	0.11	12.4	29.0	1.0	7.9	4	15	Wheat-fallow
Sultanpur	1.30	0.10	11.8	50.0	1.2	7.9	10	24	Wheat-fallow

soil were taken in centrifuge tubes and shaken with 50 ml 1N H_2SO_4 for 1 h at room temperature. After centrifugation the supernatant was discarded and the residual soil was shaken for 1 h with the extractant. The supernatant separated by centrifugation was analysed for NH_4^+-N (mineralizable *N* of Stanford and Smith 1978) by steam distillation using NaOH as alkalizer (Bremner 1965).

Pot culture

From each soil 1.5-kg subsamples in triplicate were filled in plastic pots and brought to their respective field capacity moisture by adding distilled water. The moisture level was maintained subsequently throughout the experiment. Seven seeds of wheat (*Triticum aestivum* L. cv. Sandal) were sown in each pot and the stand thinned to three seedlings after seed germination. The plants were harvested 40 days after germination, dried to a constant weight at 70°C and analysed for their N content.

Experiment 2

A garden soil having 0.6%C and 0.059%N was amended with glucose, sucrose or cellulose as a C source; KNO_3 at 150 μg N g^{-1} soil was applied as an N source. The soil was incubated for 8 weeks at 30°C and 60% WHC. Soil samples were analysed at regular intervals for NH_4^+-N and NO_3^--N.

Results

Table 2 shows the biomass C of different soils, which ranged between 21.33 and 62.00 mg 100 g^{-1} soil (426–1240 kg C ha^{-1}) and comprised from 1.64 to 6.92% of the total soil C.

Results of mineral N in fumigated and untreated soils before and after incubation for 10 days (Table 3) show that initially (after 5 days of incubation) the soils contained from 7.62 to 35.97 μg mineral N g^{-1} soil. Upon further incubation for 10 days, a net decrease in mineral N content was observed both in fumigated and untreated soils. However, some soils showed more loss of mineral N when incubated for 10 days after fumigation compared with untreated soil.

Table 4 shows the distribution of mineral N in NH_4^+ and NO_3^- before and after incubating fumigated soils. Before incubation almost the entire mineral N was in NO_3^- form. After incubation, however, a substantial increase in NH_4^+-N and a decrease in NO_3^- was observed in all the soils. Any increase in total mineral N was almost entirely attributed to increase in NH_4^+-N.

Calculations based on the ratios established by Anderson and Domsch (1980) showed that, on average, microbial biomass contained ca. 100 kg N ha^{-1}. However, biomass N calculated in this manner showed non-significant correlation with other N availability indices (Table 5). Paul and Jumma (1981) calculated the biomass by dividing NH_4^+-N (accumulated in fumigated soil after 10 days of incubation) by a factor of 0.25. In the present study NH_4^+-N estimated in soils incubated after fumigation showed a highly significant correlation with N mineralized in fumigated soils ($r = 0.7784$), 0.1N $KMnO_4$-extractable N ($r = 0.7932$) or N taken up by the wheat plants ($r = 0.6914$) and a significant correlation ($r = 0.5930$) with mineral N accumulated in soil after 8 weeks of incubation. Mineral N in fumigated soils showed the best correlations with NH_4^+-N in fumigated soils ($r = 0.7784$), 0.1N $KMnO_4$-extractable N ($r = 0.8073$), mineral N in soils incubated for 8 weeks ($r = 0.8321$)

Table 2. Microbial biomass of different soils used for agriculture

Soil location	CO_2–C evolved ($\mu g\ g^{-1}$ soil)		Biomass C mg 100 g^{-1} soil	Biomass C kg ha^{-1}	% soil C in biomass
	Fumigated soil 0–10 days	Unfumigated soil 0–10 days			
Kamoke	414	135	62.00	1240.0	6.4
Gujranwala LL	306	93	47.33	946.6	4.5
Bhalwal	249	74	38.89	777.8	4.6
Miranpur	246	75	38.00	760.0	4.6
Hafizabad	246	84	36.00	720.0	6.9
Bhalike	267	111	34.67	693.4	5.0
Gajiana	228	72	34.67	693.4	5.4
Satghra	264	111	34.00	680.0	3.5
Gujranwala UL	258	111	32.67	648.8	3.4
Pindorian D	207	66	31.33	626.6	4.2
Faisalabad	195	54	31.33	626.6	3.2
Shahpur	225	98	28.93	562.6	2.6
Shahdra	204	75	28.67	573.4	3.3
Rasulpur	168	60	24.00	480.0	2.6
Kotli	234	126	24.00	480.0	2.7
Pindorian MT	123	21	23.51	470.2	1.2
Sultanpur	231	135	21.33	426.6	1.6

