http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan
Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (13): 2426-2431, 2006
ISSN 1028-8880

Nutrients Dynamics in Komatsuna (*Brassica campestris* L.) Growing Soil Fertilized with Biogas Slurry and Chemical Fertilizer Using^{15}N Isotope Dilution Method

[1]Adel Ghoneim, [2]Hideto Ueno and [2]Azza Ebid
Environmental Soil Science Laboratory, Faculty of Agriculture, Ehime University,
3-5-7 Tarumi, Matsuyama, 790-8566, Japan
[2]Ehime University Experimental Farm, Hojo, 799-2424, Japan

Abstract: Efficient liquid slurry land application requires knowledge of nutrient content, proper application rate and crop needs. An improved understanding of the cycling of biogas slurry N is a prerequisite for making better use of this N source. The efficiency of nitrogen (N) derived from biogas slurry must be determined to optimize use of N and reduce impact on the environment. Keeping in view, a pot experiment was initiated to estimate crop N uptake from biogas slurry and chemical fertilizer that applied to Komatsuna (*Brassica campestris* L.) using ^{15}N isotope dilution method. In this experiment 180 kg N ha^{-1} of 1.0 atom % ^{15}N excess ammonium chloride was used. Nitrogen derived from biogas slurry (N_{dfs}) and recovered-N (N_{rfs}) in Komatsuna was measured to know the effects of applied slurry on the performance of the crop and nutrient dynamics in a Brown Lowland Soil, Fluvisols west Japan. It was found out that ^{15}N uptake was slightly higher in chemical fertilizer compared to slurry application. In addition, N uptake derived from chemical fertilizer (N_{dfc}) was better than that taken from soil (N_{dfs}). Fertilizer use efficiency (FUE-^{15}N) was lower in slurry treatment (47.2%) than chemical fertilizer (65.9%). The relative efficiency (slurry use efficiency/chemical use efficiency *100) was varied in Komatsuna roots and leaves.

Key words: N dynamics, derived-N, recovered-N, slurry, Komatsuna

INTRODUCTION

An important objective of the use of ^{15}N-enriched fertilizers in agricultural research is to develop criteria for the efficient use of nitrogenous (N) fertilizers. By comparing different fertilizer sources and types of application, the efficiency of this fertilizer can be assessed in terms of increasing plant uptake and reducing losses of applied N from the soil-plant system. The improved utilization of biogas slurry has a central role to decrease the environmental risks of farming. More efficient utilization of the nutrients reduces the mineral fertilizer applied and the influence of the surrounding environment (Thomsen *et al*., 1997). Biogas slurry contains valuable plant nutrients and is important for the N cycle in agricultural systems. Knowledge of the direct and the subsequent longer-term turnover of N derived from slurry are important to maximize crop utilization and minimize losses of nitrate to the environment (Sikora and Enkiri, 2001). Several studies have shown that slurry may induce immobilization of soil N after application (Opperman *et al*., 1989; Paul and Beauchamp, 1995). One of the factors affecting N immobilization is volatile fatty acid in the slurry (Kirchmann and Lundvall, 1993). The sustainable agriculture is based on adoption of farming practices that recycle biogas slurry. The rate of available nitrogen (N), which is released from organic materials in soil, is often measured by applying ^{15}N and following its recovery by the growing crop. However, the turnover of labeled N in soil modifies the ratio of labeled to unlabelled available N and thereby affects the uptake of ^{15}N by plants (Fillery and Recous, 2001). To maximize the benefit of slurry for crop use and efficient soil fertility management, it is essential to understand and quantify the dynamics of N and other nutrients from slurry. The research on plant N uptake from organic residues is limited due to methodological difficulties. Non-isotopic methods can provide useful agronomic information on the quantity of N available to the crop: N uptake by the crop is measured in the presence and absence of added residues and the difference is attributed to N mineralized from the residues. N release in practical situations is often small compared with total crop uptake so the precision of measurement is often void (Powlson and Barraclough, 1993). In addition, it is impossible to trace the flow of residue-derived N

Corresponding Author: Adel Ghoneim, Environmental Soil Science Laboratory, Faculty of Agriculture, Ehime University, 3-5-7 Tarumi, Matsuyama, 790-8566, Japan

through soil pool without isotopic labeling and losses can only be determined if all possible pathways are measured. Extensive research has been done on the impact of various organic materials such as rice straw, compost and green manure on nutrient dynamics (Eghball, 2000; Choi *et al.*, 2001). However, the detailed N uses efficiency and biogas slurry nutrient dynamics using a direct method with ^{15}N isotope dilution method is well understood.

