Biol Fertil Soils (1998) 26:323–330

ORIGINAL PAPER

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Concurrent measurements of net mineralization, nitrification, denitrification and leaching from field incubated soil cores

Received: 18 June 1997

Abstract An improved method is described for incubating intact soil cores in the field, which permits concurrent measurement of net mineralization, nitrification, denitrification and leaching. Cores were enclosed in PVC tubes with minimal disturbance to the physical state or to the natural cycles of wetting/drying, soil temperature and aeration during an incubation lasting 4–5 days. An example of the application of the method is given in which soils with contrasting drainage characteristics were compared. Over a 64-day experimental period, 58% of the mineralized nitrogen (N) in a freely drained soil was nitrified and 36% of the nitrate-N (NO_3^--N) was denitrified. In a poorly drained soil, 72% of the mineralized N was nitrified and 63% of the NO_3^--N was denitrified. In both soil types, 18% of the remaining NO_3^--N was leached. Rates of nitrification were significantly correlated with net mineralization (r^2=0.41 and 0.52) and also closely correlated with denitrification (r^2=0.67 and 0.68) in the freely and poorly drained soils, respectively. Independent measurements of these processes, using alternative techniques (for the same period), compared favourably with measurements obtained with the improved incubation method. Adoption of this method has a number of advantages with respect to field net N mineralization, and also allows interpretation of the impact this may have on other N transformation processes.

Key words Net mineralization · Nitrification · Denitrification · Leaching · Field incubation

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Introduction

Recent advances in the use of the stable isotope nitrogen-15 (^{15}N) using pool dilution methods (Barraclough 1991; Barraclough and Puri 1995) have enabled measurements to be made of *gross* rates of some of the major processes involved in N cycling in soils, particularly mineralization. It is generally accepted (Barraclough 1991; Stark and Firestone 1996) that gross rates of transformation allow a more mechanistic explanation of the processes concerned than can be derived from net rates. However, because of the requirement for expensive materials and complex analytical equipment, these methods are most effectively employed in short-term investigations. Incubation periods are also necessarily limited by the need to avoid remineralization of immobilized ^{15}N (Kirkham and Bartholomew 1954), and Barraclough (1995) has suggested that incubations lasting from 3 to 5 days are appropriate in soils with a temperature of up to 23°C. The ^{15}N technique provides a powerful tool for the researcher to add to the other methods available for field measurements, but is less suited to long-term (seasonal) evaluations which may be needed in order that balances of flows of N in agricultural systems can be constructed. Whilst gross rates provide basic mechanistic information, a better understanding of *net* rates is needed to enable practical outcomes to be demonstrated and explained.

In order, therefore, to obtain net measurements of N transformation processes and to be able to relate these to seasonal losses of N from the soil, improved field incubation techniques are required. The existing methods rely generally on an assessment of incremental (net mineralization) changes in soil inorganic N, i.e. ammonium (NH_4^+) + nitrate (NO_3^-), or decremental changes in inorganic N (net immobilization). Soil samples taken at the start of an incubation are compared with similar samples incubated in conditions that are intended to resemble closely the fluctuating environmental conditions in the field. The various approaches adopted to measure mineralization (Jarvis et al. 1996) can be divided into two distinct methods of handling the soil samples: either disturbing the soil or using

undisturbed soil cores. Disturbed soil samples, either crumbled by hand or sieved, have been incubated in the field by burying samples in polyethylene bags (e.g. Eno 1960; Westerman and Crothers 1980; Raison et al. 1987). The advantage of this approach is the prevention of losses of NO_3^- by leaching, which otherwise might be overlooked in the evaluation; however, inaccuracies can be caused by the disturbance to the soil structure. The use of relatively undisturbed soil samples as intact soil cores helps to overcome some of the disadvantages associated with the use of disturbed soil (Cabrera and Kissel 1988; Sierra 1992), but losses can still occur as a result of denitrification, which can result in an under-estimate of mineralization. For this reason, Hatch et al. (1990, 1991) used acetylene (C_2H_2) in the headspace of the incubation vessel to inhibit nitrification and, therefore, avoid the production of NO_3^-, which might otherwise be denitrified to largely unquantifiable gaseous forms, i.e., nitrogen monoxide (NO), nitrous oxide (N_2O) and dinitrogen (N_2). The assessment of net mineralization, in this case, relied upon the change in inorganic N during an incubation of 14 days.

A problem common to all these methods is that samples are often isolated during incubation from normal fluctuations in soil moisture (due to precipitation, transpiration and evaporation) and ambient soil temperature, although the effect on the latter is generally not great. Gill et al. (1995) attempted to minimize these effects by using a relatively short incubation period of 7 days, but they were unable to find any significant effect of soil water content on mineralization rates, although the literature would suggest that wetting and drying of soil is a major influence in determining process rates (Birch 1964; Stanford and Epstein 1974). In addition, the surfaces of the soil cores are exposed during incubation so that any increase in aeration may also elevate rates of mineralization. As well as a continued need to improve methods for the measurement of N mineralization in the field, there is increasing evidence in support of the proposal (Knowles 1978) that there is a close coupling between some processes (e.g. nitrification and denitrification). An improved understanding of the extent to which interdependence/regulation exists would greatly enhance our ability to predict the response of these processes under given circumstances. An improved incubation system was, therefore, developed to address some of the problems associated with existing methods and to enable simultaneous measurements to be obtained of the major N transformation and loss processes. We used the incubation system to evaluate net mineralization, nitrification, denitrification and potential leaching during the first period of winter drainage (1996/7), immediately following the return of the soil to field capacity.

