PN-ABL-437
ISN 71428

Plant and Soil 111, 81-85 (1988)
© Kluwer Academic Publishers

PLSO 7576

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

II. Incubation in laboratory

J. O. QUINTANA[1], J. PEREIRA[2], D. R. BOULDIN[1] and D. J. LATHWELL[1]
[1]*Department of Agronomy, Cornell University, Ithaca, NY 14853, USA.* [2]*EMBRAPA/CPAC, C.P. 70.0023, 73.300 Planaltina, DF, Brazil*

Received 3 November 1987. Revised June 1988

Key words: aerobic incubation, fallow plot, inorganic N, N mineralization

Abstract

Legumes managed as green manures provide a good alternative to the use of commercial N-fertilizer for non-legume crop production. A laboratory procedure based on the aerobic incubation (35 °C) of soil samples taken from plots with legumes incorporated was proposed for predicting the N supplying potential of legumes to succeeding non-legume crops. This procedure was evaluated by comparing the amount of inorganic N determined in the soil samples after incubation with N content of aboveground dry matter of maize or with inorganic N found in the soil of an adjacent fallow plot. The soil samples (0.00 to 0.15 m and 0.15 to 0.30 m) were obtained from two field experiments conducted in similar soils of the Cerrado Region of central Brazil during 1984–85 and 1986–87. Although incubation results were affected by the different pretreatments, soil samples prepared and incubated as soon as possible after being taken from the field gave the best correlations. The most convenient incubation procedure was the one-week aerobic incubation of samples previously oven dried at 50 °C. The results obtained by this procedure were significantly correlated with N content in aboveground dry matter of maize and with the inorganic N accumulated in an adjacent fallow plot.

Introduction

A great deal of work has been directed to the development of a reliable soil analysis method to predict the N supplying potential of soils (Boswell et al., 1962; Bremner, 1965; Fox and Piekielek, 1984; Norman et al., 1985). The amount of nitrogen mineralized in 7- to 14-day incubations is generally considered to be a promising method for assessing the N availability of soils (Bremner, 1965; Keeney, 1982). However, their use as a tool to establish the N supplying potential of organic materials added to the soil has been limited to a few studies (Castellanos and Pratt, 1981; Sahrawat, 1980, 1983).

Several factors affect the results of the incubation studies. Nordmeyer and Richter (1985) found that sampling the soil at different times during a crop growing period gave different amounts and courses of mineralization. The chemical composition of organic material added to the soil also affects N mineralization in incubated soil samples (Frankenberger and Abdelmagid, 1985; Nordmeyer and Richter, 1985). Many workers have reported that air-drying, oven-drying, and/or grinding of soil samples lead to significant changes in the ammonium and/or nitrate contents of soils (Bartlett and James, 1980; Beauchamp et al., 1986; Bremner, 1965; Jenkinson, 1966; Keeney and Bremner, 1966). Storage of soil samples for several months before incubation may also result in increases in the inorganic nitrogen content of the samples (Nelson and Bremner, 1972). The correlation between incubation results and N uptake by a non-legume crop may also be affected by the drying treatment received by the soil sample before incubation. The

objectives of the present study were to assess the value of the incubation method as a means to screen different legume green manures as sources of nitrogen for maize, and to determine the effect of various pretreatments of the samples on the incubation results.

Materials and methods

Origin and preparation of soil samples

The soil samples (0.00–0.15 m and 0.15–0.30 m) used were obtained from both cropped and fallow plots of two field experiments where various leguminous materials had been incorporated. The experiments were conducted in 1984–85 (Bowen, 1987) and in 1986–87 (Quintana, 1987) on Typic Acrustoxes of the Cerrado Region of Central Brazil. The legumes incorporated into the soil were mucuna [*Mucuna aterrima* (Piper and Tracy) Merr.], and soybeans [*Glycine max* (L.) Merr.] for the 1984–85 experiment, and mucuna, jack beans [*Canavalia ensiformes* (L.) DC.], crotolaria (*crotolaria paulina* Schrank), and zornia (*Zornia latifolia* Sm.) for the 1986–87 experiment. In each of the experiments, plots kept under bare fallow were sampled periodically by increments to either 60 or 120 cm and the samples were analyzed for inorganic N. Soil samples used in the incubation experiments were from the first soil sampling for each experiment.

