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Mineralization of labeled N from cowpea [*Vigna unguiculata* (L.) Walp.] plant parts at two growth stages in sandy soil

K. FRANZLUEBBERS, R.W. WEAVER and A.S.R. JUO
Department of Soil and Crop Sciences, Texas A&M University, College Station, TX 77843-2474, USA

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Abstract

Cowpea [*Vigna unguiculata* (L). Walp.] has great potential as green manure due to its rapid N accumulation and efficient N_2 fixation. The objective of this study was to measure the rate of N mineralization from cowpea plant parts harvested at onset of flowering (5 weeks) and mid pod-fill (7 weeks) under near optimum conditions. Cowpeas were grown in a greenhouse and supplied with $^{15}NH_4{}^{15}NO_3$ to isotopically label tissue. Cowpea leaves, stems, and roots were incorporated into a sandy soil (Psammentic Paleustalf) and net N mineralized was measured several times during a 10 week incubation. The amount of N accumulated in 7-week old cowpeas was more than double that in 5-week old cowpeas. The portion of N mineralized after 10 weeks was 24% for 5-week old cowpeas and 27% for 7-week old cowpeas. The rate of N mineralization from leaves and stems increased with plant age, but decreased for roots. The amount of N mineralized from 7-week old cowpeas was more than double (235%) that from 5-week old cowpeas due to greater N accumulation and a more rapid rate of N mineralization of the more mature cowpeas. The greatest amount of N was released from leaves, which amounted to 74 and 65% of total N mineralization from 5- and 7-week old cowpeas, respectively. The percentage of N mineralized by 10 weeks was linearly related to the tissue N concentration of the plant parts and to their C/N ratio. These relationships allow a quick estimation of the amount of N that would mineralize from cowpea residues incorporated into soil based on their N concentration or C/N ratio.

Introduction

There is renewed interest in using legumes as green manure to maintain soil fertility especially in areas where chemical fertilizers are costly or not readily available. Cowpea [*Vigna unguiculata* (L.) Walp.] is widely grown as a green manure crop in semi-arid regions of South Asia and as a food and fodder crop in the drier regions of Sub-Saharan Africa. It is regarded as an excellent green manure and fodder crop because of its rapid rates of N_2 fixation and accumulation of green matter and relatively low seed yield. Studies conducted in India and the Philippines showed that 6-week old cowpea can accumulate 70 to 80 kg N ha^{-1} in the above ground biomass (Morris et al., 1986; Singh et al., 1981). The crop is also known for its tolerance to drought and soil acidity (John et al., 1992).

The effectiveness of green manure as a source of N depends upon good synchronization of N release with N uptake by the main crop. Studies on kinetics of N mineralization from decomposing cowpea tissue are scarce (Frankenberger and Abdelmagid, 1985). An important management decision in the practice of green manuring is choosing the growth stage of the plant at the time at which a green manure crop is incorporated into the soil. For field-grown cowpeas, Singh et al. (1981) reported that the rate of N accumulation was constant between 4 and 6 weeks after sowing, then decreased between 6 and 7 weeks after sowing. Awonaike et al. (1991) found that soil supplied 80% of the total N assimilated in the above-ground plant parts of cowpea during the first 6 weeks of growth, while more than 80% of the N assimilated during the remaining period of growth was derived from fixation. The rate of N mineralization during decomposition of cowpea, however, may decrease with increasing age of plant tissue, as was found for white clover (*Trifolium repens* L.) (Kirchmann and Bergqvist, 1989) and for subterranean clover (*Trifolium subterraneum* L.) (Müller and Sundman, 1988).

It is often the case that not all cowpea plant parts are returned to the soil as green manure. Pods are removed for human consumption and leaves and stems may be used as fodder. Therefore information on the contribution of individual plant parts to total N mineralization is needed.

The objective of this study was to measure the rate of N mineralization from cowpea plant parts, i.e., leaves, stems and roots, harvested at the onset of flowering and at mid pod-fill.

