

Available soil nitrogen in relation to fractions of soil nitrogen and other soil properties

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Abstract

The available N of 27 soils from England and Wales was assessed from the amounts of N taken up over a 6-month period by perennial ryegrass grown in pots under uniform environmental conditions. Relationships between availability and the distribution of soil N amongst various fractions were then examined using multiple regression. The relationship: available soil N (mg kg^{-1} dry soil) = $(N_{\text{min}} \times 0.672) + (N_{\text{inc}} \times 0.840) + (N_{\text{mom}} \times 0.227) - 5.12$ was found to account for 91% of the variance in available soil N, where N_{min} = mineral N, N_{inc} = N mineralized on incubation and N_{mom} = N in macro-organic matter. The N mineralized on incubation appeared to be derived largely from sources other than the macro-organic matter because these two fractions were poorly correlated. When availability was expressed in terms of available organic N as % of soil organic N (N_{ao}) the closest relationship with other soil characteristics was: $N_{\text{ao}} = [N_{\text{inc}} \times (1.395 - 0.0347 \times \text{C:N}_{\text{mom}})] + [N_{\text{mom}} \times 0.1416]$, where C:N_{mom} = C:N ratio of the macro-organic matter. This relationship accounted for 81% of the variance in the availability of the soil organic N.

The conclusion that the macro-organic matter may contribute substantially to the available N was confirmed by a subsidiary experiment in which the macro-organic fraction was separated from about 20 kg of a grassland soil. The uptake of N by ryegrass was then assessed on two subsamples of this soil, one without the macro-organic matter and the other with this fraction returned: uptake was appreciably increased by the macro-organic matter.

Introduction

Soils differ greatly in the amounts of nitrogen made available for uptake by plants during a growing season. With established grassland, the amount of N taken up from plots receiving no fertilizer N provides a good indication of the available soil N supply and, using this method of assessment and including results from a large number of experiments in England and Wales, Richards and Hobson (1977) reported variation between 8 and $350 \text{ kg ha}^{-1} \text{ year}^{-1}$. The differences in supply reflect, in part, differences in total soil N content and, in part, differences in the proportion of the soil

organic N undergoing mineralization. This proportion is influenced by soil temperature and water status (Stanford, 1982), and also by inherent soil properties. Thus, in an investigation involving perennial ryegrass grown in pots on 21 soils under uniform environmental conditions, the proportion of the total soil N taken up over six successive harvests and including that in stubble and roots at the end of the experiment, ranged from 1.5 to 4.0% (Whitehead, 1984).

The aim of the present investigation was to examine, with a range of soils, relationships between the availability of soil N and a number of soil

properties, especially the distribution of N amongst different fractions of the soil organic matter. Available N was assessed from the amount taken up by the perennial ryegrass grown under uniform environmental conditions.

Experimental

Soils

The 27 soils (Table 1) included some from long-term pasture (> 20 years), some from areas of re-seeded grassland and some from areas of long-term arable cultivation. In all instances, they were either from the sites of multi-centre trials or from fields or experimental plots that had received known and contrasting agronomic treatments. Bulk samples of soil were taken, to a depth of 150 mm, in either September 1982 (10 soils) or

February–March 1984 (17 soils). No fertilizer N had been applied for at least one year before the samples were obtained. Each soil was sieved (< 6 mm) to remove stones and coarse fragments of plant material and then mixed. Subsamples of each soil were dried at 30 °C and ground (< 2 mm) for analysis. The remaining soil, intended for transfer to plant pots, and for the determination of macro-organic matter content and N mineralized on incubation, was frozen at –15 °C. Portions were thawed for 24 hours at 4 °C immediately before use.

Soil analyses

Organic C was determined titrimetrically (Kalembasa and Jenkinson, 1973). Total nitrogen was determined by Kjeldahl digestion with a H₂SO₄/H₃PO₄/K₂SO₄ mixture containing Se as catalyst, followed by the estimation of ammonium

