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Distribution of nitrogen pools in the soil profile of undisturbed and reseeded grasslands

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Abstract This study evaluated the effect of cultivation and reseeding on the distribution and fate of soil mineral N (SMN), soluble organic N (SON) and potentially mineralisable N (PMN) in the soil profile of two long-term grasslands in the UK. Cultivation and reseeding significantly increased the total soluble N concentration (SMN plus SON) of the soil profile (0–90 cm), with over 50 mg SON kg^{-1} observed. By contrast, the PMN pool was unaffected by cultivation and declined with increasing soil depth. The flush in SON and SMN observed in both soils disappeared within 1 year following cultivation. The fate of SON appeared to be dependent on soil type, with considerably more movement to deeper layers apparent in the profile of a silty clay loam (30% clay) than in a clay loam (49% clay). Mineralisation and/or immobilisation of SON in the topsoil probably accounted for the changes observed in the SON content of the clay loam. SON is an important N pool in grassland soils and cultivation has a significant impact on its release. Measurements of SON should therefore be included in studies of N cycling in agricultural cropping systems, so that full account may be taken of its potential as a source or sink of mobile N.

Key words Soluble organic nitrogen · Mineral nitrogen · Potentially mineralisable nitrogen · Cultivation · Grassland

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Introduction

The importance of soil mineral N (SMN: NH_4^+–N plus NO_3^-–N) in crop nutrition, and concerns over the environmental impact of NO_3^- leaching and N_2O emissions has focused attention on mineral N in agricultural soils. Measurement of the soluble organic N (SON) content of soils has largely been overlooked, partly due to lack of knowledge regarding its significance and partly due to the difficulty in measuring it by traditional Kjeldhal analysis. However, SON may contribute significantly to both crop N supply and N leaching and warrants further attention (Yu et al. 1994; Murphy et al. 1999). Development of the persulphate oxidation technique for measuring the organic N content of soil leachates and extracts (Cabrera and Beare 1993; Yu et al. 1994) has enabled the rapid determination of SON and a more widespread inclusion of SON measurements within nutrient-cycling studies (Jensen et al. 1997; McNeill et al. 1998). As a result, SON has been identified as a major pool within forest ecosystems, with the SON content of soil water extracts found to be several orders of magnitude greater than the mineral N content and contributing up to 90% of the total N leached (Qualls et al. 1991; Emmett et al. 1991; Yu et al. 1994). However, there have been few studies of the SON content of agricultural soils.

The effect of cultivation on SMN and NO_3^-–N leaching has been well documented (e.g. Silgram and Shepherd 1999), with substantial increases in N mineralisation rates, SMN and NO_3^-–N leaching observed following ploughing of grasslands (Whitmore et al. 1992; Shepherd et al. 1996). However, the effect of cultivating grasslands on the production and fate of SON has not been assessed. In addition, mineralisation studies have largely been confined to the topsoil, despite the fact that plants utilise N from deeper horizons. The topsoil has been shown to have the greatest microbial biomass and activity, residue content, easily decomposable organic matter and therefore N mineralisation (Woods 1989; Soudi et al. 1990). However, studies have shown that a significant proportion of N mineralisation can occur in subsoils (Cassman and Munns 1980; Hadas et al. 1986; Patra et al. 1998); this should therefore be included in any assessment of soil N availability.

The purpose of this study was to evaluate the effect of cultivation and reseeding on the distribution and fate of SON and other N pools [SMN and potentially mineralisable N (PMN)] in the soil profile of two long-term grasslands in the UK.

Materials and methods

Site description and experimental design

The experiment was conducted on long-term grasslands at North Wyke Research Station in Devon (UK) and ADAS Rosemaund in Herefordshire (UK). The soil at North Wyke is a poorly drained, clay-loam textured soil of the Hallsworth Series (Harrod 1981), classified as a dystric gleysol (FAO 1990), and at Rosemaund it is a stoneless silty clay loam of the Bromyard Series (Beard et al. 1986), classified as a orthic luvisol (FAO 1990). Selected soil properties of the two sites are given in Table 1. The average annual rainfall is 1035 mm at North Wyke and 669 mm at Rosemaund. Both grasslands were at least 50 years old, with a recent history of grazing and N fertiliser applications. Animals were withdrawn from the pastures in March 1993 (North Wyke) and June 1995 (Rosemaund); since then the grass has been cut at least 3 times a year for silage. N fertiliser applications were also withdrawn from Rosemaund (August 1995), but not North Wyke, where annual applications of 260 kg N ha^{-1} (split 100:80:80 in March, May and July) were maintained.

