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Combining field incubation with nitrogen-15 labelling to examine nitrogen transformations in low to high intensity grassland management systems

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Abstract The ^{15}N isotope dilution method was combined with a field incubation technique to provide simultaneous measurements of gross and net rates of N turnover in three long-term swards: unfertilized (Z) or receiving N either from N fixation as clover (C), or as 200 kg fertilizer N ha^{-1} $year^{-1}$ (F). Uniform N enrichment of soil microplots was achieved with a multi-point soil injector to measure mineralization/immobilization turnover and nitrification over a 4-day incubation. Net rates of mineralization ranged between 0.6 and 2.9 μg N g^{-1} day^{-1} and in all three treatments were approximately half the gross rates. Nitrification rates (gross) were between 1.0 and 1.6 μg N g^{-1} day^{-1}. In the F treatment, the turnover of NH_4^+-N and NO_3^--N pools was on a 2- and 4-day cycle, respectively, whereas in the N-limited treatments (C and Z) turnover rates were faster, with the NO_3^--N pools turning over twice as fast as the NH_4^+-N pools. Therefore, available N was recycled more efficiently in the C and Z treatments, whereas in the F treatment a higher N pool size was maintained which would be more vulnerable to leakage. A large proportion of the added ^{15}N was recovered in the soil microbial biomass (SMB), which represented a 4–5 times larger sink for N than the plant biomass. Although the C treatment had a significantly lower SMB than the grass-only treatments, there were no differences in microbial activity. Gross rates of nitrification increased along the gradient of N input intensity (i.e. Z<C<F), and the addition of a nitrification inhibitor (C_2H_2) tended to increase microbial immobilization, but did not influence plant N uptake. In this study, the value of combining different techniques to verify net rates was demonstrated and the improved methodology for ^{15}N labelling of soil enabled measurements to be obtained from relatively undisturbed soil under natural field conditions.

Key words Nitrogen-15 mean pool dilution · Mineralization · Immobilization · Nitrification · Field incubation

Dedicated to Prof. K. Vlassak on the occasion of his 65th birthday

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Introduction

It may be sufficient, in agronomic terms, to consider only net measurements of N cycling processes as indicators of the potential availability of N for plant uptake, or removal into the wider environment. However, to understand better the factors determining mineralization/immobilization turnover (MIT) of soil organic N, it is also important to consider the gross rates of the processes involved. Such measurements can be acquired using the ^{15}N mean pool dilution technique (Kirkham and Bartholomew 1954) and net rates may be derived from the difference between the processes that produce and consume NH_4^+-N (Davidson et al. 1991). It has been shown (Ledgard et al. 1998) that the main determinant of net N release is the extent of microbial immobilization, but this process is particularly difficult to quantify for the following reasons:

1. Consumption of the $^{15}NH_4$ label includes processes other than microbial immobilization, e.g. plant uptake, NH_3 volatilization, nitrification, chemical adsorption.
2. Estimates of the assimilation of the ^{15}N label by the soil microbial biomass (SMB) may be inaccurate because of incomplete recovery by fumigation-extraction methods and the N label may stimulate the process, being itself a source of substrate (Davidson et al. 1991).

3. Microbial uptake of organic N by the "direct" route (avoiding MIT altogether) may give erroneous values for the gross rate of mineralization (Gibbs and Barraclough 1998) and thereby underestimate the SMB component.

For these reasons, an independent measurement of net mineralization is desirable: a number of such methods for net mineralization exists (see Jarvis et al. 1996), but most perturb the soil. It is important that, as far as possible, natural conditions remain unaltered and net measurements, in particular, also require sufficient time (i.e. >24 h) to establish measurable changes. Hatch et al. (1998) recently described a field incubation method based on intact soil cores, sheathed in tubes to protect the cut surfaces, that enables in situ incubation with minimal soil disturbance. They described the use of the tube incubation method in long-term grassland systems with contrasting N-management histories. For a fuller description of the experimental site see Tyson et al. (1993). These pastures had been shown previously, using a jar incubation technique, to exhibit a wide range of net release of N (Gill et al. 1995) and nitrifying activity also differed significantly between management systems (Hatch et al. 1996). An alternative estimate of net N release can be calculated from the difference in gross rates of mineralization and immobilization obtained by mean pool dilution. Here we describe how a specially designed soil injector was constructed to ensure uniform ^{15}N enrichment of the undisturbed soil [an essential prerequisite for accurate measurements, see Davidson et al. (1991)] and how recent improvements in the methodology for recovery of ^{15}N from soil extracts (Stark and Hart 1996) enabled large numbers of samples to be processed efficiently.

We compared three methods for the measurement of net rates: tube, or jar incubations, or from ^{15}N calculation, and also describe how the tube incubation method can be combined with ^{15}N labelling techniques to provide simultaneous measurements of MIT in both gross and net terms. We also examined how variations in these processes affected the overall balance and to what extent this was influenced by nitrification.

