EFFECT OF LONG-TERM APPLICATION OF MANURE AND P FERTILIZER ON SOME PHYSICAL, CHEMICAL AND BIOLOGICAL PROPERTIES OF A BLACK CHERNOZEM

C.A. Campbell[1], V.O. Biederbeck[1], J.W.B. Stewart[2],
M. Schnitzer[3] and F. Selles[1]

[1] Research Station, Research Branch, Agriculture Canada, Swift Current, Saskatchewan. S9H 3X2
[2] Saskatchewan Institute of Pedology, University of Saskatchewan, Saskatoon, Sask. S7N 0W0
[3] Chemistry & Biology Research Institute, Agriculture Canada, Ottawa, Ontario. K1A 0C6

INTRODUCTION

Two experiments were started in the 1940s to study the effect of monoammonium phosphate and barnyard manure on wheat production on an Indian Head Black Chernozemic clay. The study showed that with time, higher rates of manure amendments gave progressively higher yield increases than did lower rates of application (Spratt and McIver 1966). In the original study Spratt and McCurdy (1966) found that high rates of P fertilizer and all manure treatments increased $NaHCO_3$-extractable P in the 0-15 cm depth but had no effect at greater depths. Furthermore, no treatment affected organic P, C or N, nor NO_3-N at any depth.

It has been observed that the soil tilth of the manured plots was visibly superior to that of unmanured plots.

The objective of this study was to determine whether the obvious changes in soil tilth, and benefits on crop yields caused by long-term application of manure could be related to some of the physical, chemical and biological properties of a Black Chernozemic soil. A second objective was to compare manure effects with those resulting from the long-term application of P fertilizer.

MATERIALS AND METHODS

Description of Experiments

A field experiment with several rates of monoammonium phosphate applications was initiated in 1945 and another experiment with various rates of barnyard manure, alone and in combination with monoammonium phosphate, was started in 1947 (Spratt & McCurdy 1966). Both experiments were established in the same 2-ha field on a level to gently undulating, well-drained Chernozemic clay of the Indian Head soil association (Mitchell et al. 1944). The plots (6 m x 19 m) were randomized within blocks in a 3-year rotation of wheat-wheat-summerfallow, and replicated three times. The wheat crop that followed summerfallow, received monoammonium phosphate fertilizer (11-48-0) drilled with the seed while the barnyard manure was spread onto fall-disced stubble plots in early spring and then disced in during the first summerfallow operation. Subsequent summerfallow tillage was done with blade implements. The manure and the phosphate fertilizer were only applied once every 3 years to any one plot.

Soil samples were taken from the wheat on fallow plots on June 30, 1982. The main treatments sampled were 11-48-0 applied at 112 kg (product)/ha (hereafter called 112 MAP treatment), three manure treatments, viz., 13.44, 20.16 and 26.88 t/ha, hereafter referred to as 6BYM, 9BYM and 12BYM (Spratt & McCurdy 1966), and the check with no P or manure applied.

Soil Physical Properties

Soil bulk density was determined on samples taken from the top 30 cm of each replicate of the 0, 6 and 12BYM and 112 MAP treatments. Hydraulic conductivities were determined on 15-cm diameter, 15-cm deep cores of soil by the constant head method (Klute 1965) after the soil was saturated with 0.001 M $CaCl_2$ in a vacuum desiccator to remove entrapped air. In each case, two cores were taken per plot.

Soil Chemical and Biochemical Properties

Phosphorus. Phosphorus transformations and availability in samples of the untreated and treated soils was assessed by two separate methods. The first assessment was based on sodium bicarbonate extractable inorganic phosphorus (IP) by the method of Olsen et al. (1954). Separate soil samples also were analyzed by an extraction method (Hedley et al., 1982) which attempts to sequentially remove the more labile inorganic and organic phosphates (IP and OP) leaving more stable phosphates which can be determined following oxidative digestion. Total phosphate in the soil extracts was determined by digestion of the soil with H_2SO_4 and H_2O_2 (Thomas et al. 1967) followed by analysis on the Technicon Autoanalyzer.

