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Nitrogen mineralization in soil from perennial grassland measured through long-term laboratory incubations

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SUMMARY

Under perennial grasslands, nitrogen contained in organic matter becomes available at varying rates via mineralization throughout the growing season. The amount of N present at any given time indicates only the quantity immediately present, and does not include N which has already been removed either by leaching or uptake into the plant system, nor the N which will become available as organic matter breaks down over time. Long-term aerobic laboratory incubation methods have been used successfully to estimate potential N mineralization under various cropping conditions. They had not been used successfully, however, to estimate potential N availability under perennial grassland.

In this research, soil samples from two long-term perennial grassland sites were taken before and after N fertilizer application at rates of 0, 175, 350 and 525 kg/ha. The soils were incubated in the laboratory at 35 °C and were eluted at 2, 4, 8, 12, 16, 22 and 30-week intervals, the length of time prescribed for determining N mineralization potential. Because a plateau had not been reached, incubation was allowed to continue for 198 weeks and 148 weeks for the pre- and post-N samples, respectively. Total N was high, as was soil organic matter in both sets of soil samples. Nitrogen mineralization potential was underestimated after 30 weeks of incubation, and overestimated after 148 weeks. The closest agreement between N measured and the estimated N mineralization potential, came after 198 weeks of incubation. This study confirmed the high N-supplying capacity of soil under long-term perennial grasslands. It also indicated that the recommended 30-week period needed to estimate N mineralization potential under other cropping systems was insufficient for a perennial grassland soil. Cumulative differences in N mineralization were found with varying rates of N fertilizer application, but these differences were rarely seen on an individual weekly basis, nor were they significant at the termination of the experiment. The response to N application differed by site.

INTRODUCTION

Under a perennial grassland, both the quantity of nitrogen (N) present and its availability influence decisions on N fertilizer recommendations. Managed perennial grasslands, which have been in continuous sod for several years and which may have received fertilizer or organic amendments, have a high organic matter (OM) component capable of releasing considerable amounts of N upon mineralization. Quantities up to 4000 kg N/ha have been estimated to become available from a permanent grassland over several years after it is ploughed (Whitmore *et al.* 1992). By contrast, soils which have been ploughed and planted with arable crops continuously, have seen their OM content decline considerably. A long-term study in England has documented a decline in soil carbon (C) from 1·5% in 1876 to 0·8% in 1959 after continuous cereal cropping (Johnston *et al.* 1994). Periodic determinations of soil N have not been helpful in estimating the amount of N which has been or will become available to a grassland during the growing season. Well-established root systems take up inorganic N in the form of ammonium (NH_4^+) and nitrate (NO_3^-) as it becomes available. The rate of its availability varies considerably, depending upon many factors, such as the quantity of OM present (Paustian & Bonde 1987), soil temperature and

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moisture (Stanford *et al.* 1973; Stanford & Epstein 1974), soil texture (Hassink 1992*b*), soil pH (Gonzalez-Prieto *et al.* 1992) and previous management practices such as grazing or cutting (Hassink 1992*a*).

Reviews of methods for measuring soil N have been described by Bremner (1965), Keeney (1982) and recently by Bundy & Meisinger (1994) who have given a comprehensive review of the current status of N-availability indices. Jarvis *et al.* (1996) have looked at N mineralization in temperate agricultural soils and given a thoughtful summary of the processes involved, as well as the laboratory, field, and modelling approaches available to measure it. Various laboratory techniques have been developed which quantify potentially mineralizable N before it is lost from the soil system. Waring & Bremner (1964) used short-term waterlogged anaerobic conditions, measuring potentially available N as NH_4^+. Stanford & Smith (1972) developed a long-term aerobic laboratory incubation method for both NH_4^+ and NO_3^- using ideal temperature and moisture conditions for N mineralization and nitrification. Their method called for N elutions over successive weeks following laboratory incubation at 35° for a total of 30 weeks. The fraction of potentially mineralizable N (N_0) was found to comprise between 5 and 40% of the total N contained in the soil, and the most reliable estimate of the rate constant k was 0·054 per week, meaning that N was released at an average rate of 5·4% of the total soil N per week. While these results were consistent for most of the soils tested, extreme variation in soil temperature and moisture conditions (Garwood & Tyson 1973), as well as organic amendments and soil type (Stanford & Epstein 1974; Griffin & Laine 1983; Chae & Tabatabai 1986) can limit the effectiveness when applying these results to estimate potentially mineralizable N in the field (Garwood & Williams 1967). Attempting to estimate field N-mineralization from laboratory incubations suffers the obvious limitation of eliminating the unpredictable flushes of N-release under drying and re-wetting conditions, as well as the static nature of the soil samples, and not accounting for N from additional OM sources (Campbell *et al.* 1988; Hatch *et al.* 1990, 1991). A recent review of the laboratory techniques for determining rates and rate constants of N mineralization (Cabrera *et al.* 1994) leaned toward a modelling approach, which emphasized soil moisture and temperature, but drew heavily on the long-term incubation method of Stanford & Smith (1972). A modelling approach based on soil thermal units has been introduced and successfully tested by Honeycutt in Maine (Honeycutt & Potaro 1990; Honeycutt 1999). Using this model, correlations between N mineralization, DM production and N turnover, with soil thermal units, have been demonstrated with data from England and New Zealand (Clough *et al.* 1998). But the challenge of quantifying the many variables which influence N mineralization, incorporating them into a model, and rapidly producing a N recommendation which a farmer could use in his current cropping system, remains.