Materials and methods

Site

The experiment was performed in the summer of 1997 using some of the Rowden field drainage experimental sites on the IGER farm at North Wyke Research Station (Devon, S.W. England). These were grazed swards which had been under long-term pasture for the last 50 years. The soil is a poorly drained, silty clay loam of the Hallsworth series, a typical pelo-stagnogley soil [England and Wales classification (Findlay et al. 1984)] and this site has been widely used in previous studies of soil N processes (Gill et al. 1995; Lovell et al. 1995; Ledgard et al. 1998). The area was divided into 1-ha treatment paddocks which had been under the present grazing management for at least 15 years.

The three base treatments used in this experiment were fertilized grass swards receiving 200 kg N ha^{-1} $year^{-1}$ (F), or swards receiving no fertilizer N containing either a mixture of grass and clover (approximately 10% by weight) (C), or grass only (Z). The C swards had been reseeded (with grass) in 1982 and slot-seeded with clover in 1988; N fixation in succeeding years averaged 110 kg N ha^{-1} $year^{-1}$ (Ledgard et al. 1998). All swards were drained by a system of field and mole drains (Tyson et al. 1993). Within each of these base treatments, individual plots (30 m^2) were sited to provide sets of three replicates. During the grazing season of 1997, animals were excluded from these plots and the swards were maintained in a three-cut silage system. In April, all the plots received P and K (25 kg ha^{-1} and 50 kg ha^{-1}, respectively) and the F plots also received 40 kg N ha^{-1}. The nine plots were cut in June, and again in July, followed by fertilizer applications of 60 kg N ha^{-1} to the F plots on both occasions. Thus, by the start of the measurement period (13 August), the F plots had received a total of 160 kg N ha^{-1}, i.e. 80% of the intended annual application.

^{15}N injection

Initial analysis of soils from the experimental sites suggested that inorganic N levels (NH_4^+ plus NO_3^-) could occasionally reach 20 mg N kg^{-1} dry soil. Therefore, an addition of an equivalent amount of ^{15}N-enriched source would result in a dilution of this source by up to twofold in the soil N pool following injection. Because rates of mineralization in these grassland soils were likely to be high (Gill et al. 1995), the initial enrichments were increased to 25 at.% $^{15}NH_4^+$ and to 20 at.% NO_3^- (since ammonification rates tend to exceed nitrification rates), which would result in a nominal starting enrichment of approximately 10 at.% N.

Microplots consisted of PVC tubes (175 mm length, 100 mm diameter), each with a chamfered cutting edge (30°) at one end. Instead of paired microplots, as used by Barraclough (1991), individual microplots were inserted in the ground (150 mm depth) using a drop-hammer device. A multi-point soil injector (Fig. 1a) was constructed which fitted onto the rim of the microplots, based on a modified design of a gas injector described by Murphy et al. (1997), but adapted for liquid additions. Injection of the liquid was via a cluster of 13 spinal needles (14-gauge stainless steel, 150 mm length), mounted in brass couplings in a perspex manifold and held in place with knurled screw caps. The pointed ends of the needles were sealed with epoxy resin and drilled with two side-port openings (10 mm behind the tips) to avoid injection beyond the depth of the microplot. Similarly, the injection ended with the needle tips 10 mm below the surface to ensure that the liquid was dispersed within the soil profile. Flow restrictors were placed over the top end of the needles to increase the back pressure within the manifold and help to maintain an even flow of liquid through the needles. The manifold and needles were supported on three polished stainless steel rods (16 mm outer diameter) which allowed the assembly to move a total distance of 140 mm. In the primed position, the needles were fully extended and a 60-ml syringe (containing 40 ml liquid) was placed in a clamp and connected to the inlet of the manifold by silicon tubing using Luer-lock fittings.

A polyethylene (100 mm diameter, 10 mm deep) template (Fig. 1b), which fitted closely inside the microplot, enabled guide holes to be made in the soil to a depth of 150 mm using a wooden handled spike. The soil injector was positioned over the microplot and the needles were then gently introduced into the soil, taking care to insert the whole needle cluster evenly, until the base plate was seated on top of the microplot rim. A handle winder, mounted on screw studding (25 mm outer diameter, 5 threads 25 mm^{-1}), was synchronized with the movement of the plunger and the motion of the needles through the soil profile. By rotating the handle, the needles were withdrawn steadily through the soil whilst the plunger was advanced into the syringe barrel to force liquid (40 ml) from the manifold. In this way, injected liquid was always uniformly distributed throughout the soil, regardless of the rate at which the needles were withdrawn.