Humic substances. Humic acids (HA's) and fulvic acids (FA's) were extracted from air-dried soil samples, separated and purified according to procedures described by Calderoni and Schnitzer (1982).

The humic constituents were characterized using several powerful modern chemical techniques, but these revealed few significant differences due to treatments, therefore they will not be presented here. One useful analysis that revealed significant treatment effects was acid hydrolysis of humin. Amino acids, amino sugars and ammonia were determined on the humic substances following hydrolysis with hot 6M HCl for 24 h, on an amino acid analyzer (Schnitzer and Hindle 1981). The samples were dissolved in Na-citrate buffer at pH 2.2. The column material was Beckman AA-10 spherical resin.

Natural ^{15}N Abundance. Four soil cores, each 3.8 cm in diameter and 2.40 m in length were removed from each replicate of the check, 6BYM, 9BYM and 12BYM. The cores were sectioned into 0-15, 15-30, and then 30 cm segments to a depth of 240 m. The different depth subsamples of each treatment plot were pooled, the soil samples air-dried immediately, lightly ground and sieved (< 2 mm). Representative samples of the first three depth increments were further ground to pass through a 100 mesh sieve for determination of total soil N including nitrate- and nitrite-N (Rennie and Paul 1971). The total N was reduced to NH_4-N for use in isotope assay as described by Selles and Karamanos (1986) and Selles et al. (1986).

Soil Biological Properties. Soil respiration, microbial biomass and rate of N mineralization were measured for the 0-7.5 cm soil segment of some manure treatments. Respiration was determined in biometer flasks on moist soil equivalent to 50 g oven-dry weight per sample as outlined by Biederbeck et al. (1984). Potentially mineralizable N (N_o) and the N mineralization rate constant k, were determined after intermittently incubating and leaching soil over 18 weeks at 35°C (Campbell et al. 1981). Soil microbial biomass was determined by the chloroform fumigation-incubation technique (Jenkinson and Powlson 1976) as modified by Biederbeck et al. (1984).

RESULTS

Soil Physical Properties

Neither bulk density nor hydraulic conductivity were significantly affected by manure or P treatments (data not shown).

Soil Chemical and Biochemical Properties

Soil phosphorus. Total phosphate whether assessed by summation of the individual results of the sequential fractionations or by digestion showed the effect of manure treatment in the 0-7.5 and 7.5-15 cm samples (Table 1). Significant changes were also apparent in the $NaHCO_3$-extractable P with addition of manure significantly increasing the amounts of P found in

Table 1. Effect of manure on soil P‡ fractions at various depths‡‡

Treatment	§Soil $NaHCO_3$-P_i			†Resin - P			†$NaHCO_3$-P_i			†$NaHCO_3$-P_o			†0.1 M NaOH-P_i		
	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30
Check	13.7	9.3	5.0	24.9	12.7	5.5	12.8	6.8	2.3	10.2	9.7	8.7	15.3	9.6	4.9
6 BYM	16.0	10.0	7.7	30.4	14.2	12.8	17.5	7.9	5.3	10.2	11.9	12.9	16.8	10.9	2.7
9 BYM	20.7	13.7	6.7	44.2	20.1	6.2	24.2	11.2	3.6	11.4	9.4	7.1	21.9	13.7	3.8
12 BYM	21.3	10.0	5.7	39.9	13.6	5.4	21.3	7.0	2.4	11.5	9.0	6.8	21.1	9.0	3.8
	M^* ; D^* ; M x D^*			M^* ; D^* ; M x D^*			M^* ; D^* ; M x D^*						D^*		