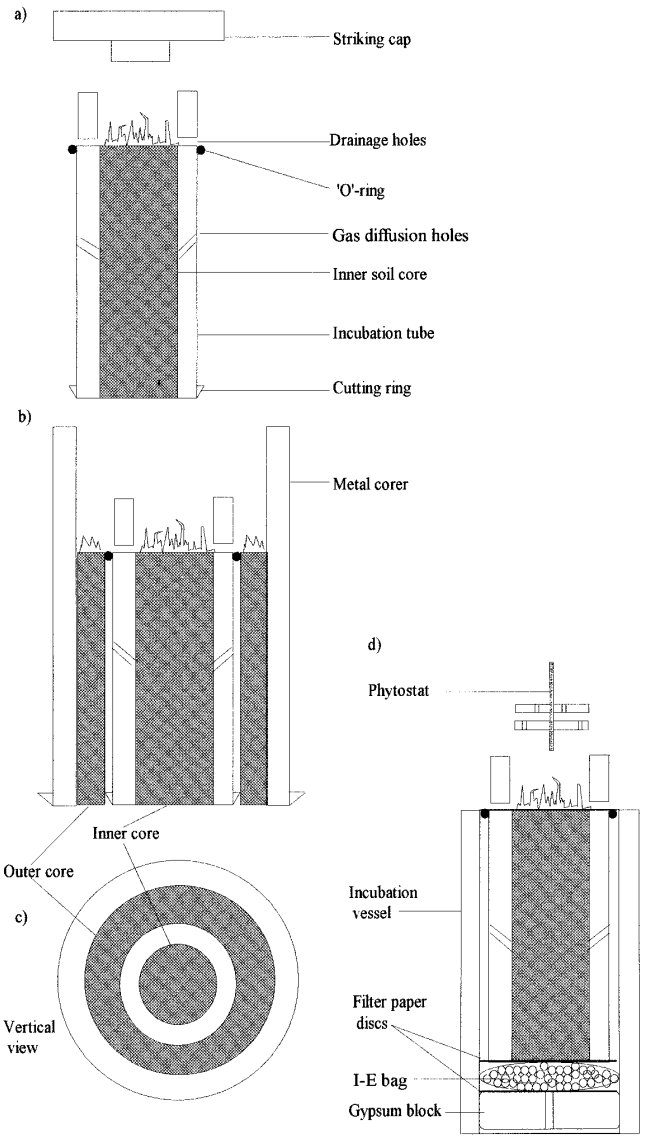

Fig. 1 a–d The field (tube) incubation system. **a** Incubation tube and soil core. **b** Metal soil corer for extracting incubation tube together with 'baseline' soil core at start of incubation. **c** Plan view of closely associated soil cores ('baseline' and incubation core). **d** Incubation tube (containing soil core and *phytostat* to suppress plant growth) fitted into incubation vessel above I-E bag and gypsum block

Materials and methods

Incubation system design features

The design features of the improved incubation system had to satisfy the following criteria: (1) in situ field incubation, (2) minimal disturbance of soil cores, (3) natural changes in soil water/temperature, (4) simultaneous measurements of processes and (5) extended (seasonal) evaluations. The incubation system (see Fig. 1) incorporated a PVC incubation tube (length 200 mm, i.d. 38 mm, o.d. 49 mm) which acted both as the coring tool (when fitted with a sharp/detachable cutting ring) and as a permanent sheath in which the soil core was held during incubation (Fig. 1a). In this way, exposure of the cut soil surfaces to abnormal aeration was avoided and compaction of the soil core was minimized. A set of four equally spaced holes (3 mm diameter) was drilled around the tube (50 mm from the top and 150 mm from the base of the tube) to provide outlets for excess surface water to drain by lateral flow from the soil, as would occur in the field. A soil core was obtained by driving the PVC tube into the ground, using a club hammer and metal striking cap (herbage was trimmed to a height of 40 mm before coring). The correct depth of core (150 mm length) was determined when these holes reached just above the surface of the soil in the tube. An O-ring (neoprene, 5 mm diameter)

was positioned in a groove cut on the outside of the tube immediately below the drainage holes. There were four further holes (3 mm diameter), equally spaced around the tube (125 mm from the top and 75 mm from the base of the tube) and inclined at 30° to the tube (sloping upwards to discourage leachate from leaking out) which permitted some exchange of gases between the core and the external soil (see below).

The PVC incubation tube containing the soil core was extracted from the ground by means of a larger, metal soil corer (80 mm i.d., 300 mm length). The metal corer was placed over the top of the protruding incubation tube and driven into the ground to the same depth as the soil core (Fig. 1b) and used to extract both the tube and an additional outer core consisting of an annular ring (30 mm width) around the incubation tube (Fig. 1c). The soil from the outer core was peeled away carefully from the PVC tube and retained as a closely representative sample of the inner soil core sample. The mineral N content of the outer core provided a baseline "starting" value for subsequent comparisons with the field incubated soil core.

An incubation vessel, consisting of a larger PVC tube (length 175 mm, 50 mm i.d., 60 mm o.d.), provided a receptacle for the tube (Fig. 1d) and was inserted into a suitably sized hole in the ground until the top of the vessel was level with the soil surface. A groove, machine cut in the top end of the incubation vessel, accepted the O-ring seal on the incubation tube so that a gas-tight connection was formed between the two tubes. A cylindrical block (47 mm diameter, 15 mm deep) of gypsum (pre-wetted under tension on a dampened sand-bed) was placed at the bottom of the vessel. The purpose of the gypsum block was to make good contact with the undisturbed soil at 175 mm from the soil surface, providing an inert medium for the transmission of soil water between the incubated core and surrounding soil. There were five holes (6 mm diameter) drilled into the gypsum: one through the centre to prevent water (leaching from the soil core) from "ponding" on top of the block, and four further holes positioned around the periphery which connected with the centre hole. These were used to supply C_2H_2 as described later.