Soil samples from the experiments of 1984 and 1985 were first air and oven dried at 40 °C and then prepared by separating first all gravel and plant materials from them, followed by grinding and sieving through a 2-mm sieve. The samples were then placed in tightly closed plastic bags and stored for a period of 9 months (samples from 1984), and 5 months (samples from 1985) before incubation. The samples from the experiment of 1986 were air dried only and stored for 3.5 months. Before incubation, the soil samples were ground, sieved, and the sieved plant material was remixed with the soil sample. One part of the 0.00–0.15 m layer samples of the first sampling date of 1986 was prepared by discarding all plant material remaining in the sieve after sieving the soil samples for comparison with the results from the comparable samples in which the sieved plant material was remixed with the soi after preparation.

Incubation procedure

The treatments used included an array of com binations of different soil sample preparation (dis carded and remixed plant material), drying (ai dried, air dried plus oven dried at 50 °C, air drie plus oven dried at 100 °C), and length of incubatio (1 and 2 weeks). The soil samples were prepared fo the incubation by placing a mixture of 10 g dry so and 30 g inert sand into a 150-ml snap cap glas bottle containing 7 ml of soil inoculant. Two rep lications were prepared of each soil sample for eac preparation, drying procedure, and length of in cubation treatments. The soil inoculant wa prepared by mixing field moist soil with water in 1-kg:2-litre proportion and allowing to settle fo about 2 hours. This was done 3 times with the sam soil; with the first 2, the supernatant was discardec while the third time it was decanted and saved fo inoculation. The sand was prepared by firs separating with sieves the particle sizes between 0. and 0.05 mm and then adding 10 litres of a 1: H_2SO_4:H_2O (volume/volume) solution to ap proximately 25 kg of sand and permitting it t stand overnight in order to eliminate $Ca(CO_3)_2$. The sand was then washed with water and air driec An electrical incubator set at 35 °C was used for th incubations.

After incubation, 50 ml of 1 *N* KCl were added t each snap cap bottle. The bottles were shaken b hand 3 times at intervals of 30 minutes befor analysis. Total inorganic nitrogen (NH_4^+-N + NO_3^--N + NO_2^--N) in the 1 *N* KCl extrac was determined by the MgO-Devarda alloy stear distillation method (Bremner, 1965; Keeney an Nelson, 1982).

The coefficients for the linear correlatio between incubation results and N content of maiz or inoganic N accumulation in fallow soil wer determined with the CORR program of SAS (SA 1982).

Results

As shown in Table 1 there was little difference correlation coefficients between samples incubat

Best Available Document

Table 1. Correlation coefficients between results of selected incubation treatments and inorganic N in fallow soil (0.0–0.6), N content in aboveground dry matter of maize

Incubation treatment[a]	Inorganic N in fallow soil		N content			Dry matter
	1984	1986	1984	1985	1986	1987
A 1W 50	0.94**	–	0.68	0.51	0.92*	0.96**
A 1W 100	0.96***	0.87***	0.63	0.66	0.95*	0.72
A 2W 50	0.95**	–	0.64	0.56	0.93*	0.89*
A 2W 100	0.97***	0.85***	0.56	0.60	0.95*	0.94*

[a] A = 0.00–0.15 m; 1W = 1 week; 2W = 2 weeks; 50 = oven dried 50 °C, 100 = oven dried 100 °C.
* Significant at $p = 0.05$.
** Significant at $p = 0.01$.
*** Significant at $p = 0.001$.

for one week compared to two week incubation. Likewise heating to 50° or 100 °C had relatively little effect. Increasing length of storage of samples prior to incubation, however, reduced the correlation between N content of above ground dry matter and the inorganic N released during the incubation period. Samples which were dried and incubated without storage resulted in the highest correlation coefficients.