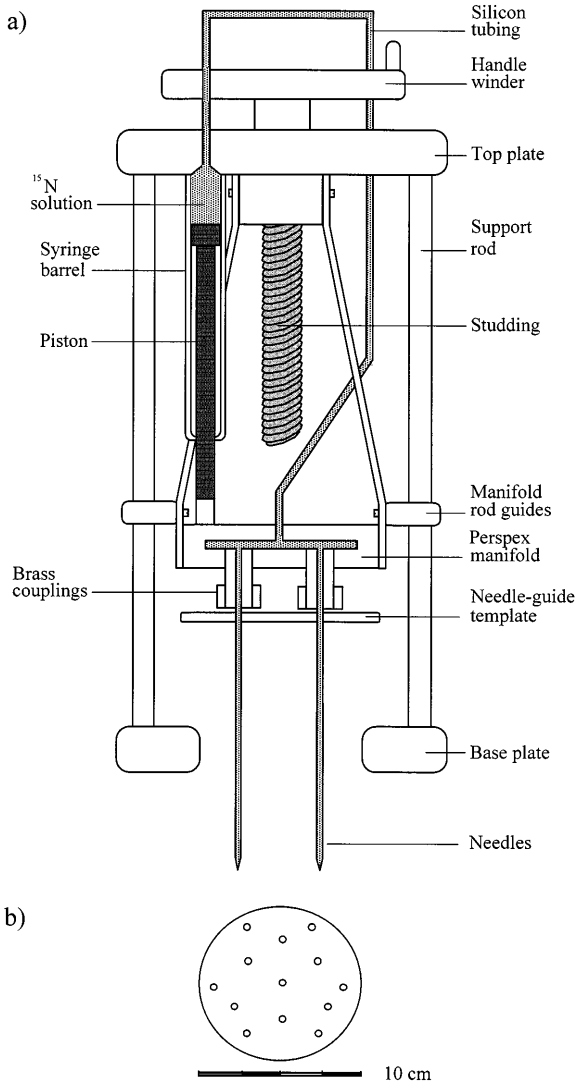

Fig. 1 **a** Diagram of the multi-point soil injector, **b** plan view of the needle-guide template which determined the location of the needles in the microplot

Experimental

One week before measurements started (6 August), 54 microplots were inserted into the soil (six microplots to each of three replicate plots × three treatments). The microplots were left for 1 week for the swards/soils to recover from any disturbance, and then on 12 August, herbage from within the microplots was cut level with the rim (i.e. 25 mm above the soil surface) to impose uniformity of plant cover between microplots (representative of cropped swards under intensive grassland management). The following day (*t*0), each microplot was injected uniformly with ^{15}N-enriched solutions throughout the soil-core profile using the soil injector. In each of the base treatments, 12 microplots received a solution containing 20 mg N kg^{-1} dry soil at 25 at.% $(^{15}NH_4)_2SO_4$ and another six microplots received 20 mg N kg^{-1} dry soil at 20 at.% $K^{15}NO_3$.

After 24 h equilibration (*t*1, 14 August), a core was removed from the centre of each microplot, using a PVC incubation tube (200 mm × 38 mm internal diameter) fitted with a cutting ring (Hatch et al. 1998). The soil remaining in the microplot, which consisted of a concentric ring of soil with a hollow centre, was removed from the microplot for analysis. The incubation tube, containing soil with the same ^{15}N enrichment as the *t*1 soil, was placed in an incubation vessel (175 mm × 50 mm internal diameter) sunk into the ground and left in the field for a further 96-h incubation. Half of the microplots that had received $^{15}NH_4^+$ were incubated in the presence of C_2H_2, a nitrification inhibitor (i.e. plus inhibitor, A+ data set; Table 1) and the other half were incubated without C_2H_2 (i.e. no inhibitor, A− data set; Table 1). The microplots receiving $^{15}NO_3^-$ were also incubated without C_2H_2 (N data set; Table 1). Infusion of the microplots with C_2H_2 was achieved with wax-coated granules of CaC_2 placed beneath the incubated soil cores which maintained C_2H_2 concentrations >0.01% in the soil pores during the incubation (Hatch et al. 1998).

At the end of the incubation period (*t*5, 18 August), the soil cores were removed and analysed within 1 h. Over the experimental period (13–18 August) the mean maximum and minimum air temperatures were 23.4 °C and 12.7 °C, respectively. The soil temperature at 100 mm depth averaged 17.3 °C, with a total of 49.2 h sunshine. Rain (3.9 mm) occurred on 12 August, but the experimental period remained dry and warm during a prolonged spell of settled weather.