This study was designed to quantify varying rates and amounts of N which could become available through mineralization in a perennial grassland. Such information could be used to predict long-term N release in permanent pasture and hay fields. We have used the long-term aerobic incubation method of Stanford & Smith (1972) to compare the potenti9al N-supplying capacity of soils supporting two perennial grasslands, before and after N-application. Stanford & Smith (1972) had performed their analyses on soils subjected to a variety of management practices, including arable crops and crop-grassland rotations. Paustian & Bonde (1987) used the method to differentiate various rates of active OM breakdown on soils having received different organic amendments, while evaluating length of incubation and treatment of the initial mineralization phase as potentially confounding the estimation of N_0 and k. Because of the high OM content of soils under perennial grasslands, and their potentially high N-supplying capacity, we sought to compare N_0 and k values after 30 weeks, both before and after various rates of N fertilizer application. Our goal was to follow the elution schedule prescribed by Stanford & Smith (1972), but to allow the incubations to continue over longer periods of time if necessary. In this way N_0 and k could be determined and compared with previous studies after 30 weeks, and the specific long-term N-supplying capacity of perennial grasslands could be compared in terms of actual N mineralized over several years, following fertilizer N treatments.

MATERIALS AND METHODS

Site procedures

The research was carried out at two sites at the University of Connecticut Plant and Soil Science Research Farm, located in Storrs, Connecticut, USA (41° 48′N, 72° 15′W). The first site had originally been seeded to reed canarygrass (*Phalaris arundinacea* L.) (RCG) and the second to tall fescue (*Festuca arundinacea* Schreb.) (TF). Both sites had been in continuous sod for more than 16 years, and had been treated prior to this experiment with *Crossbow* (2,4-dichlorophenoxy acetic acid and 3,5,6-trichloro-2-pyridinyloxyacetic acid), a broadleaf herbicide used to remove any legumes present in the plots. The soil was a Paxton fine sandy loam classified as a coarse-loamy, mixed, active, mesic Oxyaquic Dystrudept.

Soils were analysed initially (April 1996) and at the end of the growing season (October 1996). Phosphorus fertilizer was applied on 6 May 1996 to supply 49 kg

P/ha. Potassium was applied in split applications with 93 kg K/ha being applied on 6 May 1996 and 74 kg/ha being applied on 31 July 1996. Soil analysis data are presented in Table 1.

At each site, an experiment was set out using a randomized complete block design with four replications. Experimental treatments were four N rates: 0, 175, 350 and 525 kg N/ha, applied as urea. The N was applied in split applications: one half was applied as growth began in early spring, one quarter applied after the first harvest, and the remaining one quarter applied after the second harvest. The dates of N application were 6 May, 18 June and 30 July. The grass was harvested three times during the growing season, on 17 June, 29 July and 10 September.

On 4 April 1996, soil samples were taken from each of the four blocks at the two sites (8 cores per block), to a depth of 20 cm. On 10 October 1996, soil samples were taken from each replicate plot. Three cores were taken per plot. In April the cores from each block were bulked, and in October the cores from each plot were bulked, after which the samples were immediately set out to dry at room temperature to prevent further mineralization.

The soil samples taken in April were collected (at both sites from each of the blocks) before the N treatments had been applied in order to estimate the N-supplying potential of the soil. Data from these samples were subjected to a one-way analysis of variance. The October soil samples were collected after one growing season when N had been applied and the data were analysed using an analysis of variance procedure for a randomized complete block design. In this way, comparisons of rate and total N mineralization could be made between the same plots before and after N treatment, and after one full season of mechanical harvesting and removal of the plant biomass. The data from both sets of samples were further analysed using the Statistical Analysis System, NLIN (non-linear least squares) procedure. Marquardt's method (Marquardt 1963) was used to fit the data to the first order equation $N_t = N_o (1 - e^{-kt})$ where N_t = mg mineral N per kg soil mineralized during time t (weeks), N_o = nitrogen mineralization potential in µg N per g soil and k = rate of N mineralization per week (Stanford & Smith 1972).