Komatsuna (*Brassica campestris* L.) is a typical Japanese leafy vegetable. It is often called Japanese mustard spinach in the US supermarkets. Young leaves, stalks and flower shoots are used in salad and stir-fry. It is also very popular for salt pickling in Japan. Komatsu is a fast-growing vegetable and is ready for harvest 35 days after sowing in warm climates. Plants can be grown round the year in temperate and subtropical areas. An unlikely relative of the turnip family, this large leafy green is grown almost exclusively in Japan, Taiwan and Korea. The purpose of this research was to check out the effect of biogas slurry application as fertilizer for Komatsuna, nutrient dynamics and N uptake using ^{15}N isotope dilution method.

MATERIALS AND METHODS

Slurry characteristics and experimental layout: A pot experiment was carried out at the Ehime University Farm, Japan. Soil used from upland field (Low fertile, Brown Lowland Soil, Fluvisols, (in FAO/ UESCO), taken from Ehime University Farm. The soil had pH (H_2O) of 6.28, EC (dS m^{-1}) of 0.37, T-C of 1.46 %, T-N of 0.15 %, Olsen-extractable of 0.18 % and CEC of 6.40 cmol (+) kg^{-1}. The composition of biogas slurry (mainly cattle manure and waste of dairy products, which collected from Yagi-town, Kyoto, Japan) is shown in Table 1. Two treatments were set up in a completely randomized design: (1) the biogas slurry at the rate of 150 kg N ha^{-1} was applied at different intervals (8 times) and (2) the control (chemical fertilizer labeled with 1.0 atom % excess $^{15}NH_4Cl$, 99.70 atom % ^{15}N). The tracer N was applied at the rate of 0.10 g m^{-2} equivalent rate of $^{15}NH_4Cl$, 99.70 atom % injected into slurry treatment soil thoroughly. Slurry was applied in liquid form by mixing with the soil. This technique can minimize NH_3 volatilization, thereby increasing N use efficiency. Only phosphorous was applied to slurry treatment at the rate of 150 kg ha^{-1} on dry soil. Control treatment was amended with NPK fertilizers at the same rate of 150 kg ha^{-1} applied to dry soil. Plants were raised in 1/5000a Wagner's pots filled with 3.0 kg dried soil. Komatsuna (*Brassica campestris* L.) was grown in soil using a completely randomized design with five replicates. Ten seeds of Komatsuna were planted per pot. Germination was completed in 7 days. After emergence, plants were thinned to four plants per pot. The crop uptake of labeled biogas slurry N was compared with the uptake of ^{15}N-labelled mineral fertilizer in the respective treatment.

Table 1: Chemical characteristics of biogas slurry* used in the experiment

Characteristics	Values
pH	7.60
EC (mS m^{-1})	1700.00
Organic N (%)	0.11
NH_4^+-N (mg L^{-1})	1800.00
NO_2-N (mg L^{-1})	0.02
NO_3^--N (mg L^{-1})	15.00
C/N ratio	4.80
Phosphate (mg L^{-1})	180.00
K (mg L^{-1})	0.57
SO_4^{-2}	380.00
Total C (%)	1.40
Total Fe	560.00
Volatile total solids (%)	70.60

* Biogas slurry (mainly cattle manure and wastes of dairy products, which collected from Yagi-town, Kyoto, Japan

Plant harvest and chemical analysis: The harvest was taken after 47 Days After Sowing (DAS). After harvesting, Komatsuna plants were dir-dried into oven at 80°C for 48 h. Samples were weighed to determine dry matter production and cut into small pieces and pulverized for chemical analysis. A CN analyzer determined total plant N, while $^{15}N/^{14}N$-isotope ratio was determined by mass spectrometry. In addition, P, K, Ca, Mg and NO_3^- concentrations in plants were also measured. N derived from biogas slurry (N_{dfs}), from chemical fertilizer (N_{dfc}), from soil (N_{dfs}) and N recovered from biogas slurry was calculated. The soil was analyzed for total N, C and ^{15}N after harvest to determine the recovery of fertilizer N in the soil-plant system and quantify its losses.

