# **An assessment of the contribution of net mineralization to N cycling in grass swards using a field incubation method**

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Received 11 December 1990. Revised May 1991

*Key words:* acetylene, clover, grazed swards, incubation, mineralization

#### **Abstract**

Measurements of net mineralization using a field incubation method were made over a full growing season (180 d). Soil cores, taken from cut swards which for many years had been previously grazed by cattle, were placed in jars in the field for successive incubation periods of 14 d. Acetylene was added to the incubation jars to inhibit nitrification in the soil cores and thereby prevent losses of N through denitrification. Net mineralization over 180 d amounted to 415, 321 and 310 kg N ha<sup>-1</sup> under grass/ clover, unfertilized grass and grass receiving  $420 \text{ kg N} \text{ ha}^{-1} \text{ y}^{-1}$ , respectively. At the start of the growing season, an index of potentially mineralizable N in the soil was estimated by a chemical extraction method, but this index was  $<50\%$  of the estimates obtained by field incubation. The amount of N in herbage harvested regularly from the swards also under-estimated the supply of N from the soil, with apparent recoveries of 53, 82 and 74% and total yields of N of 240, 263 and 538 (kg N ha<sup>-1</sup>) from grass/clover, unfertilized grass and fertilized grass, respectively. Mineralization rates varied significantly with seasonal soil temperature fluctuations, but the incubation method was apparently less sensitive in relation to changes in soil water content. Rates of N-turnover (as % of total soil N) were highest under grass/clover (9%), but similar under fertilized and unfertilized grass swards (approximately 5%).

### **Introduction**

In studies of nitrogen (N) cycling in grassland soils, emphasis has been placed on measuring both inputs of N from fertilizer, biological fixation, excreta, atmosphere, and senescent plant material and outputs of N by removal in animal products and harvestable plant material, or as losses to waters or the atmosphere. This allows balance sheets to be constructed which describe the efficiency of utilization and ultimate fate of N (e.g. Ball, 1982; van der Meer, 1982; Whitehead et al., 1986). Another important component is the recycling of N from soil organic matter, which is the largest potential source of N with a diverse and complex nature and highly variable

rates of turnover which are difficult to quantify (see e.g. reviews by Stevenson, 1982, and Skjemstad et al., 1987). In trying to assess movements of N from organic matter, accuracy is limited by both the inadequacy of suitable methods to monitor changes and the masking effect of this large background source against which small changes are not easily detected.

The rate at which N is mineralized from the soil organic matter will vary with season, cropping, soil type and management and will be counterbalanced to a variable extent by the process of immobilization. In grassland soils this is particularly difficult to quantify because of a largely undefined equilibrium between mineralization and immobilization which alters with

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time (Barraclough and Jarvis, 1989; 't Mannetje and Jarvis, 1990). A measurement of net N release is, therefore, of value as an indication of the contribution that soil organic N can make to the inorganic N pool, although this will not necessarily provide information about the rates of the two processes since: 'a small net effect may be the result of low overall biological activity in the ecosystem, or it may be the result of high activity in which the processes work in opposite directions' (Jansson and Persson, 1982).

Information on the seasonal contribution that mineralization can make to the available N supply in grassland soils is limited, especially as it relates to inputs of fertilizer N. In a previous paper (Hatch et al., 1990), we measured net mineralization during spring using soil cores incubated in the field with acetylene to inhibit nitrification. We now describe the further application of this method extended to a full growing season, and relate the findings to changes in soil temperature and moisture, as well as comparing fertilized and unfertilized swards which had previously been grazed by cattle.

## **Methods**

## *Experimental treatments*

Field measurements of net mineralization were made during 1988 on two swards growing on soil of the Frilsham series at the farm of the AFRC Institute of Grassland and Environmental Research, Hurley, Berkshire, UK. Both sward types were established in 1976 on experimental plots (0.123ha) which were grazed rotationally each year by young beef cattle. The first sward consisted of perennial ryegrass *(Lolium perenne)*  with approximately 30% (by weight) white clover *(Trifolium repens)* which had never received fertilizer N and the second was a perennial ryegrass sward which had received 420 kg N ha<sup> $-1$ </sup> y<sup> $-1$ </sup> since the treatments began in 1976. In 1988 the cattle were excluded from areas  $(20 \text{ m}^2)$  established within both of the main experimental plots. These sub-plots were reserved for taking soil samples for mineralization measurements which were not, therefore, affected by the current season's excretal returns. One sampling area was positioned within the grass/clover plot (GC) and there were two separate areas within the grass plot: the first of these (GN) continued to receive regular inputs of fertilizer N every 28 d, i.e. at the same rate and time as the main plot, but the second (GO) remained unfertilized throughout 1988.