At Rosemaund 12 × 50 m of the pasture and at North Wyke 10 × 40 m of the pasture were sprayed with glyphosate, rotovated, subsoiled to a depth of 12.5 cm and drilled with ryegrass in August 1995 and September 1995, respectively. This procedure was repeated on a new (undisturbed) area in August 1996 at both sites to give the following treatments: undisturbed (UD), cultivated and reseeded in 1995 (RS95) and cultivated and reseeded in 1996 (RS96).

Soil sampling

Soil collection methods were the same at both sites. In June 1996 (12 June at Rosemaund and 19 June at North Wyke), approximately ten cores were taken randomly from the UD and RS95 pastures at depths of 0–10, 10–20, 20–30, 30–60 and 60–90 cm. Cores were bulked to give a single composite sample for each treatment and depth. Stones and macro-organic matter were removed by hand and subsamples taken for the determinations detailed below. This exercise was repeated on 9 November 1996 and 30 October 1996 at Rosemaund and North Wyke respectively, on the UD, RS95 and RS96 pastures. All the soil samples were stored at <4 °C and analysed within 2 weeks of sampling.

SMN and SON

SMN was determined by shaking two (October) or three (June) replicate sub-samples of field-moist soil with 2 M KCl for 1 h at a soil:solution ratio of 1:5, followed by filtration (Whatman no. 1 filter paper) and colorimetric analysis of the filtered extracts. These extracts were also used to determine the total soluble N content (TSN) by an alkaline persulphate oxidation technique described by Cabrera and Beare (1993). This involved autoclaving 4 ml of extract with 4 ml of potassium persulphate dissolved in 3.75 M NaOH, at 121 °C for 30 min. SON was calculated as the difference between TSN and SMN, but this sometimes resulted in a small negative figure because of the errors associated with the measurement of each component. In these cases, it was assumed that the amount of SON was negligible.

Table 1 Soil characteristics (0–10 cm) of long-term pastures at North Wyke and Rosemaund

Site	Clay %	Bulk density (g cm^{-3})	pH (H_2O)	Total N (%)	Organic C (%)	C:N
North Wyke	49	0.71	5.1	0.70	7.19	10.3
Rosemaund	30	1.05	5.9	0.36	3.98	11.1

Potentially mineralisable N

PMN was determined using an adaptation of the anaerobic incubation method described by Keeney (1982). Two (October) or three (June) replicate 32-g sub-samples of field-moist soil were weighed into 100-ml glass jars and 80 ml of deionised water was added. The jars were then sealed and incubated at 40 °C for 7 days, followed by determination of the NH_4^+–N content by shaking the soil suspension with 80 ml of 4 M KCl for 1 h and colorimetric analysis of the filtered extract. PMN was then calculated as the difference in soil NH_4^+–N contents between incubated and unincubated soil samples (taken from the SMN analysis).

Analyses

The concentration of NH_4^+–N and NO_3^-–N in soil extracts was determined colorimetrically using a Lachet flow injection analyser. All results were expressed on a dry weight basis, using gravimetric moisture contents determined by drying subsamples of each soil at 105 °C for 24 h.

Treatments were established on single, large field-scale plots, and were therefore not replicated. The soil sampling procedure was assumed to give samples that were representative of these treatments. The results have therefore been expressed as means of the analytical replicates with SEs giving an estimate of the size of any variation within a sample.

Results

Soil mineral N

In June 1996, the concentration of SMN in the RS95 profile was greater than that of the UD profile at both Rosemaund and North Wyke (Table 2). These differences were largely observed in the 60 to 90-cm layer at Rosemaund (Fig. 1a), but were present in the 0 to 10-cm layer at North Wyke (Fig. 1b). However, by October, differences were no longer apparent at either site between RS95 and UD treatments (Fig. 1c, d), with decreases in SMN concentrations observed in the 60 to 90-cm layer at Rosemaund, and 0 to 10-cm layer at North Wyke.