Derived and recovered N: Percentage and amount of N derived from biogas slurry, chemical fertilizer and its recovery in Komatsuna was calculated as follows:

Derived-N (%) = (^{15}N atom % excess of N)/(^{15}N atom % excess of applied N) × 100; Derived-N (mg) = (^{15}N atom % excess of N)/(^{15}N atom % excess of applied N) × total-N; and N recovery (%) = (amount of derived-N, mg)/(amount of applied-N, mg) × 100 (Hauck and Bremner, 1976; Barrachlough, 1997; Hood *et al.*, 1999). Measurement of ^{15}N in plant samples from the plots without application of slurry was used to determine the background enrichment used in the calculations.

N yield (mg pot^{-1}) of each plant part was calculated as follows:

N yield (mg pot^{-1}) = dry matter (g pot^{-1}) × N content (mg g^{-1})/100. The crop uptake of labeled biogas slurry N was compared with the uptake of ^{15}N-labelled mineral fertilizer in the reference treatment.

Statistical analysis: The data collected were subjected to the statistical analysis appropriate to the design. The availability of ^{15}N in biogas slurry was related to the availability of mineral fertilizer ^{15}N by calculating the Mineral Fertilizer Equivalent (MFE): MFE = (% uptake of ^{15}N from slurry) × 100/(% uptake of ^{15}N from mineral fertilizer) (Christensen, 1996). The significance of difference between slurry application and chemical fertilizer was estimated using the Tukey test with $p = 0.05$. A statistical analysis was performed using SAS software (SAS Institute, 1985).

RESULTS AND DISCUSSION

Dry matter and N yield of komaatsuna: Dry matter production of Komatsuna at harvest stage is given in Fig. 1. The dry weight of Komatsuna showed similar pattern in both chemical fertilizer and biogas slurry treatment. However, biogas slurry showed a higher leaves dry weight compared with chemical fertilizer treatment. Nitrogen content in plant part varied among the treatments (Fig. 2). The N content in leaves and roots were higher in labeled chemical fertilizer compared with slurry treatment. Moreover, the N concentration was highest in leaves than root.

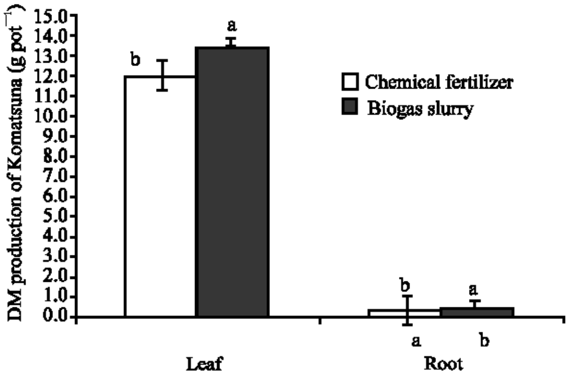

Fig. 1: Dry matter production of Komaatsuna as affected by chemical fertilizer and biogas slurry application. Means within a column following by same letter were not significantly different (Tukey-Kramer test: $p<0.05$)

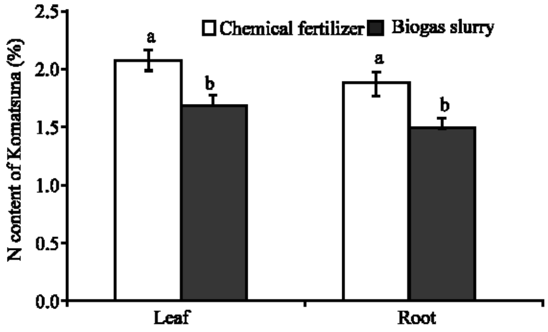

Fig. 2: Effect of chemical fertilizer and slurry application on N content of Komatsuna