# *Herbage N*

Within each of the three sampling areas a  $4-m^2$ area was reserved where soil samples were not taken so that the apparent recovery of mineralized N could be assessed from herbage harvested regularly through the growing season. Herbage was harvested every 14d from a strip  $(10 \text{ cm} \times 100 \text{ cm})$  cut at random from within the  $4\text{-m}^2$  area, using electric hand shears at a fixed height of 2 cm. The herbage remaining on the sub-plot was then cut to a uniform height of 2 cm and discarded; that on the remainder of the sampling area was also cut and removed. The sampled herbage was dried at 100°C, weighed and analyzed for total N content using a Carlo Erba NA 1500 analyzer (Erba Science, UK, Ltd).

# *Soil N*

# *( i ) Mineralization*

The measurement of net mineralization using field incubated soil cores with acetylene  $(C_2H_2)$ to inhibit nitrification has been described fully elsewhere (Hatch et al., 1990). In brief, the technique involved taking regular soil samples from the experimental areas, to a standard depth of 15 cm, using a 3.8 cm diameter corer. In the present study, four intact soil cores were placed **in a** 1-L glass jar which was sealed by a polyacetyl lid fitted with a rubber gasket and held in place by a screw cap. Acetylene  $(10 \text{ cm}^3)$  was added to the jar to give approximately  $2\%$  (v/v) in the headspace. Two replicate jars were prepared with 8 cores taken at random from each soil sampling area. The jars were placed in holes (12cm diameter, 15cm deep) in the ground adjacent to the experimental area for an incubation period of 14 d. Coring started in March, 14 d before the first fertilizer application and continued at 14 d intervals until October.

At the same time as the soil cores were obtained, a further 8 soil samples were removed at random from the soil sampling area using a 3.5 cm diameter auger. The samples were bulked together in groups of four, crumbled and stones >6 mm discarded. Duplicate extractions of each bulked sample were made by dispersing 100g fresh soil in  $200 \text{ cm}^3$  1 *M* KCl using a hand-held electric blender, followed by shaking for 1 h. The extractant was filtered and analyzed for  $NH<sub>4</sub><sup>+</sup>$  and  $NO<sub>3</sub><sup>-</sup>$  contents using standard autoanalyzer procedures to give estimates of inorganic N at the start of incubation.

After incubation of the cores, a gas sample  $(10 \text{ cm}^3)$  was removed from the headspace of the jars and analyzed for nitrous oxide  $(N, O)$  and carbon dioxide  $(CO<sub>2</sub>)$  using a gas chromatograph (Philips, PU4500) with a thermal conductivity detector. Duplicate extractions of sub-samples of the bulked soil from each jar were also carried out using the same procedure as for the augered samples. Soil water contents were determined gravimetrically on a further sub-sample of the bulked cores. The amount of inorganic N in the soil cores after incubation, less that initially present in the auger samples, was taken as the amount mineralized.

## *(ii) Effect of acetylene*

On six occasions through the growing season, soil cores were also incubated in the absence of  $C_2$ H, to assess possible effects on the measurement of mineralization. As before, two replicate jars, each with 4 intact cores were prepared, but no  $C_2H_2$  was added to the headspace during the 14 d incubation period. Extraction of the soil and analysis for mineral N were as described before.