The remaining space (10 mm) between the incubation tube and the gypsum block was occupied by a nylon bag, filled with ion-exchange resin (I-E bag) and sandwiched between two filter paper discs (47 mm diameter, grade 40; Whatman) which protected the resin and improved contact between surfaces. The function of the I-E bag was to intercept any NO_3^- which passed through the soil core, thus providing an estimate of potentially leached N. The I-E bag was constructed in the shape of a tea-bag from two circular layers of fine nylon mesh (355 μm, 48 mm diameter, Lockertex, Warrington, UK) sewn together with nylon thread. The bag contained 4 g of wet anion-exchange resin beads (Dowex 1-x 8, 20–50 mesh: a strongly basic resin supplied in the chloride form by Fluka Chemicals, Gillingham, UK). The bag was sealed before use by welding together the layers on each side of the opening, using a hot soldering iron. When the I-E bag was wetted, it occupied fully the 10 mm space above the gypsum block and helped to maintain capillarity between it and the incubated soil core. The physical separation provided by the gypsum ensured that any NO_3^- intercepted in the leachate came only from the incubated soil core and not from the undisturbed soil layer.

Controlled-release of C_2H_2

Hard-shell gelatine capsules (size 3; Farillon, Romford, UK) were used to encapsulate granules of wax-coated calcium carbide (CaC_2) and provide a controlled infusion of C_2H_2 into one set of the incubated soil cores. The capsules were pre-treated for 72 h in a desiccator by exposure to formaldehyde vapour which made them insoluble, but still porous to water. To restore their original shape and rigidity, the open halves were heated at 100°C for 30 min.

Wax-coating of the CaC_2 granules was by a simplified version of the method described by Banerjee and Mosier (1989) using either paraffin wax, beeswax, or a mixture of both. The waxes were first refrigerated and fine shavings obtained which were placed in the base of a treated gelatine capsule so that it was about 1/4 full, and CaC_2 granules (approximately 100 mg, 1–2 mm diameter) were packed above the wax to the brim of the capsule. The capsule top was also filled with wax and the two halves were pressed lightly together. Coating of the CaC_2 granules was achieved by immersing the filled capsules in a similar mixture of molten wax. The granules were coated evenly by rolling the capsules around in the molten wax until the wax inside was thoroughly dispersed (air inside the capsules escaped from between the unsealed halves). The capsules were then removed and any wax adhering to the outside was removed and the two halves were then pressed together fully to engage the built-in sealing device. After cooling, the capsules were stored in a desiccator until needed. Exposure to water softens the capsule, distorting the wax matrix so that the carbide granules become wetted progressively; the subsequent reaction leads to the release of C_2H_2 which further disrupts the wax and enables the ingress of more water. Four types of controlled-release capsules were prepared which were coated differently to produce a steady release of C_2H_2 gas throughout a 7-day incubation period:

Type 1: Uncoated, with rapid gas release from day 0 to day 1
Type 2: Coated in paraffin wax, with a delayed gas release from day 2 to day 3
Type 3: Coated in a mixture of paraffin wax/beeswax (50/50), with a delayed gas release from day 4 to day 5
Type 4: Coated in beeswax, with a delayed gas release from day 6 to day 7

The gas release times are nominal and the success of the capsules was dependent upon the persistence of C_2H_2, which acts as a nitrification inhibitor, and on a considerable degree of overlap between release times for the different types of capsules. The pre-drilled holes in each gypsum block held four capsules: one of each type, which together generated a total of 30 ml of C_2H_2. In one set of incubations, four hard-shell gelatine capsules containing CaC_2 were placed in the holes in the gypsum blocks to maintain a steady infusion of C_2H_2 into the incubated soil cores. Nitrification was inhibited by the infusion of C_2H_2 and denitrification was also curtailed when de novo NO_3^- production ceased. The resolution of both processes was then made possible from comparisons with cores which were *not* infused (see calculations). Preliminary tests on the persistence of C_2H_2 in soil cores were carried out under controlled conditions (data not shown) and demonstrated that concentrations >0.01%, which are effective at inhibiting nitrification (Berg et al. 1982), persisted within pore spaces in the soil core after a 5-day incubation at 20°C. Field incubations were arranged using four replicate cores held in incubation vessels, either with or without controlled-release capsules (i.e. $\pm C_2H_2$).

During field incubations (normally lasting <7 days, but on one occasion extended to 11 days), plant growth in the soil core was suppressed by means of a device (referred to here as a "phytostat") which prevented light from entering the incubation tube (Fig. 1d). It consisted of a pair of PVC discs (37 mm diameter) centrally mounted on a metal shaft (6 mm diameter, 50 mm length), each of which had four holes (3 mm diameter) drilled through the discs and spaced equally around the perimeter, 5 mm from the outer edge. The discs on the shaft were rotated to place them eccentrically, with no vertical alignment between the holes. This permitted water movement both into and from the tube (through precipitation and evaporation, respectively), but light was excluded and plant growth curtailed during incubation.