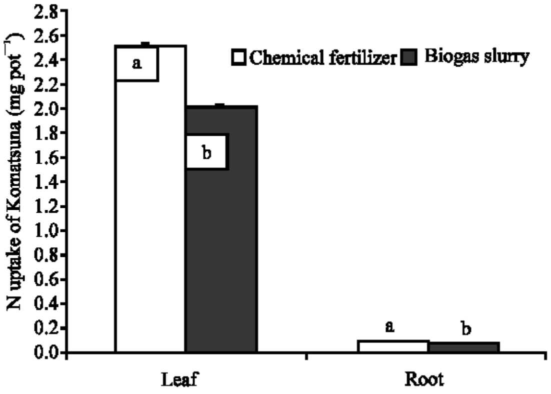

Fig. 3: ^{15}N uptake of Komatsuna at harvest stage as affected by chemical fertilizer and slurry amendments

^{15}N uptake and recovery: The ^{15}N uptake from labeled chemical fertilizer and slurry application is shown in Fig. 3. Similar to N content, ^{15}N uptake was higher in chemical fertilizer treatment than slurry. There was significant difference between the treatments. As shown in the figure that the N uptake varied between the leaves and roots. N uptake derived from chemical fertilizer (N_{dfc}) was higher than taken from soil (N_{dfs}) as shown in Fig. 4. Using the uptake rate of $^{15}NH_4Cl$ as the uptake rate of chemical fertilizer N, the relative efficiency of slurry could be defined as the relative uptake of slurry/uptake of chemical fertilizer. The calculated relative efficiency was higher in Komatsuna roots (142.3%) than leaves (73.8%). Nitrogen from many organic fertilizers often shows little effect on crop growth when applied, because of the slow-release characteristics of organically bound N. Furthermore, N immobilization can occur after application, leading to an enrichment of soil N pool. However, this process finally increases the long-term efficiency of slurry. In the present study, the application of slurry was used for only short-term. N release from organic fertilizers measured as Mineral Fertilizer Equivalent (MFE), varied greatly from 0 % (some composts) to nearly 100 % (urine). The most important indicators to be used for predicting the short-term availability of N are total and NH_4^+-N contents, C:N ratio (especially of the decomposable

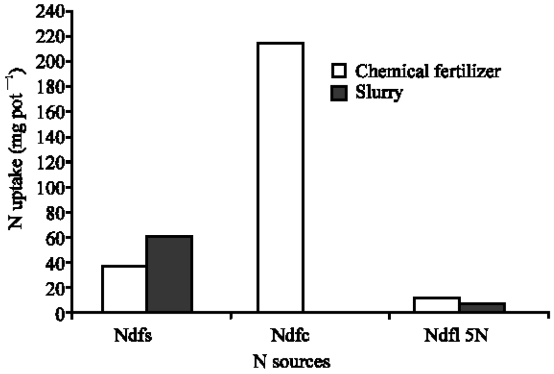

Fig. 4: Amounts of N derived from soil (N_{dfs}), chemical fertilizer (N_{dfc}) and ^{15}N (N_{df15N}) after at 47 days from sowing

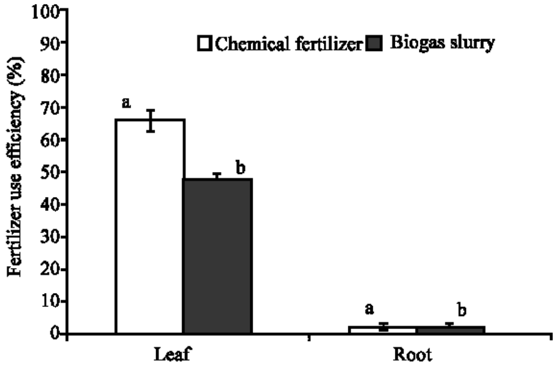

Fig. 5: Nitrogen use efficiency in soil fertilizer with chemical fertilizer and slurry