In October, the newly established reseed (RS96) at Rosemaund had a greater SMN content than UD or RS95; these differences were largely observed in the top 10 cm (Fig. 1c). At North Wyke, the SMN content of RS96 was similar to that of the other treatments (Fig. 1d).

Soluble organic N

At Rosemaund, the SON concentration in the RS95 profile (0–90 cm) was >50 mg kg^{-1} in June and contributed to ca. 90% of the TSN (i.e. SMN plus SON; Table 2). By contrast, the SON concentration of the UD profile was only 4 mg kg^{-1} SON (ca. 50% of the TSN; Table 2). The SON content declined with depth in both swards (Fig. 2a), but differences between treatments were still apparent in the subsoil, with ca. 50 mg kg^{-1} SON measured below 30 cm in RS95. It should be noted that as depth increments increased for the subsoil sampling (from 10 to 30 cm), the total amount of SON present in the subsoil of RS95 (expressed on a volume basis) would have been as great, if not greater than that measured in the topsoil. As with SMN, SON decreased from June to October in both treatments (Table 2), with the SON concentration in RS95 declining by almost 50 mg kg^{-1}. As a result, there was no difference between the SON contents of undisturbed and cultivated soils by October (Table 2). This decrease occurred in all of the soil layers sampled, but was greatest in the top 30 cm, with 60% of the total loss observed here (Fig. 2a, c). The SON content of RS96 was greater than that of RS95 and UD, but only in the top 20 cm (Fig. 2c).

Table 2 N pools (mg kg^{-1}) in the soil profile (0–90 cm) of the cultivated and reseeded in 1995 (*RS95*) and the undisturbed (*UD*) pastures at Rosemaund and North Wyke from June to October 1996. Data are means of the concentrations in each soil layer, weighted for the depth of soil sampled. *SMN* Soil mineral N, *SON* soluble organic N, *PMN* potentially mineralisable N

N form	Rosemaund		North Wyke	
	UD	RS95	UD	RS95
June				
SMN	4.5	6.8	14	17
SON	4.2	57	2.7	11
PMN	57	50	79	87
October				
SMN	4.0	3.6	12	7.7
SON	3.4	2.9	3.0	4.1
PMN	10	8.1	28	38
Balance (June–October)				
SMN	0.5	3.2	2.0	9.3
SON	0.8	54	−0.3	6.9
PMN	47	42	51	49

The SON concentrations in the soils at North Wyke were much lower than those at Rosemaund, with <15 mg kg^{-1} in the profile (0–90 cm) of both treatments (ca. 20–40% of TSN; Table 2). Despite this, the SON content of RS95 was still greater than that of UD in June, but unlike RS95 at Rosemaund, the SON content at <30 cm was negligible (Fig. 2b). In October, there was great variability in the values of SON in the 60 to 90-cm layer of RS95 and UD (Fig. 2d). This varia-