Soil/plant processing

On each day of sampling (*t*1 and *t*5), soil was removed from microplots and incubation tubes, respectively, and passed through a 6-mm mesh. Stones were discarded, but all plant material (shoots plus roots) which was retained on the sieve was carefully removed, washed clean of soil over a 200-μm mesh and dried in an oven at 105 °C. The dried plant material was then weighed and ground to a fine consistency with a hammer mill (model A20; JKA) and then a ball mill (Glen Creston). Soil sub-samples (100 g

Table 1 Treatment and ^{15}N-enrichment codes for sources of labelled N ± nitrification inhibitor (C_2H_2)

Sward	Base treatment code	Enrichment code	Nitrification inhibitor	Source	Enrichment
Fertilized grass	F	A	−	$^{15}NH_4^+$-N	25 at.%
Fertilized grass	F	A	+	$^{15}NH_4^+$-N	25 at.%
Fertilized grass	F	N	−	$^{15}NO_3^-$-N	20 at.%
Grass/clover	C	A	−	$^{15}NH_4^+$-N	25 at.%
Grass/clover	C	A	+	$^{15}NH_4^+$-N	25 at.%
Grass/clover	C	N	−	$^{15}NO_3^-$-N	20 at.%
Unfertilized grass	Z	A	−	$^{15}NH_4^+$-N	25 at.%
Unfertilized grass	Z	A	+	$^{15}NH_4^+$-N	25 at.%
Unfertilized grass	Z	N	−	$^{15}NO_3^-$-N	20 at.%

fresh) were dried at 105 °C overnight to assess the gravimetric water content. Other soil samples (50 g fresh) were shaken with 250 ml of 2 M KCl; the extracts were frozen on the day of sampling to prevent microbial activity during storage and analysed at a later date for NH_4^+ and NO_3^- (Skalar autoanalyser). SMB was determined by fumigation-extraction (Brooks et al. 1985; Vance et al. 1987) using 12-g samples of fresh soil extracted with 0.5 M K_2SO_4. The extracts were used to determine biomass C by dichromate digestion and titration against ammonium ferrous sulphate, and biomass N by Kjeldahl digestion and flow injection analysis, as summarized by Lovell et al. (1995). Biomass C and N were calculated using factors 2.64 Ec and 2.22 En, respectively (Vance et al. 1987; Jenkinson 1988) and expressed on an oven dry soil basis. SMB respiration was measured on moist soil samples incubated at 25 °C in the laboratory. CO_2 was measured against a 1% standard gas on an infra red gas analyser and basal respiration was expressed as μg CO_2–C g^{-1} dry soil day^{-1}.

Diffusion of ^{15}N-labelled solutions

The inorganic N enriched with ^{15}N was recovered from soil extracts using a diffusion technique described by Stark and Hart (1996). A minimum of 100 μg N was required to ensure a robust signal for mass spectrometry. Therefore, sufficient KCl extract to contain either 200 μg N or 140 μg N (initial tests suggested recoveries of 50% and 70% for NH_4^+ and NO_3^- contents, respectively) was placed into 250-ml wide-neck polyethylene bottles. A polytetrafluoroethane (PTFE)-encased trap consisting of two 7-mm-diameter Whatman no. 1 filter paper discs (acidified with 2 M $KHSO_4$) was added to each bottle. In the case of ^{15}N-labelled NH_4^+, the extracts were diffused for 6 days at 25 °C with 0.3 g MgO, whereas ^{15}N-labelled NO_3^- was first diffused for 7 days in open bottles (without lids) which were swirled daily to drive off NH_3. After this time, water was added to replace evaporative losses and an extra scoop (0.3 g) of MgO plus 0.5 g Devarda's alloy were added to the extracts; the bottles were sealed tightly with a screw lid and left for a further 6 days to diffuse. Losses of gaseous NH_3 via the screw caps were avoided by inverting the bottles during the diffusion process. Analysis of the SMB Kjeldahl digests (containing 140 μg N) for ^{15}N enrichment, followed the same procedure as for KCl extracts, but in this case the solutions in the diffusion bottles were made sufficiently alkaline to liberate $^{15}NH_3$ (pH>11.0), by the addition of 10 M NaOH (rather than MgO) to overcome the strong acidity of the digests.

After diffusion, the PTFE-encased traps were recovered, rinsed in deionized water, and the acidified paper discs were dried over concentrated H_2SO_4 in a desiccator. Each pair of dried discs was placed together in a single tin capsule (8 × 5 mm) and analysed for ^{15}N enrichment on a VG 602E mass spectrometer, linked to a Carlo Erba CN analyser and controlled by Europa ANCA software/interface. Gross N mineralization rates were calculated from the dilution of the ^{15}N-labelled NH_4^+ pool over 4 days (i.e. *t*5–*t*1), following the equations of Kirkham and Bartholomew (1954). Similarly, gross nitrification was obtained from the rate of dilution of the ^{15}N-labelled NO_3^- pool. Gross immobilization rates were estimated from the appearance of ^{15}N in the SMB divided by the mean ^{15}N enrichment of the NH_4^+ pool in the soil (Davidson et al 1991). Alternative estimates of immobilization were also obtained: the rate of NH_4^+ consumption was found from the rate of removal of $^{15}NH_4^+$, and NH_4^+ immobilization was also calculated by subtracting gross nitrification from the rate of NH_4^+ consumption (Davidson et al 1991). Net mineralization was calculated from the difference between the gross rates of mineralization and NH_4^+ immobilization.