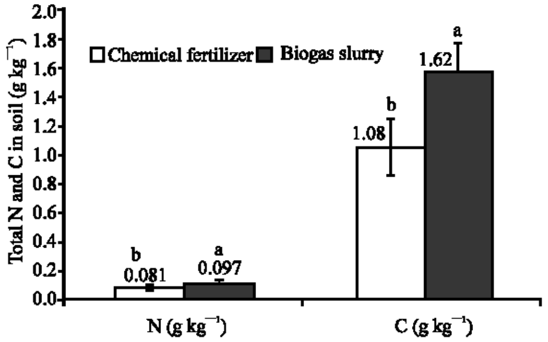

Fig. 6: Total nitrogen soil and carbon in the upper 10 cm of soil as affected by chemical fertilizer and slurry amendments

organic fraction) and stability of the organic substances. Composting reduces mineral-N content and increases the stability of the organic matter, whereas anaerobic fermentation increase NH_4^+-N content as well as the stability of organic matter, but decreases C: N ratio remarkably resulting in a product with a high content of directly available N.

^{15}N use efficiency: Nitrogen use efficiency (FUE) as affected by slurry and labeled chemical fertilizer application is shown in Fig. 5. In general, FUE calculated by ^{15}N isotope dilution method (FUE-^{15}N) varied between the plant parts. Komatsuna leaves use efficiency using ^{15}N dilution method (FUE-^{15}N) estimated to be 65.9 % on chemical fertilizer compared to 47.2 % on slurry treatment. The lower slurry efficiency mostly attributed to ammonia volatilization (Sørensen and Amato, 2002). This phenomenon usually occurs during the first few days following slurry application (Sommer and Hutchings, 2001). Similarly, Sørensen and Amato (2002) found out that NH_3 volatilization losses account for up to 40 % of slurry-added $^{15}NH_4^+$ on the day of application. In our study, the low dry matter content of the slurry, the application of liquid slurry and mixing it immediately with surface soil (1-2 cm depth) depressed the ammonia volatilization (Sommer and Hutchings, 2001). Ammonia volatilization occurs shortly following application of slurry. Up to 40% of NH_4^+-N can be volatilized even within a few hours (Meisinger and Jokela, 2000). Several slurry and soil characteristics modulate the intensity of this process and their net effect on volatilization is still hard to predict. After NH_3 volatilization, denitrification and nitrate leaching are the most likely mechanisms of slurry N loss from the soil-plant system (Chadwick *et al.*, 1998; Chantigny *et al.*, 2001).

Inorganic soil nitrogen: Measured soil NH_4^+-N after harvesting for all depths (0-5 and 5-10 cm) was between 6.0 to 17.0 mg kg^{-1} and no significant difference was found after slurry application and are therefore not presented. Chemical fertilizer and slurry tended to increase soil NO_3^--N level. However the chemical fertilizer effect on topsoil NO_3^--N level was lower than that of biogas slurry. This difference in behavior was probably due to the fact that NO_3^--N applied as inorganic fertilizer that immediately solubilized in soil and therefore more susceptible to downward movement within the soil profile.

Build-up of total N and C in the soil: Biogas slurry produced an increase of total nitrogen and total soil carbon (Fig. 6). Slurry application showed a higher total soil N (0.097 g kg^{-1}) compared to chemical fertilizer (0.081 g kg^{-1}). Total soil C in slurry treatment (1.62 g kg^{-1}) was also higher than chemical fertilizer (1.08 g kg^{-1}). This result suggest that the increase in carbon signifies an improvement in the quality of the soil and shows that the soil can help store carbon which may have implications for reducing greenhouse gases. Most of the nitrogen is organic and represents a stable pool in the soil (Choi *et al.*, 2001).

Table 2: Total P, K, Ca and Mg (g kg^{-1} DW) in Komatsuna as affected by chemical fertilizer and slurry application.