Materials and methods

Cowpea plant material

Cowpeas of the indeterminate variety 'California Blackeye' were grown in a greenhouse to onset of flowering (5 weeks) and to mid pod-fill (7 weeks) and labeled with ^{15}N. The growth medium was coarse N-free silica sand supplied with 1/4 strength Modified Evans' N-free nutrient solution (Evans et al., 1972). In an effort to obtain a uniform ^{15}N label throughout the plant, seeds were not inoculated with a *Bradyrhizobium* strain, but supplied frequently with N as $^{15}NH_4{}^{15}NO_3$ containing 1.7 atom% ^{15}N excess.

Harvest of cowpea plants of each growth stage and incorporation into moist soil occurred on the same day. Cowpea plants were separated into leaves, stems (including petioles and peduncles), and roots. Flowers, pods and nodules were not used. Peduncles were included with stems, because of the similar N concentrations of stems and large peduncles, and because their structure is more similar to that of stems than of leaves or roots. In order to obtain a representative sample of cowpea plant parts for each pot, leaves, stems, and roots were separated into two to nine subgroups according to age for leaves and according to diameter for stems and roots. The respective portions of these subgroups were weighed, cut into 1 to 2 cm pieces, and remixed before soil incorporation. An amount corresponding to two cowpea plants (Table 1) was mixed with 3 kg soil per pot (1.3 to 7.0 g cowpea dry weight per kg soil) using a kitchen type electric hand mixer. Pots containing soil without incorporated cowpea served as the control. Three replications per treatment were randomly arranged. Subsamples of plant parts were taken for determination of N concentration (Nelson and Sommers, 1973), C concentration (Nitrogen Analyzer 1500, Carlo Erba Strumentazione) and atom% ^{15}N excess (Table 1). Carbon/N ratios of leaves, stems, and roots ranged from 18 to 34 (Table 1).

Despite the precautions taken, a few large nodules had formed on cowpea roots, which were fixing atmospheric N as determined by the acetylene reduction method (Hardy et al., 1968). Fixation of atmospheric N resulted in differential dilution of the ^{15}N content of plant parts with lower ^{15}N enrichment in plant parts formed later. Atom% ^{15}N excess ranged from 0.95 to 1.56.

Soil conditions during nitrogen mineralization

A sandy soil obtained from east Texas (sandy, siliceous, thermic Psammentic Paleustalf) was used for decomposition of cowpea plant parts. It had a pH of 5.5 (H_2O, 1:2), an organic C content of 2 g kg^{-1}, a CEC of 5.0 cmol kg^{-1}, a sand content of 920 g kg^{-1} and a clay content of 60 g kg^{-1}. Phosphorus (46 mg kg^{-1} soil) and K (105 mg kg^{-1} soil) were added to the soil 2 days before cowpea incorporation. This soil was selected due to its similar physical, chemical, and

The Soil Management Collaborative Research Support Program

Management Entity Office
Box 7113, North Carolina State University
Raleigh, NC 27695-7113 USA • CGNET: TropSoils
Phone (919) 515-3922 • FAX (919) 515-3942
Email: Roger_Hanson@NCSU.EDU

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Sincerely,

Roger G. Hanson
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Enclosures

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Table 1. Dry weight, N and C concentration, C/N ratio, and atom% ^{15}N excess of plant parts from 5- and 7-week old cowpeas incorporated into soil

Plant part	Plant age (weeks)	Dry weight[a] (g)	N conc. ($g\,kg^{-1}$)	N content (mg)	C conc. ($g\,kg^{-1}$)	C/N ratio	Atom% ^{15}N excess (%)
Leaves	5	14.0	21.9	306	43.8	20.0	1.350
Leaves	7	21.0	24.4	513	44.2	18.1	1.161
Petioles	5	2.67	9.5	26	ND	ND	1.302
Peduncles	5	0.35	44.9	16	ND	ND	0.994
Stems	5	3.18	11.8	37	ND	ND	1.246
Pet + Ped + Stems	5	6.20	12.7	79	43.1	33.9	1.214
Petioles	7	4.79	10.2	49	ND	ND	1.176
Peduncles	7	5.78	19.5	113	ND	ND	1.075
Stems	7	10.3	15.0	154	ND	ND	0.970
Pet + Ped + Stems	7	20.9	15.1	316	43.8	29.0	1.039
Roots	5	3.80	22.8	87	46.5	20.4	1.480
Roots	7	8.62	20.1	173	46.4	23.1	1.431

[a] Dry weight is per two cowpea plants incorporated per pot.

mineralogical characteristics to soils typically sown to millet in Niger, Mali and Senegal (Payne et al., 1991), where cowpea has particular potential as a green manure.

