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Screening legume green manures as nitrogen sources to succeeding non-legume crops

III. The buried bag method

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Abstract

A buried bag incubation technique was proposed to monitor N release from soil and decomposing green manure. The technique would facilitate not only the screening of legumes as sources of N but also measurement of the N supplying capacity of soils. Several tropical legumes were incorporated into field plots followed either by maize (*Zea mays* L.) or by bare fallow. Soil samples from the plow layer containing the incorporated green manure were placed in low density polyethylene bags and buried within the plow layer under the maize crop for *in situ* incubation. Periodic withdrawal of the bags was accompanied by fallow soil profile sampling. Above ground N accumulation by maize was equally well correlated to N release measured by either method although the bag technique required much less labor. Supplemental experiments suggested that N accumulation in the bags was reduced due to inadequate O_2 diffusion but only when O_2 demand was high and soil water potential was high. The results show that *in situ* bag incubation alone or together with fallow soil sampling can be used to estimate the N supplying potential of soil and leguminous residues.

Introduction

The use of leguminous green manures can reduce the consumption of fossil fuel intensive N fertilizer and/or increase crop yields where N fertilizer is unavailable. A method to screen legumes for their ability to supply N to succeeding crops is needed. Ideally, the method would also have application in estimating the N supplying capacity of a soil. To this end, the method should simulate the conditions which act on the decomposition of, and N release from soil and green manure. Crop response studies in the field are the traditional choice of method but are resource intensive. A fallow soil method (Bowen *et al.*, 1988) was proposed to overcome some of the expense and space constraints of crop response studies. In a test of the method, the accumulation of NO_3^- in bare fallow soil to 120 cm depth was highly correlated to grain dry matter and aboveground N content of maize in identically treated cropped plots. However, the fallow soil method still involved substantial labor. In addition leaching of NO_3^- limited somewhat the conditions and duration for which the method might be utilized because of loss of nitrate beyond the maximum feasible sampling depth.

Buried plastic bags which prevent NO_3^- leaching have been used to monitor mineralization of soil N in agricultural soils (Campbell *et al.*, 1974, Van Schreven, 1968; Smith *et al.*, 1977; Wester-

mann and Crothers, 1980). Low density polyethylene films prevent moisture loss while allowing substantial gas exchange (Bartlett, 1965; Eno, 1960). Westermann and Crothers (1980) accurately estimated N content of aboveground maize by subtracting cropped soil NO_3^- from bag NO_3^- at harvest. An added benefit of field incubation in bags is that NO_3^- absorption by roots is prevented. This allows incubation of the soil and green manure under a crop rather than in bare fallow soil where different temperature conditions may result in different N mineralization rates.

The objective of the present work was to test the buried bag method as a means of screening leguminous green manures as sources of N for subsequent maize crops with comparison to the fallow soil method. Two additional experiments were conducted to resolve the question of whether net N mineralization in the bags was reduced due to their effect on soil aeration.

Materials and methods

Field experiments were conducted within the Savanna Research Center (CPAC-EMPRAPA; 15°35′S and 47°43′W, alt. 980 m) near Brasilia, Brazil. The average annual precipitation is about 1500 mm with 95% occurrng from October to April. The mean monthly temperatures average 22°C during the rainy and 20°C during the dry season. The soil is a Typic Haplustox which received high inputs of lime and P since clearing the savanna vegetation in 1970. Some chemical and physical properties of this soil are given in Table 1.

Low density polyethylene bags of 0.1 mm thickness were used for all studies. The bag dimensions were 8 by 60 cm which allowed burial vertically in holes left by a Dutch auger. For inorganic N determination a 15 g subsample was taken for extraction with 50 mL of 1 *M* KCl. Inorganic N in the extract was determined using the steam distillation method (Bremner, 1965; Keeney and Nelson, 1982).