Table 1. The location and some properties of the soils

| Location | Previous ^a cropping | Clay (%) | Soil pH | Organic C (%) | Total soil N (%) | C:N ratio | Macro-organic matter | | |
|----------------------------|-----------------------------------|-------------|------------|---------------------|---------------------------|--------------|-------------------------------|------------------|--------------|
| | | | | | | | % (ash-free) of total soil | N in O.M. (%) | C:N ratio |
| 1. Hurley, Berks. | L | 20.2 | 6.6 | 1.86 | 0.200 | 9.30 | 0.399 | 2.63 | 18.6 |
| 2. Drayton, Warwicks | L | 48.6 | 6.7 | 3.73 | 0.396 | 9.42 | 0.480 | 2.10 | 21.8 |
| 3. Bangor, Gwynedd | G | 25.1 | 4.7 | 3.91 | 0.306 | 12.78 | 0.267 | 2.14 | 22.4 |
| 4. Trawsgoed, Dyfed | G | 40.2 | 5.1 | 3.50 | 0.361 | 9.70 | 0.443 | 2.46 | 20.0 |
| 5. Hurley, Berks. | A | 13.5 | 5.5 | 0.79 | 0.008 | 8.98 | 0.137 | 2.65 | 19.8 |
| 6. Hurley, Berks. | G | 15.1 | 5.8 | 2.36 | 0.224 | 10.54 | 0.494 | 3.05 | 16.7 |
| 7. Burchetts Green, Berks. | G | 28.6 | 4.3 | 5.13 | 0.478 | 10.73 | 0.926 | 3.17 | 16.4 |
| 8. Hurley, Berks. | C | 19.8 | 6.4 | 1.57 | 0.171 | 9.18 | 0.336 | 3.78 | 13.4 |
| 9. North Wyke, Devon | G | 35.0 | 4.6 | 4.13 | 0.435 | 9.49 | 0.958 | 2.75 | 17.9 |
| 10. Rowden, Devon | G | 36.2 | 4.5 | 3.76 | 0.354 | 10.62 | 0.752 | 2.34 | 20.4 |
| 11. Mungrisdale, Cumbria | G | 29.2 | 5.5 | 7.44 | 0.594 | 12.53 | 0.839 | 2.47 | 19.0 |
| 12. Barnard Castle, Durham | G | 20.7 | 6.6 | 4.45 | 0.410 | 10.85 | 0.232 | 2.32 | 20.2 |
| 13. Witnerburn, Yorks. | G | 36.5 | 5.8 | 5.45 | 0.532 | 10.24 | 0.457 | 2.24 | 20.9 |
| 14. Cawthorne, Yorks. | G | 13.5 | 6.4 | 5.20 | 0.397 | 13.10 | 0.254 | 2.20 | 21.8 |
| 15. St Asaph, Clwyd | G | 24.6 | 6.4 | 4.76 | 0.398 | 11.96 | 0.463 | 2.11 | 22.6 |
| 16. Pant-y-dwr, Powys | G | 29.8 | 5.3 | 10.62 | 0.516 | 20.58 | 0.459 | 1.82 | 27.3 |
| 17. Ponterwyd, Dyfed | G | 36.2 | 4.9 | 7.8 | 0.542 | 14.17 | 0.264 | 1.91 | 25.4 |
| 18. Highclere, Berks. | G | 21.4 | 5.2 | 4.14 | 0.380 | 10.89 | 0.399 | 2.18 | 21.7 |
| 19. Selborne, Hants. | G | 24.3 | 5.0 | 3.63 | 0.376 | 9.65 | 0.360 | 2.24 | 19.7 |
| 20. Yarnton, Oxford | G | 19.7 | 4.7 | 3.66 | 0.358 | 10.22 | 0.284 | 1.86 | 24.1 |
| 21. Gt Alne, Warwicks. | G | 24.4 | 5.1 | 3.28 | 0.352 | 9.32 | 0.439 | 2.39 | 18.7 |
| 22. Exminster, Devon | G | 18.0 | 5.1 | 2.12 | 0.226 | 9.38 | 0.093 | 0.62 | 25.5 |
| 23. Chagford, Devon | G | 16.8 | 5.0 | 3.42 | 0.308 | 11.10 | 0.157 | 1.86 | 24.6 |
| 24. Bridgwater, Somerset | G | 36.8 | 5.7 | 4.44 | 0.538 | 8.25 | 0.157 | 1.97 | 23.0 |
| 25. Sonning, Berks. | A | 8.4 | 6.6 | 0.94 | 0.100 | 9.40 | 0.071 | 2.40 | 19.4 |
| 26. Sonning, Berks. | A | 11.7 | 7.0 | 2.22 | 0.208 | 10.67 | 0.176 | 2.68 | 18.2 |
| 27. Shinfield, Berks. | A | 11.7 | 5.5 | 1.41 | 0.118 | 11.95 | 0.046 | 2.33 | 21.1 |

^a A, arable for < 20 years; C, clover alone for 4 years; G, grass for > 20 years; L, grass in grass/arable rotation

in the digest using an automated colorimetric procedure involving the reaction of NH_4^+ ions with sodium phenate and sodium hypochlorite (Gehrke *et al.*, 1968). Particle size distribution (ISSS fractions) was determined by a combination of hydrometer measurements, decantation and sieving (Allen *et al.*, 1974), and soil pH was measured in a 1:2.5 suspension of soil in 0.01 M CaCl_2 after shaking for 1 hour.

In addition, the mineral N content (N_{min}) of each soil was assessed on a subsample of moist soil, taken at the time of filling the pots to be sown with ryegrass, by extraction at room temperature with 2 M KCl (Bremner, 1965). Ammonium N in the extract was measured as above (Gehrke *et al.*, 1968), and NO_3^- -N by reduction to NO_2^- on a cadmium column (Henricksen and Selmer-Olsen, 1970) followed by reaction with the azo dye Orange-I (Follett and Ratcliff, 1963).

Distribution of N in fractions of the soil organic matter

Assessments were made of the amounts of N in several fractions of the soil organic matter. In each instance, the fractions were prepared by treatment of the original soil, not by sequential treatment.