Inorganic N released from soil samples taken during the first experiment was well correlated with the quantities of inorganic N found in the fallow plots (Figure 1). Mucuna tops or tops plus roots contributed much more N than did soybean. The roots of either crop released very little N. Inorganic N released by incubation was not as highly correlated with N content of the above ground dry

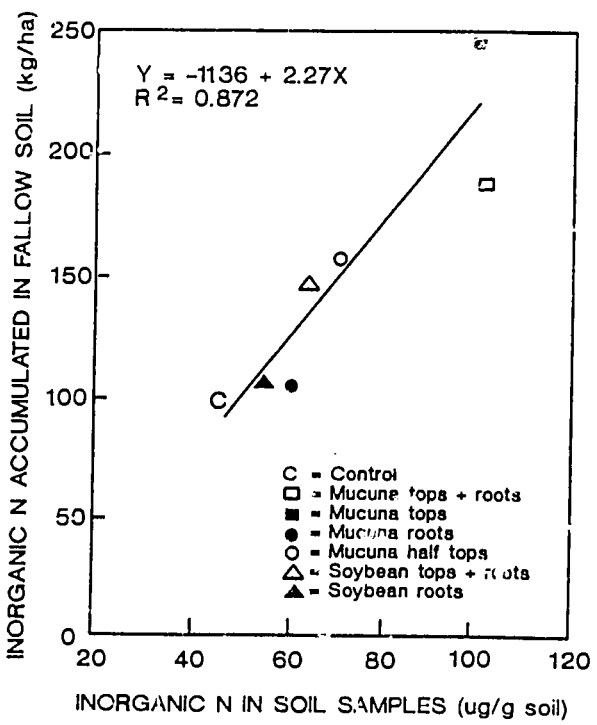

Fig. 1. Relationship between inorganic nitrogen determined in soil samples oven dried at 100 °C and incubated for 2 weeks, and inorganic nitrogen accumulated to 0.6 m in the soil profile of fallow plots 170 days after incorporation of legumes in 1984 (*first experiment*).

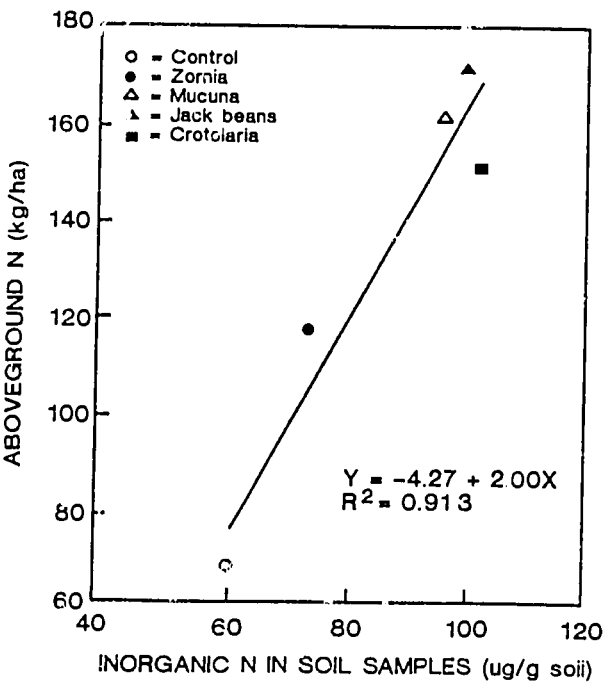

Fig. 2. Relationship between inorganic nitrogen determined in soil samples (0.00–0.15 m) taken 9 days after planting corn, oven dried at 100 °C and incubated for 1 week, and N content in aboveground dry matter of maize (*second experiment*).

