Measurement and characterisation of nitrogen release from selected types of animal manure.

Measurement and characterisation of nitrogen release from selected types of animal manure

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DECLARATION

"I hereby declare that the dissertation submitted for the degree M Tech: Agriculture, at the Tshwane University of Technology, is a result of my own investigations and it has not been previously submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references"

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Signature:

DEDICATION

This dissertation is dedicated to my parents (Gladys Shongwe and Albert Fakude), and to my uncles (James Fakude and Petros Fakude), together with their wives (Thandazile Qwabe and Rita Mzobe). I thank them for their prayers and support. I am where I am today because of their teachings and words of encouragement. Words can’t express how grateful I am to have them in my life.

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ABSTRACT

The aim of this two-part study was to contribute to improved understanding of the effects of applying animal manure on the plant- availability of N in soil. The objective of the first part was to assess the validity of the apparent relative nitrogen fertiliser (ARNFV) assay, which is a quick, relatively cheap pot-based method that uses the measurement of maize biomass production over a 42-day period to estimate the first-season NFV of organic sources of N added to soil. Comparing the nitrogen fertiliser value (NFV) estimates of nine manure samples obtained with the ARNFV assay with those provided by established methods, it was found that ARNFV assay estimates were closely correlated with net mineral N release from the manure samples during incubation and also with N yield. However, the assay had the flaw of assuming that the N content of the maize plants across treatments was constant. This assumption was shown to be invalid, because the N content of maize plants grown in the control treatment was in most instances lower than that of plants grown in soil amended with manure, which, in turn, was lower than the N content of plants grown in the chemical N treatment. Despite this flaw, the ARNFV assay could be used to good effect to obtain an indication of the first season NFV of organic sources of N. The second part of the study related to the low first-season NFV of the cattle manure samples that featured in the ARNFV assay and its objective was to determine the effect of combining the application manure and chemical N fertiliser on plant-availability of N in soil and on the NFV of a cattle kraal manure sample.The results confirmed that the strategy increased plant-availability of N in soil but quantitatively, this increase was identical to that obtained by applying the chemical source of N without manure addition, implying that the strategy had no effect on the NFV of the manure used.

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ABBREVIATIONS

NFV	Nitrogen fertiliser value
NFVs	Nitrogen fertiliser values
N	Nitrogen
P	Phosphorus
K	Potassium
g	Gram
kg	Kilogramme
ha^{-1}	Per hectare
kg ha^{-1}	Kilogramme per hectare
kg $soil^{-1}$	Per kilogramme soil
mg	Milligram
me	Milliequivalents
mm	Millimetre
^{0}C	Degrees Celsius
%	Percentage
ml	Millilitre
L	Litre
hrs	hours

Fig	Figure
NH_4^+-N	Ammonium nitrogen
NO^{3-}-N	Nitrate nitrogen
t ha^{-1}	Tonne per hectare
g pot^{-1}	Gram per pot

CHAPTER 1

1 GENERAL INTRODUCTION

Nitrogen plays a fundamental role in plant productivity, as it is a major component of amino acids, proteins, nucleic acids and chlorophyll (Haynes, 1986:24). Unkovich *et al*. (2008:3) estimated that between 150 and 200 million tonnes of mineral N are required annually to produce the world's food and animal feed. Nitrogen exists in the biosphere primarily as di-nitrogen gas (N_2), ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-) and organic N (Sandrock, 2004:18). Plants display preferences amongst these various forms of N, and for most plants, NH_4^+–N and NO_3^-–N are the two main forms of N that are actively absorbed (Näsholm, Huss-Danell & Högberg, 2000:1155).

Where water is adequately available, N deficiency is often the factor that limits plant growth in both natural and agricultural systems (Giller *et al*., 1997:152). Du Toit and Du Preez (1995:73) reported that on large-scale commercial farms in South Africa, the N content of soils has been declining from 9.5 - 103.2 mg kg^{-1} soil in virgin top soils to 4.2 - 85.8 mg kg^{-1} soil in cultivated top soils. The phenomenon of N decline is even more severe on South African smallholdings (Mandiringana *et al*., 2005:2444). Generally, African smallholders struggle to supply N to their cultivated soil in quantities that match crop N demand, which explains the decline of the N content of these soils (Kumwenda *et al*., 1996:7; Odhiambo & Magandini, 2008:357). Whilst raising the application rate of chemical N-fertiliser is typically within the means of large-scale commercial farmers, smallholders face poor access to chemical fertilisers and high levels of poverty, which prohibits them from purchasing chemical fertilisers in sufficient quantities to match crop withdrawal rates (Harris, 2002:141; Mkhabela & Materechera, 2003:152).

Besides applying chemical N-fertilisers, N can also be added to soil through biological N fixation and by applying organic fertilisers, such as animal manure or compost (Place *et al*., 2003:357-369). Amongst the various organic fertilisers, animal manure is the most abundant resource on most African smallholder farms (Giller *et al*., 1997:168; Opala, 2011:67). Animal manure is defined as the solid, semi solid and liquid by-products generated by animals raised to produce milk, meat, eggs and other products for human consumption (Sims & Maguire, 2005:402). One disadvantage of using animal manure as a source of N in crop production is that part or all of the N it contains has to be mineralised before it becomes available to plants (Janssen, 1996:39). Van der Mey, Combrink and du Preez (1994:64) defined N mineralisation as, 'a microbial process whereby organic N in the soil is converted into plant utilisable forms of N, namely NH_4^+–N and NO_3^-–N.' Knowledge of the N mineralisation dynamics of applied manure is required to predict the concentration of plant available N in the soil over time. Such knowledge is required to optimise the N nutrition of planted crops, whilst avoiding environmental pollution arising from excess mineral N, particularly in nitrate form (van der Mey, Combrink & du Preez, 1994:64; Chadwick *et al.*, 2000:159; Muñoz *et al*., 2004:719; Schröder, Jansen & Hilhorst, 2005:203).

Considerable progress has been made towards understanding the N mineralisation dynamics of applied animal manure emanating from farming systems in the developed world (Giller, Cadisch & Palm, 2002: 704; Naylor *et al*., 2005: 1621), but the same cannot be said about manure on smallholdings in developing countries (Powell *et al*., 1996:144; Mkhabela, 2006:1162). Whilst research results pertaining to the use of chemical fertilisers may be transferrable to new locations, the same does not apply to animal manure, because the N-release properties of animal manure are a function of a multitude of factors, several of which are site-specific (Giller, Cadisch & Palm, 2002: 706; Mkhabela, 2006:1161; Rufino *et al*., 2007: 278). For example, indications are that the cattle manure found in enclosures on South African smallholdings has much less value as a source of plant-available N than cattle manure generated in western farming systems (Mafongoya *et al.,* 2006:141; Mkhabela, 2006:1167; Ngwenya, 2017:54). Accordingly, there is a need to develop knowledge on the nitrogen fertiliser value of the various types of manure produced on smallholdings in the developing world. Contributing to the development of this knowledge, with specific reference to South African smallholder farming systems, was the overall aim of the current study.

The study consists of two separate parts. The first part addresses an important methodological constraint to the generation of knowledge on manure in developing countries, namely, the high cost of both instrumentation and consumables used in the study of the nitrogen fertiliser value (NFV) of manure with existing methods. To address this constraint, several research students at the Tshwane University of Technology have contributed towards the development of a cheap and quick method to assess the NFV of animal manure, called the apparent relative nitrogen fertiliser value (ARNFV) assay (Adebisi, 2015:53; Ngwenya, 2017:42; Mthimkulu, 2019:47). However, the results obtained using the ARNFV assay have not been compared with those provided by existing, validated methods. Accordingly, the objective of the first part of this study was to do such a comparison in order to assess the validity of the ARNFV assay.

The second part of the study was aimed at examining the effect of applying N to cropped soil in the form of a combination of animal manure and chemical fertilisers. This particular nitrogen application strategy has been recommended for use on African smallholdings, because research has demonstrated that applying N in chemical form can raise the NFV of manure (Kumwenda *et al*., 1996:5). However, other work has shown that the positive effect of combining chemical N-fertilisers with manure on the NFV of manure is not universal and may depend on the type of animal manure used (Bremer, Van Houtum, & Van Kessell, 1991:222). For this reason, the objective of the second part of the study was to determine the effect of adding N in chemical form to the soil on the NFV of cattle kraal manure produced on South African smallholdings.

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CHAPTER 2

2 INTERNAL VALIDITY OF THE ARNFV ASSAY

2.1 INTRODUCTION

When using animal manure to add nitrogen (N) to cultivated soils, the N-fertiliser value of the manure must be known to effectively manage plant-available N in the soil. The N-fertiliser value of animal manure refers to the proportion of the N present in the manure that acts in the same way as chemical fertiliser N in terms of plant uptake and production enhancement (Schröder, 2005;255; Hijbeek *et al.,* 2018:105). Quantifying the NFV of manure is difficult, because it tends to increase as manure application is repeated year after year due to the contribution of previous manure applications to the N uptake by the crop (Cusick *et al*., 2006:2170; Schröder, Uenk & Hilhorst, 2007:95). The residual effect of previous manure application on the NFV of manure is eliminated when the measurement is done immediately after manure is applied to soil for the first time. Under these circumstances, the first-season NFV of manure is measured. Accordingly, the first-season N-fertiliser value of manure quantifies the ability of manure to make N available to crops immediately after it has been applied to soil for the first time. By keeping constant the soil and other factors that may influence this ability, it becomes possible to quantify the N-fertiliser value of manure in a standardised manner. The current study was aimed at testing a simple method with which this can be achieved.

Several methods to determine the N-fertiliser value of manure have been developed, including a direct method and two indirect methods. The direct method involves measurement of the amount of N derived from manure that was taken up by a test crop. This can be done by labelling the manure with the stable ^{15}N isotope (Bosshard *et al*., 2009:272). One of the indirect methods, called the difference method, enables measurement of manure N taken up by the test crop by deducting N uptake by the test crop grown in an unfertilised soil from that taken up by the same crop grown in a manured soil. The N-fertiliser value of manure is then obtained by expressing this difference in N uptake as a proportion of the N applied as manure (Harmsen & Moraghan, 1988:55; Harmsen, 2003:322). The other indirect method, called the relative effectiveness method, involves determining the proportion of manure N that behaves as chemical fertiliser N. This method makes use of three treatments, namely a control treatment to which no N is applied, and two other treatments in which N is applied at a predetermined and fixed rate. In one of these two treatments, chemical fertiliser is used as the source of N and in the other manure (Cusick *et al.,* 2006:2172). N recovered by the crop in the control treatment is deducted from N recovered in the two fertilised treatments to obtain the net contribution of these two sources to crop uptake of N. The ratio of the net contribution of manure N and the net contribution of chemical fertiliser N represents the (relative) N-fertiliser value of manure as defined by Cusick *et al.* (2006:2172). In both indirect methods, N uptake is determined by measuring crop biomass, usually above-ground biomass, as well as the N content of this biomass (Roberts & Janzen, 1990:119; Muñoz *et al*., 2004:721).