Treatment	†0.1M NaOH P_o			†0.1M NaOH Son-P_i			†0.1 M NaOH Son-P_o			†0.1 M HCl-P			†Residual-P		
	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30
Check	57.9	48.7	40.0	3.6	2.9	2.2	8.3	7.1	5.8	272	264	262	363	352	326
6 BYM	58.8	40.0	25.0	4.4	2.7	2.5	9.2	7.8	7.2	278	249	247	377	367	353
9 BYM	61.3	50.9	23.5	4.4	3.2	1.7	8.0	6.5	3.9	292	285	313	377	356	316
12 BYM	62.8	49.1	27.1	4.2	3.0	2.1	8.1	5.5	3.3	280	277	296	379	359	325
	D^*			D^*			D^*						D^*		

Treatment	†Fractions			Soil digest P			% recovery of P		
	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30	0-7.5	7.5-15	15-30
Check	763	714	659	693	640	610	110	111	111
6 BYM	803	730	705	706	649	626	114	113	113
9 BYM	844	756	679	765	685	604	110	110	112
12 BYM	823	732	671	736	645	605	110	113	111
	M^* ; D^*			D^*					

* = significant $P < 0.05$; M = manure effect; D = depth effect.

§ This fraction extracted by Olsen method.

† These fractions extracted according to Hedley et al. 1982

‡ Except for recovery, all units are in µg/g soil

‡‡ All depths in cm

the first two soil depths (0-7.5 and 7.5-15). Below this depth samples tended to contain more P, but no significant trend could be found due to treatment.

A more complete assessment of the fate of applied P in BYM can be obtained from the results of the Hedley extraction (Table 1). Values of resin extractable P were increased 25% by the addition of 6 BYM in the upper soil layer (0-7.5) and by 10% in the 7.5-15 cm layer while it approximately doubled the amount in the 15-30 cm layer. Addition of a further quantity of BYM increased resin-P still further in both upper layers but there was no indication of movement into the 15-30 cm layer. Quantities of resin P extracts, were approximately twice the quantities removed by the $NaHCO_3$ Olsen technique.

Soil organic matter. Only soil from the 0-7.5 cm depth was analyzed. Organic N was unaffected by treatment, averaging 0.23% in the check, 100 MAP, 6BYM and 12BYM samples. Organic carbon was significantly ($P < 0.05$) increased by manure but not by fertilizer phosphorus. Carbon values for the check, 100 MAP, 6 BYM and 12 BYM treatments were 2.35%, 2.38%, 2.61%, and 2.58%, respectively.

Humic components. The highest rate of manure increased the HA component of the soil and also increased the proportion of C in the HA (Table 2).

The distribution of the N in HA (HA-N) among the major N components of the HA (Table 3) showed that there was about 21% more of the HA-N in the amino acids and/or proteins of HA's extracted from manure-treated soils than from the check and P fertilized soils. The manure-treated soils had 16-27% more of the HA-N as amino sugars than had the check and P-treated soils and about 17% more of the HA-N as NH_3, but they had 52% less of the unidentified HA-N. While the total N content of soils treated with manure was relatively low, these soils were richer than normal in amino acid N (56-58%).

Over 60% of the soil C and N was unextracted and remained in the humin (Table 4). The 12BYM treatments increased the proportion of the soil N in the humin and the C content of the humin fraction. In contrast to the HA, the distribution of Humin-N among its major N components was unaffected by treatment (Table 5). The HA was richer in the proportions of amino acid- and poorer in amino sugar-N and NH_3-N than the humin (Tables 3 and 5). In the humin the proportions of the total N found as amino acid-N and ammonia-

Table 2. Effect of manure and P fertilizer on weight of HA and proportion of soil C in HA of 0-7.5 cm depth

Treatment	Weight of HA (mg/125 g soil)	% of soil C in HA
Check	686	13.1
6 BYM	744	12.7
12 BYM	926*	16.1†
100 MAP	596	11.1

†, * denote significant difference from check at P < 0.10 and P < 0.05, respectively.