Principal field experiment

Seven legumes (*Canavalia brasiliensis*, *Cajanus cajan*, *Canavalia ensiformis*, *Calopogonium mucunoides*, *Crotalaria striata*, *Mucuna aterrima*, and *Pueraria phaseoloides*) and maize (*Zea mays* L.) were grown during the rainy season from December, 1986 to April, 1987. At harvest, the legumes were ground to pass a 2-cm sieve, weighed, and spread evenly on the plots of origin. Two additional treatments using mucuna (*Mucuna aterrima*) consisted of 1) chopped maize stover applied with the chopped mucuna and 2) unchopped mucuna. The maize plots were cleared of residue to be followed by no amendment or two N fertilizer rates. Total-N applied as green manure and fertilizer is given in Table 2. The same treatments except for the fertilizer rates were applied to small plots which were maintained bare by frequent hoeing. A rototiller was used to incorporate the residues to 20 cm depth about four days after harvest.

Hybrid maize ('Cargill 111s') was planted 13 days after incorporation (DAI) and grown during the dry season with irrigation. After harvesting the first crop and plowing to 25 cm a second maize crop was planted (218 DAI) and grown during the rainy season.

The bare fallow plots were intensively sampled throughout the experiment to depths determined by the depth of nitrate leaching. Three samples of each 15 or 30 cm depth increment were mixed for inorganic-N determination. The results were

Table 1. Chemical and physical properties of Typic Haplustox at experimental site

Depth (cm)	Soil pH	Extractable cations		Om content ($g\,kg^{-1}$)	Bulk density ($Mg\,m^{-3}$)
		Al ($cmol\,kg^{-1}$)	Ca + Mg		
0–20	5.0	0.04	4.3	25.6	1.21
20–40	4.8	0.22	2.8	20.5	1.23
80–100	4.8	0.02	1.4	11.4	1.08
160–180	4.9	0.00	0.4	8.1	1.08

Table 2. Large plot dry matter (kg ha^{-1}) and nitrogen content (kg ha^{-1}) of legumes and maize

Treatment	Dry matter	Total N	C–N
Canavalia brasiliensis	10,030	228	17.4
Cajunus cajan	10,410	229	19.9
Canavalia ensiformis	8,940	231	15.0
Calopogonium mucunoides	7,320	142	18.8
Crotalaria striata	13,730	306	19.0
Mucuna aterrima	6,770	152	18.3
Mucuna unchopped	6,510	146	18.3
Mucuna + maize stover	11,120	188	24.1
Pueraria phaseoloides	6,520	116	19.4
Control	0	0	---
100 kg N ha^{-1} [a]	0	100[a]	---
200 kg N ha^{-1} [a]	0	200[a]	---

[a] Rainy season rates were 80 and 160 kg N ha^{-1}. Inorganic N applied as ammonium sulfate – 20 and 80% at 21 and 45 days after planting, respectively.

corrected for soil moisture and bulk density for expression as kg ha^{-1}.

Samples of soil were collected from the cropped plots shortly after emergence of the maize (42 and 237 DAI for the dry and rainy season crops respectively). After subsampling for inorganic N determination, about 350 g of soil were placed in 0.1 mm thick, low density polyethylene bags. The bags were filled to about one fourth of their 60 cm length maintaining the bulk density approximately that of the intact field soil (Table 1). The bags were closed by constricting with a twist tie immediately above the soil sample and were buried in the original plots vertically between 5 and 20 cm depth. The rest of the bag extended above the soil surface with the open end folded twice and stapled. Bags were withdrawn periodically for determination of inorganic N.

An estimate of available N consisted of the accumulation of inorganic N in the fallow soil to 180 cm depth. Another estimate of N available to the growing maize was made by summing the accumulation of inorganic N in the bags with the accumulation in fallow soil during the period before the bags were in place. In both cases, all of the N applied as fertilizer was added to available N in control soil to estimate N available in fertilized plots. The coefficients of determination of maize N regressed on soil N were determined using the REG procedure of SAS (SAS, 1985).