Adaptations of both direct and indirect methods have been developed, usually to reduce cost and/or process duration. In studies involving animal manure, application of the direct method is cumbersome, time consuming and costly. To obtain manure that is labelled with the stable ^{15}N isotope, animals have to be fed labelled plant material that was produced by growing plants in labelled soil (Bosshard *et al*., 2009:275). Hood *et al.* (2000:42) and Hood (2001:159) proposed and tested the validity of a modification of the direct method aimed at reducing both cost and duration of the process. The design of their adaptation involves a control treatment consisting of native soil labelled with ^{15}N, and a manure treatment, in which manure is added to soil labelled with ^{15}N. In both treatments, time is allowed for the labelled N fertiliser to react with soil N to overcome errors associated with pool substitution (Hood *et al*., 2000:34). Subsequently, unlabelled manure of which the NFV is to be determined, is applied to the soil before planting the test crop in both treatments. By deducting from 1 the ratio of the atom% ^{15}N excess[1] present in the biomass produced in the manured treatment and the atom% ^{15}N excess present in the biomass produced in the control treatment, the percentage N derived from the manure (Ndfm) is obtained. Multiplying the mass of N present in the biomass produced in the manure treatment with Ndfm, the mass of N recovered from the manure is obtained. The NFV of manure is then calculated by dividing the mass of N recovered from the manure by the total mass of N added as manure (Hood, 2001:158).

The N-fertiliser equivalence method, which is an adaptation of the relative effectiveness method, eliminates the need to analyse the N content of crop harvests by using biomass as a proxy for N uptake (Muñoz *et al*., 2004:721). The N-fertiliser equivalence method was developed specifically for application in the field (Cusick *et al*., 2002:2; Kimetu *et al*., 2004:128). It involves at least five treatments, four of which comprise incremental rates of N applied in the form of chemical fertiliser, including a

^{15}N excess[1] is obtained by subtracting 0.3663 atom% from the atom% that is measured from the sample (Zapata, 1990:73; Unkovich *et al.,* 2008:127).

control (zero N), and a fifth treatment in which a specified amount of N is applied in the form of manure or any other organic source of N for which the N-fertiliser value needs to be determined. The rate of N applied as organic material is selected to fall within the range of N rates covered by the four mineral fertiliser treatments (Schröder, 2005:255; Kimani *et al*., 2008:279). The proportion of manure N that behave as chemical fertiliser N is obtained by fitting a polynomial regression curve of the second order to the biomass and N application rate data recorded in the four chemical fertiliser treatments.

In the current study, a pot-based adaptation of the N-fertiliser equivalence method, called the 'apparent relative nitrogen fertiliser value' (ARNFV) assay is described and tested for internal validity. The assay involves the growing of maize in pots over a period of 42 days to provide a measure of the 'first-season' N-fertiliser value of manure and other potential organic sources of plant-available N. The term 'apparent' refers to the use of biomass as a proxy for N uptake by plants, as in the case of the N-fertiliser equivalence method, and 'relative' to the indirectness of the method, because the N fertiliser values that are obtained are relative to a benchmark treatment, in which N is added to the soil in chemical form. Important strengths of the assay are low cost and rapidity, because NFV results are available within three months. Whilst obtaining results quickly and cheaply are important qualities of a method, they are secondary to validity and reliability, which is the main concern with the various indirect methods that have been developed (Harmsen, 2003:322; Cusick *et al.,* 2006:2172; Wagner-Riddle *et al*., 2007:4). The objective of the current study was to test the internal validity of the ARNFV assay. 'Internal' validity instead of validity in general is specified, because the results obtained from the assay rely on both soil and crop being kept constant.

2.2 GENERAL PROTOCOL OF THE ARNFV ASSAY

The ARNFV assay (Ngwenya, 2017:54) involves the growing of eight maize plants from seed planted in 7 kg soil contained in 7 litre pots for a period of 42 days. The experimental design consists of at least three treatments, namely the control treatment, the manure treatment, and the chemical N fertiliser treatment with four replications of each treatment. The number of treaments grows as additional manure samples are incorporated into the design. In both manure and chemical fertiliser treatments, N is applied at the rate of 90 kg N ha^{-1}. The rate of N application per kg of oven-dry soil is obtained by calculating the mass of a 200 mm hectare furrow slice using the dry bulk density of the soil in the pots (Schlossberg *et al.,* 2008:85). The reliability of the results obtained in different runs of the ARNFV assay depends on keeping constant the soil used to fill the pots, necessitating a source of particular soil that can be accessed indefinitely. Since the ARNFV assay equates biomass production to N uptake, all factors other than N availability, which can affect biomass production, must be non-limiting. Critical factors in this regard are the availability of plant nutrients other than N, and the availability of water. After 42 days of growth, the maize plants are cut at the base and oven dried at 60 °C until constant mass. The apparent relative nitrogen fertiliser values of the manure samples is then calculated using equation 2.1, with the biomass obtained in the control and chemical fertiliser treatments being represented by their treatment means.

$$\text{ARNFV}(\%) = \frac{\text{Ymi}-\text{Yco}}{\text{Ytc}-\text{Yco}} \text{ x } 100 \qquad \text{(Equation 2.1)}$$

whereby

ARNFV (%) = apparent relative N-fertiliser value of manure

[Ytmi] = above-ground oven-dry biomass of test crop obtained in manure treatment

[Ytc] = above-ground oven-dry biomass of test crop obtained in chemical fertiliser treatment (urea)

[Yco] = above-ground oven-dry biomass of test crop obtained in the control treatment

2.3 TESTING INTERNAL VALIDITY OF THE ARNFV ASSAY

The internal validity of the ARNFV assay was assessed in two ways. The first was by comparing the ARNFV assay results with those obtained from applying the modified direct method described by Hood *et al.* (2000) and Hood (2001) in the ARNFV assay protocol. The second was by comparing the ARNFV assay results with the net mineral N release from the same manure samples as used in the assay during a 42-day incubation period.

In both assessments a completely randomised experimental design with eleven treatments was employed. All treatments were replicated four times. Treatments included a control, a chemical fertiliser treatment (Urea 46%) and nine manure treatments. The latter consisted of three samples each of cattle kraal manure, goat manure and chicken manure. In all manure treatments as well as the chemical fertiliser treatment, N was applied at the rate of 90 kg N ha^{-1}. Selected properties of the nine manure samples used in the conduct of the two experiments are shown in Table 2.1.

TABLE 2.1: Selected properties of the animal manure samples used to assess the internal validity of the ARNFV assay

Manure	Properties								
	N	P	K	Lignin	Lignin:N	Polyphenol	Polyphenol:N	Organic Matter	C:N ratio
	%	%	%	%	ratio	Mass%	ratio	g/kg	
Cattle 1	1.66	0.84	2.67	10.70	6.40	0.20	0.12	658	20.0
Cattle 2	1.57	0.24	2.31	8.20	5.20	0.15	0.09	563	18.1
Cattle 3	1.62	0.31	2.79	9.70	6.00	0.17	0.11	644	19.1
Goat 1	2.20	0.40	4.00	7.30	3.30	0.09	0.04	467	11.4
Goat 2	2.23	0.36	4.59	8.60	3.90	0.15	0.07	461	10.3
Goat 3	2.24	0.48	3.80	11.30	5.00	0.12	0.05	509	10.9
Poultry 1	3.70	1.50	3.80	11.80	3.20	0.44	0.11	724	9.6
Poultry 2	1.69	0.62	1.22	9.70	5.20	0.27	0.14	948	16.6
Poultry 3	1.95	0.67	1.29	8.30	4.30	0.11	0.01	942	11.6

A low-fertility, loamy sand subsoil, sold commercially for building purposes, was used in both the pot and incubation experiment. Selected chemical properties of the soil are shown in Table 2.2. The dry bulk density of the soil (1 242.77 kg m^{-3}) was determined after the soil had settled in a measuring cylinder, following three wetting and drying cycles. The concomitant mass of the 200 mm hectare furrow slice of the soil was 2 485 540 kg. The plant available water capacity of the soil, defined as the difference between drained upper limit and permanent wilting point, was 48 g water per 1000 g soil. Drained upper limit of the soil in the pots was determined using the procedure described by Passioura (2006:1075). This involved applying 250 ml water to a pot filled with air-dry soil that had been settled by tapping the pot on a wooden bench; covering the pot with cling wrap; and sampling the soil water content of the wetted soil gravimetrically after 24 hrs, which was 0.100 for the soil used. The permanent wilting point of the soil in the pots was determined using the procedure described by Withers and Vipond (1974:71) with maize as the test crop. This involved growing eight maize plants under well-watered conditions for 35 days after which irrigation was discontinued, leaving the plants to subsist on stored soil water. As the plants depleted the stored water they reached the stage at which they were no longer able to extract enough water to recover full turgor overnight under atmospheric conditions of 100% relative humidity. It was at this stage that the soil in the pot was sampled gravimetrically to obtain the permanent wilting point. For maize in the soil used, the 95% confidence interval of the gravimetric water content at permanent wilting point was [0.047 – 0.056], and the mean was 0.052.

TABLE 2.2: Selected chemical properties of the test soil used in the study

Soil property	Unit	Method	Measurement	Reference
Organic Carbon (OC)	%	Walkley-Black	0.26	(Walkley & Black, 1934:29-38)
pH (H_20 $_{[Water]}$)	-	1:2.5 soil: water mass ratio	6.17	(Non-Affiliated Soil Analysis Work Committee, 1990:3/1)
Electrical conductivity	mS m^{-1}	Saturated paste extract	5.58	(US Salinity Laboratory Staff, 1954:8)
Organic N	mg kg $soil^{-1}$		18.57	(Estimated)[2]
Ammonium N	mg kg $soil^{-1}$	Extraction with K_2SO_4	4.77	Anderson and Ingram (1993:73-75)
Nitrate N	mg kg $soil^{-1}$	Extraction with K_2SO_4	9.57	Anderson and Ingram (1993:73-75)
Phosphorus (P)	mg kg $soil^{-1}$	Mehlich 3	3	(Mehlich, 1984:1411-1412)
Potassium (K)	mg kg $soil^{-1}$	Mehlich 3	76.50	(Mehlich, 1984:1411-1412)
Calcium (Ca)	mg kg $soil^{-1}$	Mehlich 3	541	(Mehlich, 1984:1411-1412)
Magnesium (Mg)	mg kg $soil^{-1}$	Mehlich 3	196	(Mehlich, 1984:1411-1412)
Sodium (Na)	mg kg $soil^{-1}$	Mehlich 3	40	(Mehlich, 1984:1411-1412)
Zinc (Zn)	mg kg $soil^{-1}$	Mehlich 3	2.12	(Mehlich, 1984:1411-1412)
Copper (Cu)	mg kg $soil^{-1}$	Mehlich 3	1.38	(Mehlich, 1984:1411-1412)
Manganese (Mn)	mg kg $soil^{-1}$	Mehlich 3	19.59	(Mehlich, 1984:1411-1412)
Sulphur (S)	mg kg $soil^{-1}$	Mehlich 3	12.47	(Mehlich, 1984:1411-1412)
Boron (B)	mg kg $soil^{-1}$	Mehlich 3	0.48	(Mehlich, 1984:1411-1412)
Iron (Fe)	mg kg $soil^{-1}$	Mehlich 3	16.58	(Mehlich, 1984:1411-1412)

[2] organic N content was estimated from OC% and a C:N ratio of 14

2.3.1 Pot experiment

The pot experiment was conducted in a greenhouse of the Tshwane University of Technology, Pretoria West. Plastic pots with a capacity of 7 L and five perforations at the bottom to allow for drainage were used in the experiments. The pots were obtained from Plastillon in Gezina. Following air-drying and screening the soil through a 2 mm gauge sieve, quantities of air-dry soil were weighed to 7 kg and transferred to the pots.