Table 3. Distribution of the HA-N among major N components

Treatment	Type of N						
	Amino acid	Amino sugar	Protein	NH_3	Unidentified	Total	Total
	- - - - - -	- - - - - -	%	- - - - - -	- - - - - -	- - - -	μg N / 10 g HA
Check	48.6	3.2	53.4	20.4	27.8	100	294
6 BYM	57.8	3.9	63.6	25.6	12.7	100	239
12 BYM	55.8	3.5	61.4	22.5	18.2	100	251
100 MAP	45.2	2.6	49.7	20.7	31.5	100	305

Table 4. Effect of manure and P fertilizer on humin C & N in 0-7.5 cm depth

Treatment	% of soil C in humin	% of soil N in humin	% C in humin	% N in humin
Check	63.0	58.5	1.85	0.17
6 BYM	57.7	62.0	1.88	0.18
12 BYM	67.0	67.1*	2.09**	0.20
100 MAP	64.4	63.7	1.80	0.17

*, & ** denote significant difference from check at $P < 0.05$ and $P < 0.01$, respectively.

Table 5. Distribution of the humin-N among major N components

Treatment	Type of N					
	Amino acid	Amino sugar	Protein	NH_3	Unidentified	Total
			%			
Check	36.8	4.5	40.5	37.8	21.3	100
6 BYM	36.7	4.3	40.4	36.0	23.1	100
12 BYM	36.8	4.5	40.5	32.6	26.1	100
100 MAP	36.7	4.1	40.4	33.8	25.4	100

N were equal; this is unusual (Table 5).

There was no effect of treatment on the amino compounds of the HA hydrolysate (data not shown). However, manure increased the absolute amounts of most of the amino acids and tended to increase the amino sugars hydrolysed from the humins (Table 6). Comparison of the molar ratios [(amino acid N/total amino acid N) x 100] of the various acids showed that the relative distribution of these compounds was not affected by treatment.

Natural ^{15}N abundance. Neither the total N (Table 7, top) nor NO_3-N to 240 cm depth (data not shown) were significantly affected by manure treatments, although manure tended to increase N in the 0-15 cm depth. The