32.61

Table 3. Mineral N content ($\mu g\ g^{-1}$ soil) of different soils before and after incubation of fumigated and untreated soils

Soil series	Mineral N before incubation	Mineral N after 10 days[a] of incubation of	
		Fumigated soil	Untreated soil
Kamoke	33.0	40.9	32.3
Gujranwala LL	36.0	6.8	15.4
Bhalwal	19.9	17.1	5.1
Miranpur	22.7	2.4	10.0
Hafizabad	20.4	36.2	2.0
Bhalike	12.1	17.5	7.8
Gajiana	20.6	6.3	20.5
Satghra	29.6	31.2	6.0
Gujranwala UL	19.9	30.1	5.7
Pindorian D	15.5	7.3	4.4
Faisalabad	27.6	17.9	7.2
Shahpur	12.1	2.3	6.0
Shahdra	27.6	31.5	8.3
Rasulpur	7.6	20.0	5.0
Kotli	20.4	12.7	14.3
Pindorian MT	12.6	3.7	2.5
Sultanpur	19.9	20.9	5.0

[a] Mineral N content of soils after incubation of fumigated or untreated soil minus that present initially in soil before fumigation and incubation

Table 4. NH_4^+–N and NO_3^-–N content of different soils before and after incubation of fumigated soils ($\mu g\ N\ g^{-1}$ soil)

Soil series	NH_4^+–N		NO_3^-–N	
	BI	AI	BI	AI
	$\mu g\ N\ g^{-1}$ soil			
Kamoke	3.9	15.6	29.1	25.3
Gujranwala LL	Nil	6.0	36.0	Nil
Bhalwal	Nil	4.0	19.9	13.1
Miranpur	Nil	Nil	22.7	2.4
Hafizabad	2.4	23.3	17.9	12.9
Bhalike	Nil	7.4	12.1	10.1
Gajiana	1.8	3.0	18.8	3.3
Saghra	2.4	8.9	27.2	22.9
Gujranwala UL	Nil	18.9	19.9	11.2
Pindorian D	1.5	7.3	13.9	Nil
Faisalabad	Nil	7.5	27.6	10.4
Shahpur	Nil	2.3	12.1	Nil
Shahdra	Nil	19.3	27.6	12.2
Rasulpur	Nil	20.0	7.6	Nil
Kotli	Nil	2.3	20.4	10.4
Pindorian MT	Nil	3.7	12.6	Nil
Sultanpur	3.9	6.8	16.0	14.1

BI and AI are before and after incubation of fumigated soils for 10 days, respectively

and N taken up by the wheat plants (0.9548). Similarly, N mineralized during 8 weeks of incubation showed a highly significant correlation with N taken up by wheat plants ($r = 0.8451$).

Discussion

Results presented in Table 2 show 426–1240 kg biomass C ha^{-1}, which comprised 1.64% in 6.92% of the total soil C. Compared with the results reported here, studies by other workers (Ayanaba et al. 1976; Jenkinson and Powlson 1976; Jenkinson and Oades 1979; Oades and Jenkinson 1979; Lynch and Panting 1980) have shown relatively high amounts of biomass C but a lower percentage of C in biomass. Low biomass C in our soils may be due to low organic C content of these soils (generally less than 1%, Table 1) whereas the soils used by workers quoted above had fairly high organic C content. Cerri and Jenkin-

Table 5. Coefficient of correlations of different N availability indices for 17 soil series

	Mineral N in fumigated soils 0–10 days	NH_4^+–N in fumigated soils, 0–10 days	Mineral N in soils, 8 weeks incubation	0.1 *N* $KMnO_4$-extractable N	N uptake by wheat plants
Biomass N	0.2995^{NS}	0.1675^{NS}	0.2993^{NS}	0.5451^{NS}	0.2459^{NS}
Mineral N in fumigated soils 0–10 days	–	0.7784^{b}	0.8321^{b}	0.8073^{b}	0.9548^{b}
NH_4–N in fumigated soils, 0–10 days	–	–	0.5930^{a}	0.7932^{b}	0.6914^{b}
Mineral N in soils, 8 weeks incubation	–	–	–	0.8012^{b}	0.8451^{b}
0.1*N* $KMnO_4$-extractable N	–	–	–	–	0.6512^{b}

NS: Not significant; [a] Significant at the 5% level; [b] Significant at the 1% level

son (1981) have shown that soil with a low C content had less biomass but more of the soil C was attributed to microbial biomass while reverse was true for soil rich in organic C. Somewhat lower biomass values obtained here may also be the result of possible error arising from the air drying of soils although the soils were preincubated for 5 days before being subjected to biomass estimations.

