

DYNAMICS OF MICROBIAL BIOMASS CARBON AND NITROGEN AND EXTRACTABLE NITRATE IN LONG TERM ROTATION STUDIES AT INDIAN HEAD

K.J. Greer and D.W. Anderson
Department of Soil Science, University of Saskatchewan, Saskatoon, Sask.

ABSTRACT

Mineralization studies in the laboratory indicate that the net turnover of nitrogen is greatly affected by crop rotation. Recently, the field applicability of such Incubation - Intermittent leaching experiments has been questioned. Therefore, field sampling was undertaken during the summer of 1988 to determine the influence of rotation history on microbial biomass C and N, and levels of extractable NO₃-N. Four long term rotation plots (Ag. Canada, Indian Head) were sampled in the fallow phase. These rotations consisted of: (1) Fallow-Wheat-Wheat, (2) Fallow-Wheat-Wheat (fertilized, straw retained), (3) Fallow-Wheat-Wheat (fertilized, straw removed), and (4) Fallow-Wheat-Wheat-Hay-Hay-Hay.

Biomass C and N, as measured by a chloroform-direct extraction technique, was found to be significantly higher in the soils from the hay rotation. The proportion of organic C present as biomass was, on average, 29% higher than in the three year rotations without hay. Similarly, nitrate levels were found to be significantly affected by rotation history and correlate strongly with the size of the microbial biomass.

The prediction of microbial biomass based on carbon added as crop residue was poor since the intrinsic assumption that all carbon is equally available for decomposition does not hold for all residues. However, the levels of biomass C and N were closely related to the N content of the residues returned. This is to be expected since the N-rich "metabolic" fraction is readily decomposed and incorporated into the microbial biomass. These relationships are clearly illustrated using a conceptual model of N turnover.

INTRODUCTION

Microbial biomass is considered to be the cumulative active population of bacteria, fungi and actinomycetes in a soil (Jenkinson and Ladd, 1981). Although comprising only a small proportion of the total organic C and N, the microbial biomass is an important portion of the soil organic matter (SOM) (Paul, 1984). The microbial biomass serves as both a source and a sink for inorganic nutrients. More importantly, however, it acts as the major agent behind SOM conversion to plant available forms of N, P, and S.

Rotation treatments have a large influence on both the size and the activity of the microbial biomass (Schnurer et al., 1985; Biederbeck et al., 1984). Cultural practices will affect the soil temperature and moisture regimes as well as the amount and type of crop residues, thereby influencing the biomass and related nutrient transformations. Measures of the size and activity of the microbial biomass have also been used as early indicators of the changes in SOM with management (Nannipieri, 1984).

Results from a laboratory mineralization study indicate that rotation has a large impact on nitrate accumulation (unpublished data). However, the field applicability of such determinations of mineralizable potentials has been questioned (Bonde et al., 1988). To substantiate the rotational affect on N dynamics, a field sampling was carried out in the summer of 1988. This study investigated the influence of crop rotation on the soil microbial biomass and accumulation of nitrate over the summerfallow period.

MATERIALS AND METHODS

Site Management and Sampling Procedure

The rotation study was set up in 1958 on the Agriculture Canada Experimental Farm, Indian Head. Over the past 30 years, eleven replicated rotation treatments have been studied on a clay textured Rego Black soil of the Indian Head Association. Prior to 1958 the plot area had been uniformly managed under a Fallow-Wheat rotation. Tillage, harvesting and straw removal were performed with field-scale equipment. Four of the original eleven rotations have been selected for this study.

Fallow-Wheat-Wheat (FWW) rotation was unfertilized in both crop years. All residues were retained on the plot after grain harvest.

Both the Fallow-Wheat-Wheat, straw retained (FWW N+P; +straw) and the Fallow-Wheat-Wheat, straw removed (FWW N+P; -straw) received an average application of 6 kg N/ha, 26 kg P₂O₅/ha in the first crop year and 40 kg N/ha, 22 kg P₂O₅/ha in the second crop year.

All residues, including the straw, stubble and roots, were retained on the FWW (N+P; +straw). The FWW (N+P; -straw) rotation did not have complete straw removal since a portion of the aboveground dry matter remained on the plot as stubble.

The six year rotation (FWWHHH) was unfertilized in all crop years. In three of the six years a 1:1 bromegrass, alfalfa hay stand was included. The hay was cut when the alfalfa was in full bloom, once or twice per year and dry matter yields measured. Breaking of the third year hay plots took place either in the late fall or spring of the Fallow year.

