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Deaminase activity in arable soils

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Abstract

Deamination of 1,2 diamino-4-nitrobenzene (DANB) was assayed in 22 arable soils. Soil texture, pH, total nitrogen and organic matter content were measured, as were the soils' nitrogen mineralisation potentials. Deaminase activity was strongly correlated with clay content, suggesting an association of deaminase with this soil fraction. There was no relationship between deaminase activity and other soil parameters. Deaminase activity was a poor predictor of nitrogen mineralisation potential. This was due to the difference in accessibility of the amino groups in DANB and of native soil organic matter.

Introduction

Understanding the supply of nitrogen from soils, and determining crop fertiliser requirements for those soils, involves the quantification of soil nitrogen mineralisation. For instance, modelling studies often rely upon values from laboratory-derived soil mineralisation potentials in order to simulate field-scale transformations of nitrogenous compounds (Addiscott and Whitmore, 1987). At present, the nitrogen mineralisation potentials of soils are usually based upon the determination of inorganic nitrogen released from the soil organic matter during laboratory incubations (Addiscott, 1983; Stanford and Smith, 1972).

These incubation studies unfortunately take many weeks to complete, and measure only a nett nitrogen mineralisation potential. Losses of mineral nitrogen due to reimmobilisation, or gaseous losses by denitrification or ammonia volatilisation are not easily quantified, although they may have significantly affected the measured mineralisation potential.

An enzymatic assay, based on the deamination of the exogenous substrate, 1,2 diamino-4-nitrobenzene (DANB), has been proposed as a means of examining soil nitrogen transformations (Killham and Rashid, 1986). This method has a comparatively short incubation time (20 h), and does not rely upon the determination of inorganic nitrogen, instead it relies on the photometric measurement of the decrease in absorbance at 405 nm, as amino groups are removed from the aromatic nucleus of DANB. The authors suggest that the deaminase method may be useful in characterising nitrogen flow and nitrogen availability in a wide range of soil types. The purpose of this work was to investigate the relationship between deaminase activity and various soil properties, and then to compare the deaminase assay with an incubation method for estimating nitrogen mineralisation potential.

Materials and methods

Top (0–30 cm) soil samples were taken from 22 sites in England in the autumn of 1987. These soils were all under arable cropping systems with various management histories. The soils were air dried and sieved (<2 mm). Representative subsamples were taken and analysed for; particle size distribution (MAFF/ADAS, 1986), Kjeldahl

Table 1. Properties of soil used in incubations

Soil	Sand[a] (%)	Silt[a] (%)	Clay[a] (%)	Organic matter (%)	Kjeldahl nitrogen (%)	pH
Royston	36	44	20	3.1	0.25	7.5
Shifnal	78	12	10	2.2	0.15	6.4
Elkesley	74	17	9	1.9	0.12	6.5
High Ercall	58	27	15	2.9	0.22	6.9
Easton	34	36	30	5.6	0.23	5.4
Escrick	85	8	7	3.3	0.17	6.4
Ridlington	40	46	14	2.2	0.12	6.9
Chasepool	81	11	8	2.9	0.13	6.0
Raskelf	80	9	11	2.8	0.12	6.5
Yaxley	22	32	46	51.4	2.30	6.7
Heslington	81	9	10	3.4	0.14	6.0
Whaplode	33	49	18	3.3	0.15	6.6
Loddon	49	30	21	2.4	0.19	6.6
Bedingfield	59	16	25	3.9	0.19	7.3
Ramsey St. Mary	6	47	47	14.1	0.90	7.2
Shereford	81	10	9	1.6	0.10	7.2
Bracebridge	60	18	22	4.1	0.20	7.3
Brancaster	76	13	11	2.2	0.14	7.1
Mareham le Fen	87	6	7	2.7	0.17	6.7
East Wretham	87	7	6	2.1	0.16	6.5
Terrington	16	54	32	3.6	0.18	7.0
Norton	15	26	17	2.3	0.15	7.3

[a] Expressed as % of mineral matter.

nitrogen (MAFF/ADAS, 1986), loss on ignition at 400°C and pH, (10 g soil in 25 ml 0.01 *M* $CaCl_2$). These properties are given in Table 1.

A further sub-sample of sieved, air dried soil (1 g) was incubated with 60 ppm DANB reagent in 200 m*M* phosphate buffer (10 mL), adjusted to pH 6.5. The soil/buffer slurry was incubated at 25°C for 20 h, with regular agitation. After incubation the slurry was extracted by shaking with methanol (2 × 15 mL) for 15 minutes, and filtering through Whatman No. 1 filter paper. The absorbance of the filtrate at 405 nm was determined and compared with suitable DANB standards made up in phosphate buffer. The absorbance of blanks containing soil and phosphate buffer but no DANB, were subtracted to minimise the effect of coloured soil components in the extract. Enzymic activity was expressed as μg substrate deaminated per 100 g of oven dry soil per hour. Five replicate assays were performed for each soil. A bacteriostatic agent was not used during the incubation.