Results presented in Table 3 show a net decrease of mineral N both from fumigated and untreated soils as a result of microbial immobilization when the soils were incubated again after preincubation. Since inoculum was to be mixed in fumigated soils, both fumigated and untreated soils were physically disturbed with a spatula, thus causing the release of some unexposed C sources for microbial utilization and therefore an immobilization of N present mainly in NO_3^- form. A comparison of fumigated and untreated soils showed that after 10 days of incubation some fumigated soils indicated more immobilization than untreated soils while others showed a reverse situation. Fumigated soils with more immobilization might have a higher fungal component in the microbial biomass. Studies by Jenkinson (1976) have shown that soils amended with fungal material cause a net immobilization of mineral N.

We have observed that before 10 days of incubation almost the entire mineral N was present as NO_3^- with a negligible content of NH_3^+-N (Table 4). During incubation, untreated soils showed a net release of mineral N which accumulated as NO_3^-. In fumigated soils, however, any increase in mineral N was due to NH_4^+-N which resulted from the mineralization of dead microbial bodies and remained un-nitrified in soil. Similar findings have been reported by Tillet (1964), Draycot and Last (1971), Ebbels (1971) and Jenkinson and Powlson (1976). According to these investigations the nitrifiers are particularly vulnerable to fumigation treatment. As a result, any mineral N released during incubation of fumigated soils stays in NH_4^+ form.

A reduction in NO_3^--N content was observed in all the soils incubated for 10 days after fumigation. Jenkinson and Powlson (1976) attributed this reduc-

Table 6. Immobilization-remineralization of NO_3^-–N during incubation of soil with glucose, sucrose or cellulose

Amendment	Nitrogen fraction	Weeks of incubation				
		1	2	4	6	8
		($\mu g\ g^{-1}$ soil)				
Glucose	NH_4^+	45.5	21.0	9.1	3.9	2.6
	NO_3^-	–	28.0	82.1	96.3	109.8
	NH_4^-	45.5	49.0	91.2	100.2	112.4
Sucrose	NH_4^+	38.5	16.1	5.6	2.8	3.1
	NO_3^-	–	11.9	40.6	67.4	75.6
	$NH_4^+ + NO_3^-$	38.5	28.0	46.2	70.2	78.7
Cellulose	NH_4^+	5.6	4.2	9.8	3.3	2.5
	NO_3^-	–	31.5	11.2	14.0	10.2
	$NH_4^+ + NO_3^-$	5.6	35.7	21.0	17.3	12.7

tion to loss of NO_3^- through denitrification during or after fumigation. Although incubation conditions in the present study were not anaerobic, denitrification might have occurred from anaerobic microsites (Ekepte and Cornifield 1964; Stefanson and Greenland 1970). Another but more probable factor responsible for the loss in NO_3^--N may be its immobilization by microbes which used dead microbial biomass as C and energy source. In addition , fumigation may also cause the release of easily decomposable organic substances which are otherwise inaccessible to microbial attack, thus causing immobilization of mineral N. Possibility of microbial immobilization of mineral N during turnover of microbial biomass killed by fumigation has been indicated by Ross et al. (1980), Sarathchandra et al. (1984) and Shen et al. (1984). Whether microbes can use NO_3^-, is, however, controversial. Reecently, Shen et al. (1984) have mentioned some exploratory experiments which revealed that microbes are unable to immobilize NO_3^--N. Some earlier reports (Jansson 1958) also show inabilitay of microbes to use NO_3^- as N source. On the contrary, Nommik (1981) reported that microbes can use NO_3^--N effectively in the presence of easily oxidizable C compounds such as glucose. Similarly, Ladd et al. (1977) reported a complete immobilization of NO_3^--N in the presence of acetate.

The results presented in Table 6 provide clear evidence of microbial immobilization of NO_3^--N. In soil amended with glucose and sucrose, the process of immobilization was complete during the 1st week of incubation followed by a net accumulation of mineral N resulting from the mineralization of microbial biomass. In cellulose amendment, however, a high amount of NO_3^- remained immobilized even after 8 weeks of incubation. Total N balance at the end of incubation showed no loss of N. Instead a gain in total N was observed in soil amended with glucose and sucrose. The results of this experiment clearly demonstrated that NO_3^- is utilized by soil microbes as a source of N provided enough available C is present. Therefore immobilization of NO_3^--N during the decomposition of dead microbial material is possible and any decrease in NO_3^--N content of the fumigated soils is attributable mainly to microbial immobilization of NO_3^--N.