Soil samples were collected from the surface (0 to 7.5 cm) of each of the fallow plots in the selected rotations on June 9, July 13 and August 12, 1988. The samples were stored field moist at 0°C for one to three days before determination of microbial biomass C and N.

A time series sampling schedule was applied to allow for analysis as a split-plot in time, thereby determining the rotation treatment (main plot) effect over the summerfallow season and minimizing the possibility of anomalies caused by environmental factors (Nannipieri, 1984).

Determination of Microbial Biomass and Nitrate

Briefly, the flush of extractable organic C or N caused by chloroform lysis of microbial cells is proportional to the amount of C or N tied up in the biomass. The size of the soil microbial biomass can be estimated from the extractable organic C flush if the efficiency of the extraction (K_c) is known. The flush of extractable organic N may also be used as an indication of biomass size, if the C:N ratio of microbial tissue is not affected by rotation treatment.

The fumigation-direct extraction in 0.5 M K₂SO₄ was similar to the methods of Vance et al. (1987) and Brookes et al. (1985). Organic C was calculated from the total and inorganic C extracted, as determined using a Beckman 935-B automatic C analyzer. Organic N was deter-

mined on the same extract using the semi-micro Kjeldahl method. The extraction efficiency used for converting the flush of extractable C (Kc) and N (Kn) to biomass C and N were 0.34 and 0.24, respectively (E. Bremer, personal communication).

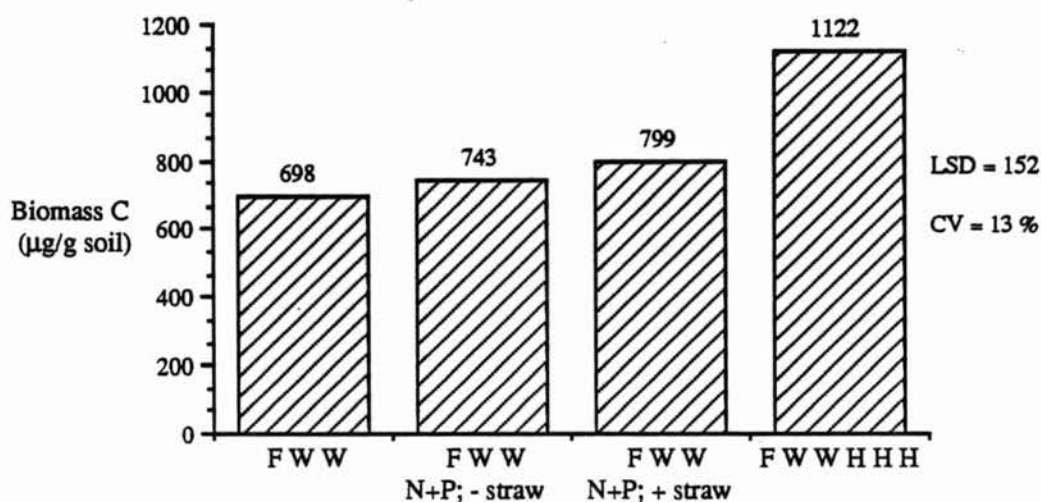
A representative portion of each soil sample was air dried and extracted with 2N KCl. Nitrate - N in the extract was measured using a Technicon autoanalyser.

RESULTS AND DISCUSSION

Crop Rotation and Microbial Biomass Size

Rotation treatment had a strong influence on biomass C (Figure 1). Fallow following brome-alfalfa hay (FWWHHH) had levels of biomass C significantly higher than all other rotations. The three year rotations had less biomass C than the hay rotation with a trend of increasing biomass from FWW, through to the FWW (N+P; -straw) and FWW (N+P;+ straw) rotations. These differences among fallow wheat rotations, however, were not significant at a 5% level.

Figure 1. Mean Microbial Biomass Carbon as Influenced by Rotation



The N in the microbial biomass followed the same trend as biomass C (Figure 2). The variability in biomass N (CV = 10%) was lower than that of biomass C (CV = 13%). Hence, all rotation treatments were significantly different from each other. The similarity in the trends of biomass C and N suggest that the composition of the microbial community, with respect to ratio of C to N in tissue, was not affected by treatment.