The amounts of C and N mineralised on incubation (C_{inc} and N_{inc}) were determined by a procedure based on that described by Clement and Williams (1962). Each soil was incubated at 30 °C for 20 days but water content was adjusted to -10 kPa and acetylene amounting to 0.5% v/v was added to each flask. The addition of acetylene was made in order to inhibit nitrification, and hence the loss of N by denitrification (Warren, 1988), since the results of preliminary incubations had indicated that substantial losses occurred from some soils.

The macro-organic matter fraction (Barley, 1955) was separated by dispersing approximately 1 kg of moist soil in 21 sodium hexametaphosphate (10 g l^{-1}). The suspension was poured on to a 0.2 mm nylon mesh, fine material washed through with a jet of tap water and the retained mixture of organic material and sand separated by repeated suspension in water and decantation of the organic material. The organic material which had passed the 6 mm sieve but was retained on the 0.2 mm mesh constituted the macro-organic matter frac-

tion. In the present investigation, and in contrast to the study of Garwood *et al.* (1972), this fraction excluded bulk of the living root material, though undoubtedly some root fragments were included. The content of actual organic matter was determined by ashing at 550 °C for 16 hours; C in this fraction was determined by an induction furnace carbon analyser (Leco model HF 10) and N by Kjeldahl digestion as described above for whole soils. The contents of C and N in the macro-organic matter fraction were expressed on an ash-free basis.

Two fractions of the soil organic N were determined by extraction of dried and ground subsamples of each soil with formic acid/acetylacetone and with 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$, solvents which have been used in other investigations of the composition of organic matter. With formic acid/acetylacetone, 10 g soil was boiled under reflux with 60 ml formic acid/acetylacetone (9:1 v/v) for 15 minutes (Jones and Parsons, 1972), the mixture centrifuged, the liquid discarded and the soil residue washed with 0.01 M CaCl_2 . With $\text{Na}_4\text{P}_2\text{O}_7$, 10 g soil was shaken with 50 ml reagent for 16 hours (Choudhri and Stevenson, 1957) and the soil residue centrifuged and washed as above. After each of these extractions, the soil residue was dried at 100 °C, weighed and its N content determined by Kjeldahl digestion. The amounts of soil N extracted by formic acid and by pyrophosphate were calculated by difference from total soil N.

Assessment of available soil N

Available soil N was defined as the amount taken up by ryegrass, including that in stubble and roots, over a period of 6 months. Visual inspection at the end of the period indicated that the pots of soil (see below) had been well explored by the roots. The available organic N was assessed by subtracting the initial mineral N (which was assumed to be completely available) from the total available soil N.

Perennial ryegrass (*Lolium perenne* L. cv. S23) was grown from seed in pots of each soil (in triplicate for soils 1 to 10 and in duplicate for soils 11 to 27) in a Saxcil controlled environment growth cabinet with a 16 h photoperiod (125 w m^{-2}) and day/night temperatures of 20/15 °C.

Approximately 10 kg of each soil was thawed and subsamples were transferred to metal cans (103 mm diameter; 190 mm depth) lined with poly-

ethylene. In order to obtain as uniform a degree of compaction as possible, the pots were tapped about 35 times during filling on to a wooden board from a height of about 120 mm. There were considerable differences between soils in the weight of dry soil per pot (from 1076 to 2640 g) reflecting differences in bulk density. At the time of sowing, the soil in each pot was brought to a soil water potential of -10 kPa (100 cm water tension), the water content at this tension having been determined as described by Andrew and Fergus (1976). Nutrients in solution were mixed with the soil before sowing to provide 100 mg K, 40 mg P, 20 mg S and 20 mg Mg per pot. No additional N was provided, and soil pH was not adjusted.

After germination, the number of seedlings per pot was reduced to 18, the soil surface was covered with a layer of black polyethylene granules, and the

pots placed on scales (Precision Engineering, Reading, Berks – scales 405 K) in the growth cabinet. The soil in each pot was restored to -10 kPa water tension three times per week using glass-distilled water. The grass was harvested by cutting 20 mm above the soil surface 42 days after sowing, and on five successive occasions at intervals of 28 days. Nutrients in solution, at the rates specified above, were applied to the surface of the pot after the first five of the six harvests. At the final harvest, stubble and roots were recovered, the roots being separated from the soil by washing over a 2-mm stainless steel sieve. All plant material was dried at 100°C , weighed and ground; it was then analysed for N by the Kjeldahl procedure described above for soil. Available soil N was then calculated by summing the amounts in the six harvests of herbage plus the amounts in stubble and roots at the final harvest.