The rates of N release were computed from the regression equations (PROC REG – SAS, 1985) for bagged and fallow soil during the periods from 42 to 140 and from 237 to 321 DAI. Comparison of the absolute rates of N release in bags vs. fallow soil was limited to the dry season incubation period because of loss of N below 180 cm in fallow plots during the rainy season. Only the net release of N from green manure was considered because the fallow soil profile was sampled to increasing depths resulting in increasing contribution of N from the subsoil organic matter (Table 1).

Supplemental field experiment

Soil was collected from the field and air-dried to slightly below the permanent wilting point. To this soil was applied either $(NH_4)_2SO_4$ to supply 100 mg N kg^{-1} dry soil, dried mucuna ground to pass a 0.5-mm sieve supplying 200 mg N kg^{-1} dry soil, or no amendment. The soils were wetted and subsampled immediately before bagging. The bags were filled to about one fourth of their length with approximatley 350 g of treated soil. The bags were closed by heat-sealing or by constricting with a twist tie immediately above the soil sample leaving no headspace. The heat-sealed bags were cut immediately above the seal and buried vertically in the soil between 5 and 20 cm depth. The twist-tied bags were knotted near the top of the bag and buried as above except that the top 40-cm extended above the soil surface. Bags were positioned in a randomized block design and withdrawn periodically over 45 days for inorganic N determination.

Supplemental laboratory experiment

Soil from the field experimental area was collected, passed through a 4-mm sieve, and maintained field moist in cold storage until use. Half of the soil was mixed with sufficient dried, ground mucuna to supply 200 mg N kg^{-1} soil. Control soil and soil plus mucuna were saturated overnight and then placed in pressure chambers for membrane desorption to −0.01 MPa. The soils were incubated for about 150 days at 30°C in 0.1 mm thick low density polyethylene bags as in the previous studies and in jars with perfor-

ated caps. The bags were filled to about one fourth of their length and laid on the incubator shelf with the open end folded under the filled part. Soil in bags was sampled periodically accompanied by less frequent sampling of soil in jars.

Results

Principal field experiment

Inorganic N content of the soil profile to 120 cm at the time of treatment incorporation was similar irrespective of previous crop (Fig. 1). In fallow soil amended with green manure, N accumulation was generally best fit to an asymptotic function of time with high initial rates declining to zero. Nitrogen accumulation in unamended fallow soil and bag incubated soil (unamended and amended) was linear.

Nitrogen availability

Nitrogen accumulated in aboveground maize was significantly correlated to soil N availability as estimated by the fallow soil ($r^2 = 0.90$) and buried bag ($r^2 = 0.92$) methods over two crops after green manure incorporation (Fig. 2). There was considerable scatter in the relationship between maize N and fallow soil N in the rainy season when loss of NO_3^- was likely and variability in the total profile NO_3^- content was high. The bag method estimates for the rainy season were better correlated to maize N although the range of N released from the residual green manures was limited.

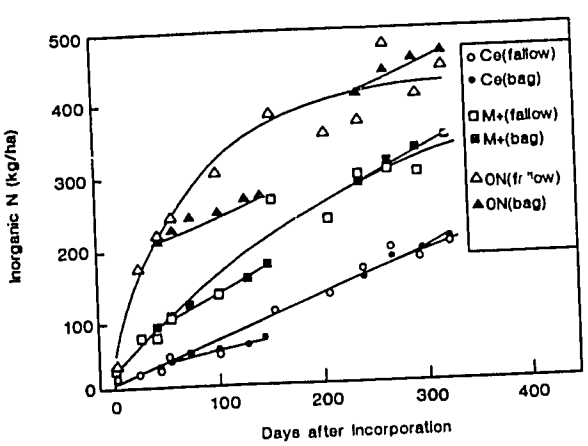

Fig. 1. Accumulation of inorganic N in bare fallow plots to a maximum sampling depth of 180 cm and in low density polyethylene bags buried in soil cropped to maize after incorporation of *Canavalia ensiformis* (Ce), *Mucuna aterrima* plus maize stover (M+), or no amendment (ON). The data for seven additional treatments fall between ON and Ce.