Crop rotation influenced the proportion of total organic C and organic N existing as biomass C and N (Table 1). In the hay rotation, the biomass C and N made up 4.7 and 8.5% of the organic C and organic N, respectively. A decreasing trend in the proportion of organic C and organic N present as biomass was observed in the three year rotations as straw was removed and

fertilization reduced. The balance of residue inputs and subsequent microbial decomposition results in a greater fraction of the soil organic matter present in an active form (Jenkinson and Ladd, 1981).

Biomass size is largely controlled by the amount and type of substrates available for growth (Alexander, 1977), and will subsequently be influenced by the residues supplied by a crop. Microbial biomass C was much greater in rotations containing legumes (Bolton et al., 1985) and where increased amounts of straw residues were available (Schnurer et al., 1985).

Figure 2. Mean Microbial Biomass Nitrogen as Influenced by Rotation

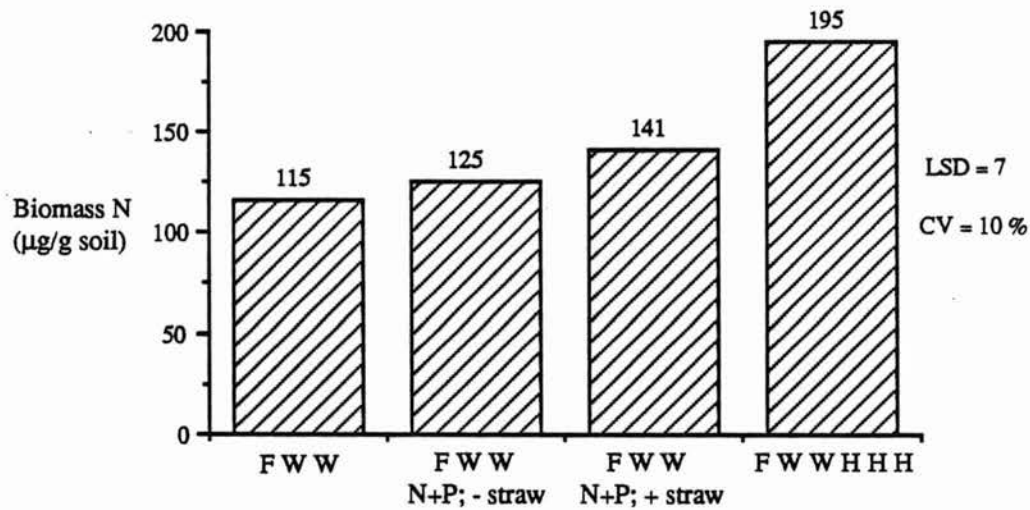


Table 1. Biomass C and N as a percent of organic C and N.

ROTATION	Biomass C (% of OC)	Biomass N (% of OC)
F W W	3.5	6.1
F W W (N+P; -straw)	3.7	6.1
F W W (N+P; +straw)	3.7	6.8
F W W H H H	4.7	8.5

McGill et al. (1986) postulated that biomass C was strongly related to the total crop yields on long term rotation plots. Intuitively, this relationship is to be expected since McGill et al. (1986) assumed that grain yield was equal to the residue returned as straw, stubble and roots. Hence, larger crop yields would result in more C added as residue and, therefore, more biomass. The simple assumption that yield is equivalent C input as residue does not hold for all rotations in this study. The FWW (N+P; -straw) had only stubble and root residue returned to the plot after harvest. Therefore part of the C present as straw residue was not available for microbial breakdown. As well, the dry matter yield of the hay crop cannot be considered to be equal to the stubble and root residue remaining. Bowren et al. (1968) reported that only one third of the total dry matter produced by alfalfa was present as roots at haying. Given these problems, an expanded set of assumptions was needed to attempt to relate levels of biomass C and N to C added as residues. Since all rotation treatments were sampled in the fallow phase, estimates of residue added from the previous crop grown should reflect more closely the level of substrate available to microorganisms.

The assumption of a 1:1 relation between grain yield and residues facilitated the calculation of C added as residue in both the FWW and the FWW (N+P; +straw) rotations. A grain yield of 1610 kg/ha (stubble wheat of the FWW) would give rise to 1610 kg/ha of straw, stubble and roots. Since dry matter is 45% C and the C:N ratio of wheat residues is about 100:1, 725 kg C/ha and 7 kg N/ha are added as residue (Table 2.).

Table 2. Estimate of C and N added as crop residue.