To enable application of the modified direct method and to determine the NFV of manure, the general protocol of the ARNFV assay was modified by pre-labelling the soil in the pots with ^{15}N. This was achieved by dissolving 21.4037 g labelled ammonium sulphate 10 atom% in 5 litre distilled water, and incorporating 50 ml of this solution into the volume of water required to raise the water content of the soil in each pot to the drained upper limit, thereby adding 6.43 mg N of labelled ammonium sulphate (^{15}N) to each kg of soil in the pots. Following ^{15}N labelling, the soil in the pots was left for four weeks, after which it had dried sufficiently to allow for its transfer to an enamel basin and incorporation of the various fertiliser materials as dictated by the particular treatments. The inorganic fertilisers used were urea (46%N), single superphosphate (8.3% P) and potassium chloride (50% K). These three chemical fertilisers were obtained from Obaro (Pty) Ltd. in Pretoria and were manufactured by Sasol. The chemical fertiliser granules as well as the manure samples were pulverised using a cast-iron pestle and mortar and then passed through a sieve with an aperture of 5 mm. All N fertilisers were applied at 36.21 mg N per kg soil except in the control treatment, which was the equivalent of applying 90 kg N ha^{-1} furrow slice. Phosphorus (P) applied at the rate of 60 kg P ha^{-1} and potassium (K), applied at the rate of 80 kg ha^{-1}, were added to the soil in all treatments, including the control. After thoroughly mixing soil with the required fertilisers and organic amendments in an enamel bowl, the soil was transferred to the designated pot, and the soil water content raised to the upper limit 24 hrs before planting. Maize (*Zea mays* var. *indentata*) was the test crop. In each pot, 16 seeds (two per hill) of the cultivar PAN 67 obtained from Pannar in Greytown were planted 45 mm deep in the soil. One week after germination, the stand in each pot was thinned to eight (one per hill) healthy looking maize seedlings.

Throughout the 42-day growing period of the maize, plant-available water in the pots was maintained above 75% of total. Watering of the pots involved weighing the pots on a daily basis during the first three weeks of growth and twice daily during the last three weeks. Weighing of the pots was done on an Adam® electronic scale with a capacity of 30 kg and an accuracy of 10 g manufactured and supplied by Adam® equipment Co Ltd in Kempton Park, weighing was followed by raising water content to the drained upper limit. Weekly, the weight of the pots with soil at drained upper limit was adjusted upwards to cater for the rising mass of the plants in the pots. The mass of the plants growing in the pots was estimated by generating a simple linear regression model that related fresh above-ground plant biomass to plant height (Appendix 2.1). The data for this regression model were obtained in a separate trial that was done before the start of the experiment.

The water used to irrigate the pots was enriched using four nutrient solutions, prepared in line with Broughton and Dillworth (1970:246), to further ensure non-limiting availability of plant nutrients other than N throughout the growing period. The first solution consisted of 147.05 g of $CaCl_2.2H_2O$ dissolved in 5 litre water. The second contained 3.35 g of $FeC_6H_5O_7$, which was dissolved overnight using a magnetic stirrer, to which were added 61.65 g $MgSO_4.7H_2O$ and 169 g $MnSO_4.H_2O$ in 5 litre water. The third solution contained 0.1235 g H_3BO_3, 0.144 g $ZnSO_4.7H_2O$, 0.05 g $CuSO_4.5H_2O$, 0.028 g $CoSO_4.7H_2O$ and 0.024 g $Na_2MoO_2.2H_2O_2$ dissolved in 5 litre water. The fourth solution consisted of 68.05 g KH_2PO_4 (68.05 g) dissolved in 5 litre water. The pH of all four solutions was 6.7 , the solutions were transfered to separate amber bottles for storage. On alternate days, the water used to irrigate the pots was enriched with solutions 1, 2 and 3, or with solution 4, all at the rate of 50 ml solution per 10 litre of irrigation water. The addition of P-containing solution 4 was necessary to prevent the possible development of P deficiency, particularly towards the end of the 42-day growing period. P deficiency might develop due to plant removal of P, fixing of P by the soil, and the formation of insoluble phosphate compounds from reaction of phosphate with micronutrients, such as Fe, Zn and Cu.

After 42 days of growth, the maize plants were pulled out of the soil, all roots were removed with a pair of scissors and the stem of each plant was brushed clean. The eight plants harvested from each pot were then transferred to labelled paper bags and oven-dried at 45 °C. Following weighing of the oven dry biomass of the plants using a Snug III digital lab scale with a capacity of 120 g and an accuracy of 0.001 g manufactured by Ohaus and supplied by Scientific United in Randburg, the plants were milled using a Fritsch pulverisette 19 supplied by Labotec South Africa, and analysed for ^{15}N and total N content using a SERCON 20-22 stable isotope ratio mass spectrometer in the Isotope Bioscience Laboratory of Ghent University, Belgium. N derived from fertiliser (Ndff) was calculated using Equation 2.2, in which ^{15}N excess expressed the ^{15}N content above the atmospheric ^{15}N standard (0.3663) of plant samples.

$$Ndff = \left(1 - \frac{Atom\ \%\ ^{15}N\ excess\ of\ fertiliser\ treatment}{Atom\ \%\ ^{15}N\ excess\ of\ control\ treatment}\right) \qquad (Equation\ 2.2)$$

The Ndff values enabled calculation of N yield (equation 2.3), quantity of nitrogen derived from fertiliser (QNdff) (equation 2.4), nitrogen fertiliser value (NFV) of the fertiliser (equation 2.5) and nitrogen fertiliser use efficiency (NFUE) (equation 2.6), with which the ARNFV of the manure samples were compared.

$$N\ yield\ (g) = \left(\frac{N\ content\ (\%) X\ biomass\ (g)}{100\%}\right) \qquad (Equation\ 2.3)$$

$$QNdff\ (g) = (Ndff\ X\ N\ yield) \qquad (Equation\ 2.4)$$

$$15N\ NFV\ (\%) = \left(\frac{QNdfm\ (manure)}{QNdfc\ (chemical\ N\ fertiliser)}\right) \times 100 \qquad (Equation\ 2.5)$$

$$NFUE\ (\%) = \left(\frac{N\ yield\ manure - N\ yield\ control}{N\ yield\ chemical\ fertiliser - N\ yield\ control}\right) \times 100 \qquad (Equation\ 2.6)$$

2.3.2 Laboratory incubation experiment

The laboratory incubation experiment involved application of the same treatments as described for the greenhouse pot experiment but this time applied in 300 g soil contained in 350 ml polystyrene cups without perforations. Before adding the fertilisers to the soil in the cups and commencement of incubation, ammonium sulphate at the rate of 6.43 mg N kg soil^{-1} was incorporated in the soil. This was achieved by dissolving 4.5496 g ammonium sulphate in 5 litre of distilled water, and incorporating 10 ml of this solution in the volume of water needed to raise the water content of the soil in the cups to the drained upper limit, the drained upper limit was determined using the method described by Passioura (2006:1075). The soil in the cups was then left for four weeks, during which the soil gradually dried. This was followed by incorporating the treatment-specific fertilisers in the soil in an enamel mixing bowl, before returning the soil to the cups and adding the volume of water needed to raise the water content of the soil in the cups to the drained upper limit. Addition of water marked the start of the 42-day incubation period. All cups were covered with a paper sheet to limit evaporation. Soil water content was maintained above 75% of plant available water by weighing the cups twice weekly and adding water when necessary.

On day 42, the experiment was terminated and the mineral N concentration in the different treatments was determined using the method described by Anderson and Ingram (1993:73-75). This involved the extraction of mineral N from the soil samples using a 0.5 molar potassium sulphate solution (Appendix 2.2). Extraction was done by transferring 7.5 ml soil from each incubation cup into 50 ml plastic extraction bottles to which 20 ml of the 0.5 molar potassium sulphate was added before shaking for 30 minutes on a Labcon Micro-processor Controlled Platform Shaker® at 160 oscillations per minute. The content of the bottles was then filtered through two Whatman™ No.1 filter papers, where after the leachate was analysed for NH_4^+-N and NO_3^--N on a Jenway 730 spectrophotometer. The sum of the NH_4^+-N and NO_3^--N in the soil was equated to the mineral N concentration, and this was used to calculate the net mineral nitrogen release value (NMNRV) of the manure samples using Equation 2.7.

$$NMNRV(\%) = \left(\frac{Nmt - Nco}{Nct - Nco}\right) \times 100\% \qquad (Equation\ 2.7)$$

whereby,

NMNRV (%) = Net mineral nitrogen release value

[Nmt] = mineral N concentration in the manure treatment after 42 days of incubation;

[Nct] = mineral N concentration in the chemical N fertiliser (urea) treatment after 42 days of incubation;

[Nco] = mineral N concentration in the control treatment after 42 days of incubation.

Analysis of variance (ANOVA) was used to assess treatment effect on the different nitrogen fertiliser values (NFV) that were obtained and treatment mean separation was done using the Fischer's protected least significant difference test (p=0.05). Comparison between the different NFVs was done by means of linear correlation analysis using Microsoft Excel® (2013).

2.4 Results

The results of the analysis of the primary data obtained in the greenhouse pot experiment and the laboratory incubation study are summarised in Table 2.3. The statistical analysis of the data appears in Appendix 2.3.