The results obtained above demonstrate that microbial biomass may contain substantial amounts of C and other nutrient elements. Since microbial biomass constitutes an important fraction of the soil organic matter and is fairly labile (Jenkinson and Ladd 1981), it is highly probable that the nutrients temporarily locked up in soil microorganisms contribute substantially to the plant-available nutrient elements in soil. Alexander (1977) suggested that microbial metabolism constitutes 1%–4% of the total soil N available to plants. In the present study, N mineralized in fumigated soil showed highly significant correlation with the N taken up by the plants, that mineralized during long-term incubation or released by acid permanganate. Therefore a major portion of plant-available N seems to be derived from the dead microbial biomass. Jenkinson and Ladd (1981) have also reported a good correlation between plant-available N and N contained in microbial biomass. Ayanaba et al. (1976) also suggested that N released in fumigated soils is a better criterion for assessing potentially available N in soil. In the present study, biomass N calculated from biomass C (Anderson and Domsch 1980) show non-significant correlation with different N availability indices. Thus it is not the biomass as such but probably its N content or C/N ratio which determines the N availability. It was also found that NH_4^+-N accumulated in fumigated soils can be used as a reliable criterion for assessing available N in soils since it also shows significant correlation with other N availability indices. Paul and Juma (1981) have used NH_4^+-N accumulated in fumigated soils to calculate biomass N. Recently Voroney and Paul (1984) have also established a k_N factor for such calculations and have suggested that N mineralization in CFIM is the most accurate measure of biomass N. Ayanaba et al. (1976) also reached similar conclusion.

The results presented here emphasize that N accumulated in fumigated soils (resulting from the mineralization of dead microbial biomass) can be used as a reliable criterion of potentially available soil N. Other indices of soil N availability have certain limitations. For example, chemical methods (Hussain and Malik 1983; Hussain et al. 1984; Stanford and Smith 1978), although quick, do not take into account the microbial immobilization of mineral N and may therefore give higher values of available N as also pointed out by Hussain and Malik (1983). On the other hand, N mineralized in fumigated soils is the end result of microbial immobilization-remineralization and gives much better correlation with plant-available N ($r = 0.95$). The incubation method also shows good correlation with other N availability indices but is time consuming. Similarly, plant experiments, although the most direct method of assessing available N, are also time consuming and cannot be used when a large number of samples are to be screened for potentially available N.

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References

Adams TM, Laughlin RJ (1981) The effects of agronomy on the carbon and nitrogen contained in the soil biomass. J Agric Res (Cambridge) 97:319–327

Alexander M (1977) Introduction to soil microbiology. Wiley, New York

Anderson JPE, Domsch KH (1978) A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biol Biochem 10:215–221

Anderson JPE, Domsch KH (1980) Quantities of plant nutrients in the microbial biomass of selected soils. Soil Sci 130:211

Ayanaba A, Tuckwell SB, Jenkinson, DS (1976) The effects of clearing and cropping on the organic reserves and biomass of tropical fores soils. Soil Biol Biochem 8:519–525

Azam F, Malik KA, Saijad M (1985) Transformation in soil and availability to plants of ^{15}N applied as inorganic fertilizer and legume residues. Plant and Soil 86:3–13

Boyoucos GT (1962) Hydrometric method improved for making particle size analysis of soils. Agron J 54:464–465

Bremner JM (1965) Inorganic forms of nitrogen. In: Black CA (ed) Methods of soil analysis. American Soc Agron Madison, pp 1179–1237

Bremner JM, Keeney DR (1965) Steam distillation methods for the determination of ammonium, nitrate and nitrite. Anal Chem Acta 32:485–495

Cerri CC, Jenkinson DS (1981) Formation of microbial biomass during the decomposition of ^{14}C labelled ryegrass in soil. J Soil Sci 32:619–626

Domsch KH, Beck TH, Anderson JPE, Soderstrom B, Parkinson D, Trolldenier G (1979) A comparison of methods for soil microbial population and biomass studies. Z Pflanzenernaehr Bodenkd 142:520–532

Draycott AP, Last PJ (1971) Some effects of partial sterilization on mineral N in a ligt soil. J Soil Sci 22:152–157

Ebbels DL (1971) Effects of soil fumigation on soil nitrogen and on disease incidence in wheat. An Appl Biol 67:235–243

Ekepte DM, Cornfield AH (1964) Losses through denitrification from soil of applied inorganic nitrogen even at low moisture contents. Nature (Lond) 201:322–323

Hussain F, Malik KA (1983) Mineralizable nitrogen as an index of soil nitrogen availability. Pak J Sci Industr Res 26:95–99