Jar incubation method

An established method for measuring net N mineralization (Hatch et al. 1990) was included as an independent method to enable a comparison with the ^{15}N tube incubation method described above. Four pairs of soil cores (37 mm diameter) were taken from each replicate plot; one core from each pair was placed in a 1-l Kilner jar (three replicate jars to each treatment) and the remaining four cores were bulked and analysed immediately for NH_4^+-N content. The Kilner jars were sealed, 2% C_2H_2 was added to the headspace, and then they were incubated in holes in the ground for 7 days. At *t*5, the cores were removed and analysed, in the same manner as for the *t*0 cores. For both jar and tube incubations, net mineralization was calculated from the difference in NH_4^+-N contents of the soil cores at the start of and after the incubation, and expressed as the mean net rate per day.

Statistical analysis

Data were examined by ANOVA and Student's *t*-test (using standard statistical software).

Results

The dry bulk density of the soils F, C and Z were 0.95 g cm^{-3}, 0.99 g cm^{-3} and 0.88 g cm^{-3} respectively. Prior to injection of ^{15}N (*t*0), the inorganic N contents of the soils were ca. 5 mg NH_4^+-N kg^{-1} soil in both F and C treatments, but were lower in treatment Z (Table 2). There was a similar NO_3^- content in F (5.9 mg N kg^{-1} soil) but only one-tenth of this amount (ca. 0.5 mg N kg^{-1} soil) in C and Z treatments (Table 2). On average,

Table 2 Inorganic N contents (mg N kg^{-1} dry soil; SE in parentheses) of NH_4^+-N pool and NO_3^--N pool, before injection of 20 mg N kg^{-1} soil (*t*0), and at 24 h (*t*1) and 96 h (*t*5) after injection ($n = 6$). For abbreviations, see Table 1

Treatment	*t*0		*t*1		*t*5	
	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N	NH_4^+-N	NO_3^--N
FA −	5.9 (0.52)	5.9 (2.44)	26.2 (1.78)	18.1 (7.92)	34.7 (5.06)	16.8 (5.44)
FA +	5.9 (0.52)	5.9 (2.44)	28.6 (2.66)	31.8 (10.31)	35.3 (2.73)	25.7 (8.68)
FN −	5.9 (2.44)	5.9 (2.44)	8.5 (1.52)	41.2 (9.70)	12.3 (4.48)	43.4 (9.14)
CA −	4.5 (0.31)	0.5 (0.15)	24.1 (1.58)	6.0 (8.20)	21.8 (2.11)	12.1 (1.66)
CA +	4.5 (0.31)	0.5 (0.15)	26.7 (2.21)	7.1 (1.73)	32.3 (2.53)	6.5 (3.33)
CN −	4.5 (0.31)	0.5 (0.15)	9.9 (0.78)	28.2 (2.20)	10.3 (2.56)	26.5 (2.13)
ZA −	3.7 (0.44)	0.3 (0.07)	24.4 (2.21)	2.4 (0.68)	36.7 (8.92)	7.4 (2.83)
ZA +	3.7 (0.44)	0.3 (0.07)	20.6 (1.04)	3.2 (2.18)	26.7 (5.65)	1.0 (0.11)
ZN −	3.7 (0.44)	0.3 (0.07)	8.8 (1.51)	21.2 (0.58)	6.9 (0.76)	22.8 (1.26)

Table 3 Mean ^{15}N enrichments and percentage recovery of ^{15}N from NH_4^+ ($A \pm C_2H_2$) and NO_3^- ($N–C_2H_2$) pools ($n=6$). For abbreviations, see Table 1

Treatment	^{15}N enrichment of soil		Recovery of ^{15}N (%)							
	at.%	^{15}N	Soil		SMB		Plant[a]		Total	
	*t*1	*t*5	*t*1	*t*5	*t*1	*t*5	*t*1	*t*5	*t*1	*t*5
FA−	14.5	9.0	64.0	50.3	5.2	11.7	2.7	4.8	72	67
FA+	15.1	8.8	71.5	51.9	5.2	15.2	2.9	4.8	80	72
FN−	10.8	9.4	64.6	61.1	0.3	0.0	1.4	3.0	66	64
CA−	14.9	6.5	60.1	22.3	18.3	10.8	5.0	7.8	83	41
CA+	14.5	8.5	64.8	44.9	25.1	18.7	3.4	5.2	93	69
CN−	13.5	11.2	63.2	49.6	12.6	0.0	3.9	8.8	80	58
ZA−	13.7	8.6	55.7	54.4	15.8	19.5	5.1	7.9	77	82
ZA+	13.9	8.9	48.1	42.2	45.1	22.8	5.7	10.9	99	76
ZN−	16.0	13.4	57.2	51.3	0.1	0.0	5.1	7.3	62	59

[a] Shoot plus root

each needle in the soil injector delivered 2.99 ± 0.275 ml solution, which was estimated to have increased the total soil water content of the microplots by not more than 5% (w/w). At *t*1, following injection of ^{15}N, the inorganic N in all treatments increased ($P<0.001$) by approximately 20 mg N kg^{-1} soil, as intended (the exception was FN), and these concentrations persisted through to the *t*5 sampling.