### *( iii ) Chemical extraction of potentially mineralizable N*

Samples of soil  $(0-15 \text{ cm})$  were collected in March 1988, from all 3 treatment areas, before the samples for incubation were taken, air-dried and ground to pass a 2-mm sieve. Mineralizable N was then estimated by the method described by Whitehead (1981), i.e. by boiling the dried soil with  $1M$  KCl and analyzing the extract for inorganic N. The value obtained was then adjusted by a factor derived by Whitehead (1986) to take account of rainfall and soil temperature

(354 mm and a mean of 13.5°C at 10 cm, respectively, for the period April to September 1988) to give an index of potentially mineralizable N.

## *Root N*

The possibility of N being prematurely released from roots during incubation was investigated. Soil cores (48) were taken at random from the GO treatment in March, and grouped into 12 replicates, each consisting of 4 bulked cores. The roots from each replicate group of cores were separated from the soil by washing over a 2-mm sieve, dried at 100°C and analyzed for N and carbon (C) content, as for herbage. At the same time, twelve jars with  $2\%$  (v/v)  $C_2H_2$  and twelve jars without  $C_2H_2$  and each containing 4 cores were incubated in the field for 14 d. After incubation, the roots from the 4 bulked soil cores from each jar were separated and analyzed as before. Total N and C contents of the roots were then compared, before and after incubation.

## *N: fixation*

The amount of  $N_2$  fixed symbiotically in the grass/clover (GC) sward was estimated using the  $C_2$ H<sub>2</sub>-reduction method (Hardy et al., 1973). Each week (March to October, 1988) eight cores (2.5 cm diameter, 10 cm deep) were removed at random from the adjacent grazed area of the main experimental plot and placed in a 1-L jar with 10%  $C_2H_2$  in the headspace for an incubation period of 2h in 15cm deep holes in the field. A  $10$ -cm<sup>3</sup> sample of air in the headspace was then removed using a syringe. The gas sample was analyzed for ethylene  $(C_2H_4)$  using a gas chromatograph (Pye Unicam, GCD) fitted with a flame ionisation detector. For every 3 moles of  $C_2H_4$  produced, 1 mole of N<sub>2</sub> was assumed to have been fixed by the clover plants in the GC sward (Hardy et al., 1973).

## **Results**

The total N and C contents of the soil had increased appreciably between 1976 when the swards were sown and March 1988 before the start of the present study (Table 1). Net miner-

*Table 1.* Total N and C contents of Frilsham soil (0-15 cm) at sowing in 1976 and when mineralization measurements began in 1988

	Sward	$\%\rm N$	%C	C/N	$kg$ N ha <sup>-1</sup>	$kgC$ ha <sup>-1</sup>
1976	$\overline{\phantom{0}}$	$0.21^{b}$	$1.91^{\circ}$	9.1	3780 <sup>a</sup>	$34380^a$
1988	Grass/clover	0.22	2.42	11.0	4620 <sup>a</sup>	$50820^a$
1988	Grass	0.29	3.24	$\sim$ .	$6090^{\circ}$	68040 <sup>a</sup>

<sup>a</sup> Calculated using bulk density values for Frilsham soil of 1.20 (1976) and 1.40 (1988): the same value was obtained for soils under both grass/clover and grass treatments.

<sup>b</sup> Tyson (personal comm.).

alization was then evaluated over the growing season from April to September, inclusive (180 d). Mean daily rates of net mineralization  $(kg N ha^{-1} d^{-1})$ , assessed by successive 14-d incubations in the presence of  $C_2H_2$  over the 180-d period, ranged from 0.7-4.1 (GC, Fig. la), 0.8-3.2 (GO, Fig. lb) and 1.1-3.8 (GN, Fig. lc). The overall mean daily rates  $(kg N ha<sup>-1</sup> d<sup>-1</sup>)$  for the 180 d in the three treatments were very similar, i.e.  $2.3 \pm 0.45$  (GC),  $1.8 \pm 0.31$  (G0) and  $1.7 \pm 0.82$  (GN).