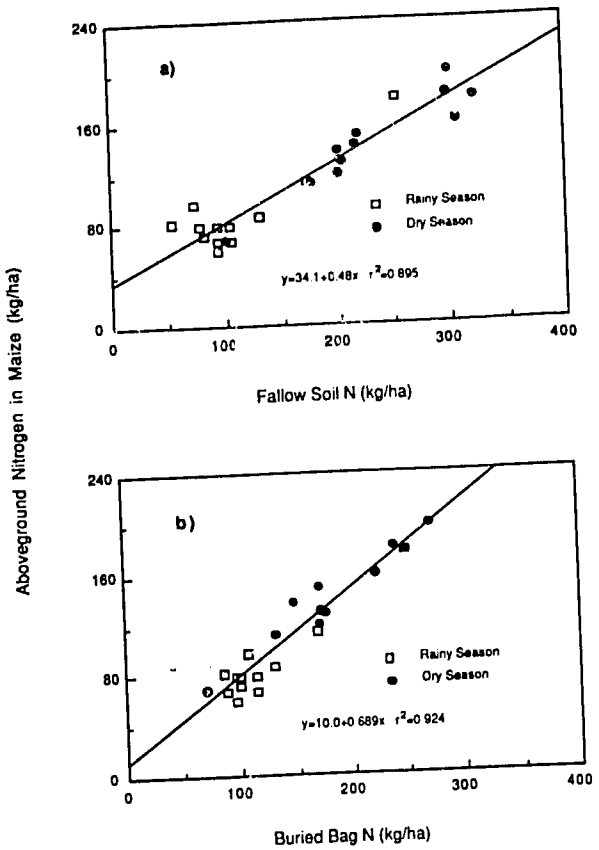

Fig. 2. Relationship between N content of aboveground maize and N availability estimated by a) fallow soil and b) buried bag methods. The fallow soil estimate is the sum of the residual profile inorganic N at planting and the accumulation of N in the bare fallow profile until harvest. The bag estimate is the sum of the accumulation of inorganic N in bags and the accumulation in fallow soil while the bags were not in place.

The buried bag incubation method involved much less labor for sampling and for inorganic N determination. At the time of bag incubation unit establishment, additional operations beside sampling and N determination were required (*i.e.* bagging soil and bag burial). This was compensated by ease of sampling thereafter.

In spite of the reduction of N accumulation observed in the bags during the dry season incu-

bation, the correlation between aboveground maize N and available N estimated by the bag method is highly significant. The regression equation shows that 69% of the N estimated to be available was found in the aboveground maize (Fig. 2). Fertilizer N recovery in the above ground maize over two crops was 66%. Taking recovery into account, all of the N estimated to be available by the bag method was found in the aboveground maize. The fallow soil method overestimated slightly the N available to the crop but only at high levels of fallow soil N. This implies either 1) that more N was released in the bare fallow than in the cropped soil or 2) that more N was lost in the cropped than in the bare fallow soil. It is probable that excess N accumulated in bare fallow soil due to higher soil temperatures than under the crop. In addition, more optimal soil moisture conditions prevailed in the fallow plots because there was much less evapotranspiration than in the cropped soil. It might be suspected that some NO_3^- may have leached beyond the rooting depth of the maize. However, excavation during the grain filling period revealed roots to 180 cm at a time when there had been little apparent NO_3^- leaching loss below that depth in the fallow plots. Since there was less potential for leaching in the cropped plots because of higher evapotranspiration, leaching of NO_3^- beyond the reach of maize roots was unlikely. Furthermore, cropped profile sampling at harvest revealed very little inorganic N. It appears that the fallow soil method slightly overestimated N available to maize because more N was mineralized in bare fallow plots.