Laboratory procedures

After drying, the soils were sieved to pass a 1·65-mm mesh screen, and duplicate 15 g samples of soil were combined with equal weights of 20-mesh quartz sand and mixed thoroughly. This procedure allowed for homogeneous mixtures to be transferred to leaching tubes. The soil was poured into 50 ml leaching tubes and held in place by means of a glass wool pad at the bottom. A thin glass wool pad was also placed over the top to avoid dispersing the soil when liquid was poured into the tube. Any mineral N initially present in the soil was removed prior to incubation, by leaching with 100 ml of 0·01 M $CaCl_2$ in 5 to 10 ml increments, followed by a nutrient solution containing calcium sulphate, magnesium sulphate, calcium phosphate and potassium sulphate, but devoid of N (i.e. minus-N solution). Excess moisture was removed by vacuum (60 cm Hg vacuum). The tubes were covered with perforated parafilm to reduce water loss, while simultaneously allowing for the gaseous exchange of CO_2 and O_2, and incubated at 35 ± 1 °C. After 2 weeks, mineral N was recovered by leaching with 0·01 M $CaCl_2$ and the minus-N solution, followed by applying suction. Tubes were returned to the incubator for successive incubation periods with intervals of 2, 4, 4, 4, 6 and 8 weeks (cumulative: 2, 4, 8, 12, 16, 22 and 30 weeks) and intermittent leaching of mineral N and restoration of soil water contents after each interval. Both in their original paper and in subsequent studies, Stanford & Smith (1972) and Stanford *et al.* (1974) had advocated an initial pre-incubation period of 2 weeks. The results from this period were considered by Stanford *et al.* (1974) to be meaningless and were therefore discarded, due to the initial soil disturbance causing rapid decomposition of plant residues and an artificial flush of N-mineralization. Other studies disagreed with this premise, and found more accurate results when the first 2 weeks were included (Smith 1966; Paustian & Bonde 1987). Because in the present study, N_o and k values wee calculated both with and without the first 2 weeks of incubation and no apparent difference was noted, the entire 30-week period was included in our calculations. In the model proposed by Stanford & Smith (1972) N mineralization did not cease by the time the fraction characterized as N_o had been reached, but it had reached a small constant value which appeared to plateau when plotted cumulatively over time. Bonde & Lindberg (1988) in their mineralization studies with various organically amended soils, found that such a plateau had not been reached after 30 weeks and proposed allowing the mineralization to continue. This was based on the work of Sorensen (1981) who allowed labelled C and N mineralization studies to continue for 1600 days (229 weeks), before the full mineralization potential had been reached. Because the perennial grassland soils we analysed had not reached a plateau, the samples were returned to the incubator after the prescribed 30-week period. They were eluted 16 times at various intervals over a total 198-week period for the April samples, and a total of 13 times over a 148-week period for the October samples.

Both sets of soil samples were analysed for OM using the weight loss-on-ignition (LOI) method (Ball 1964). They were analysed for total N and total C using a LECO FP–2000 Carbon/Nitrogen Analyser (LECO, St. Joseph, Michigan).

Table 1. *Soil* fertility levels at each site throughout the sampling period*

Date	Site†	pH	Ca	Mg	P	K
			—kg/ha‡—			
Apr 1996	RCG	6·2	2163 (MH)	374 (H)	< 1·12 (VL)	75 (VL)
	TF	6·3	1924 (MH)	469 (VH)	< 1·12 (VL)	76 (VL)
Nov 1996	RCG	6·0	2311 (MH)	345 (H)	2·46 (VL)	66 (VL)
	TF	6·1	2001 (MH)	463 (VH)	1·8 (VL)	69 (VL)

* All soils extracted with a Modified-Morgan $NH_4(OAc)$ extractant, pH 4·8, 4 gm/20 ml.
† RCG, Reed canarygrass; TF, Tall fescue.
‡ MH, medium high; H, high; VH, very high; VL, very low.