Soil water content was maintained between 50 and 75% of water holding capacity ($0.24\,kg\,kg^{-1}$) by daily weighing of pots and adding distilled water. The mean soil temperature during cowpea decomposition was 28 °C, with a mean daily maximum of 37 °C and a mean daily minimum of 20 °C.

Measurement of nitrogen mineralization

Two weeks after cowpea incorporation two sorghum [*Sorghum bicolor* (L.) Moench, var. 'Sureno'] plants were planted per pot to act as a sink for mineralized N. Mineral N in the soil and N in shoots and roots of sorghum (if present) were determined 13, 26, 48, and 70 days after cowpea incorporation. The content of each pot was passed through a 2 mm sieve to facilitate removal of sorghum roots and undecomposed cowpea pieces. Mineral N was extracted from moist soil with 2 *M* KCl on the same day as plant harvest and determined by steam distillation after reduction of nitrate by addition of Devarda's Alloy. Sorghum roots were cleaned of adhering soil and from undecomposed cowpea pieces by flotation in distilled water and hand separation. Sorghum shoots and roots were analyzed for total N including nitrate (Nelson and Sommers, 1973).

Nitrogen mineralized from cowpea plant parts (N_t) was estimated by the isotope dilution method using the following equation (Vose and Victoria, 1986):

$$N_t = (\text{at\% }^{15}\text{N excess of mineral N in soil/at\% }^{15}\text{N excess in cowpea}) \times (\text{mineral N in soil}) + (\text{at\% }^{15}\text{N excess of N in sorghum/at\% }^{15}\text{N excess in cowpea}) \times (\text{N in sorghum})\,.$$

Statistical analysis

Differences in net N mineralization among plant parts and growth stages were evaluated using the GLM procedure of SAS (SAS Institute, Inc., 1990). The fraction of cowpea N released was described by exponential decay models of the form, $N_t = N_0(1 - e^{-kt})$, where N_t is net cowpea N mineralized at time t, N_0 is the fraction of cowpea N that is potentially mineralized, and k is the fractional N mineralization rate. Models were fitted using the NLIN procedure of SAS.

Results and discussion

Nitrogen accumulation

Nitrogen accumulation in 5- and 7-week old cowpea is given in Table 1. The amount of N accumulated in 7-week old cowpeas was more than double (213%) that in 5-week old cowpeas, not including pods that had formed on the 7-week old plants. Seven-week old cowpea leaves, stems, and roots contained 68, 302, and 100%, respectively, more N than 5-week old cowpea plant parts. Relative and absolute increases in N accumulation were greatest for stems, mainly because of stem elongation from approximately 0.3 to 1.0 m and due to formation of peduncles during the two additional weeks of growth. Similarly, Awonaike et al. (1991) reported that N accumulation of field-grown cowpea was greater during the early pod-fill stage than during the vegetative stage. Nitrogen concentration of 7-week old cowpeas was greater for leaves and stems, but lower for roots compared to 5-week old plants (Table 1).