The ^{15}N enrichments of the soils at *t*1 were broadly similar across all treatments (range 10.8–16.0 at.%; Table 3), and had only declined by about one-third at *t*5. Consequently, of the total ^{15}N recovered from all treatments at *t*5, the soil retained the major component (22–61%), with more variable amounts recovered in the SMB (0–23%) which in some treatments declined with time; the minor component was in plant material, i.e. 3–11% in shoots and roots. Of the ^{15}N added, therefore, on average 68%, 56% and 72% in total was recovered at *t*5 in F, C and Z treatments, respectively. Recovery of ^{15}N by plants (shoots plus roots) was similar in all three treatments and increased by about 50% between *t*1 and *t*5.

Although the lowest values for gross mineralization were found in the Z treatments (Table 4), no significant differences were found between the treatments. Conversely, the lowest rate of immobilization of NH_4^+ (calculated) was found with the F treatments, and this trend was repeated in the immobilization values found in the chloroform-labile pool of the SMB, but the data were highly variable and no significant differences were established.

Net rates of mineralization (Table 5) obtained with the tube and jar incubation methods were in general agreement and fell within the range calculated from ^{15}N measurements (i.e. 0.6–2.9 μg N g^{-1} day^{-1}). Net rates of mineralization were approximately half the gross rates found for each of the treatments. Estimates of net mineralization in the C treatment were intermediate between those calculated for treatments F and Z, but there were no significant differences overall between treatments. The highest rate of gross nitrification was found with the F treatment, but this differed from Z only at a low level of significance ($P=0.09$); rates with C were intermediate. Gross nitrification was also balanced with NO_3^- consumption. The rate of nitrification in the F soil was similar to the SMB immobilization rate, but about 70% in treatment C and only <40% in treatment *Z*.

SMB data were variable [coefficient of variation (CV) = 93.1%], with immobilization rates which increased in the order: treatments F<C<Z, but the differences were not significant (Table 4). In terms of SMB size, biomass C was ca. 2000 μg C g^{-1} dry soil in treatments F and Z, but 30% lower ($P<0.05$) in treat-

Table 4 Gross rates (μg N g^{-1} day^{-1}; SE in parentheses) of mineralization, NH_4^+ consumption and immobilization obtained from ^{15}N mean pool dilution ($n=6$). *SMB* Soil microbial biomass; for other abbreviations, see Table 1

Treatment	Gross mineralization	NH_4^+ consumption	NH_4^+ immobilization[a]	SMB immobilization
$FA-C_2H_2$	4.1 (1.41)	2.8 (1.26)	1.2 (1.34)	1.5 (0.35)
$FA+C_2H_2$	4.2 (0.73)	2.5 (0.79)	0.9 (0.91)	2.0 (0.43)
$CA-C_2H_2$	5.4 (1.14)	6.0 (1.21)	4.8 (1.25)	1.7 (0.34)
$CA+C_2H_2$	4.1 (0.63)	2.7 (0.48)	1.5 (0.58)	2.4 (0.74)
$ZA-C_2H_2$	3.6 (0.55)	4.0 (0.54)	3.0 (0.54)	2.8 (0.48)
$ZA+C_2H_2$	2.5 (0.65)	4.3 (1.06)	3.3 (1.06)	3.0 (0.61)

[a] Calculated from Davidson et al. (1991) (NH_4^+ consumption − gross nitrification = NH_4^+ immobilization)

Table 5 Different techniques used to estimate (μg N g^{-1} day^{-1}; SE in parentheses) net mineralization and gross NO_3^- production (nitrification) and consumption ($n=6$, except jar: $n=3$). For abbreviations, see Table 1

Base treatment	Net mineralization			NO_3^-	
	Tube	Jar	Calculated[a]	Production (nitrification)	Consumption
F	2.3 (0.66)	1.9 (0.32)	2.9 (1.95)	1.6 (0.45)	1.5 (0.42)
C	2.1 (0.84)	1.8 (0.13)	0.6 (1.69)	1.2 (0.33)	1.6 (0.47)
Z	0.6 (1.80)	1.3 (0.28)	0.6 (0.77)	1.0 (0.06)	0.8 (0.16)

[a] Calculated from the difference between gross mineralization and NH_4^+ immobilization (using A-data sets)

ment C; C:N ratios were 6.8, 6.7 and 6.0, respectively. Basal microbial respiration rates (μg CO_2–C g^{-1} dry soil day^{-1}) were lowest ($P<0.01$) in treatment C (39.1 ± 1.70), but similar in treatments F and Z (57.7 ± 2.71 and 53.9 ± 3.77, respectively). Microbial activity, assessed by specific respiration, was similar in all three treatments, with a mean value of 29.3 ± 2.60 μg CO_2–C mg^{-1} microbial C g^{-1} day^{-1}.