RESULTS

April 1996 samples: pre-N applications

A mean total of 558 mg/kg NO_3^--N were nitrified from the soil at the RCG site, and 563 mg/kg NO_3^--N from the soil at the TF site (Table 2). Using the convention that there is approximately 2·2 million kg soil/ha (Brady & Weil 1996) to a normal plough depth (15 cm furrow slice), approximately 1230 kg/ha NO_3^--N at the RCG site, and 1238 kg/ha NO_3^--N at the TF site, could become available over 3·8 years. This would be the equivalent of 323 and 325 kg N/ha/year at the RCG and TF sites, respectively, if N mineralized on a 12 month per year basis. This estimate exceeded the relative amount of NO_3^--N projected by Whitmore *et al.* (1992). They had determined as much as 4000 kg N/ha was potentially mineralizable over 20 years from a perennial grassland after ploughing (or approximately 200 kg N/ha/year). Other researchers have estimated as much as 400 kg N/ha/year could be available from a perennial grassland in the first year after ploughing (Clement & Back 1969; Johnston *et al.* 1994). Soil analyses indicated that the soil from the RCG site contained 3003 mg/kg or 6620 kg/ha total N, and 2580 mg/kg, or 5673 kg/ha total N at the TF site, both within the range found under perennial grasslands by Gill *et al.* (1995), and Hatch *et al.* (1991). Given the amount of NO_3^--N accounted for, these results indicated that 18·7 and 21·9% of the total N contained in the RCG and TF soils, respectively, had been nitrified after 198 weeks (Table 2). After 30 weeks (210 days), only 231 mg/kg NO_3^--N had been nitrified at the RCG site, and 223 mg/kg at the TF site (Table 2). Hence after 30 weeks, 7·6% of the total N had been nitrified at the RCG site, and 8·6% at the TF site, which was within the range of 5

Table 2. *Long-term incubations from April and October 1996 samples: N nitrified, total N contained within the sample, and proportion of total N nitrified*

Date	D.F.	Site*		N applied kg/ha	N nitrified mg/kg		Total N mg/kg	% total N nitrified %	
					30 weeks	198 weeks		30 weeks	198 weeks
April		RCG		—	231	558	3003	7·6	18·7
		TF		—	223	563	2580	8·6	21·9
	6		S.E.		14·3	14·0	139·1	0·3	1·0
					30 weeks	148 weeks		30 weeks	148 weeks
October		RCG		0	190	531	3192	6·0	16·2
				175	201	576	3052	6·6	18·9
				350	216	596	3017	7·2	19·6
				525	219	585	2853	7·7	20·5
				Mean	207	571	3029	6·9	18·8
	9		S.E.		6·3	19·1	131·9	0·4	0·7
		TF		0	154	487	2708	5·7	18·0
				175	144	439	2776	5·2	15·8
				350	166	517	2698	6·2	19·1
				525	172	542	2801	6·2	19·5
				Mean	159	496	2746	5·8	18·1
	9		S.E.		10·3	30·0	120·8	0·4	0·9

* RCG, Reed canarygrass; TF, Tall fescue.

Table 3. *Values for N mineralization potential (N_0) and N mineralization rate constant (k) of soils from both sites for the April and October 1996 long-term incubation samples after 30 and 198 weeks, and 30 and 148 weeks, of incubation*

Date	Site*	N applied kg/ha	N_0 µg/g	k	N_0% total N %	N_0 µg/g	k	N_0% total N %
				—30 weeks—			—198 weeks—	
April	RCG	—	273	0·059	9·1	527	0·018	17·5
	TF	—	239	0·076	9·3	536	0·017	20·8
	Mean	—	256	0·067	9·2	532	0·018	19·1
				—30 weeks—			—148 weeks—	
October	RCG	0	267	0·040	8·4	638	0·0114	20·0
		175	257	0·048	8·4	698	0·0110	22·9
		350	253	0·058	8·4	689	0·0126	22·8
		525	237	0·071	8·3	652	0·0137	22·9
		Mean	254	0·054	8·4	669	0·0122	22·1
				—30 weeks—			—148 weeks—	
	TF	0	215	0·038	7·9	701	0·0077	25·9
		175	264	0·026	9·5	599	0·0084	21·6
		350	218	0·046	8·1	716	0·0081	26·5
		525	205	0·055	7·3	749	0·0083	26·7
		Mean	226	0·041	8·2	691	0·0081	25·2

* RCG, Reed canarygrass; TF, Tall fescue.