ROTATION	1987 Grain / Hay yield (kg/ha)	Total residue (kg/ha)	C added (kg/ha)	N added (kg/ha)
F W <u>W</u>	1610	1610	725	7
F W <u>W</u> (N+P; -straw)	3242	2161	973	10
F W <u>W</u> (N+P; +straw)	3079	3079	1386	14
F W W H H <u>H</u>	3930	2620	1179	30

Removal of the straw in the FWW (N+P; -straw) was considered to reduce the litter addition by one-third (Van Veen and Paul, 1981). Hence the C and N returns were lower than the three year fertilized rotation on which straw was retained. Similarly, the removal of the top growth as hay in the FWWHHH rotation resulted in C additions that were intermediate of the FWW fertilized rotations. However, the N addition was the highest since an average C:N ratio of the hay was assumed to be 40:1.

No clear trend is revealed when biomass C is plotted against C additions (Figure 3). However, if the hay rotation is ignored, a trend toward higher levels of biomass C with increasing wheat residues is apparent. The lack of fit of the biomass C and C added in the hay rotation suggests that the decomposability of C added as hay residues is substantially different.

Microbial decomposition is greatly influenced by the N content of the residues added (Millar et al., 1936). Recent models of biomass turnover and soil organic matter decomposition have partitioned residues into readily and more slowly decomposable fractions (Van Veen et al., 1984 and Parton et al., 1983). The readily decomposable fraction (Figure 4) has been suggested to be composed of cellulose, hemicellulose as well as N rich metabolic compounds (such as amino acids). Since the bulk of the N in plant residues is in this readily decomposable fraction, one would expect biomass size to relate well to N added as residue.