Plant N uptake data (calculated from ^{15}N recoveries within base treatments) were also variable (CV = 60.3%), with only low rates which averaged 0.24 ± 0.051, 0.44 ± 0.055 and 0.51 ± 0.063 kg N ha^{-1} day^{-1} for F, C and Z treatments, respectively. These results may not be wholly representative of the swards in equilibrium with the management regimes, but demonstrated a more rapid response to recently applied N in those swards containing N-starved plants (viz treatments C and Z), compared with the lower uptake rate ($P<0.01$) found in the higher N-status plants (treatment F). There was no effect of either the form of N (NH_4^+ or NO_3^-) or inhibitor (C_2H_2) on the relative uptake rates within the base treatments, but between treatments, NO_3^- uptake rates were lower in treatment F ($P<0.05$) than in treatments C or Z.

Discussion

The ^{15}N soil injector performed well and achieved enrichments in the soil N pools at *t*1 which were broadly within a range suitable for direct measurement on the mass spectrometer, i.e. ^{14}N spiking of the samples was unnecessary. However, variability between replicates, and in particular in ^{15}N recovered in the SMB, tended to be high. This would have arisen from variations in the soil N pool size, incomplete mixing of the enrichment solution with the N pools and inconsistent recovery of ^{15}N immobilized in the SMB. Such problems are recognized in studies involving undisturbed soil, particularly in field experiments where data can be difficult to interpret because of spatial variation (Barraclough and Puri 1995). However, the alternative of using sieved soil to achieve homogeneous soil N pools would almost certainly have disturbed the natural equilibria of the measured process rates. The recoveries in this experiment were in line with total recoveries of added ^{15}N in other studies (Bristow et al. 1987; Schimel et al. 1989), with a similar range in recovery of ^{15}N in the SMB (viz 9–15%) to that reported by Hart et al. (1993). A potential source of error in attributing the initial, rapid removal of ^{15}N (due to abiotic reactions) to microbial assimilation, was largely avoided by delaying the first sampling for 24 h after ^{15}N injection, as recommended by Barraclough (1991).

Gross rates of mineralization and NH_4^+ consumption were similar in all treatments, and the amounts of N processed each day were of the same order of magnitude as the concentrations of the soil inorganic N pool prior to injection. Thus, inherently low inorganic N levels would be expected to remain under "steady state" conditions, unless amended by fertilizer inputs. However, differences in gross rates do not necessarily correspond to differences in N turnover rates (Tietema and Wessel 1992). A useful indication of the relative turnover rates in these treatments can be obtained from calculations of pseudo-residence times, defined as the ratio of the NH_4^+-N pool size to the NH_4^+-N consumption rate (Frissel 1981). Ratios of 2.1, 0.8 and 0.9 for the NH_4^+-N pool and 3.9, 0.3 and 0.4 for the NO_3^--N pool were found with the F, C and Z treatments, respectively. Therefore, turnover of N pools was faster in the N-limited swards (C and Z), with the NO_3^--N pools turning over twice as fast as the NH_4^+-N pools. An explanation for this might be that plants experiencing only a limited mobile-N supply, become effective "scavengers" of sources of available N (Parsons et al. 1991). In the F swards, the turnover of the NH_4^+-N pool was on a 2-day cycle, whereas the NO_3^--N pool took twice this time to be processed. Thus, different N inputs over the longer term resulted in different N turnover rates. In the case of the fertilized swards, the increased size of the available N pools and the lower turnover rates (particularly NO_3^-) would have increased the potential for losses to the wider environment, as reported by Scholefield et al. (1993). The relatively high N-turnover rates in all three treatments raises the possibility of remineralized labelled N entering the labelled pool, which can cause an "underestimation" of actual rates. However, an incubation period lasting 3–5 days at temperatures up to 23 °C has been suggested as still valid (Barraclough 1991), and during this period replenishment of

the N pool is likely to come chiefly from native N sources. It is clear then, that high fluxes and a rapid turnover of the inorganic N pools are features of these grazed grassland soils, as demonstrated in a previous study (Ledgard et al. 1998) which showed similar rates of net mineralization, but higher gross rates.