TABLE 2.3: Effect of source of N on the above-ground biomass and N content of maize grown in pots for 42 days, on N yield and quantity of N derived from soil pool (qNdfsp) and fertiliser (qNdff), and on the mineral N concentration in the soil after 42 days of incubation

Source of N	**Above-ground oven-dry biomass**	**N content**	**N yield**	**Ndff**	**qNdff**	**qNdfsp**	**Mineral N in soil after 42 days of incubation**
	(g pot^{-1})	(%)	(g pot^{-1})	ratio	(g pot^{-1})	(g pot^{-1})	(mg N kg soil^{-1})
Control	6.94^{e}	0.55^{c}	0.038^{f}	0.00	0.000	0.038^{e}	17.36^{e}
CM1	5.72^{f}	0.62^{bc}	0.035^{f}	0.43^{b}	0.015^{cd}	0.020^{f}	13.33^{f}
CM2	6.21^{f}	0.65^{b}	0.040^{f}	0.42^{b}	0.017^{cd}	0.023^{f}	16.36^{e}
CM3	7.56^{de}	0.62^{bc}	0.047^{ef}	0.22^{d}	0.010^{d}	0.037^{e}	16.31^{e}
GM1	7.86^{d}	0.69^{b}	0.054^{de}	0.24^{d}	0.013^{d}	0.041^{de}	19.56^{d}
GM2	9.99^{c}	0.64^{b}	0.064^{cd}	0.37^{bc}	0.024^{bc}	0.040^{de}	20.07^{d}
GM3	10.24^{c}	0.67^{b}	0.069^{c}	0.35^{c}	0.024^{bc}	0.045^{cd}	22.72^{c}
PM1	10.85^{c}	0.68^{b}	0.074$_{bc}$	0.42^{b}	0.031^{b}	0.043^{de}	22.96^{bc}
PM2	12.40^{b}	0.67^{b}	0.083^{b}	0.39^{bc}	0.032^{b}	0.051^{bc}	23.79^{bc}
PM3	12.89^{b}	0.67^{b}	0.086^{b}	0.39^{bc}	0.032^{b}	0.054^{b}	24.23^{b}
Urea	21.05^{a}	0.93^{a}	0.196^{a}	0.56^{a}	0.110^{a}	0.086^{a}	52.27^{a}
LSD (p=0.05)	**0.684**	**0.079**	**0.0141**	**0.057**	**0.0094**	**0.0066**	**0.882**

CM: Cattle manure; GM: Goat manure; PM: Chicken manure

The effect of source of N on the above-ground oven-dry biomass was highly significant ($p < 0.001$), with biomass ranging from 5.72 g pot^{-1} in the CM1 treatment to 21.05 g pot^{-1} in the urea treatment. All sources of N except CM1 and CM2 increased ($p \leq 0.05$) biomass production of maize relative to the control treatment. Among the nine manure treatments, chicken manure (PM samples) had the greatest positive effect on biomass production, followed by goat manure (GM samples). The effect of applying cattle kraal manure (CM) on biomass production varied between marginally positive (CM3) and marginally negative (CM1 and CM2).

The effect of source of N on the N content of maize was highly significant ($p < 0.001$). Treatment means ranged from 0.55% in the control treatment to 0.93% in the urea treatment. Whilst this result negated the assumption that N content of the plants grown in the pots would be constant across treatments, an assumption on which the ARNFV assay is based, the magnitude of the differences in N content was four times less than the magnitude of the differences in above-ground biomass. N content tended to be positively associated with biomass, with plants in the control treatment recording the lowest N content and plants in the urea treatment the highest ($p \leq 0.05$) but differences in N content among the nine manure treatments, which ranged between 0.62% and 0.69%, were not significant ($p > 0.05$).

The effect of source of N on the N yield was highly significant ($p < 0.001$). N yield ranged from 0.035% in the CM1 treatment to 0.196% in the urea treatment. The pattern in the N yield data was similar to that observed in the above-ground biomass data (poultry manure> goat manure > cattle manure).

The effect of source of N on the Ndff (proportion of plant N derived from fertiliser) was highly significant ($p<0.001$). Ndff values ranged between 0.22 in the CM3 treatment to 0.55 in the urea treatment. However, there appeared to be no clear pattern in the Ndff results. For example, cattle kraal manure treatments recorded both the highest (CM1) and lowest (CM3) Ndff values.

The effect of source of N on the qNdff (quantity of plant N derived from fertiliser) was highly significant ($p<0.001$), with values ranging between 0.015 g N per pot in the CM1 treatment to 0.110 g N per pot in the urea treatment. The pattern in the qNdff data was similar to the pattern in the above-ground biomass and N yield data (poultry manure> goat manure > cattle manure).

The effect of source of N on the qNdsp (quantity of plant N derived from soil pool) was highly significant ($p<0.001$). The qNdsp values ranged from 0.020 g N pot^{-1} in the CM1 treatment to 0.086 g N pot^{-1} in the urea treatment. The qNdsp value obtained in two of the three cattle manure treatments (CM1 and CM2) was significantly lower than the value obtained in the control treatment. For the other two types of manure, the qNdsp value was significantly higher ($p<0.05$) than in the control treatment. The pattern in the qNdsp data was similar to the pattern in the above-ground biomass, N yield and qNdff data (poultry manure> goat manure > cattle manure).

The effect of source of N on the concentration of mineral N in the soil after 42 days of incubation was highly significant ($p<0.001$). Values ranged from 13.33 mg N kg soil^{-1} in CM1 to 52.27 mg N kg soil^{-1} in the urea treatment. For the manure samples, the pattern in the data resembled that observed for above-ground biomass, with cattle kraal manure treatments (CM) recording the lowest concentrations of mineral N and chicken manure treatments (PM) the highest. All three cattle manure treatments tended to reduce mineral N concentration in the soil relative to the control treatment but this reduction was only significant in the CM1 treatment. Goat and chicken manure treatments all increased ($p \leq 0.05$) mineral N concentration in the soil relative to the control treatment. Noteworthy was that the mineral N concentration after 42 days of incubation in the urea treatment (52.27 mg N kg soil^{-1}) was almost identical to the sum of the mineral N concentration in the control treatment (17.36 mg N kg soil^{-1}) and the quantity on N added as urea (36.21 mg N kg soil^{-1}), as would be expected.

Estimates of the first-season NFV of the different manure samples were calculated using the data presented in Table 2.3. The manner in which these NFVs were obtained for each of the methods used was explained in section 2.3.1 of this manuscript. The different NFVs are presented in Table 2.4 and the statistical analysis appears in Appendix 2.4.

The effect of source of N on NFV was highly significant ($p<0.001$). NFV was lowest for the cattle manure samples, ranging between -1.86% and 4.38%, intermediate for the goat manure samples, ranging between 8.6% and 19.82%, and highest for the chicken manure samples, ranging between 23.75% and 30.75%. The effect of method on the NFV of the manure samples was also highly significant ($p<0.001$). The net mineral nitrogen release value, obtained by means of the laboratory incubation study, produced the lowest mean NFV (7.35%). The mean of the nitrogen fertiliser use efficiency values, which was second lowest (14.95%), and the mean of the ARNFVs obtained by means of the assay (16.75%), were reasonably similar.

TABLE 2.4: Nitrogen fertiliser value of nine animal manure samples obtained using four different methods

Source of N	NFV method				
	ARNFV%[1]	**^{15}N NFV%[2]**	**NFUE%[3]**	**NMNRV%[4]**	**Mean**
CM1	-8.63^{o}	13.72^{gh}	-1.66^{mn}	-11.54^{o}	-1.86^{f}
CM2	-5.19^{no}	15.21^{g}	1.43^{lm}	-2.86^{n}	2.26^{e}
CM3	4.38^{kl}	9.46^{ij}	5.90^{jk}	-3.01^{mn}	4.38^{e}
GM1	6.54^{jk}	11.76^{ghi}	10.39^{hij}	6.31^{jk}	8.69^{d}
GM2	21.63^{cd}	21.51^{cd}	16.60^{ef}	7.77^{jk}	17.02^{c}
GM3	23.41^{c}	22.01^{cd}	19.86^{de}	11.35^{ghi}	19.82^{c}
PM1	27.73^{b}	28.14^{b}	23.00^{cd}	16.05^{fg}	23.75^{b}
PM2	38.68^{a}	29.10^{b}	28.77^{b}	18.43^{f}	28.85^{a}
PM3	42.19^{a}	29.24^{b}	30.30^{b}	19.69^{f}	30.75^{a}
Mean	**16.75^{b}**	**20.02^{a}**	**14.95^{c}**	**7.35^{d}**	**14.83**

LSD ($p = 0.05$) Source of N = 3.365
LSD ($p = 0.05$) Method = 1.061
LSD ($p = 0.05$) Source of N x Method = 4.285

[1] ARNFV%: Apparent relative nitrogen fertiliser value assay;
[2] ^{15}N NFV%: Modified direct method;
[3] NFUE%: Nitrogen fertiliser use efficiency;
[4] NMNRV%: Net mineral nitrogen release value of the laboratory incubation study

The highest mean NFV was obtained by means of the modified direct method (20.02%). The source of N x method interaction effect on the NFV of the manure samples was highly significant ($p<0.001$) as well. Of particular importance was that the modified direct method yielded substantially higher NFV estimates for the cattle manure samples than any of the other methods but not for the chicken manure samples, and that the ARNFV assay yielded the widest range of values.

2.4.1 Relationship between the nitrogen fertiliser values obtained with the different methods

The relationships between the nitrogen fertiliser values obtained with the different methods (Table 2.4) were tested using linear regression analysis. The results of the analyses are presented in Figures 2.1 to 2.6. Figure 2.1 shows the relationship between the ARNFV% of the nine manure samples and their corresponding nitrogen fertiliser use efficiency (NFUE%).