Figure 3. Trend in Biomass Carbon and Estimated Carbon addition as Crop Residue

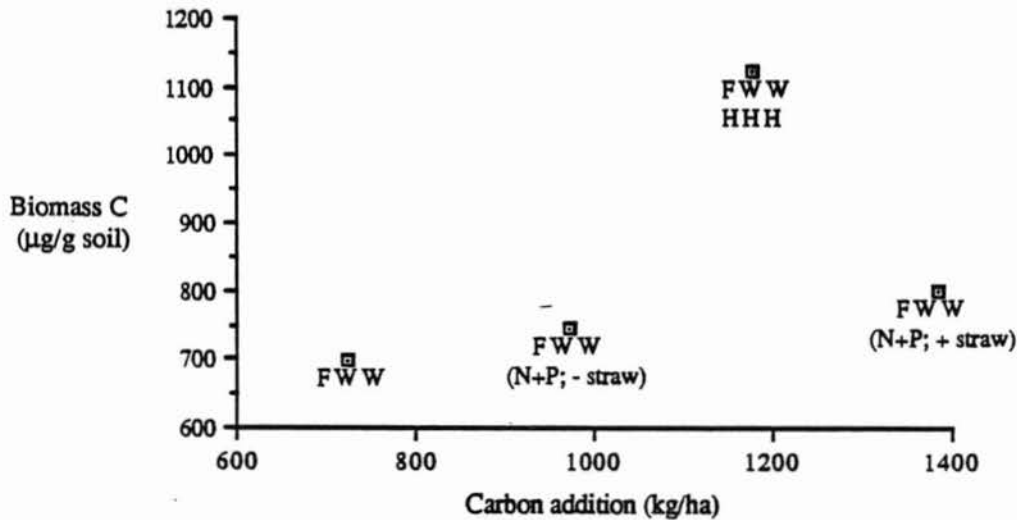
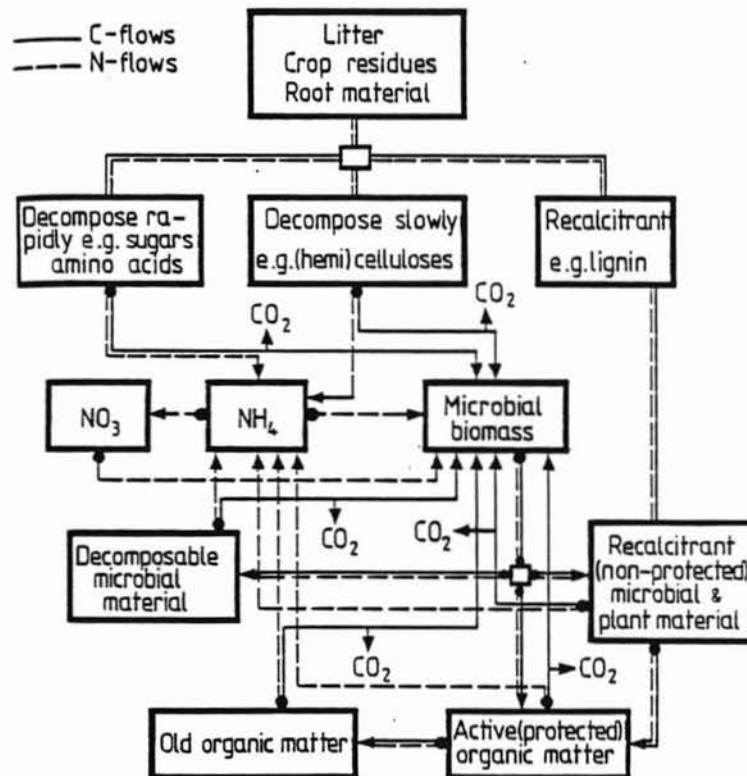
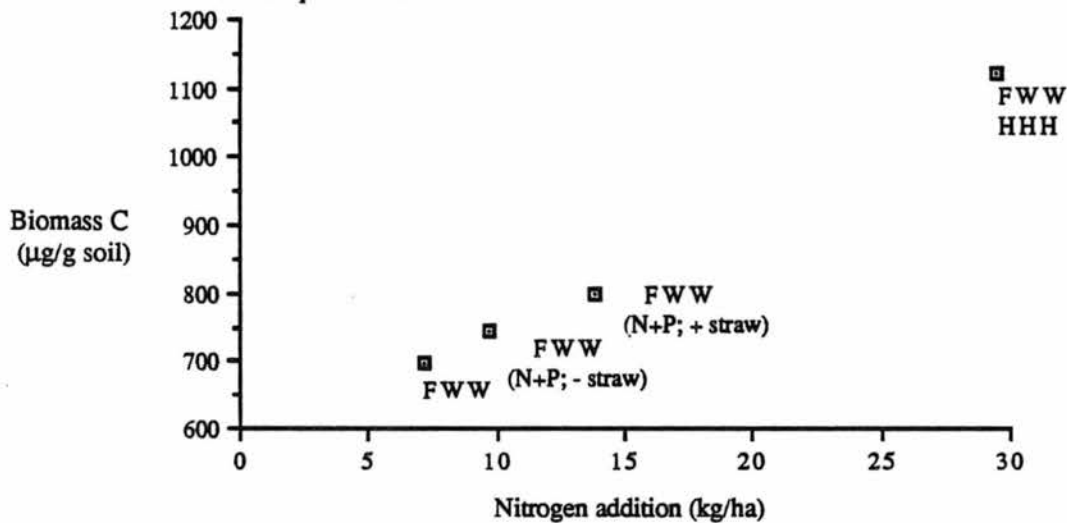


Figure 4. Scheme (Version 4) of the N-mineralization-immobilization model (taken from van Veen et al., 1984).



The amount of N added as residues is, in fact, closely related to the size of the microbial biomass (Figure 5). The increased levels of wheat residues added from FWW to FWW (N+P; +straw) (Table 2) result in increasing the total amount of readily decomposable, N-rich, metabolic fraction. The hay rotation, although having a lower amount of residue added, had a narrow C:N ratio and therefore, proportionally higher levels of readily decomposable residues. This larger proportion of metabolic substrates is responsible for the high biomass C levels observed in the hay rotation (Figure 1).

Figure 5. Trend in Biomass Carbon and Estimated Nitrogen addition as Crop Residue



The response of biomass C is, therefore, dependent on the quantity of residues added only when the quality of residues is equivalent. The quality of the residue is related to the proportion of the N-rich metabolic fraction, which can be estimated using the C:N ratio of the litter returned (Alexander, 1977). The C:N ratio of the residue also has a large impact on N turnover since C and N transformations are interdependent (Jansson and Persson, 1982). Clearly, an increase in the size of the readily decomposable fraction (Figure 4) will enhance the turnover of C and N via a larger microbial biomass.

Nitrate Accumulation

The accumulation of inorganic N as nitrate was greatest in the hay rotation (Figure 6). Nitrate in the three year wheat rotations, although not significantly different, increased slightly with fertilization (6.5 to 7.1 µg/g soil) and straw removal (7.7 µg/g soil).