Temperature and soil water effects

Tests on the effect on temperature within the soil cores in the PVC tubes were carried out. Platinum resistance (2 kΩ) thermometers were inserted into two separate cores at 100 mm depth from the soil surface and readings were compared with those of thermometers placed at the same depth in the undisturbed soil. Continuous recordings, made over two separate 7-day periods, showed that the mean temperature of the sheathed soil cores was 0.25°C ($P < 0.001$) lower than that of the undisturbed soil: this discrepancy occurred following the peak in diurnal fluctuations of temperature (noon). Incubated soil cores were, therefore, slightly less buffered against heat loss than undisturbed soil. If the Q_{10} of the rate of nitrification is taken to be from 1.7–3.0 (Belser 1979), then this process could be slowed by up

Table 1 Soil particle size distribution (%) in the Crediton and Hallsworth soil series (0–150 mm depth). *OM* Organic matter (not including living roots) assessed by loss of mass on ignition

Soil series (drainage)	Clay (<0.002 mm)	Silt (0.002–0.02 mm)	Fine sand (0.02–0.2 mm)	Coarse sand (0.2–2.0 mm)	OM (%)
Crediton (freely)	24.2	10.3	14.9	51.7	5.6
Hallsworth (poorly)	48.5	12.7	16.3	7.7	14.1

Table 2 Percentage by volume of soil, stone, water- and air-filled pore spaces at field capacity in freely and poorly drained soils at IGER, North Wyke, UK (January 1997)

Soil series (drainage)	Fine soil (%)	Stone >2 mm	Pore space (% volume)			Dry bulk density (g soil cm^{-3})
			Water	Air	Total	
Crediton (freely)	44.9	5.0	35.7	14.4	50.1	1.19
Hallsworth (poorly)	27.2	0.5	46.8	25.5	72.3	0.72

Table 3 Some properties of the freely and poorly drained soils (0–150 mm depth) measured at the end of the experimental period (oven dry weight basis)

Soil series (drainage)	Inorganic N (μg N g^{-1})	NH_4^+:NO_3^-	Total N (mg N g^{-1})	Organic C (mg C g^{-1})	C:N	pH
Crediton (freely)	3.4	3.8	3.3	24.0	7.3	5.3
Hallsworth (poorly)	5.9	3.9	6.3	57.8	9.2	5.1

to 8% during that part of the day when the temperature of the incubated soil core falls by ca. 0.25°C.

The effect of gypsum blocks on the water-holding capacity of incubated soil cores was also investigated using four replicate tubes over 28 days. During this period, 152.3 mm precipitation was recorded, and no significant difference in water contents was found between incubated cores and undisturbed soil.

Experimental

Two soil types of SW England, with contrasting drainage characteristics, were selected at the farm of the Institute of Grassland and Environmental Research, Okehampton, to test the performance of the method. The first was a freely drained, reddish gravelly loamy soil over Permian breccia from the Crediton Series: a typical brown earth (England and Wales classification, Findlay et al. 1984); USDA dystrochrept or eutrochrept; FAO dystric or eutric cambisol. The second was a poorly drained clay loam soil of the Hallsworth series, derived from the carboniferous Culm measures: a typical pelo-stagnogley soil (England and Wales classification, Findlay et al. 1984); USDA typic haplaquept; FAO dystric gleysol (USDA SCS 1975; FAO 1990). Both soil types had been under a three-cut grass silage management system with similar inputs of N for the last 5 or 4 years, but previously had been under continuous arable cropping and long-term grassland, respectively. Some important physical characteristics of the soils are presented: soil particle distribution (Table 1), water/air-filled porosities (Table 2) and relevant chemical properties (Table 3). Each soil type was represented by four replicate plots (500 m^2). In 1996, N applications to freely drained and poorly drained soils amounted to 285 kg fertilizer-N ha^{-1} and 297 kg fertilizer-N ha^{-1}, respectiveley and 128 kg slurry-N ha^{-1} and 98 kg slurry-N, respectively. Three silage cuts were made in 1996, the last being on 1 October for both soil types. From 31 October 1996 to 3 January 1997, two paired soil cores (each including associated outer cores) were taken at random from within each of the field plots every 4–5 days (11 days on one occasion) in incubation tubes: one to be incubated with C_2H_2 and the other without C_2H_2. The 16 cores were then incubated in the field in incubation vessels with an I-E bag and gypsum block placed beneath each core: half of the blocks (8) contained controlled-release capsules (incubations with C_2H_2). At the end of each incubation period, the soil core was removed and NH_4^+-N and NO_3^--N were extracted using 2 M KCl extractant [5:1, KCl:fresh soil (v/w)]. The NH_4^+-N and NO_3^--N of a new set of outer cores were extracted in the same way to provide baseline values for the succeeding incubation. Recovery of absorbed NO_3^- from the I-E bag was by extraction with 50 ml of 1.5 M H_2SO_4 for 1 h with shaking, followed by two further rinses with 50 ml batches of the extractant; the samples were made up to standard volume (200 ml) with rinses of deionized water. This procedure was found to consistently recover 90% of the absorbed NO_3^-, under controlled laboratory conditions. A factor of 1.11 was used to adjust estimates of leaching, based on those from I-E bags, to take account of the NO_3^- which was recovered.

N transformation calculations

The major processes of net mineralization, nitrification and leaching were determined by direct measurements. An estimate of denitrification was obtained by the difference in the levels of NO_3^- accounted for in paired incubations ($\pm C_2H_2$). Thus, denitrification was inhibited in the first ($+C_2H_2$) and allowed to proceed in the second ($-C_2H_2$) incubation, as shown in Fig. 2. The following relationships could then be derived:

$$M = m + D + L, \text{ therefore } D = M - (m + L),$$

also

$$M = [\text{increase in } NH_4^+\text{-N } (+C_2H_2)],$$

and

$$N = [\text{increase in } NH_4^+\text{-N } (+C_2H_2)] - [\text{increase in } NH_4^+\text{-N } (-C_2H_2)],$$

(this assumes immobilization is the same for incubations $\pm C_2H_2$), where M = net mineralization; m = increase in inorganic N (NH_4^+ +