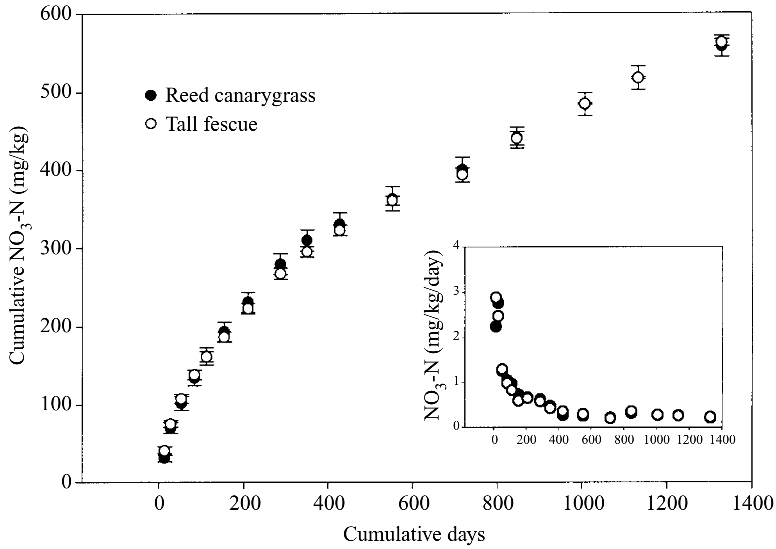

Fig. 1. Cumulative NO_3-N mineralized during long-term incubation of two soils, and NO_3-N mineralized at each sampling time (inset). Samples collected in April 1996, before N treatment application (vertical bars show S.E.).

to 40% of total N estimated as N_o according to Stanford & Smith (1972) after 30 weeks of incubation. Using the formula described by Stanford & Smith (1972), N_o at the RCG site was 273 µg/g ($k = 0·059$), which would be 9·1% of total N. At the TF site N_o was 239 µg/g ($k = 0·076$) (Table 3), which represented 9·3% of total N. When allowed to continue for 198 weeks, the N_o and k values were 527 µg/g and 0·018 at the RCG site, and 536 µg/g and 0·017 at the TF site. In agreement with Paustian & Bonde (1987), our data indicated that k values decreased and were less variable with increasing length of incubation. The

Table 4. *Weekly and cumulative nitrate-N nitrified during the long-term incubation of soil samples taken from the reed canarygrass (RCG) site in October 1996* (D.F. = 9)

		Weekly					Cumulative				
		N applied (kg/ha)					N applied (kg/ha)				
		0	175	350	525		0	175	350	525	
Week	S.E.	mg/kg				S.E.	mg/kg				Mean
2	3·8	16·6	22·4	32·5	46·2	3·5	16·6	22·4	32·5	46·2	29·4
4	3·3	28·9	31·3	29·0	23·5	2·9	45·4	53·7	61·5	69·7	57·6
8	2·2	33·8	33·0	38·4	36·4	4·1	79·2	86·7	99·8	106·1	93·0
12	1·3	23·2	24·2	25·6	24·4	4·8	102·3	110·9	125·5	130·5	117·3
16	0·9	19·1	19·5	19·9	18·0	4·9	121·5	130·4	145·4	148·5	136·5
22	0·9	31·9	32·3	31·7	30·9	5·5	153·3	162·6	177·1	179·4	168·1
30	1·2	37·3	38·1	39·5	40·0	6·3	190·6	200·8	216·6	219·4	206·9
44	3·7	54·6	61·2	67·3	64·5	7·6	245·2	262·0	283·9	283·9	268·8
55	2·9	45·4	44·6	47·1	45·3	8·5	290·6	306·6	331·0	329·2	314·4
78	5·6	61·8	75·7	78·0	69·7	11·0	352·4	382·2	409·8	398·9	385·8
101	8·5	75·6	66·6	72·3	73·1	13·5	428·0	448·9	481·4	472·0	457·6
120	9·2	62·7	76·8	69·8	69·6	15·7	490·7	525·6	551·2	541·6	527·3
148	5·3	40·6	50·7	44·8	43·4	18·8	531·3	576·3	596·0	585·0	572·2

rate and amount of NO_3^--N nitrified over the course of the study varied very little between the two sites (Fig. 1). When the cumulative amount for each time period was measured, there was no significant difference between the amounts measured at the two sites throughout the entire incubation period.

Ammonium-N was also measured throughout the duration of this experiment. Generally, amounts of NH_4^+-N measured were negligible since the incubation conditions favoured rapid nitrification of the NH_4^+ mineralized, and the data have therefore not been reported here.