The similarity between nitrate accumulation and biomass size (Figures 6 and 1) indicates that the net inorganic N mineralized increases with the size of the biomass. This relationship is to be expected since the microbial biomass drives the turnover of N (Paul and Juma, 1981). A strong correlation between biomass size and nitrate accumulated is, therefore, not surprising (Table 3). However, the interpretation of such correlations must be done with caution since accumulation of inorganic N is the net result of microbial mineralization and immobilization (Jansson and Persson, 1982).

Figure 6. Mean Extractable Nitrate Nitrogen as Influenced by Rotation

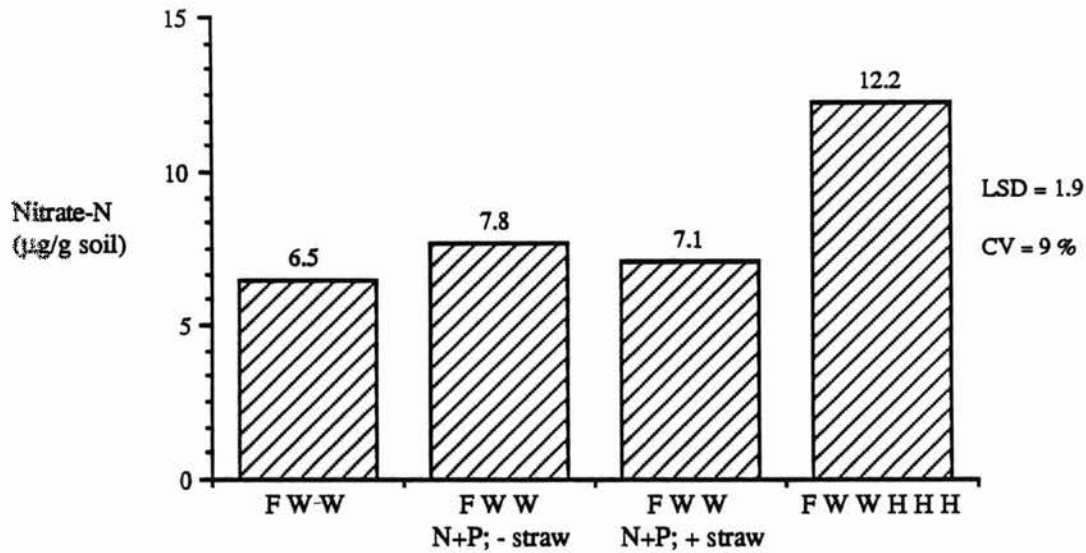


Table 3. Dependence of nitrate on biomass carbon and nitrogen.

Independent	n	r ²	Slope	Sign. level
Biomass C	12	0.82	0.016	0.001
Biomass N	12	0.77	0.076	0.001

NOTE : Y- intercepts not significantly different from 0.

The type of substrate decomposed has been known to have a great impact on the balance of mineralization or immobilization. Residues with narrower C:N ratios have higher proportions of metabolic components and are more likely to result in the accumulation of nitrate or ammonium (Heard, 1965). The significantly higher nitrate accumulation in the hay rotation is a result of the larger proportion of N-rich components returned mainly as root residues (Ferguson and Gorby, 1971). Ferguson and Gorby (1964) reported that the addition of wheat residues may reduce nitrate accumulation during the fallow period. The trend in the three year rotations (Figure 6) seems to support this. However, one should keep in mind that the differences observed were not significant at $P > 0.05$.

CONCLUSIONS

The size of the soil microbial biomass is greatly affected by rotation treatment. Management practices that increase the amount and the decomposability of residues returned will, thereby increase the size of the microbial biomass.

Estimated residue additions suggest that the size of the soil microbial biomass corresponds closely to the amount of easily decomposable N-rich residues. The estimate of total carbonaceous material is related to the biomass size only when the relative proportions of metabolic and slowly decomposable fractions are constant.

Nitrate accumulation is significantly affected by cropping sequence. The inclusion of hay prior to the fallow year resulted in higher proportions of N rich substrates, larger microbial biomass and, therefore, significantly greater nitrate levels.