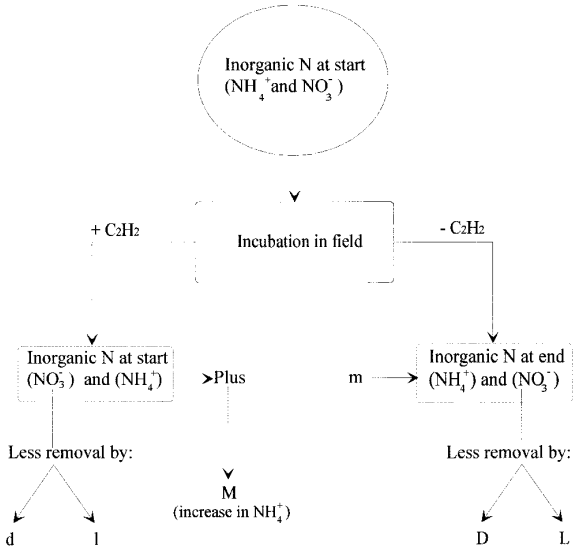

Fig. 2 Diagram showing paired incubations ($\pm C_2H_2$) used to derive N transformation rates of net mineralization (*M*), denitrification (*D*) and leaching (*L*). Increase in inorganic N content (*m*) at end of incubation ($-C_2H_2$) is net value (after removal of NO_3^--N by *D* and/or *L*). Change in original NO_3^--N content ($+C_2H_2$) is net of any removal in denitrification (*d*) and/or leaching (*l*), where de novo NO_3^--N production is inhibited

NO_3^-) less removal in *D* and *L*; *D* = denitrification (of original plus new sources of nitrate)#; *L* = leaching potential (obtained from I-E bags)*; *N* = net nitrification.

For controls: #*D* is *not* adjusted by *d* (to give *actual* N denitrified in measurement period); **L* is adjusted by l (to give *potential* leaching of new NO_3^- only), defined as (see Fig. 2): *d* = denitrification (confined to original NO_3^- at start of incubation); *l* = leaching of original NO_3^- (from I-E bags to check effectiveness of C_2H_2).

Denitrification was also measured independently over the experimental period by the jar incubation method (Ryden et al. 1987), using eight cores (100 mm deep × 25 mm diameter) per jar, and four replicate jars per soil type. At the end of the experiment, winter herbage production from the swards on the two soil types was assessed by taking four replicate quadrats (1 m^2) cut to a height of 40 mm. The herbage was dried at 85°C for 12 h in a forced-draught oven, ground and analysed for total N content using a Carlo Erba CN analyser. Potential nitrifying activity was estimated in soil obtained at the end of the experiment using a soil corer (four replicates of ten bulked cores; the cores were 150 mm deep × 37 mm diameter). The soil was sieved (6 mm mesh) and fresh soil (equivalent to 10 g dry weight) was incubated in a water bath at 25°C as a slurry (50 ml) of 2 mM $(NH_4)_2SO_4$, 2 mM phosphate buffer (pH 7.5) and 15 mM $NaHClO_3$ (an inhibitor of the oxidation of NO_2^- to NO_3^-). The rate of NO_2^- production over 8 h was evaluated (Belser and Mays 1980) using a simple linear regression. Total C and N and pH of oven-dried sub-samples of the bulked soils were also measured.

Leaching of NO_3^--N was independently assessed using ceramic suction cups (25 mm diameter, 55 mm length) inserted at a depth of 600 mm in the freely drained soil and at 300 mm in the poorly drained soil (12 replicates for each soil type). Estimates of net mineralization were also independently obtained using the method of Hatch et al. (1990) with a 7-day field incubation of four cores (125 mm deep × 37 mm diameter) per incubation vessel, with C_2H_2 to inhibit nitrification. There were four replicate incubations for each soil type. The original method was altered slightly so that the assessment of net mineralization was based only on the change in soil NH_4^+-N during incubation, rather than the overall change in inorganic N (i.e. $NH_4^+ + NO_3^-$). This was based on the assumption that, as shown in previous studies (Hatch et al. 1991; Gill et al. 1995), C_2H_2 is an effective inhibitor of nitrification and, thus, there should be no increase in NO_3^- levels.

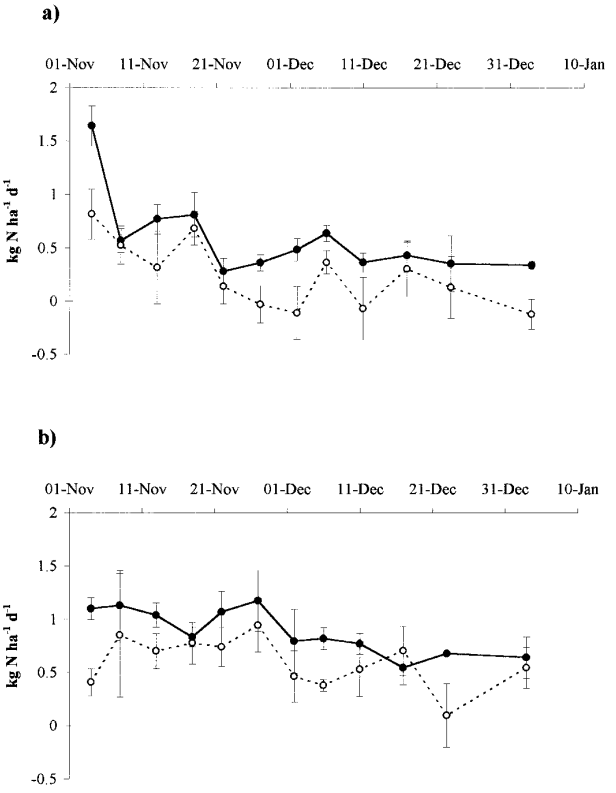

Fig. 3 a, b Mean daily rates (kg N ha^{-1} day^{-1}) of net mineralization (*closed circles* ± SEM) and nitrification (*open circles* ± SEM) in: **a** freely and **b** poorly drained soils over an experimental period (64 days) in autumn/winter 1996/7 ($n = 4$)