Table 2. Distribution of soil N in fractions related to availability

| Soil no. | Organic N as % of total soil N | Percentage of soil organic N | | | | |
|----------|--------------------------------|---------------------------------|--|-------------------------|---------------------------|-----------------------|
| | | soluble in HCOOH/acetyl-acetone | soluble in 0.1 M $\text{Na}_4\text{P}_2\text{O}_7$ | in macro-organic matter | mineralised on incubation | available to ryegrass |
| 1 | 99.6 | 14.0 | 12.7 | 4.95 | 0.92 | 1.71 |
| 2 | 99.4 | 13.0 | 14.6 | 2.36 | 0.42 | 0.98 |
| 3 | 99.5 | 33.9 | 12.2 | 1.83 | 1.82 | 1.75 |
| 4 | 99.2 | 29.6 | 9.3 | 2.82 | 1.65 | 2.15 |
| 5 | 98.3 | 5.3 | 10.9 | 4.15 | 2.38 | 1.60 |
| 6 | 99.7 | 23.1 | 11.9 | 6.30 | 1.44 | 2.14 |
| 7 | 99.6 | 25.4 | 17.4 | 5.83 | 2.43 | 2.61 |
| 8 | 99.1 | 11.0 | 11.2 | 7.37 | 2.69 | 3.19 |
| 9 | 98.1 | 41.5 | 19.5 | 5.87 | 2.73 | 3.60 |
| 10 | 99.6 | 32.6 | 12.1 | 4.57 | 2.83 | 2.07 |
| 11 | 99.0 | 34.5 | 21.5 | 3.35 | 3.62 | 3.87 |
| 12 | 99.1 | 34.1 | 27.4 | 1.29 | 1.33 | 1.10 |
| 13 | 99.4 | 27.2 | 16.4 | 1.82 | n.d.* | 1.77 |
| 14 | 99.2 | 21.1 | 10.7 | 1.39 | 2.07 | 1.35 |
| 15 | 98.4 | 28.6 | 18.6 | 2.43 | 1.20 | 0.61 |
| 16 | 98.5 | 40.2 | 28.3 | 1.57 | 1.39 | 0.45 |
| 17 | 98.7 | 38.3 | 20.0 | 0.89 | 1.39 | 0.46 |
| 18 | 98.4 | 30.5 | 18.6 | 2.23 | 1.73 | 1.29 |
| 19 | 98.8 | 17.4 | 13.2 | 2.09 | 2.47 | 1.74 |
| 20 | 99.2 | 26.7 | 19.7 | 1.47 | 1.56 | 1.43 |
| 21 | 98.7 | 33.1 | 29.5 | 2.96 | 2.97 | 2.56 |
| 22 | 99.3 | 27.6 | 17.2 | 0.67 | 1.47 | 1.41 |
| 23 | 99.0 | 37.2 | 25.3 | 0.95 | 1.33 | 1.19 |
| 24 | 99.2 | 14.9 | 12.1 | 0.55 | 1.56 | 0.71 |
| 25 | 98.6 | 12.4 | 15.7 | 1.72 | 1.07 | 0.98 |
| 26 | 98.9 | 23.3 | 13.7 | 2.26 | 3.65 | 3.24 |
| 27 | 98.8 | 10.5 | 16.1 | 0.90 | 2.14 | 1.28 |

*n.d. – not determined.

Table 3. Simple correlation coefficients (*r*) between fractions of soil N and soil properties, expressed on soil weight basis (mg kg⁻¹) where possible

| <i>Between different fractions of soil N</i> | | | | | | | |
|--|------------------|---------------------|-----------|----------------------|-----------------|---|------------|
| | Available soil N | Available organic N | Mineral N | N min. on incubation | N ext. by HCOOH | N ext. by Na ₄ P ₂ O ₇ | Macro-OM N |
| Available soil N | – | | | | | | |
| Available organic N | 0.934*** | – | | | | | |
| Mineral N | 0.643*** | 0.327 | – | | | | |
| N min. on incubation | 0.919*** | 0.895*** | 0.521** | – | | | |
| N extracted by HCOOH | 0.716*** | 0.504** | 0.808*** | 0.677*** | – | | |
| N extracted by Na ₄ P ₂ O ₇ | 0.623*** | 0.401* | 0.790*** | 0.603** | 0.894*** | – | |
| Macro-OM N | 0.728*** | 0.776*** | 0.255 | 0.644*** | 0.429* | 0.320 | – |
| <i>Between soil N fractions and soil properties</i> | | | | | | | |
| | Available soil N | Available organic N | Mineral N | N min. on incubation | N ext. by HCOOH | N ext. by Na ₄ P ₂ O ₇ | Macro-OM N |
| Total soil N | 0.668*** | 0.498** | 0.717*** | 0.684*** | 0.856*** | 0.826*** | 0.412* |
| Soil organic N | 0.665*** | 0.498** | 0.709*** | 0.684*** | 0.853*** | 0.822*** | 0.412* |
| Soil organic C | 0.511** | 0.287 | 0.737*** | 0.531** | 0.871*** | 0.858*** | 0.258 |
| C min. on incubation | 0.619*** | 0.507** | 0.579** | 0.695*** | 0.550** | 0.597** | 0.212 |
| Soil C:N ratio | 0.111 | –0.091 | 0.472* | 0.138 | 0.554** | 0.550** | –0.059 |
| Soil pH | –0.355 | –0.307 | –0.279 | –0.436* | –0.468* | –0.308 | –0.306 |
| % clay | 0.400* | 0.316 | 0.398* | 0.362 | 0.518** | 0.451* | 0.410* |
| N conc. in macro-OM | 0.089 | 0.271 | –0.353 | 0.126 | –0.317 | –0.351 | 0.488* |
| C:N of macro-OM | –0.194 | –0.399* | 0.346 | –0.163 | 0.357 | 0.386 | –0.532** |

*, **, ***; *r* values significant at *P* < 0.05, 0.01 and 0.001 respectively.