Total net mineralization (Table 2) was calculated as the cumulative amounts of mineralized N for each 14-d period and was highest under the grass/clover (GC) sward, but was very similar under the two grass swards (GO and GN). Mineralization rates in all three treatments (Fig. la, b, c) were lower in early spring  $(\leq 1 \text{ kg N ha}^{-1} d^{-1})$ , but increased during the summer months and then declined again with the onset of autumn. Variations in rates were greatest in the GN treatment, but these changes did

*Table 2.* Nitrogen supplied through mineralization and utilized by swards

	Unfertilized	Fertilized	
	GC	G0	GN
Mineralized N $(kg ha^{-1})$	415	321	310
Herbage yield $(t$ DM ha <sup>-1</sup> )	8.3	9.0	13.3
Yield N $(kg ha^{-1})$	240	263	538
Range % N in herbage	$2.5 - 4.0$	$2.2 - 4.5$	$3.4 - 5.1$
Apparent recovery $(\%)$	53 <sup>a</sup>	82	74ª

<sup>a</sup> Apparent contribution from  $N<sub>2</sub>$  fixation and fertilizer has been accounted for.

not appear to correspond to the timing of fertilizer applications. An apparent net immobilization occurred only in this treatment (Fig. lc), i.e. on 21 April  $(-0.4 \text{ kg N ha}^{-1} d^{-1})$ , and also on 25 August  $(-4.0 \text{ kg N ha}^{-1} d^{-1})$ , but with no obvious environmental reason for this. Soil temperature at 10-cm depth (Fig. 2) was positively correlated with mean daily mineralization rates and the effects were statistically significant in GO  $(p<0.01)$  and GC  $(p<0.05)$ , but not in the GN treatment. Changes in mineralization rates appeared to follow a similar pattern of changes to those for soil water content, particularly in GN, but there was no significant relationship in any of the treatments. The estimates of potentially mineralizable N by chemical extraction, adjusted for seasonal weather conditions, were 131, 140 and 134 kg N ha<sup>-1</sup> for GC, G0 and GN. respectively, and were, therefore, very much less than those from the in situ field measurements over the 180-d period (Table 2).

An apparent recovery of mineralized N in the herbage was also calculated (Table 2). There was a similar pattern of herbage production in all three swards during spring, but yields from GC and GO fell more rapidly than from GN over the remainder of the growing season (Fig. 3). The highest yield of herbage was thus achieved in the well-fertilized treatment (GN); only approximately 60% of this yield occurred in the two unfertilized treatments (GC and GO). Herbage N concentrations were also lower in GC and GO, resulting in total yields of N less than half of that obtained in the GN treatment. However, when the recovery of herbage N was expressed as a percentage of available N (i.e. mineralized  $+$ fertilizer N), a higher proportion was recovered in GO than in GN (Table 2). Although mineralization was highest under the GC sward, this



*Fig. 1.* Mean daily rates of net mineralization for Frilsham soil (1988) Under a. grass/clover, **b**. unfertilized grass and c. fertilized grass receiving 420 kg N ha<sup>-1</sup> y<sup>-1</sup>. Each point is the mean of 2 replicates.  $S.E.M. = 0.50$ .

sward had the poorest recovery of N in the harvested herbage (Table 2).

For most of the growing season, concentrations of inorganic N  $(NO<sub>3</sub><sup>-</sup> + NH<sub>4</sub><sup>+</sup>)$  in both the unfertilized treatments (GC and GO), in the soil sampled before incubation, were remarkably stable and remained below 10  $\mu$ gNg<sup>-1</sup> dry soil throughout the 180d (Fig. 4a, b). Even with added fertilizer in the GN treatment, only on one occasion (11 August), when a value of 57  $\mu$ g N g<sup>-1</sup> dry soil was recorded (Fig. 4c), was there a marked perturbation in inorganic N levels. However, soil sampling on this occasion took place within a few days of fertilizer application, during an exceptionally dry period, so it is possible that undissolved fertilizer granules were included in the sample. Inorganic N levels  $(\mu g N g^{-1}$  dry soil) over the whole season ranged from 2.3-7.1 in GC, 1.6-6.0 in GO and 1.4-56.6 in GN treatments. Towards the end of the growing season, inorganic N levels in GN increased steadily and exceeded 20  $\mu$ g N g<sup>-1</sup> dry soil, but only small increases were detected in the unfertilized treatments (GC and GO). Although the proportions of  $NH<sub>4</sub>-N$  and  $NO<sub>3</sub>-N$ varied in all treatments, the ratio  $NH<sub>4</sub>:NO<sub>3</sub>$  in both GO and GN treatments was on average 4:1, whereas in the GC it was approximately 8:1. With the exception of the single high value of inorganic N found in GN, the  $NO<sub>3</sub><sup>-</sup>$  component remained low throughout the 180 d in all treatments.