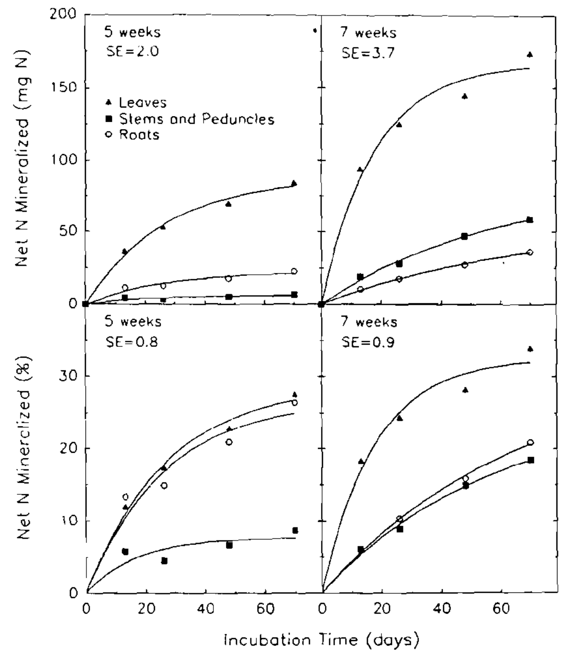

Fig. 1. Amount and percentage of net N mineralized from cowpea plant parts harvested at onset of flowering (5 weeks) and at mid pod-fill (7 weeks) decomposing in sandy soil. Symbols are measured data points, lines represent non-linear regression equations. Standard errors (SE) are for individual data points (n = 3).

Nitrogen mineralization

Sorghum growth and N uptake, as well as mineral N content in the soil were closely related to the amount of N released from cowpea plant parts incorporated into the soil. Sorghum plants in soil without cowpea green manure showed poor growth and acute N deficiency symptoms (data not shown). Nitrogen released from cowpea plant parts was present as mineral N in the soil 13 days after incorporation. At 26 days after incorporation (12 days after planting of sorghum), 13 to 26% of the released N was present in sorghum shoots and roots, with the remaining N being present as mineral soil N. After this date, essentially all N released from cowpea was present in sorghum plants.

The amount of N mineralized from 7-week old cowpea plants after a period of 10 weeks was more than double (235%) that mineralized from 5-week old cowpea plants (Fig. 1). The relative increase in the amount of N mineralized was most pronounced for stems combined with peduncles, for which N mineralization increased by a factor of 8.5. The absolute amount of N mineralized, however, increased most for leaves.

The greatest amount of N was released from leaves, which amounted to 74% of the total N release for a 5-week old plant and 65% for a 7-week old plant. The total above ground biomass contributed 80 and 87% of N released from 5- and 7-week old plants, respectively. This indicated that the contribution of N to the following crop from decomposing cowpea roots will be approximately one fifth of the whole cowpea plant used as green manure. In a field study, when above ground material was removed, cowpea roots increased soil mineral N content but not the yield of the following upland rice crop (John et al., 1992). When cowpea tops were used as green manure, however, rice yield was increased.

The percentage of N mineralized after a period of 10 weeks was 24% for 5-week old cowpea plants, and 27% for 7-week old plants. (The difference was significant at $p = 0.058$). When 5-week old cowpea parts were incorpo-

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rated, relative release of N from leaves and roots were similar and greater than for stems combined with peduncles (Fig. 1). For 7-week old cowpeas the percentage of N mineralized from stems combined with peduncles and that from roots were similar at all sampling dates, whereas the percentage of N mineralized from leaves was larger, and reached 34% at the end of 10 weeks. Frankenberger and Abdelmagid (1985) found 36 and 47% net N mineralized from mature cowpea leaves and roots, respectively, at 20 weeks, while incorporation of stems resulted in net N immobilization. Nitrogen mineralization from 7-week old cowpea leaves in our study was very similar to that found for leaves of mature cowpeas (Frankenberger and Abdelmagid, 1985). For mature cowpea roots, however, a greater fraction of N was mineralized than for younger cowpea roots in our study. Similar or even greater net N mineralization of mature cowpea plant parts compared to young cowpeas was not expected. The higher values found for mature cowpea by Frankenberger and Abdelmagid (1985) may have been a result of using a different cowpea variety or different growth conditions (leaves and roots of mature cowpeas had lower C/N ratios than leaves and roots of young cowpeas used in our study), the removal of mineralized N by frequent leaching during incubation making N reimmobilization unlikely, or the fact that there was no crop growing during decomposition. Janzen and Radder (1989) reported lower net N mineralization of residues while a crop was growing and attributed this, in part, to enhanced N immobilization in the rhizosphere. Stems of mature cowpeas showed net N immobilization (Frankenberger and Abdelmagid, 1985), while in our study net N mineralization of 9 and 18% was observed for stems of 5- and 7-week old cowpeas, respectively, at 10 weeks. In support of these observations, Munoz et al. (1983) observed that in vitro dry matter digestibility of soybean stems decreased substantially beginning with pod development, while digestibility of soybean leaves remained approximately constant.