Table 4. Simple correlation coefficients (*r*) between fractions of soil N, expressed as a proportion (%) of total organic N where possible, and between fractions and soil properties

| <i>Between different fractions of soil N</i> | | | | | |
|--|---------------------|----------------------|-----------------|---|------------|
| | Available organic N | N min. on incubation | N ext. by HCOOH | N ext. by Na ₄ P ₂ O ₇ | Macro-OM N |
| Available organic N | – | | | | |
| N min. on incubation | 0.796** | – | | | |
| N extracted by HCOOH | 0.109 | 0.108 | – | | |
| N extracted by Na ₄ P ₂ O ₇ | –0.134 | –0.055 | 0.578*** | – | |
| Macro-OM N | 0.658*** | 0.342 | –0.087 | 0.255 | – |
| <i>Between soil N fractions and soil properties</i> | | | | | |
| | Available organic N | N min. on incubation | N ext. by HCOOH | N ext. by Na ₄ P ₂ O ₇ | Macro-OM N |
| Soil organic N ^a | –0.041 | 0.044 | 0.621*** | 0.364 | –0.168 |
| Soil organic C ^b | –0.185 | –0.012 | 0.647*** | 0.487* | –0.228 |
| C min. in incubation ^c | 0.108 | 0.125 | –0.399* | –0.119 | –0.068 |
| Soil C:N ratio | –0.315 | –0.069 | 0.438* | 0.430* | –0.287 |
| Soil pH | –0.111 | –0.265 | –0.489* | –0.233 | 0.002 |
| % clay | –0.014 | –0.125 | 0.350 | 0.050 | 0.060 |
| N conc. in macro-OM | 0.642*** | 0.505** | –0.325 | –0.352 | 0.832*** |
| C:N of macro-OM | –0.730*** | –0.479* | 0.340 | 0.376 | –0.803*** |

*, **, ***; *r* values significant at *P* < 0.05, 0.01 and 0.001 respectively.

^a mg N kg⁻¹ soil

^b mg C kg⁻¹ soil

^c % of organic C

Multiple regression analysis of results

In order to take into account the additive effects of more than one fraction of soil N on availability, multiple regression models were constructed. It was possible to include soil properties which did not have a large enough influence on available N to be correlated significantly on their own. The multiple regression models were computed stepwise, with combinations of fractions being selected to minimise the residual variation, and optimum regression coefficients for each term being calculated. The addition of terms to the regression equation was stopped when the improvement to the fit of the equation became so small that the regression coefficients of all potential additional terms would not be significantly different from zero. A constant was normally included in the regressions in order to account for fractions of soil N not explicitly stated.

Results and discussion*Available soil N and available organic N*

The available soil N, defined as the amount taken up by ryegrass, ranged from 24 to 290 mg kg⁻¹ dry soil, amounts representing between 1.46 to 5.43% of the total soil N. The available organic N amounted to between 0.45 and 3.87% of the total organic soil N (Table 2).

Amounts and proportions of the soil N in a range of soil fractions

Total soil N ranged from 0.088 to 0.594% of dry soil, and the C:N ratio of the whole soil from 9.2 to 20.6 with a mean of 10.9 (Table 1). The mineral N content of the soils at the time of filling the pots ranged from 7.0 to 82.5 mg kg⁻¹ dry soil. With all soils, at least 98% of the total N was present as organic N (Table 2).

The amounts of N mineralized during incubation at 30°C for 20 days ranged from 11 to 213 mg kg⁻¹ dry soil, representing from 0.42 to 3.65% of the soil organic N (Table 2), while the amounts of N in the macro-organic matter fraction (N_{mom}) ranged from 15 to 278 mg N kg⁻¹ dry soil, 0.55 to 7.37% of the soil organic N. The C:N ratio of the macro-organic

matter fraction ($C:N_{\text{mom}}$) ranged from 13.4 for soil 8, which had grown white clover alone for 4 years, to 27.3 for soil 16 which was from an area of long-term pasture: the mean value was 20.8.

The amounts of N in the fraction soluble in formic acid ranged from 60 to 2120 mg kg⁻¹ dry soil, between 7 and 43% of the total soil N, while the organic N soluble in formic acid represented between 5 and 40% of the soil organic N. Jones and Parsons (1972) reported that the amounts extracted by formic acid from a range of soils were proportionately least with neutral or slightly alkaline soils low in organic matter, and similar relationships with organic matter content and pH were observed in the present investigation (Table 4).

The N soluble in Na pyrophosphate ranged from 109 to 1517 mg kg⁻¹ dry soil, representing between 10 and 30% of the total soil N, and between 9 and 29% of the soil organic N.