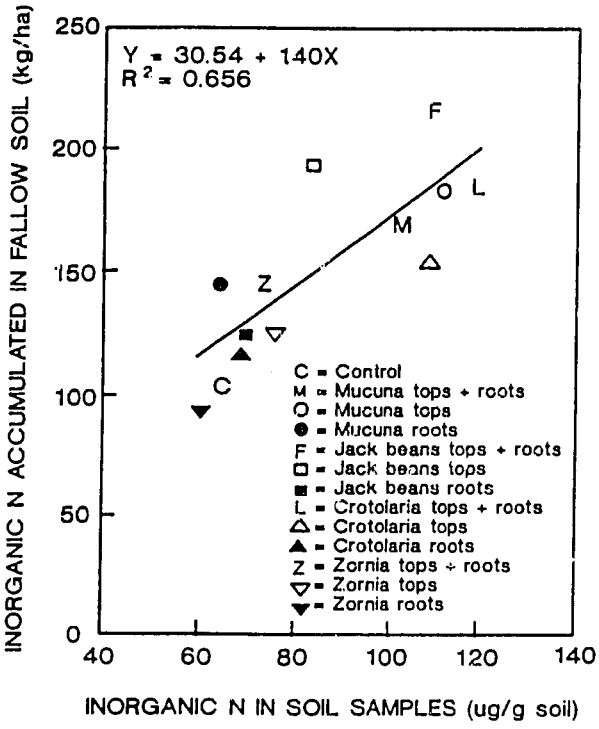

Fig. 3. Relationship between inorganic nitrogen determined in soil samples oven dried at 100 °C and incubated for 1 week, and inorganic nitrogen accumulated to 0.6 m in the soil profile of fallow plots 167 days after incorporation of the legumes in 1986 (*second experiment*).

matter of maize even though mucuna released more N by incubation and more was found in the above ground dry matter compared to soybean.

In the second field experiment (1986–87) inorganic N released by incubation of samples with different legumes incorporated was linearly related to the N content of the above ground dry matter of maize (Figure 2). In addition inorganic N released by incubation was well correlated with inorganic N accumulated in the fallow plots (Figure 3). Jack bean was particularly effective in supplying N as shown by either N content of dry matter of maize or by N accumulated in the fallow plot. Zornia was a poor supplier of N. In agreement with the first experiment the roots of all crops released little inorganic N to the fallow plots.

Discussion

Although the amount of mineral N determined in soil samples after incubation varied significantly amoung the various pretreatments and length of incubation (Bremner, 1965; Castellanos and Prat 1981), the correlations among them were uniform highly significant. The simplest procedure from th standpoint of convenience is oven drying soil sam ples (0.00–0.15 m) at 50 °C and subsequent inc bation for 1 week. Results obtained with this in cubation treatment were significantly correlate with N content of above ground dry matter of th 1986 experiment but the correlations with N con tent in aboveground dry matter in 1984 (r = 0.6 and 1985 (r = 0.51) were not significant. Diffe ences in r values for the different years are believe to be the result of length of storage (Nelson an Bremner, 1972). Samples prepared for incubatio soon after being taken from the field gave the be correlations. Incubation treatments, however, wer well correlated (r > 0.85) with the inorganic accumulated to several depths in fallow soil various times after incorporation of the legumes i both the 1984–85 and the 1986 experiments.

Our results show that laboratory incubation ca be used as a quick screening procedure to evaluat differences in N supplying potential of legum green manures to succeeding non-legume crop Small field plots where legumes have been grow and/or incorporated are used. Soil samples shoul be taken between 2 to 3 weeks after incorporatio of legumes and immediately air dried, ground sieved (discarding the sieved plant material), an subsequently oven dried at 50 °C and incubated fo 1 week. Oven drying at 50 °C is a simple means t ensure uniform sample treatment prior to incu bation. The samples should be prepared and in cubated as soon as possible after being taken fron the field. Once legumes with the highest potential a N sources have been identified, their effectivenes as N sources for specific non-legume crops can b determined in field experiments.