Results

Net rates of mineralization (kg N ha^{-1} day^{-1}) in the soils measured by tube incubation (Fig. 3a, b) ranged from 0.28 to l.64 (freely drained) and 0.55 to 1.17 (poorly drained). The mean daily rates of mineralization (kg N ha^{-1} day^{-1}) for the two soils over the measurement period were significantly different ($P<0.001$): 0.59 (±0.064) compared with 0.88 (±0.056), respectively. Cumulative totals for the net release of mineralized N from soil organic matter (Table 4) compared favourably with independent measurements using the jar incubation technique over the same measurement period. Coefficients of variation were high, but of the order expected with field measurements (mean values of 52% and 38%, for freely and poorly drained soils, respectively). In the freely drained soil, 58% of mineralized N was nitrified, compared with 72% in the poorly drained soil. Daily rates of nitrifying activity (kg N ha^{-1} day^{-1}) ranged from 0.03 to 0.82 (Fig. 3a) and from 0.37 to 0.95 (Fig. 3b), with mean daily rates for the whole period of 0.24 (±0.072) and 0.60 (±0.074), respectively. No independent method for assessing nitrifying activity in the field was available, but measurements of potential nitrification made in the laboratory gave rates of 14.7 and 8.3 nmol NH_4^+ oxidized g^{-1} (dry soil) h^{-1} in the freely and poorly drained soils, respectively. Thus, higher nitrifying rates were found in the field in the poorly drained soil, but the

Table 4 Comparison between the tube incubation method and alternative methods of measuring several N transformation processes over 64 days (31/10/96–3/1/97). Data are means with ± standard error of the mean shown in parentheses

Process	Freely drained soil (kg N ha^{-1})		Poorly drained soil (kg N ha^{-1})	
	Tube incubation/ resin bags	Jar incubation/ ceramic cups	Tube incubation/ resin bags	Jar incubation/ ceramic cups
Net mineralization	34.8 (1.29)	56.1 (0.58)	53.9 (0.39)	43.2 (1.17)
Nitrification	20.2 (2.42)	–	38.7 (0.97)	–
Denitrification	7.2 (3.39)	5.3 (0.15)	24.2 (3.51)	7.1 (1.76)
Leaching	3.7 (0.71)	3.7 (1.73)	6.9 (0.88)	3.8 (2.05)

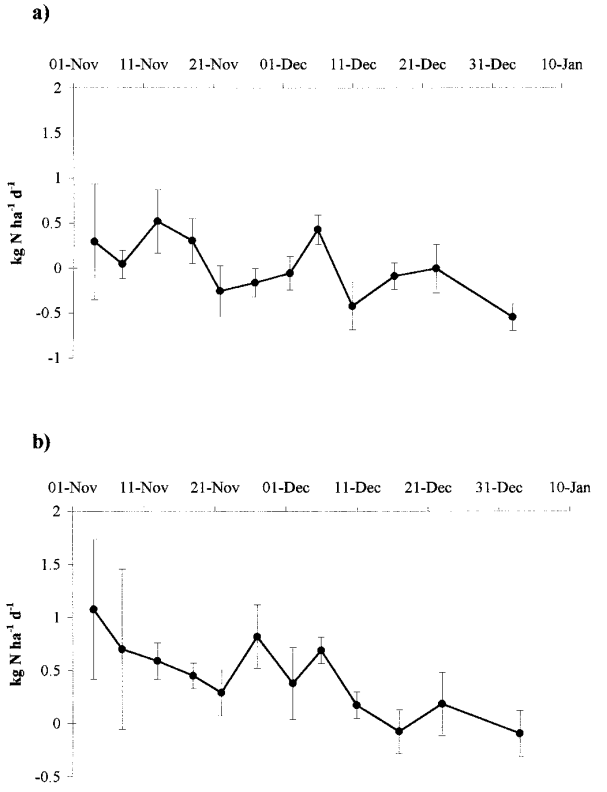

Fig. 4 Mean daily rates (kg N ha^{-1} day^{-1}) of denitrification in **a** freely and **b** poorly drained soils over an experimental period (64 days) in autumn/winter 1996/1997. *Vertical bars* are ± standard error of the mean (n = 4)

greater potential activity was in the freely drained soil (P<0.001). Other factors which might have been expected to correspond with different rates of nitrification were similar (Table 3), e.g. soil pH and the ratio of NH_4^+:NO_3^-. As might be expected, nitrification was related to rates of mineralization (P<0.001) in both the freely drained (r^2=0.41) and in the poorly drained (r^2=0.52) soils, with 40% and 51% of the variance accounted for, respectively. Also, NH_4^+ contents exceeded those of NO_3^- in both soils, which confirmed that the rate of nitrification lagged behind that of net mineralization.

Daily rates of loss of N by denitrification (kg N ha^{-1} day^{-1}) (estimated by differences in inorganic N from tube incubations $\pm C_2H_2$) ranged from 0.04 to 0.52 (Fig. 4a: freely drained) and from 0.17 to 1.08 (Fig. 4b: poorly drained). The mean daily rates of denitrification (kg N ha^{-1} day^{-1}) differed between the two soils (P<0.01): i.e., 0.05 (±0.086) compared with 0.40 (±0.079), respectively. Daily rates of denitrification were closely correlated with rates of nitrification in the freely drained soil (P<0.001), with r^2=0.67 and 66% of the variance accounted for. However, rates of denitrification also included some negative values (Fig. 4a) which were assumed to be zero (i.e. at or near a rate below the level of detection) in the calculation of the cumulative total (Table 4). In the poorly drained soil, there were fewer negative values (Fig. 4b) and the two processes were significantly correlated (P<0.001), with r^2=0.68 and 68% of the variance accounted for. The cumulative total was >3-fold that of the freely drained soil (Table 4).