Relationships between available soil N and measured fractions of soil N

Available soil N, expressed in mg per kg soil, was highly correlated with N mineralised on incubation ($r = 0.919^{***}$), and also with the N in macro-

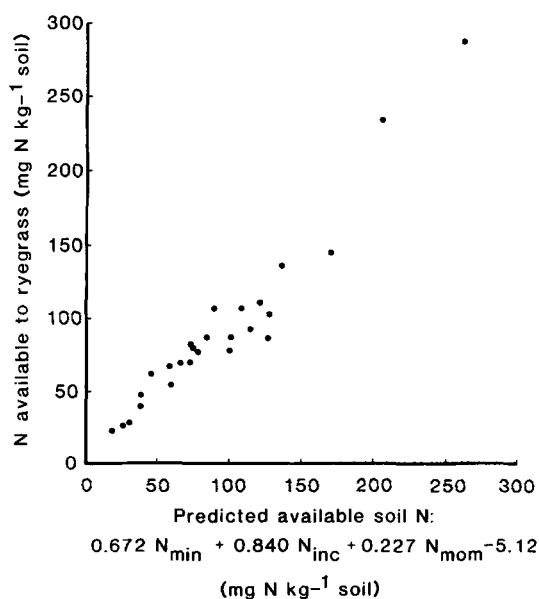


Fig. 1. Relationship, for 27 soils, between the amount of N available to ryegrass and 'available soil N' predicted by a linear combination of mineral N, N mineralized on incubation and macro-OM N, with coefficients optimised by regression.

organic matter ($r = 0.728^{***}$), the N soluble in formic acid ($r = 0.716^{***}$), and the N soluble in pyrophosphate ($r = 0.623^{***}$), as indicated in Table 3. As would be expected, the amount of available soil N, was positively correlated with total soil N ($r = 0.668^{***}$) and with the amount of initial mineral N ($r = 0.643^{***}$).

Multiple regression analysis showed that a combination of fractions of soil N accounted for variations in the available soil N, better than any single fraction. Thus, 91.3% of the variance was accounted for in the regression equation describing available soil N, expressed in mg N kg⁻¹ dry soil, given below (Figure 1):

$$\begin{aligned} \text{Available soil N} = & 0.672 N_{\text{min}} + 0.840 N_{\text{inc}} \\ & + 0.227 N_{\text{mom}} - 5.12 \end{aligned}$$

The constant term was not significantly different from zero, but all other regression coefficients were significant at the 1% or 0.1% levels.

The supply of available N can be regarded as having two components, the initial mineral N which, normally, is completely available, and the potentially mineralizable N which is only partially available over a period of months (Stanford, 1982). In the present investigation, the potentially mineralizable N was assessed by measurement of the N mineralized during incubation. On the assumption that the mineral N fraction was entirely taken up by the grass, the relationship between available organic N and soil fractions was examined more closely.

Relationships between available organic N and measured fractions of soil N

Available organic N, expressed in terms of mg N per kg dry soil (Table 3), was correlated closely with N mineralised on incubation ($r = 0.895^{***}$) and N in macro-organic matter ($r = 0.776^{***}$), and correlated moderately well with total soil N, soil organic N, and N extracted by formic acid, and rather less well with N extracted by pyrophosphate.

When the data were expressed in terms of the proportions of soil organic N (Table 4), thereby eliminating the effect of variations in total soil N on the amount of available N, available organic N was correlated with N mineralized on incubation and N in macro-OM according to the relationships:

$$\begin{aligned} \text{Available organic N} = & 0.930 N_{\text{inc}} - 0.048 \\ (r = 0.796^{***}, \% \text{ variance accounted for} = & 61.9) \end{aligned}$$

$$\begin{aligned} \text{Available organic N} = & 0.325 N_{\text{mom}} - 0.829 \\ (r = 0.658^{***}, \% \text{ variance accounted for} = & 40.9) \end{aligned}$$

$$\begin{aligned} \text{Available organic N} = & -0.219 C:N_{\text{mom}} \\ & + 6.303 \\ (r = -0.730^{***}, \% \text{ variance accounted for} = & 51.3) \end{aligned}$$

Relationships between different fractions of soil N

The amounts of N (in mg per kg soil) released on incubation, and extracted by formic acid and sodium pyrophosphate were well correlated with each other, the correlation coefficient between any pair of these quantities always being significant at better than the 0.1% level (Table 3). Each quantity was also well correlated with both mineral N and soil total N (Table 3). The amount of macro-OM N (mg per kg) was highly correlated with N mineralised on incubation ($r = 0.644^{***}$), but was poorly correlated with N extracted by formic acid and total N, and was not significantly correlated with N extracted by pyrophosphate. The amounts of N extracted by formic acid and by pyrophosphate were more closely related to total and organic N than to available soil N or available organic N, whereas N mineralized on incubation and N in macro-OM were more closely related to available N than to total or organic N.