Acknowledgements

Joint publication done as part of a Title XI Collaborative Research Support Program betwee CPAC/EMBRAPA, Planaltina, Brazil and Cornel University, Ithaca, New York carried out a CPAC. The financial support of USAID throug the Title XII CRSP Subgrant SM-CRSP-01 fron North Carolina State University, in carrying ou this research, is gratefully acknowledged.

References

Bartlett R and James D 1980 Studying dried, stored soil samples – some pitfalls. Soil Sci. Soc. Am J. 44, 721–724.

Beauchamp E G, Reynolds W D, Brashe-Villeneuve D and Kirby K 1986 Nitrogen mineralization kinetics with different soil pretreatments and cropping histories. Soil Sci. Soc. Am. J. 50 1478–14843.

Boswell F C, Richer A C and Casida L E 1962 Available soil nitrogen measurements by microbiological techniques and chemical methods. Soil Sci. Soc. Am. Proc. 26, 254–257.

Bowen W T 1987 Estimating the Nitrogen Contribution of Legumes to Succeeding Maize on an Oxisol in Brazil. Ph.D. Thesis. Cornell Univ., Ithaca, New York.

Bremner J M 1965 Inorganic forms of nitrogen. *In* Methods of Soil Analysis. Part II. Ed. (A Black). Agronomy 9, 1179–1237.

Castellanos J Z and Pratt P F 1981 Mineralization of manure nitrogen-correlation with laboratory indexes. Soil Sci. Soc. Am. J. 45, 354–357.

Fox R H and Piekielek W P 1984 Relationship among anaerobically mineralized nitrogen, chemical indexes, and nitrogen availability to corn. Soil Sci. Soc. Am. J. 48, 1087–1090.

Frankenberger, Jr W T and Abdelmagid H M 1985 Kinetic parameters of nitrogen mineralization rates of leguminous crops incorporated into the soil. Plant and Soil 87, 257–271.

Jenkinson D S 1966 Studies on the decomposition of plant material in soil. II. Partial sterilization of soil and the soil biomass. J Soil Sci. 17, 280–302.

Keeney D R 1982 Nitrogen-availability indexes. *In* Methods of Soil Analysis. Part II. Ed. Page et al. 2nd ed. Agronomy 9, 711–733.

Keeney D R and Bremner J M 1966 Comparison and evaluation of laboratory methods of obtaining an index of soil nitrogen availability. Agron. J. 58, 498–502.

Keeney D R and Nelson D W 1982 Nitrogen – inorganic forms. *In* Methods of Soil Analysis. Part II. Eds. A L Page et al. 2nd ed. Agronomy 9, 643–698.

Nelson D W and Bremner J M 1972 Preservation of soil samples for nitrogen analysis. Agron. J. 64, 196–199.

Nordmeyer H and Richter J 1985 Incubation experiments on nitrogen mineralization in loess and sandy soils. Plant and Soil 83, 433–445.

Norman R J, Edberg J C and Stucki J W 1985 Determination of nitrates in soil extracts by dual-wavelength ultraviolet spectrophotometry. Soil Sci. Soc. Am. J. 49, 1182–1185.

Quintana J O 1987 Evaluation of Two Procedures for Screening Legume Green Manures as Nitrogen Sources to Succeeding Corn. Ph.D. Thesis. Cornell Univ. Ithaca, NY. 81 pp.

Sahrawat K L 1980 Nitrogen supplying ability of some Philippine rice soils. Plant and Soil 55, 181–187.

Sahrawat K L 1983 Correlations between indexes of soil nitrogen availability and nitrogen percent in plant, nitrogen uptake, and dry-matter yield of rice grown in the greenhouse. Plant and Soil 74, 223–228.

SAS Institute Inc. 1982 SAS user's guide: Basics. SAS Institute Inc., Cary, North Carolina.