Nitrogen release in bags vs. fallow

Inorganic N accumulated in bags at a lower rate than in fallow plots during the dry season incubation period and at a higher rate during the rainy season (Fig. 1). Rates in fallow soil during the rainy season are apparently lower because of loss of NO_3^- below the sampling depth of 180 cm. During the dry season incubation period, a fair comparison of the rates can be made.

There were several possible factors which may have resulted in the differences observed in N accumulation in fallow and bagged soil. Soil temperature was certainly a factor since bags were buried in soil cropped to maize where the soil temperature was lower. The mean of the daily maximum and minimum soil temperature at 10 cm depth was 2.5°C lower under the crop than under bare fallow over the dry season incubation period. This would explain a constant 25% increase across treatments ($Q_1O = 2$, Stanford *et al.*, 197[illegible]) in N accumulation in the bare fallow soil. However, the degree to which the net rates (control subtracted) of N accumulation in fallow soil exceeded those in bag-incubated soil was highly variable, ranging from 5 to 175%. The magnitude of the difference appeared to be most related to the magnitude of the rates. As the rate of inorganic N accumulation increased, the rate of accumulation in bags fell increasingly behind the fallow soil rate. This suggested that O_2 diffusion may have been the factor limiting microbial activity in the bags. It appears that the rate of N accumulation in bags decreased in relation to that in fallow soil as the rate of N release from soil and green manure exceeded 1 kg ha^{-1} day^{-1} (Fig. 3).

It is doubtful whether differences in soil moisture between bare fallow soil and soil incubated in buried bags can explain an important part of the difference observed in N accumulation. While the soil moisture in the bags was maintained constant at −0.02 to −0.03 MPa potential, the fallow plots were irrigated frequently so that soil water potential fluctuated on a weekly basis from saturation to about −0.05 MPa. Any

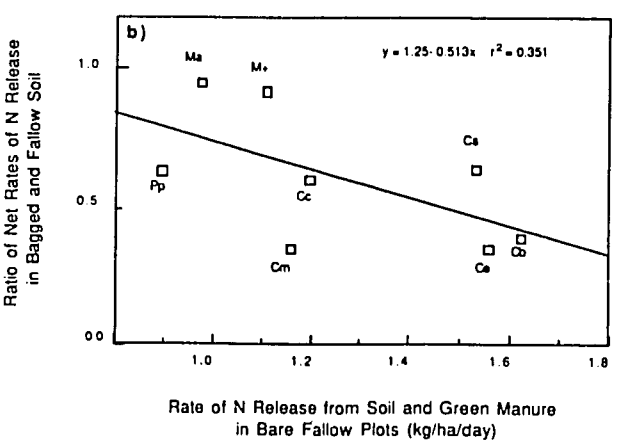

Fig. 3. Ratio of net rates of N release from green manure in plastic bags and fallow soil regressed on rates of N release from green manure and soil. All rates measured from 42 to 140 DAI.

differences due to soil moisture would have a constant effect across green manure treatments. A variable effect may occur if some leguminous residues released compounds inhibitory to further mineralization. These would be retained in the bags but not in fallow soil. No evidence of inhibitory substances is available.

Supplemental field experiment

The data in Figure 4 show that 100 mg NH_4^+-N kg^{-1} soil incubated in bags was completely transformed to NO_3^- in three weeks whether the bags were closed by heat seal or twist tie. On the other hand, when 200 mg mucuna-N kg^{-1} soil was incubated, differences were observed between the sealed and tied bags (Fig. 5). First, there was a major loss of NO_3^- in the sealed bags during the first week of incubation. Second, while NH_4^+-N was completely nitrified by four weeks in the tied bags, almost half of the inorganic N was still in NH_4^+ form in the sealed bags at six weeks. Although the differences were not statistically significant and the mucuna dry matter was added at the rate of 17 Mg ha^{-1} which is higher than would normally occur in the field, there was sufficient evidence to avoid the use of sealed bags in further work.