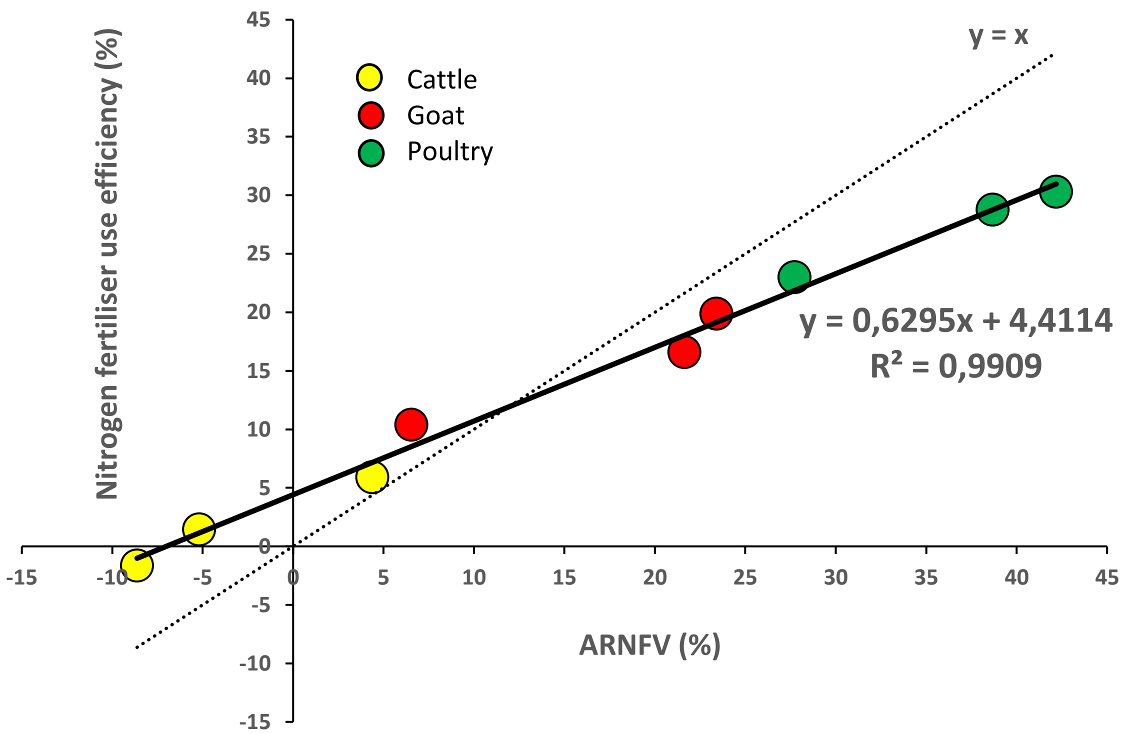

FIGURE 2.1: Relationship between the apparent relative nitrogen fertiliser value (ARNFV%) of nine manure samples and their corresponding nitrogen fertiliser use efficiency (NFUE%)

Both nitrogen fertiliser value estimates were correlated closely and signicantly (r=0.9954)**. The regression line equation had an intercept of +4.4 and a slope of 0.63. This meant that the model predicted that both methods would yield exactly the same NFV for manure with an ARNFV% of 11.1% and that differences between the two NFV estimates would widen as the ANRFV% increased above or dropped below this value.

Figure 2.2 shows the relationship between the ARNFV% of the nine manure samples and their corresponding NMNRV% obtained using incubation.

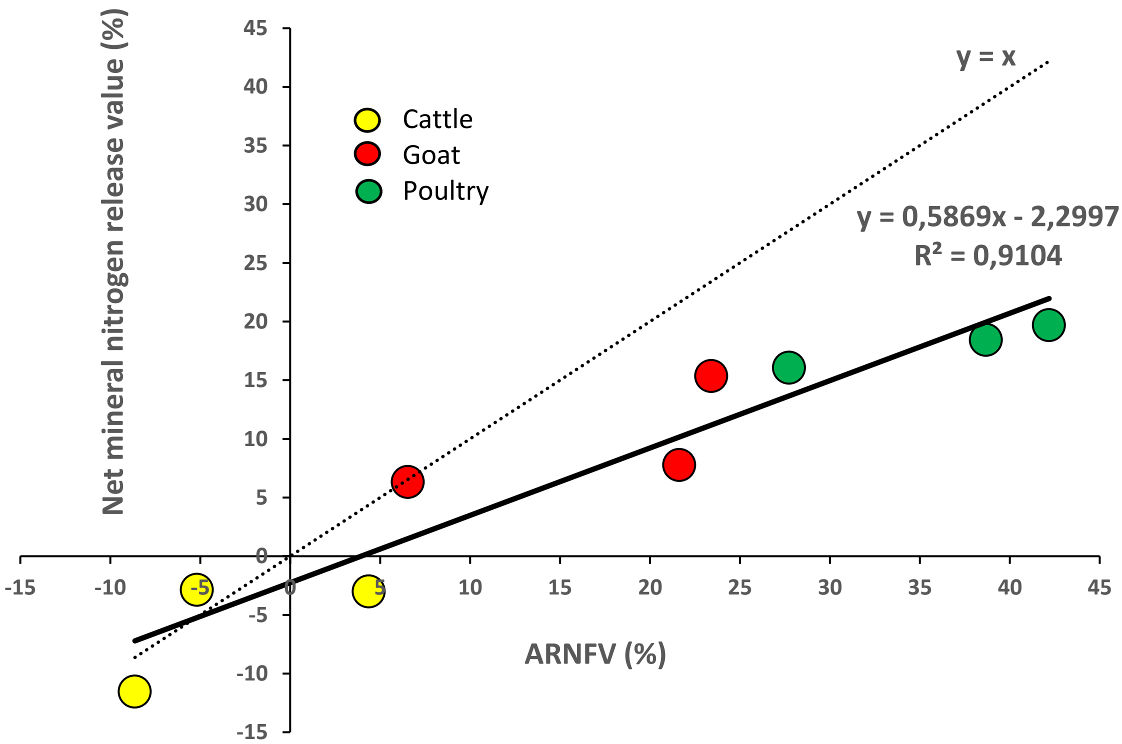

FIGURE 2.2: Relationship between the apparent relative nitrogen fertiliser value (ARNFV%) of nine manure samples and their corresponding net mineral nitrogen release value (NMNRV%)

Both NFV estimates were correlated closely and signicantly (r=0.9541)**. The regression line describing the linear relationship between NMNRV% and ARNFV%

had a small, negative intercept (-2.3) and a slope of 0.59. This meant that the model predicted the ARNFV% of manure to be persistently higher than the NMNRV% for any manure with an ARNFV% greater than -5.6%.

Figure 2.3 shows the relationship between the ARNFV% of nine manure samples and their corresponding NFV estimate obtained with the modified direct method (^{15}N NFV%).

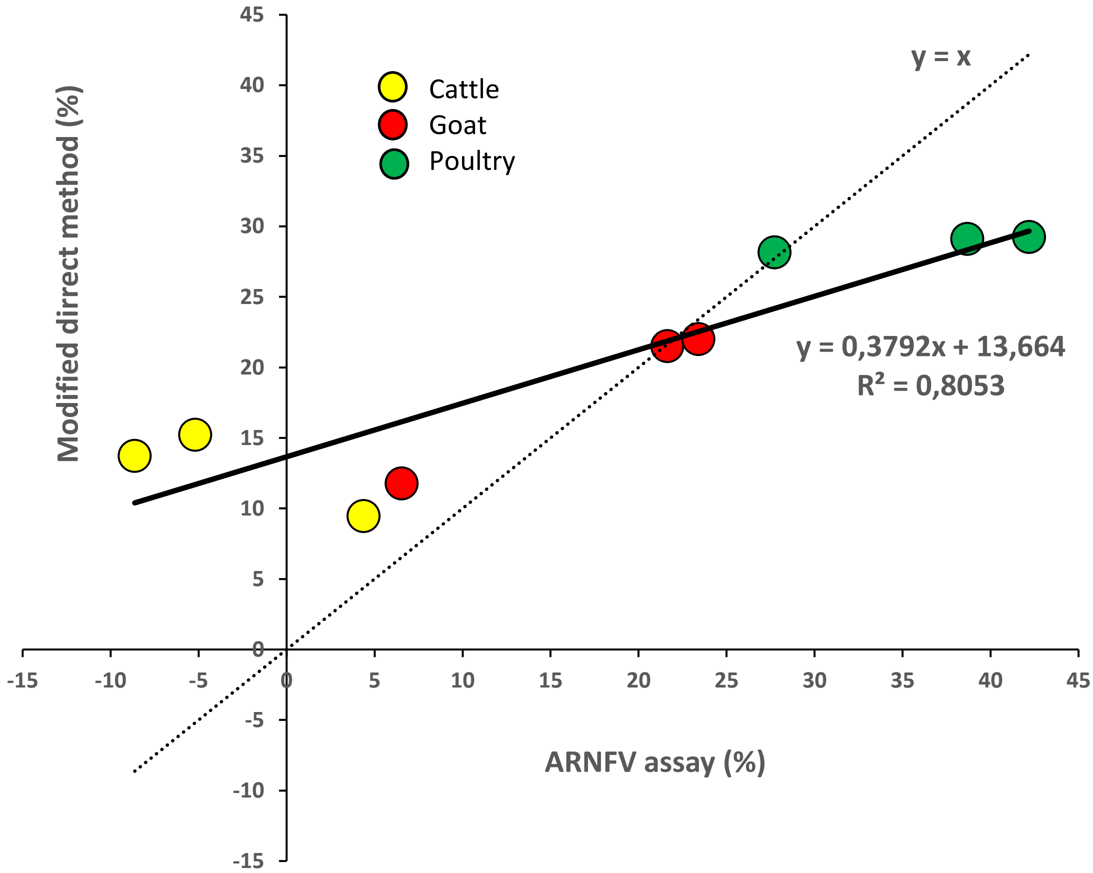

FIGURE 2.3: Relationship between the apparent relative nitrogen fertiliser value (ARNFV%) of nine manure samples and their corresponding nitrogen fertiliser value (^{15}N NFV%) obtained with the modified direct method

The correlation between the two NFV estimates was close and signicant (r=0.8053)** The regression line describing the linear relationship between the ^{15}N NFV% and ARNFV% had a large positive intercept (+13.6) and a gentle slope (0.38). Generally, this reflected relatively high ^{15}N NFV% values for samples with a low ARNFV% and relatively low ^{15}N NFV% values for samples with a high ARNFV%.

Figure 2.4 shows the relationship between the nitrogen fertiliser use efficiency (NFUE%) of nine manure samples and their corresponding net mineral nitrogen release value (NMNRV%).