The nitrogen mineralisation potential of each soil was also determined using an incubation method (Addiscott, 1983). Representative sub-samples (15 g) of field moist soil from each site were sieved <3 mm and placed in glass vials. The soils were then incubated in a box lined with moistened filter paper, at a constant 20°C. Duplicate samples were removed after 0, 2, 4, 6, 8 and 10 weeks of incubation. Their inorganic nitrogen content was determined with a Technicon Auto Analyser following extraction with 2 *M* KCl. Regression analysis of accumulated mineral nitrogen against time was used to determine each soil's nitrogen mineralisation potential, expressed as μg nitrogen per 100 g of oven dry soil per hour.

Results and discussion

Rates of deamination and mineralisation are shown in Tables 2 and 3. Deaminase activities were similar to those reported by other workers (Killham and Rashid, 1986). Mineralisation potentials were similar to those obtained for other soils (Adiscott, 1983). Generally, there were no clear relations between mineralisation potentials and soil properties or previous man-

Table 2. Deaminase activity

Soil	Deaminase activity (μg DANB 100 g^{-1}h^{-1} ± S.E.M.)
Royston	2420 ± 24
Shifnal	1170 ± 22
Elkesley	550 ± 40
High Ercall	840 ± 25
Easton	2810 ± 85
Escrick	480 ± 44
Ridlington	1400 ± 22
Chasepool	590 ± 17
Raskelf	1150 ± 27
Yaxley	3870 ± 69
Heslington	630 ± 1
Whaplode	1840 ± 15
Loddon	1590 ± 77
Bedingfield	1510 ± 49
Ramsey St. Mary	3150 ± 16
Shereford	2030 ± 43
Bracebridge	2860 ± 9
Brancaster	2190 ± 17
Mareham le Fen	690 ± 18
East Wretham	1820 ± 23
Terrington	2520 ± 53
Norton	1310 ± 16

agement history. The soils from Ramsey St. Mary and Yaxley did however have the highest organic matter content and mineralisation rate.

Deaminase activity was significantly correlated with the clay content (Fig. 1). The correlation was not improved by incorporating other soil factors into the regression equation, or by considering previous management, (for instance, a history of leys, farm yard manure or slurry application).

Previous work (Killham and Rashid, 1986) had shown that toluene had little effect on the DANB assay of deaminase. It was inferred that the enzyme system was extracellular and was unaffected by cell proliferation during the incubation. It was also suggested that the thermal stability of the deaminase system was due to colloid protection. Data from Fig. 1 would support this.

The association of other soil enzymes with the colloidal fraction of soil has been well documented. It has been found (Ladd and Butler, 1972) that protease activity is positively correlated with

Table 3. Mineralisation potential as determined by laboratory incubation. Data fitted to straight line equation $y = bx + c$

Soil	Intercept μgN 100 g^{-1} soil	Slope μgN 100 g^{-1}h^{-1}	R^2 %	Variance Ratio F
Royston	1085.0	1.316	92.4	121.8
Shifnal	1004.3	0.647	79.4	38.5
Elkesley	1122.6	0.644	86.2	62.4
High Ercall	1605.2	0.975	98.9	863.7
Easton	1098.6	0.707	85.9	61.1
Escrick	1005.0	0.670	96.8	271.2
Ridlington	448.3	0.732	96.9	307.8
Chasepool	979.1	0.630	89.2	82.7
Raskelf	679.0	1.043	93.8	121.5
Yaxley	6133.8	4.411	99.7	3803.8
Heslington	671.7	0.738	85.0	56.6
Whaplode	557.9	1.258	99.3	1461.7
Loddon	801.9	0.805	66.4	19.7
Bedingfield	664.1	0.505	89.7	87.2
Ramsey St. Mary	1298.1	1.418	94.8	182.4
Shereford	842.0	0.686	90.2	74.1
Bracebridge	1363.8	0.631	95.7	222.7
Brancaster	548.1	0.430	78.0	35.5
Mareham le Fen	568.0	1.033	96.9	247.7
East Wretham	830.7	1.356	94.2	162.7
Terrington	715.5	0.384	96.2	251.2
Norton	723.1	0.376	61.1	15.7

All regressions were highly significant, $P < 0.01$.

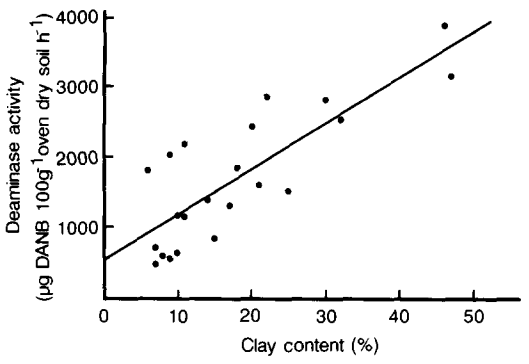

Fig. 1. The relationship between deaminase activity and clay content. The regression equation was Deaminase activity = 564.5 + 64.9 (Clay %). The regression accounted for 66.1% of the variation, and was significant at the 0.1% level.