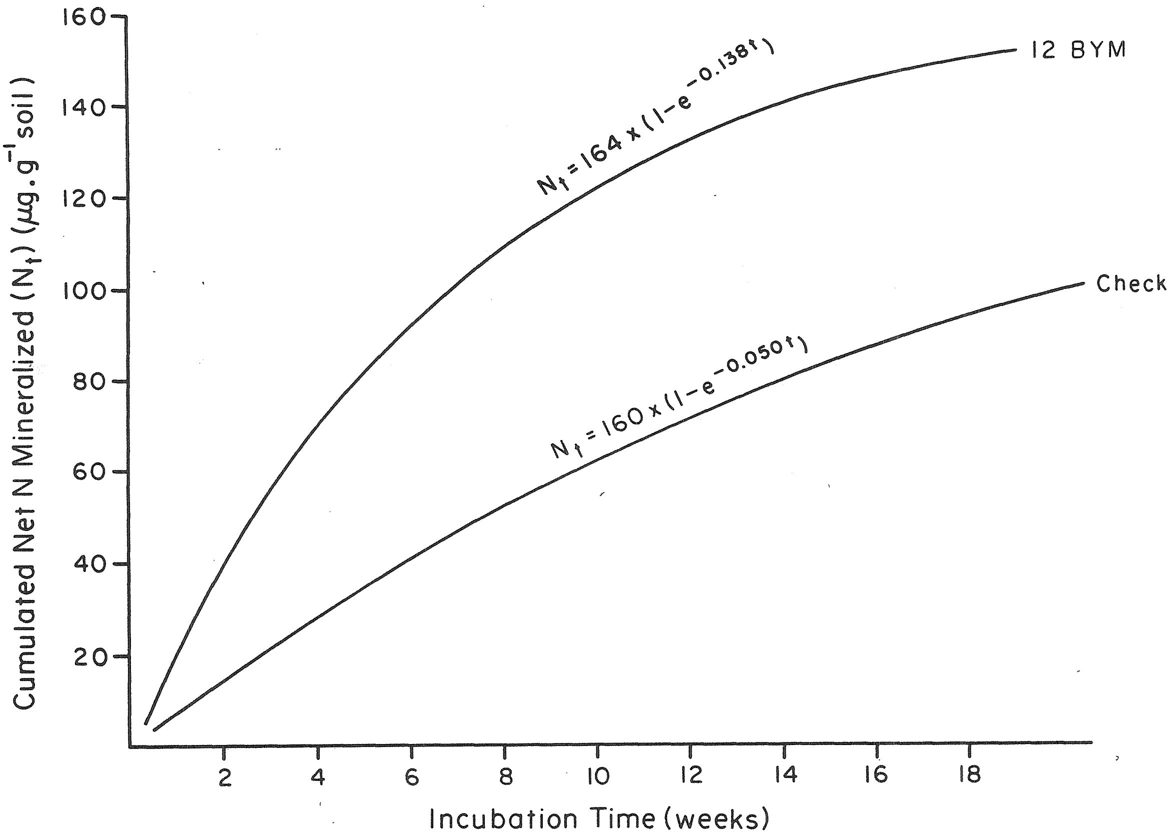

Fig. 1. Effect of manure on potentially mineralizeable N and rate constant for 0-7.5 cm depth incubated at 35°C.

delta ^{15}N of total N in the 12BYM treatment was increased significantly over ^{15}N abundance in the check in the top 30 cm of soil (Table 7, bottom).

Soil Biological Properties

Neither microbial biomass C or N, or CO_2 respiration for the top 7.5 cm of soil was influenced by manure or P treatments (data not shown). The average biomass C for the check, 100 MAP, 6BYM and 12BYM treatments were 46.9, 43.7, 44.6 and 44.2 mg C/100 g soil, respectively; respiration in the same order was 100.6, 98.2, 105.5 and 105.7 µg CO_2-C/g soil/10 days.

Nitrogen mineralization for soil from the 0-7.5 cm depth was greater ($P < 0.05$) for 12BYM treatment than for the check. Calculations showed that

Table 6. Effect of manure and P fertilizer on amount of amino acids and amino sugars in humin hydrolysates in 0-7.5 cm depth

Amino acids	Treatment							
	Check		6 BYM		12 BYM		100 MAP	
	μg N / g soil	molar ratio†	μg N / g soil	molar ratio	μg N / g soil	molar ratio	μg N / g soil	molar ratio
Aspartic acid	81.5	13.1	89.8	13.5	90.2	12.4	83.7	13.4
Threonine	27.6	4.4	29.9	4.5	32.3	4.5	28.3	4.5
Serine	38.4	6.2	38.3	5.8	45.7	6.3	41.3	6.6
Glutamic acid	54.5	8.8	59.6	9.0	65.4	9.0	55.3	8.8
Proline	26.0	4.2	28.6	4.3	31.6	4.4	26.3	4.2
Glycine	80.7	13.0	88.2	13.3	93.7	12.9	82.0	13.1
Alanine	57.3	9.2	62.0	9.3	65.5	9.0	57.7	9.2
Valine	31.3	5.0	34.5	5.1	39.2	5.4	31.3	5.0
Methionine	7.4	1.2	7.7	1.2	8.5	1.2	6.7	1.1
Isoleucine	16.8	2.7	18.9	2.8	21.2	2.9	16.7	2.7
Leucine	27.9	4.5	31.1	4.7	35.4	4.9	27.7	4.4
Tyrosine	0.5	0.1	1.7	0.3	2.0	0.3	0.7	0.1
Phenylalanine	14.0	2.3	15.8	2.4	17.9	2.5	11.3	1.8
Histidine	39.9	6.4	32.0	4.8	40.3	5.6	41.3	6.6
Lysine	60.1	9.7	61.0	9.2	67.1	9.3	58.3	9.3
Arginine	57.3	9.2	64.8	9.8	68.9	9.5	57.7	9.2
Total	621		664		725**		626	
Amino Sugars								
Glucosamine	48.2		53.6		58.5		42.5	
Galactosamine	26.3		24.6		30.4		28.0	
	74.5		78.2		88.9		70.5	

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† Molar ratio = (amino acid N/total amino acid-N) x 100

potentially mineralizable N was the same for both treatments (164 vs 160 μg/g soil) but the rate constant was much greater for the 12BYM treatment (Fig. 1).