October 1996 samples: post-N applications

The soil samples taken on 10 October 1996 were incubated in the laboratory for a total of 148 weeks. On average a total of 571 mg/kg NO_3^--N, or 1256 kg NO_3^--N/ha, were eluted from the soil at the RCG site, and 496 mg/kg, or 1091 kg NO_3^--N/ha from the soil at the TF site (Table 2). Since the incubation continued for 2·85 years, this would be the equivalent of 442 kg/ha/year at the RCG site, and 383 kg/ha/year at the TF sites, respectively, assuming a 12 month period of mineralization per year. There was no significant difference between the cumulative total NO_3^--N mineralized, nor in the total N present at either site in response to N treatment. Since the sites contained a mean total N of 3029 mg/kg, or 6662 kg/ha (RCG) and 2746 mg/kg, or 6040 kg/ha (TF) respectively, 18·8 and 18·1% of the total N in the soil had been nitrified after 2·85 years. There was a significant N linear ($P < 0·05$) response among N treatments for percentage total N mineralized in the RCG soils, but no significant difference in the TF soils (Table 2). After 30 weeks, a mean total of 207 mg/kg NO_3^--N had been nitrified at the RCG site, and 159 mg/kg at the TF site. This represents 6·9 and 5·8% of the total N at the RCG and TF sites, respectively. Again this is within the 5–40% range characterizing N_o as a per cent of total N estimated after 30 weeks by Stanford & Smith (1972), but less than the 30 week total measured from the pre-N treatment samples from April 1996. Using the formula described by Stanford & Smith (1972) the potential mean N_o at the RCG site was 254 μg/g at a k rate of 0·054, and at the TF site it was 226 μg/g, at a k rate of 0·041 (Table 3), representing 8·4 and 8·2% of total N at the two sites, respectively. When allowed to continue for 148 weeks, the mean N_o values were 669 μg/g ($k = 0·0122$) at the RCG site, and 691 μg/g ($k = 0·0081$) at the TF site. As in the initial soil samples, NH_4^+-N was measured throughout the course of this experiment, but since the amounts were negligible, they have not been reported.

Except for weeks 2, 4, 44 and 78 at the RCG site (Table 4), and weeks 2, 55, 78 and 148 at the TF site (Table 5) there was no significant difference between the N treatments from week to week. This was consistent with the effect of fertilizer N on N mineralization reported by Hassink (1992*a*). However, when analysed cumulatively with time, treatments were consistently and significantly different at the RCG site, with the exception of the cumulative total at week 148 (Table 4). By contrast, at the TF site

Table 5. *Weekly and cumulative nitrate-N nitrified during the long-term incubation of soil samples taken from the tall fescue (TF) site in October 1996* (d.f. = 9)

		Weekly					Cumulative				
		N applied (kg/ha)					N applied (kg/ha)				
		0	175	350	525		0	175	350	525	
Week	s.e.	mg/kg				s.e.	mg/kg				Mean
2	6·5	11·7	8·8	21·8	31·5	7·1	11·7	8·8	21·8	31·5	18·5
4	2·2	21·1	16·3	21·1	20·7	8·0	32·8	25·1	42·9	52·2	38·3
8	3·3	23·1	27·6	26·5	22·4	8·7	55·9	52·7	69·3	74·6	63·1
12	0·9	20·5	19·6	20·5	20·2	8·7	76·4	72·3	89·9	94·8	83·4
16	0·9	18·3	17·5	18·8	18·8	8·9	94·7	89·8	108·7	113·6	101·7
22	1·6	27·2	24·5	25·4	26·1	9·7	121·9	114·3	134·1	139·6	127·5
30	1·5	31·9	29·9	32·2	32·4	10·3	153·8	144·2	166·3	172·0	159·1
44	3·9	47·4	44·0	44·6	51·4	12·7	201·2	188·2	211·0	223·4	206·0
55	3·1	37·6	32·0	35·4	40·3	13·8	238·8	220·2	246·3	263·7	242·3
78	5·7	62·0	40·3	61·3	70·3	16·8	300·8	260·5	307·6	334·0	300·7
101	8·8	61·5	63·5	68·5	69·9	18·9	362·3	324·1	376·1	403·9	366·6
120	10·2	80·3	70·1	80·2	87·9	26·1	442·5	394·2	456·3	491·7	446·2
148	6·3	44·2	44·5	60·3	49·8	30·0	486·7	438·7	516·6	541·5	495·9