Mean values ( $\mu$ g NO<sub>3</sub>-Ng<sup>-1</sup> dry soil) at the start/end of incubation with  $C_2H_2$  were  $0.9 \pm 0.33/0.3 \pm 0.10$  (GC),  $0.9 \pm 0.28/0.4 \pm 0.4$ 0.18 (G0) and  $1.1 \pm 0.32/0.5 \pm 0.21$  (GN, the high value in August being omitted). The presence of  $C_2H_2$  was, therefore very effective in inhibiting nitrification, despite substantial increases in inorganic N  $(NH_4^+ + NO_3^-)$  at the end of incubation. However, in the present soil there was no consistent effect of the presence or absence of  $C_2H_2$  on measured rates of mineralization and only on one occasion (8 September) in GC treatment was there a significant difference ( $p = < 0.05$ ) between the two treatments. Losses of nitrogen through denitrification (as measured by  $N_2O$  concentration when  $C<sub>2</sub>H<sub>2</sub>$  was added) were not observed in GC and GO at all, and only on two sampling dates in the



*Fig. 2.* Mean soil temperature at 10 cm and soil water contents (0-15 cm) for each incubation period during 1988.

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**(a)** 

GN treatment were denitrification losses recorded, i.e. on 25 August  $(0.13 \text{ kg N} \text{ ha}^{-1} \text{ d}^{-1})$ and on 22 September  $(0.12 \text{ kg N ha}^{-1} d^{-1})$ ; this was only coincident with an increase in soil water content in the latter case (Fig. 2). Discrepancies through the reduction of  $NO_3^-$  were unlikely, therefore, to have affected the measurement of mineralization in the present soils, but the effectiveness of  $C_2H_2$  in preventing denitrification losses was not fully tested. However,  $C_2H_2$  was shown both here and in the previous study (Hatch et al., 1990) to inhibit nitrification in this and heavier textured soils. Furthermore, in another investigation using this technique on a poorly drained soil of the Halstow series, consis-





*Fig. 3.* Dry matter herbage yields from 14-d harvests taken throughout 1988 growing season from swards of grass/clover  $(\Box)$ , unfertilized grass  $(\bigcirc)$  and fertilized grass receiving 420 kg N ha<sup>-1</sup> y<sup>-1</sup> ( $\bullet$ ).

*Fig. 4.* Inorganic N ( $NO_3^- + NH_4^+$ ) in Frilsham soil (0-15 cm) in 1988 under grass clover  $(\Box)$ , unfertilized grass  $(\bigcirc)$ and fertilized grass receiving  $420 \text{ kg N} \text{ ha}^{-1} \text{ y}^{-1}$  (.). Each point is the mean of 2 replicates.  $S.E.M. = 2.84$ .

tently higher values for mineralization were obtained with  $C_2H_2$  (Titchen, personal comm.), indicating the need to restrict nitrification in the first instance and denitrification in the second.

One of the problems associated with the present technique is a possible change in the oxygen  $(O<sub>2</sub>)$  status of the soil samples, both when the samples were taken and during the incubation period.  $O<sub>2</sub>$  levels in the incubation jars were monitored indirectly by measuring the  $CO<sub>2</sub>$  evolved at the end of 14d incubation. On three occasions (30 June, 25 August and 22 September),  $O<sub>2</sub>$  levels were calculated to have fallen below 6% in the air space of the jars with soils from all three treatments, which is the level below which Parkin and Tiedje (1984) found a rapid increase in the rate of denitrification. For all the remaining periods,  $O<sub>2</sub>$  concentration at the end of incubation, was never less than 50% of that at the start. Thus, at no time during the incubation was the  $O<sub>2</sub>$  status of the soil system likely to have been greatly changed from that in the natural state of the freely draining Frilsham soil. For heavier soils a refinement of the method would be to sheath the cores in a polyethylene sleeve or perforated plastic tube to help preserve the aeration status of the soil during incubation.