clay content for the substrate benzyloxycarbonyl phenyl alanyl leucine. No significant correlation was found between protease and total organic nitrogen or carbon with this substrate. However, with the substrate benzoyl arginine amide, stronger correlations were found with organic carbon and nitrogen than with the clay content. Other workers (Burns *et al.*, 1972) showed that soil urease activity is associated with the clay fraction of soil. These workers also showed that the urease enzyme was made resistant to pronase attack by association with soil organic matter. They also noted that urease activity was initially increased by absorption onto bentonite. Whilst a significant correlation was found between urease activity and clay content, a better correlation was obtained with soil organic carbon (Zantua *et al.*, 1977). In the present work the relationship between deaminase activity and soil organic matter was weak. Below about 6% organic matter, deaminase activity was independent of organic matter content, though above 6% there appeared to be some correlation. However the two high organic matter soils, from Yaxley and Ramsey St. Mary, may however have given undue weight to this relationship. Similarly there was no relationship between deaminase activity and total nitrogen. Variation in urease activity was accounted for by clay and organic matter content in the presence of toluene. When toluene was absent only consideration of organic carbon levels could explain variation in urease activity (Dalal, 1975). This suggests the association of the ureolytic biomass with the soil organic carbon fraction. Other workers (Kanazawa and Filip, 1986) separated an arable soil into organic and mineral particle size fractions and then investigated β-glucosidase, β-acetylglucosaminidase and proteinase activities. Whilst the highest activities were associated with the largest organic particles, the most active mineral fraction contained the silt-clay particles. The results shown in Figure 1 support the hypothesis that deaminase is closely associated with the soil colloids (Killham and Rashid, 1986), which protect it from biological and physical/chemical degradation. The exact nature of this association is not clear, although there are many possible mechanisms (Weethall, 1975).

Deaminase activity and mineralisation rate as determined by laboratory incubation were poorly correlated (Fig. 2). There was no correlation if the results from the Yaxley soil were removed from the data analysis. The rate of nitrogen release from DANB was two orders of magnitude greater than that from soil organic matter, suggesting DANB was much more accessible to the deaminase system than were amino groups within native soil organic matter.

It has been suggested (Burns *et al.*, 1972) that the exchange of substrate and products to the urease/organic matter complex is by diffusion. A similar mechanism may operate for DANB. Most soils contain only trace amounts of access-

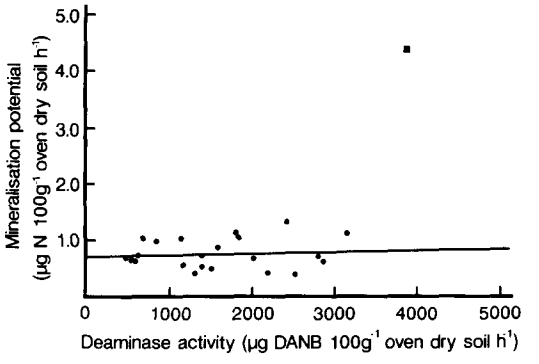

Fig. 2. The relationship between mineralisation potential and deaminase activity. The Mineralisation potential (with Yaxley, ■ symbol) = 0.180 + 0.00047 (Deaminase activity). The regression accounted for 28.3% of the variation and was significant at the 5% level. The Mineralisation potential (without Yaxley) was 0.698 + 0.00007 (Deaminase activity). The regression accounted 3.2% of the variation and was not significant.

ible amino groups, principally as free amino acids or amino sugars. The coloured KCl extracts of the peat soils, Ramsey St. Mary and Yaxley, do suggest however that there may be more soluble carbon (and nitrogen) available. Most amino nitrogen will occur combined in stable polymeric compounds, protected from enzymic attack by association with other soil components. Consequently, before a colloid-bound deaminase system could deaminate a substrate, the substrate would first have to be converted into a more mobile form. This process would rely on an array of enzymic and abiotic processes, and is likely to be the rate limiting step for mineralisation. The high mineralisation rate found for Yaxley may be due to its having a large amount of nitrogen in a soluble and consequently available form.

The suitability of this method for determination of deaminase activity may depend on the pH of the buffer. At low pH's (<5.5) DANB becomes unstable and loss of colour may occur by non-enzymatic mechanisms. This process may in part explain the residual deaminase activities found in autoclaved soils (Killham and Rashid, 1986), and also noted in this work (data not shown). Autoclaving did however remove some 90–95% of the deaminase activity, showing that this was still a largely biologically mediated reaction. The pH of the phosphate buffer used in this work should have minimised any effect due to substrate instability.

In conclusion it would appear that the deaminase system is associated with the colloidal fraction of the soil. The deaminase system whilst being an important component of the nitrogen mineralisation process, is only one of many such components and it does not satisfactorily represent the combined effect of all the components. From preliminary nitrogen mineralisation studies (Killham and Rashid, 1986), it was suggested that the deaminase method may be of use in studying nitrogen flow and availability. The present work would suggest that the deaminase assay cannot predict nitrogen mineralisation potential *per se*. Determination of accumulated inorganic-nitrogen during incubation, despites it's faults, is a more useful method.

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