The cores remained tightly fitted in the incubation tubes during incubation, so that leakage down the sides was unlikely and shrinkage of the soil within the tubes did not occur. Both methods for leaching (by I-E bags or ceramic suction cups) and for denitrification (by difference in the tube incubation or by jar incubation) gave results which were in close agreement, in the case of the freely drained soil (Table 4). In the poorly drained soil, however, the estimates of leaching and denitrification were greater with the tube incubation method (Table 4). The N which was unaccounted for in our methodology (i.e. net mineralization *minus* losses from denitrification and leaching) amounted to 24.0 kg N and 22.8 kg N, respectively, of which 26.2 (±1.60) kg N and 55.8 (±6.81) kg N were recovered in the herbage at the end of the measurement period. It is possible that more herbage N was present in regrowth immediately following the last silage cut on the poorly drained soil (due to the wetter soil conditions), and was therefore over-estimated, whereas growth on the drier soil had ceased after cutting, and regrowth only occurred after the soil moisture level had returned to field capacity. This discrepancy would have been carried forward into the assessment of N taken up during the winter from N mineralized during the measurement period, but nevertheless provides an estimate of N removed from the soil by the herbage. Losses of N through senescence and transport into roots, which were not accounted for, would also contribute to errors in the estimates for both soil types.

Discussion

The tube incubation method allows concurrent measurements of the major N transformation processes to be made which would not be possible using existing techniques. Foremost amongst the advantages associated with this method is the opportunity to extend the period of measurement indefinitely; this would not be practicable when using other methods, particularly those using ^{15}N methodology for gross measurements. An additional benefit of the improved incubation method is that integrated estimates of the various processes are obtained for defined periods, in contrast to estimates obtained by extrapolating from "spot" measurements, when using the alternative methods. The present experiment, which was designed to illustrate the use of the technique under field conditions, showed that the processes of net mineralization, nitrification and denitrification were closely associated. Such associations might be expected, given the substrate/product relationships which exist, although supporting evidence is limited. It has been shown recently (Hatch et al. 1996) that antecedent fertilizer inputs can lead to an increase in the rate of nitrification; this has implications for the supply, distribution and fate of N in soils. Further possible correlations might also have been expected between nitrifying activity and the removal of nitrate through leaching. However, the lack of drainage in December 1996 (driest December since 1959) precluded meaningful comparisons between these data.

Errors associated with the measurements were generally within acceptable limits for field experimentation; however, some negative values were found for nitrification, denitrification and leaching (negative rates for mineralization are correctly defined as net immobilization). The reason for this was probably the combination of inherently low rates in the measured processes and the high spatial variation between replicates. Greater replication (e.g. 6–8 incubation tubes to each treatment) would be required to better resolve temporal changes in these processes. In overall terms, the values obtained with tube incubation and other methods compared well, with the exception of denitrification in the poorly drained soil. Lower than expected values in heavier soils are a recognized problem with the jar incubation method (Smith and Arrah 1990) because of enhanced aeration and an inability to ensure satisfactory infusion of C_2H_2 to inhibit fully nitrous oxide reductase activity (i.e. reduction of N_2O to N_2). Marked advantages of the tube incubation method are, therefore, the enclosure of the soil core in a sheath to prevent premature exposure of soil surfaces, and the improved method of providing a sustained infusion of C_2H_2. Degradation of C_2H_2 by C_2H_2-metabolizing populations is a recognized problem (with the possibility of wider effects on C cycling processes), but is one which usually only develops after a lag phase of up to 8 days (Terry and Duxbury 1985). The greater variability in the data relating to denitrification in the freely drained soil, however, was probably caused by an inadequate supply of C_2H_2 from the controlled-release capsules, because of very dry soil conditions. It may be necessary in such circumstances to re-wet the gypsum blocks periodically to ensure that the reaction with the CaC_2 granules proceeds satisfactorily.

Differences in assessments of nitrifying activities (actual and potential) are less easy to reconcile. The greater potential nitrifying activity in the freely drained than in the poorly drained soil suggests that a more active and/or abundant population of nitrifiers may have developed in the former in response to aerobic conditions more favourable for growth of the bacteria. The higher (actual) field rate of nitrification found with the poorly drained soil is, however, compatible with having a higher rate of net mineralization (since the supply of NH_4^+ substrate will be the rate-limiting step). It is also possible that the pH in the microsites of the undisturbed, poorly drained soil were higher, which would also favour nitrification despite the apparent similarity in pH of the two soils, measured on disturbed soil samples. Such differences would not be a factor in the laboratory incubations, which were based on soil slurries buffered at pH 7.5.

Estimates of N leached using both I-E bags and cup-samplers were low; they reflected the generally lower losses associated with cut (as opposed to grazed) swards (Ryden et al. 1984) and were also due to the unusually dry conditions of the 1996/7 winter. It would, therefore, be of interest to assess leaching under grazed swards in a more typical drainage period, but a considerable increase in the number of replicates would be required to overcome the greater variability in the soil inorganic N content induced by N returned via excreta. The paired sampling approach, adopted in the present method, has an advantage over random sampling since spatial variation is largely accounted for by closely linking the cores taken initially with the incubated cores. I-E bags also have the advantage over cup-samplers of operating in a passive mode with respect to the passage of soil water, whereas cup-samplers may modify the flow characteristics of soil water and are less satisfactory in heavier soils (Hatch et al. 1997).