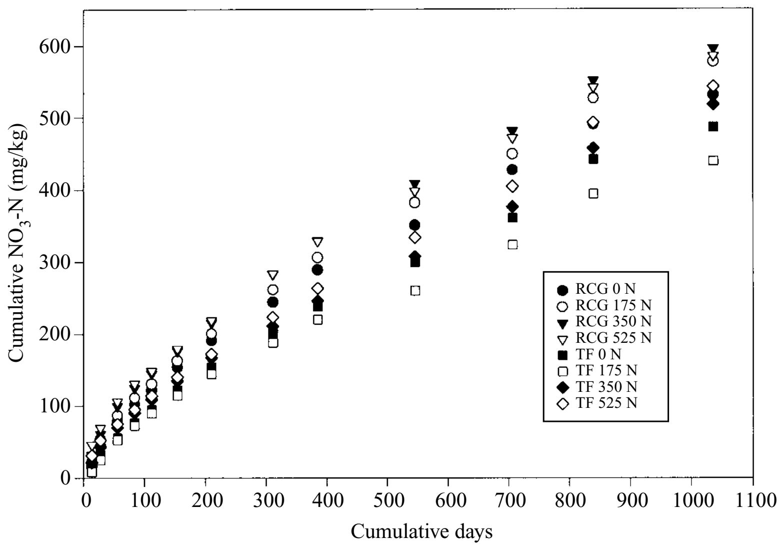

Fig. 2. Cumulative NO_3-N mineralized during long-term incubation of two soils fertilized at four N rats. Soil samples collected in October 1996. RCG, Reed canarygrass; TF, Tall fescue.

the cumulative difference among N treatments was only significant after the first elution, but remained non significant for the remainder of the sampling period (Table 5). The RCG soils consistently contained more cumulative NO_3^--N than did the TF soils (Fig. 2).

Soil samples taken from both sites in October were analysed for OM. There was no significant difference in OM as a result of N treatment at either site. The mean OM value at the RCG site was 84 g/kg and at the TF site it was 80 g/kg. Although the samples for April 1996 and October 1996 were not statistically compared due to the difference in N treatment, there was a slight decrease in OM from 89 to 84 g/kg at the RCG site, whereas the OM at the TF site remained constant at 80 g/kg.

DISCUSSION

The N-supplying capacity of the soil from both sites was established both before and after N application (Table 2), and was high, as would be expected from soils with a high OM content under perennial grassland (Clement & Back 1969; Whitmore *et al.* 1992; Clough *et al.* 1998). In all cases, the first order kinetics model described by Stanford & Smith (1972) successfully fitted the data generated from the perennial grassland soil in this study and both N_o and k values were determined (Table 3). The initial soil samples, incubated before N fertilizer had been applied, indicated no significant difference in the N-supplying capacity at both sites. However, since there was a significant difference in the total N between sites (Table 2), the fact that the same amount of N was mineralized can be attributed to the different rates of mineralization. The k values determined after 30 weeks were 0·059 at the RCG site, and 0·076 at the TF site (Table 3). This is in agreement with Griffin & Laine (1983) who indicated that N_o values alone did not correlate well with actual NO_3^--N measured, but only when combined with the specific k value for that site.

Prior to incubation, any existing NO_3^--N differences in the soil samples were removed by eluting with 100 ml of 0·01 M $CaCl_2$ before the long-term incubation began. By the end of week 30 when N_o would typically be measured according to Stanford & Smith (1972), there was a significant linear response to N in cumulative NO_3^- measured at the RCG site ($P < 0{\cdot}01$), but no significant difference at the TF site (Tables 4 and 5). Similarly, the k rate had increased in response to N treatment, ranging from 0·04 to 0·071 at the 0 and 525 kg N/ha treatments at the RCG site, and from 0·026 to 0·055 at the 175 and 525 kg N/ha treatments at the TF site. The mean k value at the RCG site after N treatment was 0·054, the same value determined as typical of the 39 soils tested by Stanford & Smith (1972) after 30 weeks, compared to 0·059 prior to N application. By contrast, the k value at the TF site decreased from 0·076 in the April samples, to a mean of 0·041 after N application. The N_o value as a percentage of total N (Table 3) was 9·1 and 9·3 at the RCG and TF sites in the April samples, and a mean of 8·4 and 8·2 at the two sites in the October samples after N had been applied. This was at the low end of the range of 5–40 % originally determined by Stanford & Smith (1972), but more consistent and not demonstrating the same variability. The total N content averaged 3003 and 2580 mg/kg (0·30 and 0·26 %) pre-N treatment, compared with 3029 and 2746 mg/kg (0·30 and 0·28 %) after N treatment, at the RCG and TF sites respectively. This was considerably higher than the total N measured in either of the previous studies where the mean total N had been 0·104 % (Stanford & Smith 1972) and 0·068 % (Stanford *et al.* 1974). However the Mollisol soils in their original study (Stanford & Smith 1972), which had the highest total N (0·19 to 0·29 %), showed relatively low fractions of N_o (11·5 to 13·5 %) as a percentage of total N. This relationship between high total N and N_o representing a lower percentage of total N is consistent with our results. The decrease in actual N_o and k values was greater at the TF site than at the RCG site after N application which suggests the need for site differentiation in predicting N mineralization response to fertilizer application.