Hussain F, Malik KA, Azam F (1984) Evaluation of acid permanganate extraction as an index of soil nitrogen availability. Plant and Soil 79:249–254

Jenkinson DS (1966) Studies on the decomposition of plant material in soil II – Partial sterilization of soil and the soil biomass. J Soil Sci 17:280–302

Jenkinson DS (1976) The effecsts of biocidal treatments on metabolism in soil. IV – The decomposition of fumigated organisms in soil. Soil Biol Biochem 8:203–208

Jenkinson DS, Ladd JN (1981) Microbial biomass in soil. In: Paul EA, JN Ladd (eds) Soil biochemistry, vol 5. Marcel Dekker, New York

Jenkinson DS, Oades JM (1979) A method for measuring adenosine triphosphate in soil. Soil Biol Biochem 11:193–199

Jenkinson DS, Powlson DS (1976) The effects of biocidal treatments on metabolism in soil. V – A method for measuring soil biomass. Soil Biol Biochem 8:209–213

Ladd JN, Parsons JW, Amato M (1977) Studies of nitrogen immobilization and mineralization in calcareous soils. I -Distribution of immobilized nitrogen in soil fractions of different particle size and density. Soil Biol Biochem 9:309–318

Lynch JM, Panting LM (1980) Cultivation and the soil biomass. Soil Biol Biochem 12:29–33

Malik KA, Bhatti NA, Kauser F (1979) Effect of salinity on the decomposition and humification of organic matter by some cellulolytic fungi. Mycologia 71:811–820

Nommik R (1981) Fixation and biological availability of ammonium on soil clay minerals. In: Clark FE, Rosswall T (eds) Ecol Bull 33. Terrestrial nitrogen cycles, Sweden, pp 272–281

Oades JM, Jenkinson DS (1979) The adenosine triphosphate content of soil microbiol biomass. Soil Biol Biochem 11:210–204

Paul EA, Van Veen J (1978) The use of tracers to determine the dynamic nature of organic matter. Trans. 11th Internat. Congr Soil Sci 1:61–102

Paul EA, Voroney RP (1980) Nutrient and energy flows through soil microbial biomass. In: Ellwood DC, Hedger Jn, Latham MS, Lynch JM, Slater JH (eds) Contemporary microbial ecology. Academic Press, London, pp 215–237

Paul EA, Juma NG (1981) Mineralization and immobilization of soil nitrogen by microorganisms. In: Clark FE, Rosswall T (eds) Terrestrial nitrogen cycles: processes, ecosystem strategies and management impacts SNSRC(NFR). Stockholm, Sweden, pp 179–195

Ross DJ, Tate KR, Kairns A, Pansier EA (1980) Microbial biomass estimation in soil from tussock grasslands by three biochemical procedures. Soil Biol Biochem 12:375–383

Sarathchandra SU, Perrott KW, Upsdele MP (1984) Microbiological and biochemical characteristics of a range of New Zealand soils under established pasture. Soil Biol Biochem 16:177–184

Shen SM, Pruden G, Jenkinson DS (1984) Mineralization and immobilization of nitrogen in fumigated soil and its measurement. Soil Biol Biochem 16:437–444

Standford G, Smith SJ (1978) Oxidative release of potentially mineralizable soil nitrogen by acid permanganate extraction. Soil Sci 126:210–218

Stefanson RC, Greenland DJ (1970) Measurement of nitrogen and nitrous oxide evolution from soil-plant system using sealand growth chambers. Soil Sci 109:203–206

Stotzky G (1965) Microbiol respiration. In: Black CA (ed) Methods of soil analysis. Am Soc Agron Madison, pp 1179–1237

Tillett EK (1964) The effects of different fumigants on the mineralization of soil nitrogen. Rhodesian J Agric Res 2:13–16

USDA (1954) Diagnosis and improvement of saline and alkali soils. USDA Handbook No. 60

Van Veen JA, Ladd JN, Amato M (1985) Turnover of carbon and nitrogen through the microbial biomass in a study loam and a clay soil incubated with [$^{14}C(4)$] glucose and [^{15}N] $(NH_4)_2SO_4$ under different moisture regimes. Soil Biol Biochem 17:747–756

Voroney RP, Paul EA (1984) Determination of K_c and K_n in situ for calibration of the chloroform fumigation – incubation method. Soil Biol Biochem 16:9–14

Watanabe FS, Olson SR (1965) Test of an ascorbic acid method for determining phosphorus in water and $NaHCO_3$ extracts from soils. Soil Sci Soc Am Proc 29:677–678

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