Net N mineralization from decomposing cowpea plant parts was described by first order decay models (Fig. 1 and Table 2). The models predict that 25% of the N in 5-week old cowpeas will potentially be released and 30% of the N in 7-week old cowpeas, when potential N release of each plant part was added. Most plant parts had almost reached their potential N release after an incubation period of 10 weeks. However, N mineralization from 7-week old stems and roots was still increasing at the end of the incubation period and models predicted greater potential N mineralization than that observed at 10 weeks. In laboratory studies conducted at near-optimum temperature and moisture conditions, between 70 and 80% of the total amount of potentially mineralizable N was mineralized within 11 weeks after soil incorporation (Frankenberger and Abdelmagid, 1985; Fu et al., 1987). Due to this finding and due to the first order decay nature typically observed for N mineralization, Vigil and Kissel (1991) assumed that the amount of N mineralized after 11 weeks approximates the amount of N mineralized in one growing season under field conditions. When N mineralization after 11 weeks is predicted using the derived regression equations (Table 2), 56 and 132 mg N would be released per cowpea plant for 5- and 7-week old plants, respectively, in one growing season. When above-ground plant material is used as fodder, 5- and 7-week old cowpea roots would release 11 and 19 mg N per plant, respectively, in one growing season.

The growth stage of cowpea, i.e., onset of flowering (5 weeks) versus mid pod-fill (7 weeks), significantly ($p < 0.05$) affected the rate of N mineralization of all plant parts at all retrieval dates, except for stems at the first retrieval. The percentage of N that was mineralized from leaves and from stems at 10 weeks

Table 2. Parameters of first-order regression equations of the form $N_t = N_0(1 - \exp(-kt))$, describing net N mineralization from cowpea plant parts at two growth stages estimated by the isotope dilution method

Plant part	Plant age (weeks)	N_0 (%)	k (day^{-1})	RMSE (%)
Leaves	5	28.9	0.0368	1.03
Leaves	7	32.6	0.0564	1.91
Stems	5	7.82	0.0591	1.52
Stems	7	25.7	0.0179	0.587
Roots	5	26.9	0.0378	2.36
Roots	7	31.2	0.0154	0.401

was greater for the more mature plants, but lower for roots. The difference in N mineralization between the two growth stages was related to the N tissue concentration of the plant parts. The N concentration of 7-week old plants was higher than of 5-week old plants in leaves and stems, but lower in roots.

Effect of N concentration and C/N ratio

The percentage of N mineralized at 10 weeks was linearly related to the tissue N concentration of the plant parts and to their C/N ratio (Fig. 2). The relationship with C/N ratio was similar to that found by Vigil and Kissel (1991) who emphasized that these relationships allow for a quick and simple way of arriving at a quantitative estimate of the amount of N that will mineralize from soil incorporated crop residues. In our study, the critical N concentration and C/N ratio at which neither net mineralization nor immobilization occurs after 10 weeks, numerically determined from the derived equations, were a N concentration of 8.1 g N kg^{-1} residue and a C/N ratio of 39.4. These values were again similar to those found by Vigil and Kissel (1991). From data reported by Frankenberger and Abdelmagid (1985) critical N concentrations as low as 8.0 g kg^{-1} can be calculated. Lower values for critical C/N ratios (Iritani and Arnold, 1960) can be explained by