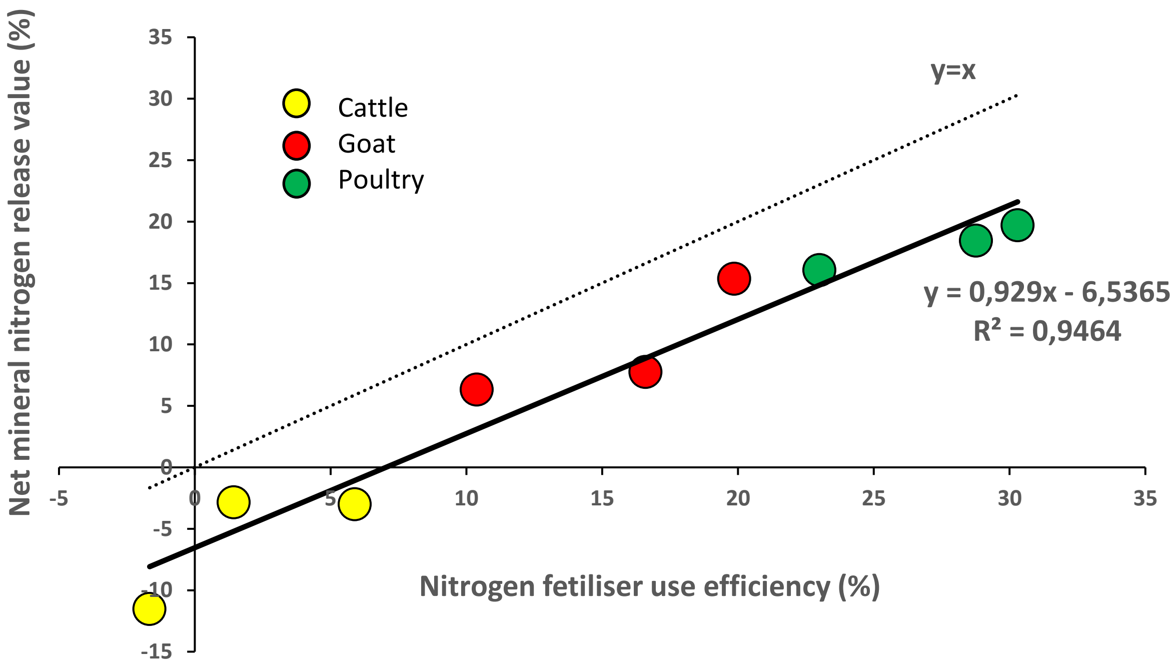

FIGURE 2.4: Relationship between the nitrogen fertiliser use efficiency (NFUE%) of nine manure samples and their corresponding net mineral nitrogen release value (NMNRV%)

Both estimates of the NFV of manure were correlated closely and signicantly (r=0.929)**. The regression line describing their linear relationship had a negative intercept (-6.5) and a slope of 0.93. The negative intercept and the slope close to 1 of the model meant that the model predicted that the NMNRV% of manure samples lagged their corresponding NFUE% by a fairly constant precentage (6.5%), with a slight widening of the difference between the two estimates as the NFUE% increased above zero.

Figure 2.5 shows the relationship between the nitrogen fertiliser use efficiency (NFUE%) of the nine manure samples and their corrresponding ^{15}N NFV% obtained using the modified direct method.

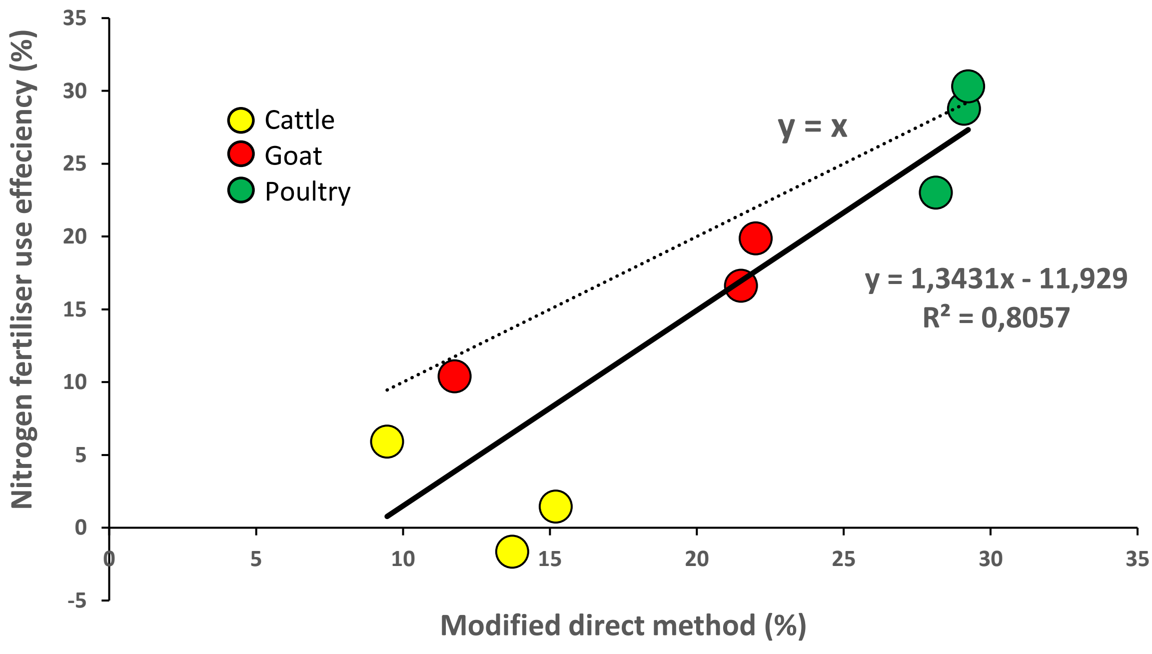

FIGURE 2.5: Relationship between the nitrogen fertiliser value of nine manure samples obtained with the modified direct method (^{15}N NFV%) and their corresponding nitrogen fertiliser use efficiency (NFUE%)

Both NFV estimates were correlated closely and signicantly (r=0.8057)**. The model had a large negative intercept (-11.9) and a slope greater than one (1.34). The intercept meant that that the model predicted that manure with an NFUE% of zero had an ^{15}N NFV% of 11.9% but the slope of 1.34 meant that differences between the two estimates narrowed as the NFUE% of manure rose above zero. The model predicted that both methods would yield the same value for manure with an NFUE% of 34.8%.

Figure 2.6 shows the relationship between the nitrogen fertiliser value of nine manure samples obtained with the modified direct method (^{15}N NFV%) and their corresponding net mineral nitrogen release values (NMNRV%).

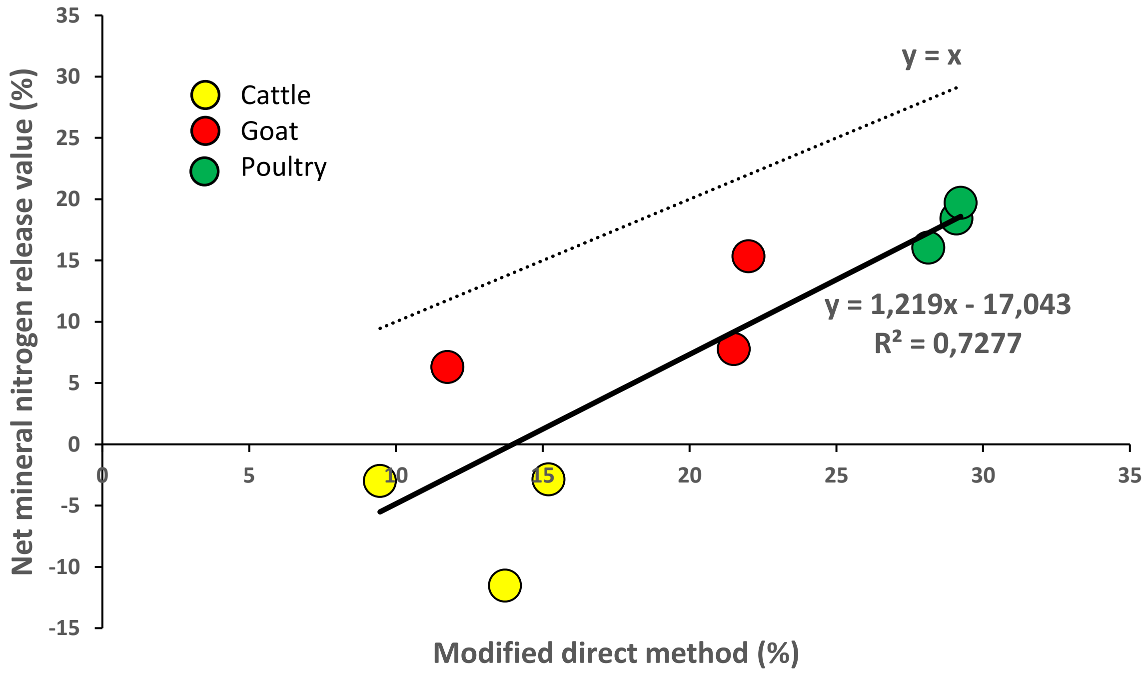

FIGURE 2.6: Relationship between the nitrogen fertiliser value of nine manure samples obtained with the modified direct method (^{15}N NFV%) and their corresponding net mineral N release values (NMNRV%)

Both NFV estimates were correlated closely and signicantly (r=0.7277)**. The regression line describing the linear relationship between them had a negative intercept (-17) and a slope of 1.2. As a consequence, when manure had a zero ^{15}N NFV%, the model predicted the corresponding NMNRV% to be about -17%. Differences between the two estimates tended to decrease as the ^{15}N NFV% of manure increased, but over the full range of manure samples tested, the ^{15}N NFV% remained greater than the NMNRV%.

2.5 DISCUSSION

The validity of the apparent relative nitrogen fertiliser value assay (ARNFV) was tested by using the assay to determine the nitrogen fertiliser value (NFV) of nine manure samples and comparing the values obtained with the NFV values recorded using three other established methods. The findings of the study showed that the four methods yielded different NFV estimates. Comparing the results of the different methods showed that in terms of the slope of the linear regression equation, the closest fit (slope of 0.929) was between the nitrogen fertiliser use efficiency (NFUE) and the net mineral nitrogen release value (NMNRV). The latter estimate was obtained through incubation, which was the only method that did not rely on plants to provide an NFV estimate of manure. This method also produced the lowest NFV estimates of the four methods used (Table 2.4). One possible reason for these low NFV estimates could be that the single measurement taken after 42 days of incubation did not capture the dynamics of the mineral N concentration in soil amended with organic fertilisers. Kaleem *et al*., 2007:1700; Azeez and Van Averbeke (2010:5650), Ngwenya (2017:62-64) and Mthimkulu (2019:67-68) all reported that mineral N in soil amended with organic fertiliser varied considerably as incubation progressed, showing intermittent periods of net mineralisation and net immobilisation of N. It can be expected that plants growing in the soil make the most of periods of net mineralisation to take up N, thus promoting their growth. Ngwenya (2017:67), who measured the mineral N release form nine manure samples at weekly intervals, demonstrated the effect of the mineral N release dynamics on the NMNRV of manure. Compared to when the NMNRV of manure was based on a single measurement after 42 days of incubation, the reasecher reported that the NMNRV of the manure samples increased by an average of 5.1% when the mineral release N dynamics during incubation period were taken into account. When this 5.1% increase is added to the NMNRV estimates obtained in the current study (Fig. 2.4), the intercept of the linear regression model that describes the relationship between the NMNRV and NFUE estimates is reduced from -6.5365% to -1.4365%. Accordingly, the regression equation that relates the results of the two methods would become NMNRV% = 0.929 NFUE% -1.4365%, which suggest a nearly perfect match between these two NFV estimates. A second possible reason for the low NFV estimates obtained with the incubation method could be that some of the N taken up by the maize plants was in organic forms. Näsholm, Huss-danell and Hogberg (2000:1155), Paungfoo-Lonhienne *et al*. (2008:4526), Jones *et al*. (2005:415) and Vonk *et al*. (2008:548) all provided evidence of plants taking up dissolved organic forms of nitrogen with low molecular weight.