Supplemental laboratory experiment

Control soil and soil plus mucuna supplying

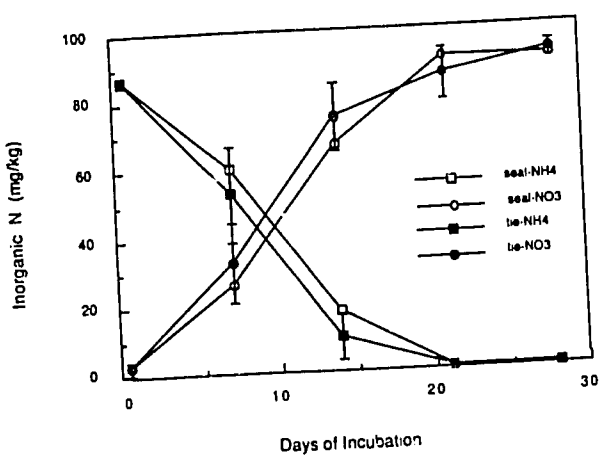

Fig. 4. Nitrification of 100 mg NH_4^+-N kg^{-1} soil added as $(NH_4)_2SO_4$ and incubated in the field in buried plastic bags closed by heat seal or twist tie.

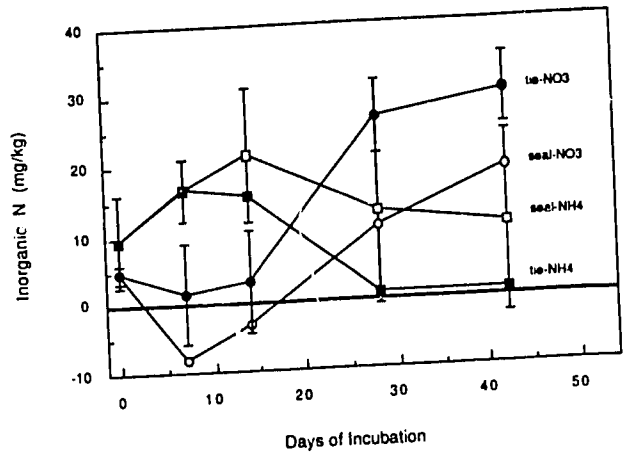

Fig. 5. Mineralization of N from 200 mg N kg^{-1} soil as mucuna incubated in field buried plastic bags closed by heat seal or twist tie. *Data points* are means of four replications with standard errors shown for NO_3^- means in tied bags and NH_4^+ means in sealed bags.

200 mg N kg^{-1} soil were incubated in bags and in jars with perforated caps as a control on aeration. While N release from unamended soil was identical whether in bags or jars, release of N from soil plus mucuna was significantly greater in jars than in bags during the first 28 days of incubation when the N accumulation rate was approximately 5 kg ha^{-1} day^{-1} (Fig. 6). During the next 80 days when the rate was only about 1 kg ha^{-1} day^{-1}, there was very little difference

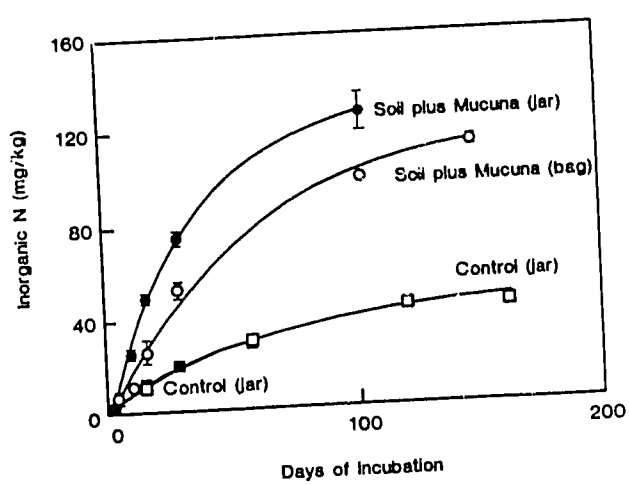

Fig. 6. Release of N from control soil and soil plus mucuna supplying 200 mg N kg^{-1} soil incubated at 30°C and −0.01 MPa water potential in glass jars with perforated caps and in plastic bags.