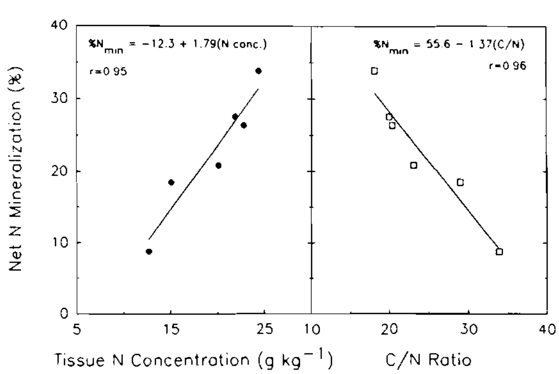

Fig. 2. Relationships between percentage of N mineralized from decomposing cowpea plant parts after 10 weeks and their tissue N concentration and C/N ratio. Symbols are measured data points, lines represent linear regression equations.

the shorter duration of the studies. Janzen and Kucey (1988) showed a decrease in critical residue N concentration for net N mineralization to occur with increasing incubation time. These relationships between N mineralization and N tissue concentration or C/N ratio of the plant material may have to be extended to include other plant quality descriptors, when the range of species is extended. Tropical legumes, for example, can contain considerable amounts of polyphenolics, which reduce the rate of N mineralization (Palm and Sanchez, 1991).

Comparison of analytical methods

Nitrogen mineralization from cowpea plant parts was also estimated by subtracting the sum of mineral N in soil and N in sorghum shoots and roots in the control treatment from those observed in the green manure treatments. Estimates of N mineralization by the difference method were consistently higher compared to estimates by the ^{15}N dilution method. Averaged over plant parts and growth stages, N mineralization estimated by the isotope dilution method was 68, 77, 93, and 89% of that estimated by the difference method, after 13, 26, 48 and 70 days of incubation, respectively. The difference was significant ($p < 0.05$) in 11 out of the 24 plant part × growth stage × incubation time combinations. The absolute difference in the estimates for N mineralization by the two methods, however, was small, ranging from 2.7 to 14.9 mg N, and averaged 6.8 mg N per 3 kg soil.

The discrepancy in the results obtained with the two methods has generally been attributed to microbial immobilization-mineralization turnover (Jansson and Persson, 1982), also referred to as pool substitution (Jenkinson et al., 1985), to an increased rate of N mineralization of soil organic matter in amended soil compared to in unamended soil, i.e., positive priming (Hallam and Bartholomew, 1953), and to greater uptake of soil N by a more extensive root system in amended soil compared to unamended soil (Jansson and Persson, 1982). These mechanisms were not likely to be significant under the experimental conditions of our study, because of very low soil mineral N concentration and very low soil organic matter content in the control

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treatment, and because of confinement of roots in pots. Non-uniform labeling of cowpea plants due to the formation of some N_2-fixing nodules on cowpea roots was observed with plant parts formed later having lower ^{15}N enrichment. These younger plant parts had significantly greater tissue N concentrations than plant parts formed earlier and were therefore expected to release a greater portion of their N and at a faster rate. Greater release of N from less ^{15}N enriched plant parts would lead to underestimation of N mineralization. From our experience, it is very difficult to grow legumes that are commonly grown in the area, in a greenhouse without the formation of some nodules, even when pots and growth medium are autoclaved. Plant parts are usually not analyzed separately for their ^{15}N content in order to evaluate the uniformity of the label. This mechanism may be important in many studies on N mineralization from ^{15}N labeled legumes.

Conclusions

Net N mineralization from cowpeas grown to mid pod-fill was more than double that for cowpeas grown to onset of flowering during decomposition in a sandy soil. Greater N mineralization from more mature cowpeas was a result of more N available for mineralization (213%) and a higher percentage of N mineralized (27 vs. 24%) of 7-week old cowpeas compared to 5-week old cowpeas. Due to the greater N accumulation and high rate of N mineralization it is of advantage to grow green manure cowpeas until mid pod-fill rather than to the onset of flowering. The benefit of allowing a green manure to grow for additional N accumulation must be measured against resources that may be limiting, such as soil moisture, time before planting of the main crop, and time available for N release and crop N uptake.

Nitrogen mineralization rates of cowpea leaves, stems, and roots were linearly related to their N concentration and C/N ratio, indicating net N mineralization for plant parts with N concentrations above 8 mg kg^{-1} or a C/N ratio less than 39 after 10 weeks.

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