Comparing ARNFV estimates with NFUE estimates of the NFV of manure (Fig. 2.1), the slope (0.63) of the linerar regression indicates that NFUE estimates were on average 0.63 times lower than ARNFV estimates. Part of the over-estimation of the NFV of manure by the ARNFV assay was due to the difference in the N content of maize plants in the manure treatments, which ranged between 0.62% and 0.69% with a mean of 0.66%, and the N content of the plants in the urea treatment (0.93%), as shown in Table 2.3. The ratio of the avarage N content of the plants grown in the nine manure treatments (0.66%) and the N content of those grown in the urea treatment (0.93%) is 0.71. This ratio approximates the slope of the regression equation of 0.63. The postive intercept of 4.41% of the regression equation is explained by the low N content of the plants in the control treatment (0.53%) compared to the manure (0.66%) and the urea treatments (0.93%). This meant that when calculating the ARNFV of manure, both the biomass of the plants in the manure treatment and the urea treatment were reduced by a value (biomass in the control treatment) that was too high. As a result, when the ARNFV of a manure sample equalled zero, the NFUE was still positive (4.41%). It follows that the ARNFV assay has a major flaw, which arises from the assumption that the N content of maize plants grown over a period of 42 days is constant. This was shown not to be true, confirming other work that demonstrated the dependence of the N content of maize plants during the early vegetative stages of growth on level of plant-available N in the soil (Alexandrova & Donov, 2003:42).

The ^{15}N NFV values obtained using the modified direct method results were least related to the results obtained with the other three methods. Generally, the modified direct method was less sensitive to differences among manure samples and produced fairly high NFV estimates for samples which the other three methods identified as lacking any NFV (zero value). The reason for this abberation was that the ^{15}N NFV, as defined in this study, did not take into account the effect of the added source of N on the quantity of nitrogen which maize plants derived from the soil pool. Table 2.3 shows that this effect was considerable.

2.6 CONCLUSION

The internal validity of a pot-based adaptation of the N-fertiliser equivalence method, called the ‘apparent relative nitrogen fertiliser value’ (ARNFV) assay was assessed. The results showed that the assay was flawed because it assumes N content of the maize plants grown in the pots to be independent of the level of plant-available N in the soil, thus enabling the use of plant biomass as a suitable proxy for N yield. This assumption was shown to be invalid as significant differences in N content were observed among plants grown in the control soil, soil amended with manure and soil amended with chemical N fertiliser. This caused an underestimation of the NFV of manure samples with low NFV (cattle manure) and an overestimation of manure samples with a high NFV (chicken manure). Net mineral N release during incubation and N yield, obtained by measuring the N content of the maize test crop, provided NFV estimates that were closely correlated but NFV estimates using incubation were 6.5% lower than estimates based on N yield. Measuring net N mineralisation on a weekly basis instead of once at the end of the incubation period could narrow this gap. Despite its flaw, the ARNFV assay provided NFV estimates of manure that were closely correlated to those based on incubation and N yield and provided a useful indication of the NFV of manure, making it an appropriate choice under circumstances where instrumentation for the application of alternative methods is not available.

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CHAPTER 3

3 EFFECT OF MINERAL N APPLICATION ON THE NITROGEN FERTILISER VALUE OF CATTLE KRAAL MANURE

3.1 INTRODUCTION

Chadwick *et al*. (2000:166) defined the N-fertiliser value of manure as the sum of its inorganic N content and its organic N-fraction that is mineralised during a specified period of time. Generally, the plant-availability of N in manure is low in comparison to that in chemical fertilisers (Harris & Yusuf, 2001:325). For this reason, combining the application of manure and mineral N-fertilisers is widely used as a strategy to ensure adequate N availability for crops (Kumwenda *et al*., 1996:5; Harris, 2002:144). Palm, Myers and Nandwa (1997:212) and Opala (2011:69) developed a decision tree that uses selected properties of the organic source of N to decide whether the source can be applied directly to the soil as is, needs to be combined with the application of mineral N fertiliser, or should rather be applied as a surface mulch to ensure adequate plant-availability of N. From the decision tree presented in Figure 3.1, it can be seen that the properties of the organic source that underpin the decision tree are N-content of the organic source and its lignin and polyphenol content. The decision tree indicates that incorporation of an organic source of N into the soil should be combined with the application chemical N when the organic source has a low N content (<2.5%) and contains relatively low levels of lignin (<15%) and polyphenol content (<4%) or when both its N content (>2.5%) and lignin (>15%) and polyphenol content (>4%) are high.

The guidelines also indicate that when the organic source of N has both a low N content and a high lignin (>15%) and polyphenol content (>4%), combining application with chemical N fertiliser is expected to have little positive effect on N availability, and for this reason, it is recommended that the organic material is not incorporated in the soil but rather applied as a mulch.

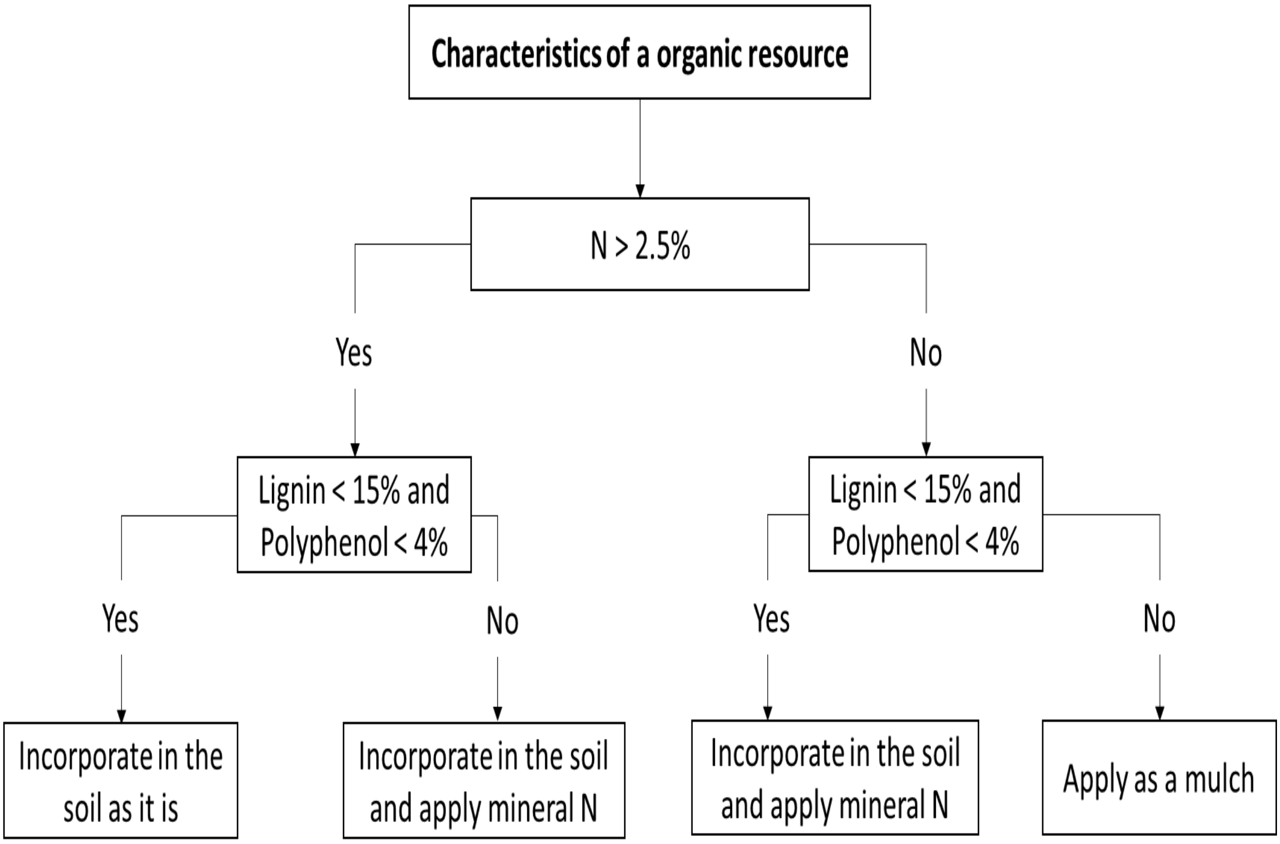

FIGURE 3.1: A tree diagram guiding the use of organic resources (Palm, Myers & Nandwa, 1997:212; Opala, 2011:69).

Combining mineral and organic sources of N to enhance plant-availability of N in soil may not be the only benefit derived from this practice. It could also augment mineralisation of N from the organic source as suggested by Myers *et al*. (1994:97).

Accordingly, the objective of this study was to determine the effect of combining the application of cattle kraal manure and chemical N fertiliser on the plant-availability of soil N and on the nitrogen fertiliser value (NFV) of the cattle kraal manure.

3.2 MATERIALS AND METHODS

3.2.1 Treatments and experimental design

A pot experiment with five treatments was conducted in the green house of the Tshwane University of Technology (Pretoria campus) using maize pan 67 as a test crop. The first treatment was the control, in which no source of N was added to the soil. The second treatment involved the application of N at the rate of 90 kg N ha^{-1} as limestone ammonium nitrate (LAN), and in the third treatment cattle kraal manure was applied to the soil at the rate of 90 kg N ha^{-1}. In the fourth treatment, the application of N at the rate of 90 kg N ha^{-1} as cattle kraal manure was combined with the application of limestone ammonium nitrate (LAN) at the rate of 30 kg N ha^{-1}. The fifth treatment consisted of the application of (LAN) at the rate of 30 kg N ha^{-1}. These five treatments were arranged in a completely randomised design and were replicated four times.

3.2.2 Materials

The cattle kraal manure used in the study contained 1.62% N, 9.71% lignin and 0.17% polyphenol. According to the decision tree presented in Figure 3.1, the cattle manure should be applied in combination with chemical N fertiliser to optimise plant available N, because the N content of the manure was less than 2.5% but both its lignin and polyphenol contents were below the critical limits of 15% and 4%, respectively. A summary of the properties of the cattle kraal manure that was used in the study are presented in Table 2.2 under the entry 'Cattle 3'. The same low-fertile sandy clay loam subsoil as described in section 2.3.1 of this dissertation was used to fill the pots.