There were few significant correlations between fractions when the data were expressed in terms of proportions of soil organic N (Table 4). The N mineralized on incubation (% of organic N) was not correlated with any other fraction of soil N, but was significantly correlated with the N concentration in macro-OM ($r = 0.505^{**}$) and the C:N ratio of macro-OM ($r = 0.479^*$).

Models for available organic N constructed using combinations of soil N fractions

Available organic N expressed as a percentage of soil organic N was closely correlated with N mineralized on incubation and also with macro-OM N.

Table 5. Models for available organic N, based on combinations of soil fractions (significance of regression coefficients indicated under each coefficient)

| | |
|--|------|
| <i>Model 1</i> | |
| Available organic N (mg N kg ⁻¹ soil) = | |
| 0.904 N _{inc} + 0.211 N _{mom} - 0.000503 C _{org} + 0.26 | |
| *** ** * | NS |
| % variance accounted for = | 89.3 |
| <i>Model 2</i> | |
| Available organic N (% soil organic N) = | |
| 0.756 N _{inc} + 0.2160 N _{mom} - 0.322 | |
| *** *** | NS |
| % variance accounted for = | 78.5 |
| <i>Model 3</i> | |
| Available organic N (mg N kg ⁻¹ soil) = | |
| (2.427 - 0.0775 [C:N _{mom}]). N _{inc} + 441 | |
| *** ** | NS |
| % variance accounted for = | 89.6 |
| <i>Model 4</i> | |
| Available organic N (% soil organic N) = | |
| (1.395 - 0.0347 [C:N _{mom}]). N _{inc} + 0.1416 N _{mom} | |
| ** * | * |
| % variance accounted for = | 80.6 |
| <hr/> | |
| N _{inc} = N mineralised on incubation (mg N kg ⁻¹ soil). | |
| N _{mom} = N in macro-OM (mg N kg ⁻¹ soil). | |
| C _{org} = Organic C (mg C kg ⁻¹ soil). | |
| C:N _{mom} = C:N ratio of macro-OM. | |

However, these two fractions were not significantly correlated with each other, suggesting that they represented different components of the soil organic N. In order to take into account both these fractions and also others, which individually were not significantly correlated with available soil N, multiple regression models were constructed (Table 5).

Models 1 and 2 were found by stepwise multiple regression, using the fractions and properties given in Table 3. In model 1, with data expressed in mg kg⁻¹ soil, N mineralized on incubation was the dominant fraction but organic C significantly affected the correlation, higher levels of soil organic C being associated with lower levels of available N. In model 2, with data expressed as proportions of soil organic N, only two fractions occurred, N mineralized on incubation and macro-OM N. The fit obtained with this model (variance accounted for = 78.5%) was much better than for either fraction individually (61.9 and 40.9% respectively), supporting the hypothesis that these two fractions were largely independent contributors to available N.

Models 3 and 4 were based on the concept that N was made available from the fraction mineralized on incubation and from macro-OM, but that the release of N was modified by the C:N ratio of the macro-OM. The fraction of soil N was multiplied by a factor expressing this effect. An expression of the form: $a - b \times C:N_{mom}$ was highly correlated with available organic N (proportional basis), and was used to make the adjustment. Thus, the adjusted N mineralized on incubation was: $a(N_{inc}) - b(C:N_{mom})(N_{inc})$, optimum values of *a* and *b* being found by regression. These models used all three independent variables that were highly correlated with available organic N. Adjustment of N_{inc} by the C:N ratio of macro-OM gave only small improvements in the overall % variance accounted for. With data expressed on a weight basis (model 3), the adjusted N mineralized on incubation accounted for almost the same % variance as model 1. The constant term was non-significant in models 1 to 3, and if the regression procedure was repeated, specifying no constant term, there was no change to the combination of fractions selected and little difference to the % variance accounted for. With data expressed on a proportional basis, the inclusion of a constant and adjustment of N_{inc} gave the same combination of fractions as model 2, but if a constant term was not included, model 4 resulted. In this model, adjustment of N mineralised on incubation by a factor based on the C:N ratio of macro-OM gave a small improvement in goodness-of-fit over model 2, which used the same two fractions of soil N. The possibilities of including an adjustment of macro-OM N, or adjusting N_{inc} or N_{mom} by soil C:N ratio, were also examined, but had no appreciable effect. Nor were the correlations improved by other soil properties, with the exception of organic C in model 1.

The role of macro-organic matter

The macro-OM isolated in the present work was defined as material having a specific gravity < 1.0 and a particle size > 0.2 mm and < 6 mm, and in the grassland soils was composed largely of root residues. The C:N ratio of grass roots is generally in the range 25 to 45 (Whitehead, 1970), whereas the C:N ratio of macro-OM obtained in this work

ranged from 16.4 to 27.3 (excluding soil 8 which had a macro-OM C:N ratio of 13.4). This indicates that partial humification of the root material had taken place, as the C:N ratios were intermediate between those of roots and those of the whole soils (8.7 to 17.6).