Ledgard et al. (1998) also found that immobilization was more rapid in unfertilized swards, which ultimately determined the differences found in net mineralization rates. In the present study, the effect of the inhibitor, C_2H_2 (A+ data set), was to produce higher values for the immobilization of ^{15}N in the SMB in all treatments. This would suggest that NH_4^+ uptake was enhanced when this N form predominated, and confirmed the affinity of components of microbial-uptake mechanisms for NH_4^+-N. The smaller size of the SMB found in treatment C, may have reflected the length of time since the plots were reseeded: treatments F and Z were long-term pastures, undisturbed for >50 years, whereas treatment C was ploughed in 1982 and may not have reached a new equilibrium.

The competitive success of the SMB for available N could, in turn, represent a major controlling factor over the general availability of N for plant uptake. Plant biomass was found to be a more important sink for added N than SMB by Bristow et al. (1987). However, in the present study with recently cut swards, and as reported by other workers (Jackson et al. 1989), microbial uptake was found to be the dominant factor in the removal of added N. The rapid immobilization of fertilizer N (Bristow et al. 1987) is likely, in the short term, to limit the efficiency of fertilizer N use. In the longer term, it can enhance the N supply by saving N which might otherwise have been lost, and ultimately increases the active organic N pool (Recous and Mary 1988).

Whilst plants can absorb NH_4^+ and NO_3^- equally (Clarkson et al. 1986), the dependence of roots on mass flow (mobile ions) and diffusion gradients (concentration dependent) to acquire N (Nye and Tinker 1977), suggests that the form and concentration of inorganic N in soil solutions will influence uptake patterns. Recous et al. (1988) showed that plant uptake of NO_3^- was more efficient than that of NH_4^+. In the present study, microbial uptake of NH_4^+ outstripped plant uptake, but where NO_3^- was supplied, microbial recovery was low and uptake was more efficient in the unfertilized swards, confirming that plants can compete effectively when the more mobile form of N (i.e. NO_3^-) predominates.

Measurements of net nitrification (by tube incubation) were not consistent with other estimates and may have been adversely affected by additions of N (as $^{15}NH_4^+$), since the assessment relied on differences in NH_4^+ produced in the presence and absence of the nitrification inhibitor (C_2H_2). Also, the 24-h delay in introducing the nitrification inhibitor (equilibration period, *t*0–*t*1) would have allowed some nitrification to occur before the *t*1 sampling, and this may have obscured any differences between the treatments. The immediate stimulatory effect on nitrifying activity by the injection of $^{15}NH_4$ could be largely overcome by including C_2H_2 in the enrichment solution. Other workers have used C_2H_2 dissolved in solution (Kilham 1987; Jackson et al. 1989) and an alternative approach would be to equilibrate the ^{15}N solution with C_2H_2 prior to injection. Nitrification of the newly added $^{15}NH_4^+$ would then be prevented and the effect could be sustained using wax-coated CaC_2 granules and tube incubation.

It was interesting to note that within each treatment, the presence of C_2H_2 increased the rate of immobilization of $^{15}NH_4^+$ into the SMB by 33%, 41% and 7% for F, C and Z (A+ data sets), respectively. This suggested that the addition of C_2H_2 had the greatest effect on the treatments with the highest nitrifying activities, since there would be correspondingly less N in the more mobile form (NO_3^-) available for plant uptake which would then favour microbial immobilization. In the present study, 39%, 25% and 33% of the NH_4^+ that was mineralized was nitrified (based on gross nitrification rates) in F, C and Z treatments, respectively, compared with 72% found with the same soil type in a previous autumn/winter study (Hatch et al. 1998). Similar gross rates of nitrification (12–46%) were also measured during the growing season of annual grasses in California (Davidson et al. 1990). Thus, the oxidizing capacity of the nitrifiers was exceeded in the summer conditions of the present study, which suggests that the Q_{10} of nitrifiers must be somewhat lower than that of heterotrophs involved in organic matter decomposition.

The results from our experiment suggest that nitrifying activity could be an important factor in determining the equilibria between the competing processes of microbial and plant sinks for mineral N. Such differences were also reflected in the relative amounts of NH_4^+ to NO_3^- in the inorganic N pools, with ratios of approximately 1, 10 and 12 for F, C and Z treatments, respectively. Furthermore, the microbial sinks in the three sward types were some four- to five-fold larger than the plant sinks, with proportionately higher rates of immobilization compared (on a per day basis) with plant uptake rates.

Whilst large field variability has been a recognized problem in the measurement of MIT (Stockdale et al. 1994), in this study the value of combining different techniques to provide simultaneous estimates of MIT processes was demonstrated. Although the deficiencies in each approach were evident in high variation in some of the data, and a degree of disturbance of the soil/plant equilibria was inevitable, the general agreement between net measurements showed that these were probably within acceptable limits. Comparing measurements in the presence and absence of process inhibitors is a useful approach in determining the consequences of changes in major soil processes, and is one that could be applied more widely. Finally, the advantage of obtaining supplementary information from the expensive and time-consuming ^{15}N labelling technique makes the combined ^{15}N tube incubation method a more attractive option for field experimentation.

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