3.2.3 Methods

Before planting, the soil water content was raised to the upper limit using 1000 ml of water per pot. In each pot 16 seeds of maize (*Zea mays* var. *indentata*) employing the cultivar PAN 67 obtained from Pannar in Greytown, were planted in each pot. After emergence, about a week after planting, the seedlings were thinned to leave eight healthy looking plants in each pot. The experiment was irrigated according to the ARNFV protocol described in section 2.3.1 of this dissertation. Harvesting was done 42 days after planting, whereby the eight maize plants were cut at soil surface level with a pair of scissors. The maize plants were then placed in labelled brown paper bags, and oven dried at 60 ˚C until constant mass. Weighing was done a Snug III digital lab scale with a capacity of 120 g and an accuracy of 0.001 g. Biomass recorded in the various treatments were used to calculate the NFV of the cattle kraal manure for the two treatments in which this organic source of N was applied. The NFV of the cattle kraal manure in the manure only treatment was calculated using equation 3.1.

$$\text{NFV (\%)} = \left(\frac{\text{M 90N} - \text{C}}{\text{LAN 90N} - \text{C}}\right) \times 100 \text{ (Equation 3.1)}$$

Whereby;

NFV (%) = nitrogen fertiliser value of manure expressed as a percentage;

M 90N = Oven-dry above-ground biomass obtained in the treatment in which N was applied as cattle kraal manure at the rate of 90 kg N ha^{-1}; and

C = Oven-dry above-ground biomass obtained in the control treatment;

LAN 90 N = Oven-dry above-ground biomass obtained in the treatment in which N was applied as limestone ammonium nitrate (LAN) at the rate of 90 kg N ha^{-1}.

The NFV of the cattle kraal manure in the treatment that combined its application was combined with the application of limestone ammonium nitrate (LAN) was calculated using equation 3.2.

$$\mathrm{NFV}\ (\%) = \left(\frac{((\mathrm{M\ 90N + LAN\ 30N}) - \mathrm{C})) - (\mathrm{LAN\ 30N} - \mathrm{C})}{\mathrm{LAN\ 90N} - \mathrm{C}}\right) \times 100\ (\text{Equation 3.2})$$

Whereby;

NFV (%) = nitrogen fertiliser value of manure expressed as a percentage;

M 90N + LAN 30N = Oven-dry above-ground biomass obtained in the treatment in which N was applied as cattle kraal manure at the rate of 90 kg N ha^{-1} and as limestone ammonium nitrate at the rate of 30 kg ha^{-1};

C = Oven-dry above-ground biomass obtained in the control treatment;

LAN 30 N = Oven-dry above-ground biomass obtained in the treatment in which N was applied as limestone ammonium nitrate (LAN) at the rate of 30 kg N ha^{-1}; and

LAN 90 N = Oven-dry above-ground biomass obtained in the treatment in which N was applied as limestone ammonium nitrate (LAN) at the rate of 90 kg N ha^{-1}.

3.2.4 Statistical analysis

The biomass and NFV data were captured on a spread sheets using Microsoft Excel® (2013). Treatment effects were assessed using analysis of variance (ANOVA), and when such effects were significant ($p \leq 0.05$), the Fischer's protected least significant difference (LSD) test (p=0.05) was used to separate treatment means. The Genstat software version 11.1 was used for the ANOVA and LSD tests.

3.3 RESULTS

3.3.1 Biomass production

The biomass data and statistical analysis of the data are presented in Appendix 3.1. The analysis of variance shows that the effect of fertiliser on biomass production was highly significant ($p<0.001$). Table 3.1 presents the biomass means obtained in the different treatments.

TABLE 3.1: Effect of fertiliser on the oven-dry above-ground biomass (g) of maize after 42 days of growth

Treatment	**Oven-dry above-ground biomass (g)**
Control	4.01^{c}
M 90N	4.40^{c}
M 90N + LAN 30N	9.54^{b}
LAN 30N	9.34^{b}
LAN 90N	14.50^{a}
LSD (p=0.05)	**1.085**

The highest biomass production was observed in the chemical fertiliser treatment in which LAN was applied at the rate of 90 kg N ha^{-1} (LAN 90N). The lowest biomass was obtained from the control treatment but biomass produced in the treatment in which cattle kraal manure was applied at the rate of 90 kg N ha^{-1} (M 90N) did not differ significantly ($p>0.05$) from that recorded in the control. Combining the application of cattle kraal manure at the rate of 90 kg N ha^{-1} with the application of LAN at the rate of 30 kg N ha^{-1} (M 90N + LAN 30N) more than doubled biomass production of maize compared to when manure only was applied (M 90N). However, there was no significant difference ($p>0.05$) between the biomass obtained from theM 90N + LAN 30N treatment and the treatment in which only LAN was applied at the rate of 30 kg N ha^{-1} (LAN 30N).

3.3.2 Nitrogen fertiliser value of cattle kraal manure

The effect of combining cattle kraal manure and chemical N fertiliser on the nitrogen fertiliser value (NFV) of the cattle kraal manure is shown in Table 3.2. The statistical analysis of the data is presented in Appendix 3.2.

TABLE 3.2: Effect of mineral N application on the NFV (%) of cattle kraal manure

Treatment	**Nitrogen fertiliser value (%)**
M 90N	3.69[a]
M 90N + LAN 30N	2.97[a]
	Difference not significant ($p=0.05$)

The analysis of variance indicates that combining application of the cattle kraal manure with N in chemical form had no significant effect ($p=0.730$) on the NFV of the manure. The NFV of the manure (CKM 3) was equally low in both treatments (3.69% and 2.97%) and similar to the value of 4.38% reported for CKM 3 in the ARNV assay assessment (Table 2.4).

3.4 DISCUSSION

The results of the study showed that maize production was increased by combining application of cattle kraal manure with chemical fertiliser N. This confirms the findings of other studies, including Ghosh and Sharma (1999:464) in India and Nyamangara, Piha, and Giller (2003:292) and Nyamangara, Mudhara and Giller (2005:61) in East Africa. The positive effect on crop yield of combining mineral and organic sources of N could be solely due to the N added in mineral form but there could also be an improvement in the NFV of the organic source. Bremer, van Houtum and van Kessel (1991:222) argued that the effect of adding mineral N on net N release from the organic source would most probably vary, because organic materials have unique qualities and the effect of added N depends on the chemical composition of the organic material. Fog (1988:456) further explained that positive effects on net N release from the organic source were likely to occur when the organic carbon compounds present in the organic source were easily degradable, whilst negative effects were likely when these organic carbon compounds were recalcitrant, as in the case of lignin and polyphenol. The observation that the NFV of CKM 3 was not affected by the addition of chemical N suggests that CKM3 is a source that falls between these two extremes.

3.5 CONCLUSION

The objective of this study was to determine the effect of combining the application of cattle kraal manure and chemical fertiliser N on plant availability of N in soil and on the NFV of the cattle kraal manure. The results showed that adding both chemical fertiliser N and cattle kraal manure increased plant available N in the soil as evidenced by a substantial increase in biomass production of the test crop. The magnitude of this increase appeared to be propotional to the quantity of chemical N that was applied, indicating that the addition of chemical N had no effect on the nitrogen fertiliser value of manure. Further work involving organic fertilisers with widely varying compositions is needed to determine whether the absence of this effect on the NFV of organic materials is universal or it is simply a function of the type of organic material applied to soil.

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CHAPTER 4

4 SUMMARY AND CONCLUSIONS

Incorporating organic material, particularly animal manure, into cultivated soil can contribute to meeting the nitrogen (N) requirements of crops. For the majority of smallholder farmers in South Africa, application of animal manure is the main way in which N removed from soil by harvesting crops is replenished. Animal manure is a term that encompasses a wide range of materials with varying chemical and physical properties. Providing smallholders with application guidelines when using animal manure as a fertiliser requires improved understanding of the effect various types of animal manure have on the plant-availability of nutrients when applied to soil. The aim of this study was to contribute to improved understanding of the effects of applying animal manure on the plant- availability of N in soil. N is widely regarded as the most limiting nutrient in smallholder farming in South Africa.

The study was made up of two parts. The objective of the first part was to assess the validity of the ARNFV assay, which is a quick, relatively cheap pot-based method that uses the measurement of maize biomass production over a 42-day period to estimate the first-season NFV of organic sources of N added to soil. The assay involves three treatments, namely native soil (control), a treatment in which the organic source is applied at a particular rate of N (90 kg N ha^{-1}) and a treatment in which N is applied in chemical form at the same rate as the organic source. Obtaining NFV estimates of organic sources of N is important, because it enables prediction of crop response to the application of organic sources of N to soil at particular rates. Comparing the NFV estimates of nine manure samples obtained with the ARNFV assay with those provided by established methods it was found that ARNFV assay estimates were closely correlated with net mineral N release from the manure samples during incubation and also with N yield but the assay had the flaw of assuming that the N content of the maize plants across treatments was constant. This assumption was shown to be invalid, because the N content of maize plants grown in the control treatment was in most instances lower than that of plants grown in soil amended with manure, which, in turn, was lower than the N content of plants grown in the chemical N treatment. As a result, when compared with the N yield method, the ARNFV assay tended to progressively underestimate the NFV of manure samples as the NFV of manure samples dropped below 11.1%, and progressively overestimate the NFV of manure samples as the NFV increased above this value. Despite this flaw, the ARNFV assay was shown to be a method that could be used to good effect to obtain an indication of the first season NFV of organic sources of N.

Application of the assay clearly identified significant differences in first-season NFV among cattle kraal manure, goat manure and chicken manure samples, which were also identified by the established methods. Of particular significance was the low NFV of the three cattle manure samples, because cattle manure is the main type of manure used by South African smallholders to improve the fertility of their cropped lands.

In terms of ease of use and cost, the ARNFV assay has the weakness or requiring knowledge of the N content of the organic source. Obtaining such information is fairly costly and may not always be practically possible. Further work aimed at removing this requirement is, therefore, recommended.

The second part of the study related to the low first-season NFV of the cattle manure samples that featured in the ARNFV assay. Elsewhere, particularly in East Africa, the strategy of combining low-quality cattle kraal manure with chemical N fertilisers to improve the plant-availability of N in soil has been used to good effect. The positive effect on crop yields of this strategy arises primarily from the N added in chemical form but could also partly be due to an increase in the NFV of the manure. Accordingly, the objective of the second part of the study was to determine the effect of combining the application manure and chemical N fertiliser on plant-availability of N in soil and on the nitrogen fertiliser value (NFV) of a cattle kraal manure sample with an NFV of 4%. The results confirmed that the strategy increased plant-availability of N in soil but quantitatively, this increase was identical to that obtained by applying the chemical source of N without manure addition. By implication, the strategy had no effect on the NFV of the manure used.

From a practical perspective, the study raises important questions about the usefulness of cattle kraal manure as a supplier of plant available N in cultivated soils. The results suggest that the ability of cattle kraal manure to provide crops with nitrogen is limited but the scope of the study was limited to investigating the first-season NFV of manure. It is quite possible that the NFV of cattle kraal manure increases over time as a result of the residual effects of previous applications. An enquiry into such possible residual effects is, therefore, recommended.