A potential immediate source of mineralizable N is that which may have been released from living root tissues during incubation. However, there was no evidence of any difference between the dry matter yield of roots separated from soil cores before and after a 14-d incubation (Table 3). Further, incubation, whether  $C_2H_2$  was present or not, did not result in any significant reduction in the N (or C) contents of the roots, with little likelihood, therefore, of a release of mineral N from this source.

*Table 3.* The effect of the presence or absence of C<sub>2</sub>H<sub>2</sub> on the N and C contents of roots during 14-d incubation

Incubation period	Mean yield of roots per jar (g dry wt)	$\%$ N in roots	% $C$ in roots
$0 d (-C_2 h_2)$	4.2	1.93	40.0
14 d $(-C_2H_2)$	4.1	1.95	39.2
14 d $(+C,H, )$	3.9	1.89	40.1
S.E.M. (33 d.f.)	0.42	0.040	0.77

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Interpolation from weekly measurements of N, fixation as assessed by the  $C_2H_2$ -reduction method indicated an apparently very low input of N to the GC sward. Thus,  $N_2$  fixation was estimated to have contributed only  $23 \text{ kg N}$  to the GC treatment over the growing season. This may have been an underestimate, due to the well-known deficiencies in the  $C_2H_2$ -reduction assay (Minchin et al., 1983) and the problems associated with interpolation from a 7-d sampling regime. Contributions of N from fixation were unlikely, however, to have been a major component of the N supply to the sward.

#### **Discussion**

The swards used in the present study in 1988 had been established for 12 years, before the present measurements of net mineralization were made. In that time, soil C had increased by  $34$  t ha<sup>-1</sup> and  $16 \text{ tha}^{-1}$  and soil N by  $2.3 \text{ tha}^{-1}$  and  $0.8$  t ha<sup>-1</sup> under fertilized grass and grass/clover, respectively (see Table 1). Nevertheless, despite these wide differences in accumulated C and N, there was an identical shift in C/N ratios in the soils under both sward types from 9 in 1976 to approximately 11 in 1988. Even with major changes in cultivation from arable to woodland and a doubling of the organic C after 18 years, the C:N ratio in the topsoil in the Broadbalk Wilderness at Rothamsted increased only slightly, from 9.0 to 10.5 (Jenkinson, 1988). In our study, annual N-turnover rates in 1988, defined here as: turnover =  $(N \text{ mineralized/total soil})$  $N \times 100$ , were very similar in the soil under fertilized grass  $(5.1\%, \text{ GN})$  or unfertilized grass (5.3%, GO), but were considerably higher under grass/clover (9.0%, GC). It is possible, therefore, that the forms of N accumulated in the soil organic matter under grass/clover in the present study, were mineralized more easily than those under grass. Our values are in the middle to upper end of the range of N-turnover rates reported in the literature e.g. 1-3% (Bremner, 1965), 2.5% (Tyson et al., 1990), 3.6% (Macduff and White, 1985), 2-6% (Hassink et al., 1990),  $3-7.5\%$  (Ball and Ryden, 1984), and up to  $10\%$ (Bartholomew, 1965), assessed by various techniques for different soil types.

In another study, also on Frilsham soil, the N content of the soil under a grazed grass/clover sward reached an equilibrium after approximately 10 years (Tyson et al., 1990), so that the high value which we obtained for net mineralization with GC  $(415 \text{ kg N ha}^{-1})$  may be a feature of a well-established mixed sward. Certainly, high productivity has been achieved from unfertilized mixed swards grazed by sheep (Parsons et al., 1991) which was attributed to rapid cycling of relatively large quantities of N through the soil/ plant/animal system: mineralization rates comparable to those in our study may have contributed to this rapid cycling.