After 30 weeks, only a relatively small percentage of the total N had actually been mineralized, and a plateau marked by a small stable constant (Bonde & Lindberg 1988) had not yet been reached. Therefore, the incubation process was continued over 198 weeks for the April samples, and over 148 weeks for the October samples. Whereas a shorter incubation period using this method could be meaningless (Dendoveen 1990), a longer incubation period for these perennial grassland soils was shown to be necessary. Matus & Rodriguez (1994) had adapted the Stanford & Smith (1972) method to calculate a K_1 value based on year 0–1 of incubation, and a K_2 value based on years 0–10. Even with this adjustment however, N_o values were underestimated for perennial grassland soils (Matus & Rodriguez 1994). Clearly, our results indicated that the N_o determined after 30 weeks, for both the April and October samples, underestimated the amount of N which would become available over a long period of time. The April samples, which were incubated for 198 weeks, indicated that the actual N mineralized (Table 2) was fairly close to the estimated N_o (Table 3). The fact that there is a greater discrepancy between the N_o and actual measured N mineralized in the October samples after 148 weeks incubation, could indicate that the longer incubation was necessary to give a more accurate estimate of the N mineralization potential.

An important aspect of this study was that cumulative NO_3^--N measured over time as a result of N treatment varied between sites and/or species. A significant response was noted continually until the last week at the RCG site, but no significance was noted at the TF site after the first week. Greater cumulative N mineralization resulting from the substantial period of incubation at the RCG site was in agreement with the results of Gill *et al.* (1995) who found greater N mineralized on drained sites with fertilizer inputs of 200 kg/ha N. This variation in N-mineralization, according to site and species, was in agreement with the results of Clough *et al.* (1998). They found no correlation between N turnover and soil thermal units when all data sets were pooled, but a high degree of correlation when plotted as subsets according to region and species composition. Potentially more NO_3^--N was available in the soil for plant uptake for at least 120 weeks at the RCG site where N had been applied, but no subsequent fertilizer was added during the course of the incubation. Gill *et al.* (1995) likewise found no decrease in N mineralization in plots which had been previously fertilized, but had no fertilizer N application during the year of the field incubation. Ultimately, however, after 148 weeks (2·8 years), the differences in our study dissipated. This information could be useful when considering changes in management practices. If a grassland had been in intensive production with fertilizer-N or organic amendments added over years, N-mineralization could continue to contribute substantial amounts of N, gradually decreasing over time. In some instances, as at the RCG site, more cumulative N could become available for a period of time where more N had been added. These results agreed with a study in the Netherlands, which measured a chronosequence of N mineralization under different grasslands which had not been fertilized for 2, 6, 19 and 45 years. That study indicated that relative mineralization strongly decreased over time to rates of 88, 75, 54 and 51 %, respectively (Olff *et al.* 1994). The fact that different cumulative N mineralization responses occurred at the RCG and TF sites in our study indicates that such results may be site and/or species specific. In their review of the current relationship between the predictive value of laboratory and field incubation studies and N actually mineralized and available to a particular crop, Jarvis *et al.* (1996) pointed out the need for standardization in the systems used, and the need to develop rapid methods for estimating actual N availability from any mineralization study. This study suggests that under a perennial grassland the contribution from mineralized organic matter is constant and steady, and can accumulate into substantial quantities over a long period of time. The decreasing k rates over time are consistent with the decrease in OM as it is mineralized over time in the closed system of a laboratory study. However, the same soil left in the field would have even greater potential for N mineralization with drying and rewetting cycles (Hatch *et al.* 1990, 1991), and continual input from additional OM breakdown. A possible means of making this information practical may be to coordinate it with discrete measurements of soil sample N at particular points in the growing season and critical levels of NO_3^--N in relation to optimum yield (Collins 2000).

CONCLUSIONS

This experiment clearly indicated the high N-supplying capacity of perennial grassland soils which had been in sod for several years. Without any fertilizer additions, the high OM content provided an abundant source of N which became available over time. After N fertilization, there were few significant responses to N rate in NO_3^--N mineralized and measured on a weekly basis. However, differences in cumulative N as a result of N treatment were observed over time, but varied by site. Incubation of soil removed from grassland sites (following N fertilization and a harvest sequence) revealed varying mineralization responses. An incubation period of 30 weeks underestimated potentially mineralizable N, whereas incubation periods of 148 weeks overestimated N_o compared with actual NO_3^--N measured. Incubation periods of 198 weeks resulted in better estimations of mineralizable potential. These results indicate the difficulty in predicting N-mineralization potential from a short-term laboratory incubation of perennial grassland soils. They seem to defy modelling in a typical sense and could point instead toward the need for an index of soil N at particular points in time correlated with optimum yield, as a means of discerning the need for additional N application.

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