REFERENCES

- Alexander, M. 1977. Introduction to Soil Microbiology. (2nd edition) John Wiley and Sons, New York.
- Biederbeck, V.O., Campbell, C.A. and Zentner, R.P. 1984. Effect of crop rotation and fertilization on some biological properties of a loam in southwestern Saskatchewan. *Can. J. Soil Sci.* 64: 355-367.
- Bolton, H.(Jr.), Elliott, L.F., Papendick, R.I., and Bezdicsek, D.F. 1985. Soil microbial biomass and selected soil enzyme activities: effect of fertilization and cropping practices. *Soil Biol. Biochem.* 17: 297-302.
- Bonde, T.A., Schnurer, J., and Rosswall, T. 1988. Microbial biomass as a fraction of potentially mineralizable nitrogen in soils from long-term field experiments. *Soil Biol. Biochem.* 20: 447-452.
- Bowren, K.E., Cooke, D.A. and Downey, R.K. 1968. Yield of dry matter and nitrogen from tops and roots of sweetclover, alfalfa and red clover at five stages of growth. *Can. J. Plant Sci.* 49: 61-68.
- Brookes, P.C., Landman, A., Pruden, G. and Jenkinson, D.S. 1985. Chloroform fumigation and the release of soil nitrogen: A rapid direct extraction method to measure microbial biomass nitrogen in soil. *Soil Bio. Biochem.* 17: 837-842.
- Ferguson, W.S. and Gorby, B.J. 1964. Effect of straw on availability of nitrogen to cereal crops. *Can. J. Soil Sci.* 44: 286-291.
- Ferguson, W.S. and Gorby, B.J. 1971. Effect of various periods of seed-down to alfalfa and brome grass on soil nitrogen. *Can. J. Soil Sci.* 51: 65-73.
- Heard, A.J. 1965. The effect of the nitrogen content of residues from leys on the amounts of available soil nitrogen and on yields of wheat. *J. Agric. Sci. (Camb.)* 64: 329-334.
- Jansson, S.L. and J. Persson, 1982. Mineralization and Immobilization of soil Nitrogen. In F.J. Stevenson (ed.) Nitrogen in agricultural soils. ASA, CSSA and SSSA Monograph No.22, Madison, WI.
- Jenkinson, D.S. and Ladd, J.N. 1981. Microbial biomass in the soil: Measurement and turnover. In E.A. Paul and J.N. Ladd (eds.) Soil Biochemistry. Vol. 5. Marcel Dekker, New York.
- McGill, W.B., Cannon, K.R., Robertson, J.A. and Cook, F.D. 1986. Dynamics of soil microbial biomass and water-soluble organic C in Breton L after 50 years of cropping to two rotations. *Can. J. Soil Sci.* 66: 1-19.
- Millar, H.C., Smith, F.B. and Brown, P.E. 1936. The rate of decomposition of various plant materials in soils. *Am. Soc. Agron. J.* 28: 914-923.

- Nannipieri, P. 1984. Microbial biomass and activity measurements in soil: Ecological significance. In M.J. Klug and C.A. Reddy (eds.) Current Perspectives in Microbial Ecology. Proc. 3rd Int. Symp. on Microbial Ecology. Amer. Soc. Microbiology, Washington, D.C.
- Parton, W.J., Persson, J. and Anderson, D.W. 1983. Simulation of organic matter changes in Swedish soils. In W. K. Lauenroth, G.V. Skogerboe and M. Klug (eds.) Analysis of Ecological Systems: State-of-the-Art in Ecological Modelling. Elsevier, Amsterdam.
- Paul, E.A. 1984. Dynamics of organic matter in soil. *Plant Soil* 76: 275-285.
- Paul, E.A. and Juma, N.G. 1981. Mineralization and immobilization of soil nitrogen by microorganisms. In F.E. Clark and T. Rosswall (eds.) Terrestrial nitrogen cycles. Ecol. Bull. 33, Stockholm.
- Schnurer, J. Clarholm, M. and Rosswall, T. 1985. Microbial biomass and activity in an agricultural soil with different organic matter contents. *Soil Bio. Biochem.* 17: 611-618.
- van Veen, J.A. and Paul, E.A. 1981. Organic carbon dynamics in grassland soils. 1. Background information and computer simulation. *Can. J. Soil Sci.* 61: 185-201.
- van Veen, J.A., Ladd, J.N., and M.J. Frissel. 1984. Modelling C and N turnover through the microbial biomass in soil. *Plant Soil* 76: 257-274.
- Vance, E.D., Brookes, P.C. and Jenkinson, D.S. 1987. An extraction method for measuring soil microbial biomass C. *Soil Biol. Biochem.* 19: 703-707.