between accumulation of nitrate in the bags and the jars. The soil plus mucuna treatment was repeated at lower water potentials (−0.02, −0.03, −0.04, and −0.1 MPa). At −0.02 MPa, N accumulation was only slightly greater in jars than in bags. At −0.03 and −0.04 MPa, N accumulation in jars and bags was identical. Finally, at −0.1 MPa, soil plus mucuna released more N in bags than in jars. Evidently, moisture plays a role in the performance of the bags. This follows since moisture films also are relatively impermeable to gases. Again, the amount of mucuna dry matter was higher than would occur in most field situations (17 Mg ha^{-1}).

Discussion

In the present work, the fallow soil method (Bowen *et al.*, 1988) was again shown to be appropriate for screening leguminous green manures as sources of N for non-legume crops. A modification involving incubation of soil from the plow layer containing the incorporated green manures in low density polyethylene bags was an equally good predictor of N availability to maize.

The first buried bag incubation units were established 42 DAI. Sampling of cropped plots was adequate to monitor release of N until the maize emerged and roots developed sufficiently to influence soil N levels. Indeed, separate fallow plots were not needed because the cropped plots could be sampled for a short period. This is convenient because bag burial may not be possible immediately after green manure incorporation for several reasons. There may be additional tillage operations or mechanized planting which might damage the incubation units. Many green manure materials may be difficult to sample adequately until they are at least partially decomposed. Undecomposed plant materials may demand high rates of O_2 diffusion which the bags may not allow. By the time bag incubation unit establishment becomes possible, substantial inorganic N will have mineralized and leached beyond the plow layer in the presence of adequate irrigation or rain water as was the case in this study. Therefore, sampling of the soil profile was necessary to estimate the total N available to the crop.

As the season progresses, sampling the cropped profile becomes inappropriate because of crop uptake. Bare fallow profile sampling would become inadequate as NO_3^- is leached to depths where labor requirements and the chance of N loss are high. In the present study and in the work of Bowen (1987), the fallow soil estimate of N released from mineralizing residual green manure during the second maize crop was inaccurate. This is because the amounts of N released were low, there were losses below the sampling depth, and the data for the whole profile inorganic N content were variable.

Bag incubation allows estimation of N release from unamended soil or soil with small amounts of residues. To facilitate sampling and mixing after large amounts of residues are applied, some decomposition prior to the first sampling for N mineralization and bagging should occur. Sampling, however, must be done before major NO_3^- losses from the soil profile can occur. Buried bag incubation can be used during high rainfall periods without the occurrence of leaching losses. The bags can be buried under a growing crop where temperature conditions are more appropriate than in bare fallow plots to estimate N mineralization.

There have been few reports of restricted aeration from workers using low density polyethylene bags but also little work with treatments which demanded high O_2 diffusion. Bremner and Douglas (1971) found all plastic films including polyethylene to be too impermeable to gases for incubation work. However, their tests were conducted using small plastic surface areas. The results of the principal field experiment indicate that the use of low density polyethylene bags 0.1 mm thick should be restricted to cases in which N release does not exceed 1 kg ha^{-1} day^{-1}. Results from the supplemental experiments did not contradict this threshold. High rates of N release occur when labile soil N or organic amendment N are high, especially soon after organic additions. Incubation in 0.05 mm thick bags during the first incubation period of the principal field experiment did not result in substantially different N accumulation. The bag thicknesses employed in this work are in the upper part of the range of bag thickness commonly used (Gordon, 1988). It is possible that

reducing the bag thickness would result in increasing mineralization rates. In one of few studies with variable bag thickness, Gordon *et al.* (1987) observed no differences in N mineralization from forest floor material in bags whose thickness ranged from 0.015 to 0.032 mm. Further research should be conducted in which soil with variable green manure additions is incubated in bags of widely varying thickness.

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