Amendments	Leaf	Root	Total
Chemical fertilizer	0.462b	0.348b	0.810b
Biogas slurry	0.888a	0.438a	1.326a
Chemical fertilizer	59.800a	47.900a	107.700a
Biogas slurry	52.300a	39.800b	92.100b
Chemical fertilizer	27.300a	12.800a	40.100a
Biogas slurry	22.500b	11.700b	34.200b
Chemical fertilizer	3.410a	3.380a	6.790a
Biogas slurry	3.08b	3.210b	6.290b

DW: Dry Weight. Means within a column followed by same letter(s) were not significantly different (Tukey-Kramer test; $p<0.05$)

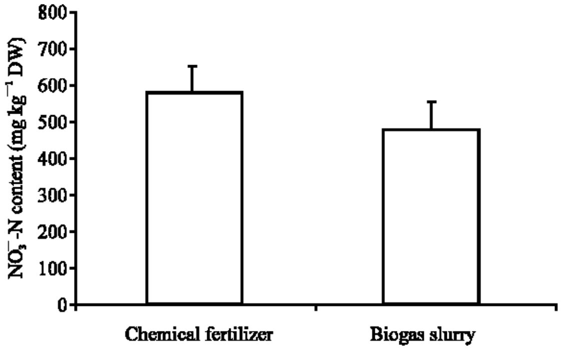

Fig. 7: Effect of chemical fertilizer and slurry application on NO_3^--N content in Komatsuna leaves

Availability of plant nutrients: Liquid slurry contains macro- and micronutrients that enhance the soil chemical and physical properties. The total P, K, Ca and Mg concentrations in Komatsuna leaf and root are mentioned in Table 2. The data showed that the total K, Ca and Mg concentrations were higher in chemical fertilizer compared to slurry application and there was significant difference between the treatments. On the other hand, the application of the slurry increased the plant concentration of P. Biogas slurry contains significant concentrations of N, P and K and their utilization for crop production is beneficial in terms of nutrient recycling and reducing commercial fertilizer use. The distribution of moisture, degradable C and N after application of slurry can affect the turnover and plant availability of slurry N. There were many factors affecting the release of nutrient availability for Komatsuna such as, chemical composition and C to N ratio of added slurry and such external factors as soil and climatic conditions where the experiment was conducted (Constantinides and Fownes, 1994). Nitrogen is the main limiting factor for most field crops and nitrate is the major form of nitrogen absorbed by crop plants. Farmers often use nitrogen fertilizers to increase crop yields. Consequently, many vegetables and forage crops accumulate high levels of nitrate. The effect of application of chemical fertilizer and slurry application on NO_3^- plant content is shown in Fig. 7. Nitrate content in Komatsuna leaf ranged from 460 mg NO_3 kg^{-1} DW in slurry compared to 580 mg NO_3 kg^{-1} DW in chemical fertilizer. Nevertheless, the conventional Komatsuna contained significantly greater nitrate than organic spinach, suggesting that nitrogen fertilizer applications might be the main cause of the difference. As slow release nitrogen fertilizers, some organic fertilizers may reduce nitrate accumulation in plants. However, easily decomposable organic fertilizers such as blood meal and guano might increase nitrate accumulation in the same way as conventional chemical fertilizers (Termine *et al.*, 1987).

REFERENCES

Barrachlough, D., 1997. The direct or MIT route for nitrogen immobilization: A^{15}N mirroe image study with leucine and glycine. Soil Biol. Biochem., 29: 101-108.

Chadwick, D.R., Van der Weerden, J. Mattinez and B.F. Pain, 1998. Nitrogen transformation and losses following pig slurry applications to a natural soil filter system (Solepur Process) in Brittany, France. J. Agric. Eng. Res., 69: 85-93.

Chantigny, M.H., P. Rochette and D.A. Angres, 2001. Short-term C and N dynamics in a soil amended with pig slurry and barely straw: A field experiment. Can. J. Soil Sci., 81: 131-137.