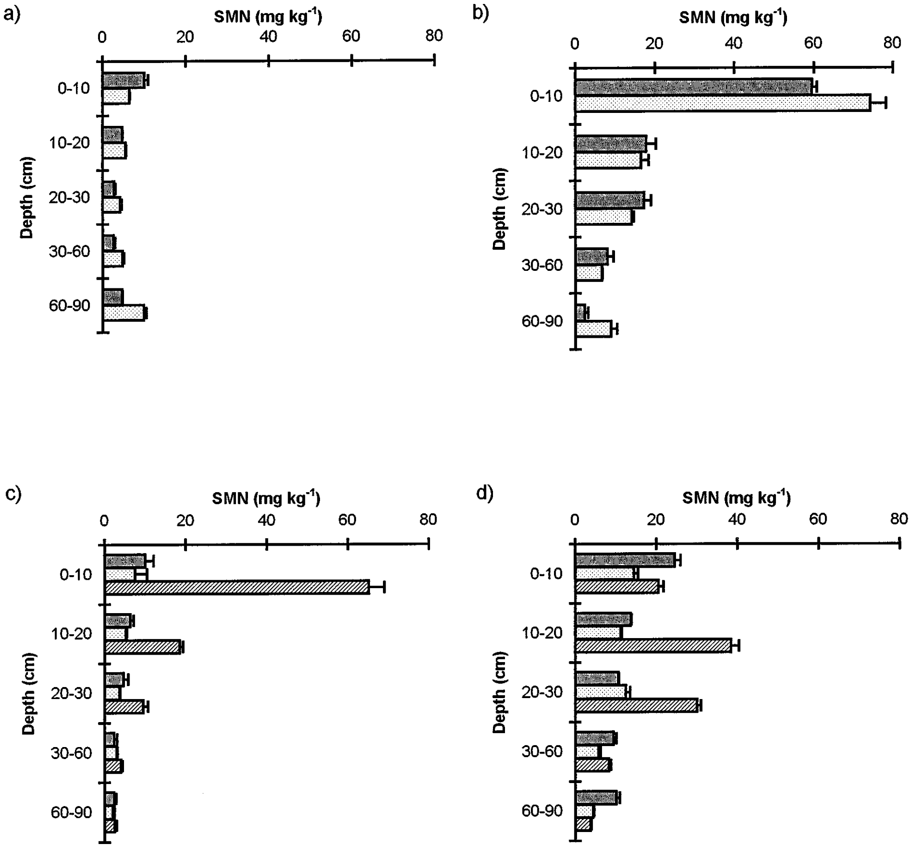
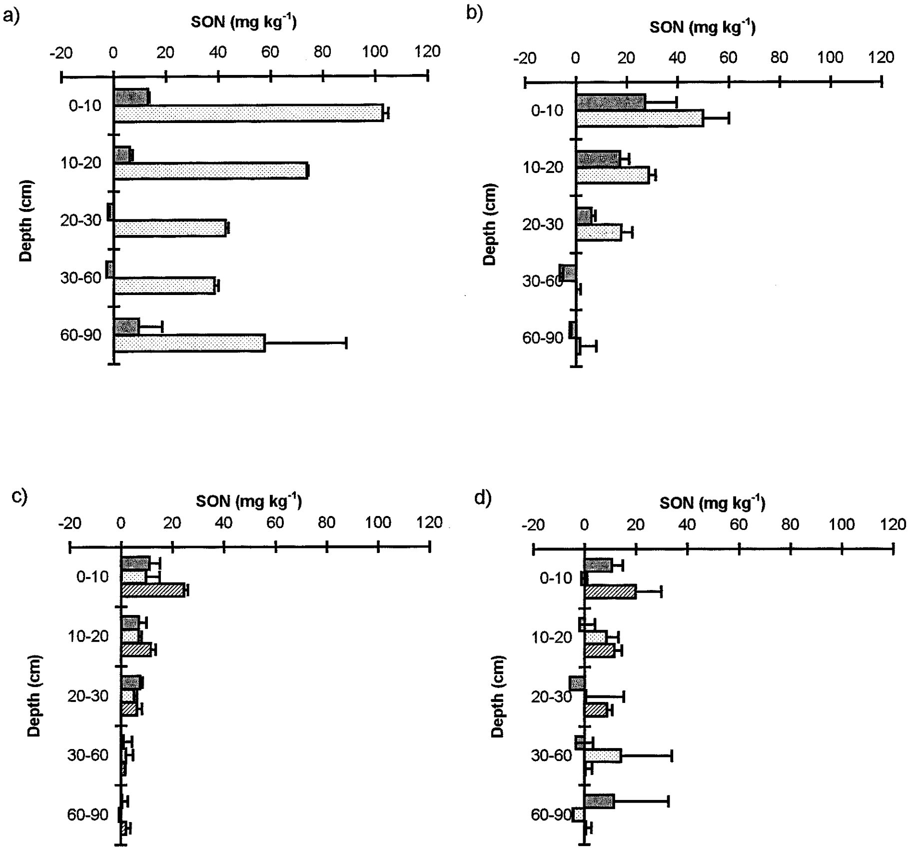


Fig. 1 Effect of cultivation on the soil mineral N content (*SMN*) of the soil profile at: **a** Rosemaund in June 1996, **b** North Wyke in June 1996, **c** Rosemaund in October 1996 and **d** North Wyke in October 1996. *Filled bars* represent the undisturbed (UD) treatment, *dotted bars* the cultivated and reseeded in 1995 (RS95) treatment, *shaded bars* the cultivated and reseeded in 1996 (RS96) treatment

Fig. 2 Effect of cultivation on the soluble organic N content (*SON*) of the soil profile at: **a** Rosemaund in June 1996, **b** North Wyke in June 1996, **c** Rosemaund in October 1996 and **d** North Wyke in October 1996. *Filled bars* represent the UD treatment, *dotted bars* the RS95 treatment, *shaded bars* the RS96 treatment



tion was largely associated with the measurement of TSN (by persulphate oxidation) rather than SMN, and may have been the result of contamination of the 60 to 90-cm sample. These results should therefore be treated with caution. Again, SON concentrations declined from June to October (Table 2), with the decrease predominantly observed in the top 20 cm. The SON content of RS96 was similar to that of RS95 and UD (Fig. 1d) and declined with depth.