Although concentrations of N were lower in macro-OM than in humified organic matter, the correlations between available organic N and macro-OM N, expressed as proportions of soil organic N, in both simple and multiple regressions, suggested that macro-OM contributed substantially to the available N. The four long-term arable soils included in the investigation fitted the correlations obtained although the amounts of macro-OM and hence the relative importance of macro-OM as a source of N were small. The importance of macro-OM was supported by the results of two supplementary experiments. In the first experiment, macro-OM was separated from each of soils 1 to 10, and duplicate subsamples, calculated to contain 1.1 g ash-free organic matter (oven dry basis), incubated moist in a sand matrix (150 g) for 28 days at 25 °C. Variation amongst the 10 soils in the amounts of CO₂-C evolved from the macro-OM was relatively small (Table 6), showing that respiration and decomposition proceeded at similar rates. Mineral N was released from all samples of macro-OM, indicating that macro-OM N could be mineralized during a short decomposition period. However, the amounts of N mineralized differed widely amongst the soils (Table 6), supporting the view that the availability of the soil organic N was influenced by the composition of macro-OM. The

Table 6. Amounts of C and N mineralized from macro-organic matter isolated from 10 soils, during 28 days' incubation in sand at 25 °C (means of duplicate sub-samples)

| Soil | CO ₂ -C (mg g ⁻¹ macro-OM) | Mineral N (µg g ⁻¹ macro-OM) |
|---------|---|--|
| 1 | 47.4 | 36 |
| 2 | 39.6 | 35 |
| 3 | 56.0 | 653 |
| 4 | 42.4 | 633 |
| 5 | 46.2 | 763 |
| 6 | 39.9 | 287 |
| 7 | 47.9 | 720 |
| 8 | 60.7 | 2348 |
| 9 | 49.2 | 71 |
| 10 | 43.7 | 696 |
| Mean SE | ± 1.34 | ± 94 |

second supplementary experiment was carried out under the same conditions and in parallel with the main pot experiment. Macro-OM was first separated from 20 kg of soil 6, a grassland soil, and the remaining soil recovered, dried and reconstituted by mixing. Separated macro-OM was then added back to half the reconstituted soil, and ryegrass was grown for 6 months on subsamples of reconstituted soil with and without macro-OM. Plant uptake of N was 30% greater from the soil with added macro-OM, although the increase in total soil N was only 6.25%. This result indicated that the N in macro-OM was more readily available than the N in humified organic matter.

Little attention has previously been given to the physical fractionation of soil in the context of the availability of soil N to plants, and there is little information on the transformations of N during the decomposition of roots (Smith and Peterson, 1982). In grassland soils, an annual input of dead material from roots and unharvested herbage takes place, estimated by Whitehead (1986) to provide up to 320 kg N ha⁻¹ under intensively managed grassland, and much of this must undergo mineralization.

As stated above, the correlation of available N with the N mineralized on incubation was greatly improved by the inclusion of macro-OM N in the regression, both with data expressed on a proportional basis and as mg N kg⁻¹ soil. While the reviews of Stanford (1982) and Kenney (1982) show that the correlations of N mineralized on incubation with available N are much improved by including initial mineral N as a separate term, there appear to be no examples of an improvement resulting from the inclusion of an independent soil fraction such as N in macro-OM.

Emphasis was placed on the plant available organic N, expressed as a proportion of the soil organic N, in order to avoid the influences of initial mineral N and total soil N. With data expressed in this way, the C:N ratio of macro-OM was inversely correlated with the available organic N, consistent with the observation (Power, 1968) that the mineralization of N during the incubation of grass roots was inversely related to C:N ratio. Although attempts to improve the degree of correlation in the regression equations through adjustment for C:N ratio of macro-OM were not successful, in models 3 and 4 (Table 5) adjustment of the N mineralized

on incubation for the C:N ratio of the macro-OM did produce a small improvement in the goodness-of-fit.

Conclusions

The investigation demonstrated that macro-OM has an important role in the supply of available N in grassland soils. Although macro-OM had a lower concentration of N than did more humified organic matter, it was, at least in part, a labile fraction of soil N. The C:N ratio of macro-OM was correlated highly with the proportion of available organic N, suggesting that the C:N ratio of macro-OM influenced its decomposability. However, the regression relationships between N_{ao} and N_{mom} showed little improvement when C: N_{mom} was included in the regression.

Nitrogen mineralized on incubation also represented a potentially labile fraction of organic N and this fraction appeared to be largely distinct from the macro-OM. Multiple regression models of the supply of N from pre-existing mineral N, N mineralized on incubation and macro-OM N could account for most of the variation in available soil N and available organic N, relationships which should have value in the construction of more realistic models of the turnover of soil N.

Recognition of the importance of decomposition rates of roots, and the nature of the decomposition products has increased as a result of the development of simulation models of nutrient cycles. The model of McGill et al. (1981), for example, simulated the turnover of C and N in soil, and included 'metabolic' and 'structural' fractions of litter material derived from roots and shoots. Many assumptions were required about the rates and pathways of the inputs and outputs of C and N from the litter material. Macro-OM determined by a procedure that excludes living roots so far as possible, is a directly measurable fraction of the soil, that could be explicitly included in such a model, improving its realism and ease of validation.

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