Choi, W.J., S.A. Jin, S.M. Lee, H.M. Ro and S.H. Yoo, 2001. Corn uptake and microbial immobilization of ^{15}N-labeled urea-N in soil as affected by composted pig manure. Plant Soil, 235: 1-9.

Christensen, B.T., 1996. Nitrogen Added to Soil in Crop Residues and Animal Manure. In Nitrogen Supply and Nitrogen Fixation of Crop for Cool and Wet Climates. Samuelsen *et al.* (Eds.), Office for official publication of the European communities, Luxembourg, pp: 117-133.

Constantinides, M. and J.H. Fownes, 1994. Nitrogen mineralization from leaves and liter of tropical plants: Relationship to nitrogen, lignin and soluble polyphenol concentrations. Soil Biol. Biochem., 26: 49-55.

Eghball, B., 2000. Nitrogen mineralization from field-applied beef cattle feedlot manure or compost. Soil Sci. Soc. Am. J., 64: 2024-2030.

Fillery, I.R.P. and S. Recous, 2001. Use of Enriched ^{15}N Sources to Study Soil N transformation. In: Stable Isotope Techniques in the Study of Biologocal Processes and Functioning of Ecosystems. Unkovish *et al.* (Ed.). Kluwer Acad. Publ., Boston, MA., pp: 167-194.

Hauck, R.D. and J.M. Bremner, 1976. Use of tracers for soil and fertilizer nitrogen research. Adv. Agron., 28: 219-266.

Hood, R.C., N. Goran, K.M. Aigner and G.A. Hardarson, 1999. Comparison of direct and indirect ^{15}N isotope techniques for estimating crop N uptake from organic residues. Plant Soil, 208: 259-270.

Kirchmann, H. and A. Lundvall, 1993. Relationship between N immobilization and volatile fatty acids in soil after application of big and cattle slurry. Biol. Fertil. Soils, 15: 161-164.

Meisinger, J.J. and W.E. Jokela, 2000. Ammonia volatilization from dairy and poultry manure. In: Managing nutrients and pathogens from animal agriculture. Proc. Conf. Nutr. Manage. Consultants, Extension Educators and Producer Advisors, Camp Hill, PA. 28-30 Mar. 2000. NRAES Cooperative Extension, Ithaca, NY., pp: 334-354.

Opperman, M.H., M. Wood and P.J. Hrris, 1989. Changes in inorganic N following the application of cattle slurry to soil at two temperatures. Soil Biol. Biochem., 21: 319-321.

Paul, J.W. and E.G. Beauchamp, 1995. Availability of manure slurry ammonium for corn using ^{15}N-labelled $(NH_4)_2SO_4$. Can. J. Soil Sci.., 75: 35-42.

Powlson, D.S. and D. Barraclough, 1993. Mineralization and Assimilation in Soil-plant Systems. In: Nitrogen Isotope Techniques. Knowles, R. and T.H. Blackburn (Eds.). Academic Press Inc, San Diego. pp: 209-242.

SAS Institute, 1985. SAS/STAT Guide for personal Computers Version 6. SAS Institute, Gary, NC.

Sikora, L.J. and N.K. Enkiri, 2001. Uptake of ^{15}N fertilizer in compost-amended soils. Plant Soil, 235: 65-73.

Sommer, S.G. and N.J. Hutchings, 2001. Ammonia emission from field applied manure and its reduction. Eur. J. Agron., 15: 1-15.

Sørensen, P. and M. Amato, 2002. Remineralisation and residual effects of N after application of pig slurry to soil. Eur. J. Agron., 16: 81-95.

Termine, E., D. Lailon, B. Taupier-Letage, S. Gautier, R. Lafont and H. Lafont, 1987. Yield and content in nitrates, minerals and ascorbic acid of leeks and turnips grown under mineral or organic nitrogen fertilizations. Plant Foods Human Nutr., 37: 321-332.

Thomsen, I.K., V. Kjellerup and B. Jensen, 1997. Crop uptake and leaching of ^{15}N applied in ruminant slurry with selectively labeled faeces and urine fractions. Plant Soil, 197: 233-239.