Potentially mineralisable N

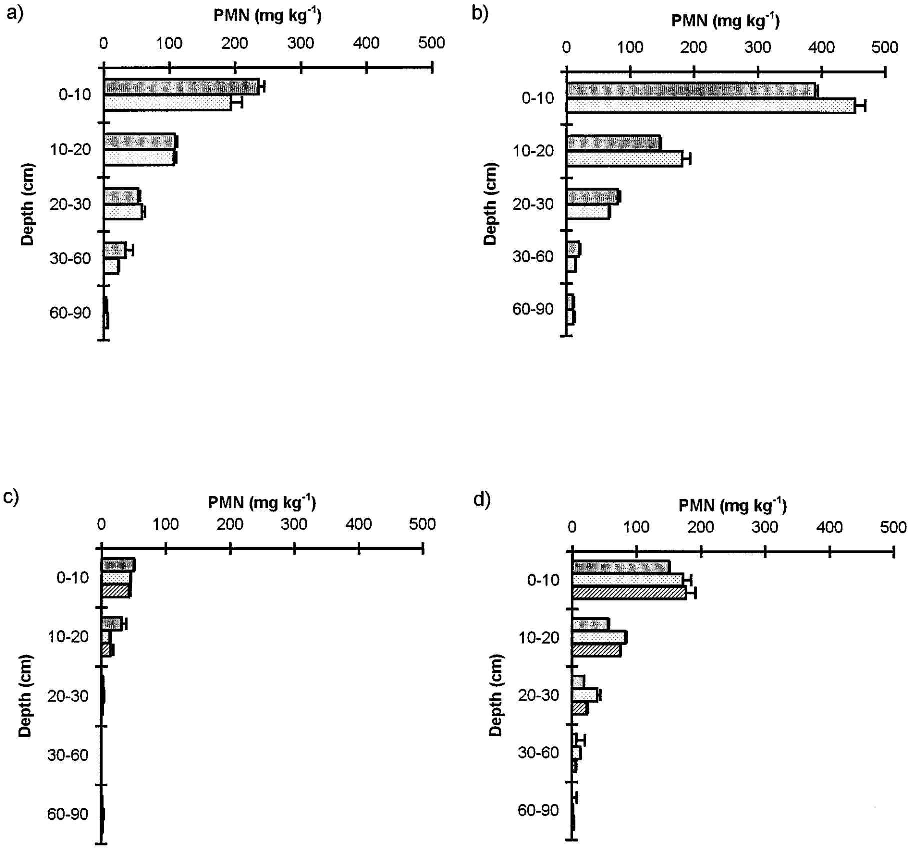
PMN behaved very differently to SMN and SON and decreased with depth at all sites and at both sampling times (Fig. 3). There was also no difference between the PMN contents of RS95 and RS96 and the UD treatment on any occasion. However, the PMN concentration in all soils declined from ca. 50–90 mg kg^{-1} in June to <40 mg kg^{-1} in October (Table 2), with the majority of the decrease (ca. 40–50%) observed in the top 10 cm. This decrease was greater than the decline in TSN observed in both the UD and RS95 grasslands at North Wyke and the UD grassland at Rosemaund (Table 2), but similar to that in the RS95 grassland at Rosemaund.