It is also interesting to note that the unfertilized grass sward (GO) out-yielded GC in both N and herbage and was also the most efficient in terms of the recovery of mineralized N in herbage. Whether this high productivity could have been sustained in subsequent growing seasons is uncertain and may have only been a short-term legacy of the high N inputs from fertilizer and grazing returns of previous years. Unfertilized swards in The Netherlands were reported to generally yield  $100-250$  kg N ha<sup>-1</sup> y<sup>-1</sup>, but higher values were not uncommon, up to a maximum of  $528$  kg N ha<sup>-1</sup> (van der Meer and van Uum-van Lohuyzen, 1986). In the UK, values for N offtake in unfertilized cut swards ranged from 8 to 351 kg N ha<sup>-1</sup> (Richards and Hobson, 1977) so that the 321 kg N ha<sup> $-1$ </sup> estimated to have been supplied through mineralization in GO is within the range suggested by these published values, although lower offtakes have been found, i.e. 11 to  $136$  kg N ha<sup>-1</sup> (Morrison et al., 1980).

Of concern in measuring components of the N cycle is the lack of reliable data to account for all of the various pathways of loss. When allowance was made for the contribution of N from fixation (GC) or fertilizer (GN) and the amounts of N removed in herbage from these swards, 198 kg N and 192kgN, respectively, were unaccounted for, compared with only 58 kg N in GO. Excess soil N, which exceeds the immediate requirements of a growing crop, is vulnerable to gaseous and leaching losses. On the same swards on this freely draining soil, over-winter leaching of nitrate (kg N ha<sup> $-1$ </sup>) was estimated to be 199 and 3 (1987 and 1988) and 20 and 4 (1988 and 1989) in GN and GC, respectively (Macduff et al., 1990).

Some of the N which was unaccounted for in our studies may, therefore, have moved below the root zone to be subsequently leached in the following winter, but this is unlikely to have been the major route for the disappearance of N from either GC or GO. Since the N contents of the unharvested fractions of the plants were not measured, some of the N which was unaccounted for may have been incorporated into roots and stubble, particularly in GC, with high levels of unaccounted for N and low leaching losses. An alternative explanation for the disappearance of mineralized N is immobilization by the soil microbial biomass. In another study, by far the largest component of mobile N in the present soils was in the microbial biomass (Bristow and Jarvis, 1990), which can compete effectively with plant roots for available N (Jackson et al., 1989) and has the capacity to immobilize N rapidly (Bristow et al., 1987). The lack of detectable change in soil inorganic N levels when N fertilizer was added to GN, is indicative of a high buffering capacity for N, perhaps through a rapid uptake into plants and especially into the soil microbial biomass.

In the previous study (Hatch et al., 1990) and again here, the chemical extraction method for potentially mineralizable N (Whitehead, 1981) gave a poor indication of the amount of N mineralized as compared with the in situ field incubation technique, viz. 32, 44 and 43% of the field estimates for GC, GO and GN, respectively. This shortfall in the chemical index may have been due to the existence of forms of organic N which were resistant to chemical extraction at the start of the growing season, but which subsequently underwent transformation and became susceptible to mineralization as the season progressed. Further, additional inputs of N to the soil, which will not be included in an initial chemical extraction and which may represent a substantial source of relatively easily mobilized N, may have come from unharvested, senescent plant material. Returns of N by this means have been estimated to amount to  $252 \text{ kg N} \text{ ha}^{-1}$  in a comparable grass sward on the same soil type receiving 420 kg N ha<sup>-1</sup> y<sup>-1</sup> which was continuously grazed by sheep (Parsons et al., 1991).

Mineralization is highly dependent upon environmental conditions (see Haynes, 1986). Mea**surements of net mineralization using the field incubation technique were positively correlated with seasonal temperature fluctuations, but were less sensitive to soil water content. In this respect, a shortened incubation period may help to improve the resolution of the effects of soil water. It is unlikely, however, that variation in these two variables alone could be used to explain variation in mineralization rates and as our results are derived from a single growing season, further information is required of these and other factors, and their interactions which may influence mineralization. Despite these shortcomings, the present method provides a rapid and convenient means of assessing the contribution that this important process makes to the overall N budget in grassland soils.** 

#### **Acknowledgements**

We thank D H Roberts for maintaining the field experiments, A M Cornell for some of the chemical analysis and H Shaikhani for statistical advice. The work was commissioned by the Ministry of Agriculture, Fisheries and Food, London.

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