DISCUSSION

Our inability to show any differences in bulk density or hydraulic conductivity might not be so surprising since large and frequent (> 20 tons/ha/yr) applications of manure for many years (> 10 yr) are required before such differences become measurable (Sweeten and Mathers 1985; Russell 1973; Khaleel et al. 1981).

Table 7. Effect[†] of manure on soil N and natural ^{15}N abundance in soil profile

Treatment	Depths (cm)			
	0-15	15-30	30-60	60-90
		Total-N ($mg.g^{-1}$)		
Check	1.82	1.55	1.21	0.90
6 BYM	2.03	1.48	1.10	0.89
9 BYM	2.15	1.53	1.11	0.87
12 BYM	2.01	1.45	1.02	0.80
		Natural ^{15}N abundance ($\delta a^{15}N$) (‰)		
Check	6.85	6.60	5.94	5.35
6 BYM	7.00	6.36	6.05	5.26
9 BYM	7.10	6.30	5.78	5.03
12 BYM	8.57	7.80	6.64	6.27

[†] Manure significant ($P < 0.05$); depth significant ($P < 0.01$); LSD = 0.76.

The extra P supplied in the manure accumulated mainly in labile inorganic P compounds that were extracted with the resin and bicarbonate. The effect of additional BYM on % C was small, though significant; therefore, no significant changes were expected or noted in organic P forms. These results are similar to those reported earlier by Spratt and McCurdy (1966) except that, using the Hedley extraction, we were able to identify manurial induced increases in phosphates even in the 15-30 cm depth.

We were only able to show small increases in organic C and no changes in total N resulting from manure treatments. Again, this can be credited to the relatively low rate and infrequent applications of manure and the relatively high level of organic matter already in this soil (Anderson and Peterson 1973).

There was convincing evidence of manure effects through its influence on amino compounds. In a somewhat similar study and analysis, Khan (1971) reported that the application of manure at 44.8 metric tons/ha every 5 years over a 39-year period to a fallow-wheat and a 5-year grain-legume rotation, increased the amount of amino sugars in the soils but did not affect the amino acids (soils were hydrolyzed with 6 M HCl). Khan (1971) also found no effect of annual applications of N-P-K-S on the amino compounds in his study. Our results were similar to Khan's with respect to the effect of mineral fertilizers, but we obtained significant increases in amino acids and to a lesser extent for amino sugars due to manure applications. Our results are in agreement with related findings of others (Bremner 1955; Stewart et al. 1963; Sowden and Ivarson 1959; Gupta amd Reuszer 1967) who reported that most of the N immobilized in soil organic matter enters the acid soluble fraction which contains amino acids and amino sugars. However, as observed by Khan (1971), the chemical nature of the soil organic matter was unchanged since the relative distribution of the amino acids (molar ratios) was not affected by manuring or P fertilization.

Although manure treatments had no significant effects on total N nor on NO_3-N in the soil profile, use of the natural ^{15}N abundance technique confirmed that manure comprised an integral, though not substantial part of the soil N in the 0-30 cm depth.

Although the potentially mineralizeable N was not affected by manuring, net N mineralization was increased through the effects on the mineralization rate constant (k). We expected to find further supporting evidence in terms of an increase in microbial biomass N but did not. This may be due to the timing of sampling; microbial biomass is dynamic and closely dependent on weather changes (Lynch and Panting 1979), thus we may have missed a period when differences were greater. Martyniuk and Wagner (1978) have shown that manure significantly increases microbial numbers (plate counts) but changes vary markedly with time.

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