Discussion

TSN pool

The SON content of soils has been determined using a number of different extractants, including water (Qualls and Haines 1991), K_2SO_4 (Cabrera and Beare 1993) and KCl (Murphy et al. 1999). The type of extractant used has been found to affect the quantity of TSN extracted, with KCl shown to remove more SON and SMN than water. This may be due to the removal of N from exchange surfaces and the lysing of microbial cells by such a strong salt solution. Cabrera and Beare (1993) found that determination of TSN by persulphate oxidation of KCl extracts was overestimated at low N concentrations, but underestimated at high N concentrations, compared to extraction with K_2SO_4 and analysis by Kjeldhal digestion. However, Murphy et al. (1999) found no significant difference between the SON content of a sandy loam soil under arable cultivation, whether extracted by water, KCl or K_2SO_4. Subsequent comparison of KCl and water extraction of air-dried soil samples from each of the grassland soils studied here, showed that KCl extracted ca.15–25% more TSN than a water extraction of the North Wyke clay loam soil, but ca.10% less TSN than a water extraction of the Rosemaund silty clay loam soil (W. Wilmer, personal communication). The effectiveness of the extrac-

Fig. 3 Effect of cultivation on the potentially mineralisable N (*PMN*) content of the soil profile at: **a** Rosemaund in June 1996, **b** North Wyke in June 1996, **c** Rosemaund in October 1996 and **d** North Wyke in October 1996. *Filled bars* represent the UD treatment, *dotted bars* the RS95 treatment, *shaded bars* the RS96 treatment



tant therefore appears to be influenced by soil type. Despite these differences, concentrations of SON in the profile of the grassland soils measured here were 20–90% of the TSN pool, with exceptionally high values observed in the reseeded treatments in June. Murphy et al. (1999) measured ca. 2 and 5 mg kg^{-1} of KCl-extractable SON in the 0 to 25-cm layer of a sandy loam soil under continuous arable, and an 8-year grass ley, respectively, representing approximately 33–60% of the TSN. Similar SON contents were observed by Jensen et al. (1997), who measured concentrations of up to 13 mg kg^{-1} in the top 15 cm of a sandy loam soil, following incorporation of oilseed-rape straw.

The effect of cultivation on N dynamics is well documented, with the increases in mineralisation, SMN and leaching observed largely attributed to the physical disruption of aggregates, thus exposing previously protected organic matter to the processes of decomposition (Jarvis et al. 1996). Few studies have looked at changes in SON after cultivation. At Rosemaund, cultivation and reseeding led to an increase in the TSN content of the 0 to 10-cm layer in autumn 1996 (RS96). Both the SON and SMN pools were increased, probably as a result of physical disruption of soil aggregates and incorporation of grass residues following cultivation, leading to enhanced mineralisation. Measurements were not taken immediately following cultivation in 1995, but in June 1996 the TSN content of RS95 was still greater than that of UD, which suggested cultivation had a similar effect here. The differences in June were largely associated with the SON pool, which was significantly greater throughout the whole profile of RS95, with over 50 mg kg^{-1} measured in the subsoil (>30 cm). This subsoil SON probably originated from that released immediately after cultivation the previous autumn, with ca. 250 mm of rainfall or 180 mm of drainage giving rise to leaching over the intervening winter. However, it is surprising that topsoil SON concentrations were still enhanced over 10 months following cultivation. SON can arise in soils by several processes: substrate fragmentation, depolymerisation and solubilisation, microbial lysis, faunal grazing of soil microbes and freeze/thawing (Wang and Bettany 1994). It is possible that the increased pool of easily decomposable material resulting from cultivation may not have been fully utilised prior to winter, when biological activity tends to decrease with a fall in temperatures. Resumption of decomposition processes in spring may therefore have led to an increased pool of SON in these soils in June. However, these differences were not reflected in the topsoil SMN pool.

The release of TSN after cultivation in 1995 must have been much greater than that observed in 1996, with over 50 mg kg^{-1} TSN measured in the profile of RS95 in June 1996. This compared with only 5 mg kg^{-1} in the profile of RS96 in October 1996. The difference in magnitude of the N flush was probably the result of differences in the management of the grass swards prior to cultivation, with N fertiliser additions and sheep grazing (and therefore excretal inputs) prior to reseeding in 1995, compared to no N additions or grazing in 1996. The return of N to the soil following cultivation would therefore have been much greater following reseeding in 1995 than in 1996 when no N fertiliser was applied to the sward and the grass was cut rather than grazed. Net N mineralisation (measured by in-situ core incubation) was also greater following cultivation and reseeding in autumn 1995, compared to autumn 1996 (data not shown). By October, the increase in TSN following cultivation had virtually disappeared, probably as a result of immobilisation or mineralisation and plant uptake in the topsoil and leaching in the subsoil.