The tube incubation method also overcomes many of the other problems associated with enclosed incubation vessels, by causing only minimal disturbance to the soil which allows natural wetting/drying processes to proceed and avoids large changes in the soil atmosphere/temperature status. Finally, the low cost of the equipment involved in the tube incubation method enables large-scale monitoring for prolonged periods of experimentation to be undertaken, as well as providing the opportunity for examining the linkage between N cycling processes.

Acknowledgements This project was funded by the Ministry of Agriculture, Fisheries and Food, London. The Institute is supported by the Biotechnology and Biological Sciences Research Council. The authors wish to thank P. Owen, M. Davies and A. Stone for technical assistance and A. J. Rook for statistical advice.

References

Banerjee NK, Mosier AR (1989) Coated calcium carbide as a nitrification inhibitor in upland and flooded soils. J Indian Soc Soil Sci 37:306–313

Barraclough D (1991) The use of ^{15}N tracer experiments. 1. Theory. Plant Soil 131:89–96

Barraclough D (1995) ^{15}N isotope dilution techniques to study soil nitrogen transformations and plant uptake. Fertil Res 42:185–192

Barraclough D, Puri G (1995) The use of ^{15}N pool dilution and enrichment to separate the heterotrophic and autotrophic pathways of nitrification. Soil Biol Biochem 27:17–22

Belser LW (1979) Population ecology of nitrifying bacteria. Annu Rev Microbiol 33:309–333

Belser LW, Mays EL (1980) Specific inhibition of nitrite oxidation by chlorate and its use in assessing nitrification in soils and sediments. Appl Environ Microbiol 39:505–510

Berg P, Klemedtsson L, Rosswall T (1982) Inhibition effect of low partial pressures of acetylene on nitrification. Soil Biol Biochem 14:301–303

Birch HF (1964) Mineralization of plant nitrogen following alternate wet and dry conditions. Plant Soil 20:43–49

Cabrera ML, Kissel DE (1988) Potentially mineralizable nitrogen in disturbed and undisturbed soil samples. Soil Sci Soc Am J 52:1010–1015

Eno CF (1960) Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci Soc Am Proc 24:277–279

FAO (1990) FAO-UNESCO Soil map of the world. Vol I Revised legend. World Soil Resources Report 60, UNESCO, Paris

Findlay DC, Colborne GJN, Cope DW, Harrod TR, Hogan DV, Staines SJ (1984) Soils and their use in South West England. Bulletin no 14. Soil Survey of England and Wales, Harpenden

Gill K, Jarvis SC, Hatch DJ (1995) Mineralization of nitrogen in long-term pasture soils: effects of management. Plant Soil 172:153–162

Hatch DJ, Jarvis SC, Philipps L (1990) Field measurement of nitrogen mineralization using soil core incubation and acetylene inhibition of nitrification. Plant Soil 124:97–107

Hatch DJ, Jarvis SC, Reynolds SE (1991) An assessment of the contribution of net mineralization to N cycling in grass swards using a field incubation method. Plant Soil 138:23–32

Hatch DJ, Jarvis SC, Parkinson R (1996) Nitrification in grassland soils. Transactions of the 9th Nitrogen Workshop, Braunschweig, September 1996, pp 239–241. Schmidt Buchbinderei und Druckerei, Braunschweig

Hatch DJ, Jarvis SC, Rook AJ, Bristow AW (1997) Ionic contents of leachate from grassland soils: a comparison between ceramic cup samples and drainage. Soil Use Manage 13:68–74

Jarvis SC, Stockdale EA, Shepherd MA, Powlson DS (1996) Nitrogen mineralization in temperature agricultural soils: Processes and measurement. Adv Agron 57:188–235

Kirkham D, Bartholomew WV (1954) Equations for following nutrient transformations in soil, utilizing tracer data. Soil Sci Soc Am J Proc 18:33–34

Knowles R (1978) Common intermediates of nitrification and denitrification, and the metabolism of nitrous oxide. In: Schlesinger D (ed) Microbiology 1978. American Society of Microbiology, Washington DC, pp 367–371

Raison RJ, Connell MJ, Khanna PK (1987) Methodology for studying fluxes of soil mineral-N in situ. Soil Biol Biochem 19:521–530

Ryden JC, Ball PR, Garwood EA (1984) Nitrate leaching from grassland. Nature 311:50–53

Ryden JC, Skinner JH, Nixon DJ (1987) Soil core incubation system for the field measurement of denitrification using acetylene-inhibition. Soil Biol Biochem 19:753–757

Sierra J (1992) Relationship between mineral N content and N mineralization rate in disturbed soil samples incubated under field and laboratory conditions. Aust J Soil Res 30: 477–492

Smith KA, Arah JRM (1990) Losses of nitrogen by denitrification and emissions of nitrogen oxides from soils. Proc Fertil Soc No 299

Stanford G, Epstein E (1974) Nitrogen mineralization-water relations in soils. Soil Sci Soc Am Proc 38:103–107

Stark JM, Firestone MK (1996) Kinetic characteristics of ammonium-oxidizer communities in a California oak woodland-annual grassland. Soil Biol Biochem 28:1307–1317

Terry RE, Duxbury JM (1985) Acetylene decomposition in soils. Soil Sci Soc Am J 49:90–94

USDA SCS (1975) Agricultural Handbook No 436. Soil taxonomy: A basic system for soil classification for making and interpreting soil surveys. USDA Soil Conservation Service

Westerman DT, Crothers SE (1980) Measuring soil nitrogen mineralization under field conditions. Agron J 72:1009–1012