The increase in TSN following cultivation and reseeding at North Wyke in 1995 was much smaller than at Rosemaund, with no apparent movement of the peak down the profile (despite over 400 mm of rainfall from December 1995 to April 1996). Again, this flush did not persist for longer than 1 year. Approximately 250 mm of rain fell between the June and October samplings in 1996, but there was no evidence of movement of TSN down the profile. SON could therefore have been lost by lateral flow, immobilised into more stable organic matter forms or fixed onto clay minerals. Alternatively, mineralisation of SON in the upper layers, followed by plant uptake of the mineral N released, may have been responsible for the decline in TSN of RS95 between June and October 1996. The absence of SON movement down the profile was not surprising on this poorly drained clay soil, where drainage predominantly occurs via lateral flow (Hatch et al. 1997). However, a greater flush in TSN would be expected at North Wyke where soils have a higher organic matter and total N content than those at Rosemaund. Mineralisation rates have been shown to decrease with increasing clay content in undisturbed soils, because of increased physical protection of the soil organic matter (Verberne et al. 1990). However, when clay soils are disturbed, the opposite is true, with greater increases in SMN observed compared with sandy soils, because of exposure of a larger pool of physically protected organic matter (Hassink 1992). That this did not happen here, maybe due to different degrees of disruption achieved by the cultivation operations at each site, with a finer tilth possibly achieved on the silty clay loam at Rosemaund.

The flush in SON following cultivation was large and appeared to be dependent on previous management practices, particularly N inputs from fertiliser or animal excreta. Although sampling was limited to only two occasions during the year, measurement of the distribution of SON within the soil profile gave insight into the possible fate of SON between sampling. This differed across soil types, with considerably more leaching apparent in the silty clay loam at Rosemaund than in the clay loam at North Wyke. Interestingly, the flush in SON and SMN disappeared after just 1 year following cultivation. This was in contrast to other studies, which showed enhanced mineralisation rates, after cultivation of grassland, which continued for several years (Whitmore et al. 1992). These studies however, considered grasslands cultivated for arable production, with cultivations repeated each year, thereby potentially inducing a repeated flush in activity. The effect of a single cultivation and reseeding operation compared with multiple cultivations following conversion to arable cultivation, on the turnover of organic N needs further research.

PMN pool

The differences in the SON content of the cultivated and undisturbed pastures were not observed in the PMN pool, which was very similar in RS95, RS96 and UD, and declined with depth on all occasions at all the sites. Stockdale and Rees (1994) observed that the anaerobic incubation technique was capable of detecting small differences in PMN resulting from the application of residues and manures. It is therefore surprising that there was no difference in the PMN content associated with cultivation and reseeding of the present sites. However, Patra et al. (1998) also observed no significant effect of grassland management (reseeding and N fertiliser applications) on the size of the PMN pool.

Determination of the PMN content of soils may be expected to include SON. However, no relationship was observed between the SON and PMN pools. This was also observed by Murphy et al. (1999) for a range of soil types. Determination of PMN by anaerobic incubation may therefore not be sufficiently sensitive to discriminate between N that will be mineralised compared to N that will be converted to soluble forms (SON). Alternatively, not all the SON may be readily mineralisable, so that changes in the SON pool of RS95 could have been due to immobilisation or leaching rather than mineralisation. Direct uptake of low molecular weight compounds by soil microbes (and plants) can occur (Barraclough 1997) and could also partly explain the discrepancy.

The decline in the size of the PMN pool from June to October at all sites did not solely result from mineralisation, as comparable increases in the SMN pool were not observed. Plant N uptake during this period ranged from 80–170 kg N ha^{-1} and represented only ca. 20–55% of the decline in PMN. It is probable, therefore, that a substantial proportion of this pool was converted into more stable organic forms through immobilisation.

Conclusions

SON is a significant N pool in grassland soils and cultivation has been shown to have a major impact on its release. Leaching of SON following cultivation could make a significant contribution to the NO_3^- pollution of water courses (if mineralised and nitrified in surface or groundwaters). Alternatively, mineralisation of SON within the soil profile could contribute to the crop N supply. As a result, the measurement of SON should be included in studies of N cycling in agricultural cropping so that full account may be taken of its potential as a source or sink of mobile N. Much remains unknown about the mobility and decomposability of SON, so further research into its role in N transformations is required.

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