Project Report No. 489 April 2012 £9.24

Rapid analysis of manure and organic recyclables for sustainable agriculture via Near Infrared Reflectance Spectroscopy (NIRS)

April 2012
Cost £9.24

Project Report No. 489

Rapid analysis of manure and organic recyclables for sustainable agriculture via Near Infrared Reflectance Spectroscopy (NIRS)

by
Ken Smith

ADAS Wolverhampton, Woodthorpe, Wolverhampton WV6 8TQ

This is the final report of a 42 month project (RD-2005-3169) which started in October 2007. This was a Defra LINK project (LK0988) with a contract for £65,000 from HGCA.

While the Agriculture and Horticulture Development Board, operating through its HGCA division, seeks to ensure that the information contained within this document is accurate at the time of printing, no warranty is given in respect thereof and, to the maximum extent permitted by law, the Agriculture and Horticulture Development Board accepts no liability for loss, damage or injury howsoever caused (including that caused by negligence) or suffered directly or indirectly in relation to information and opinions contained in or omitted from this document.

Reference herein to trade names and proprietary products without stating that they are protected does not imply that they may be regarded as unprotected and thus free for general use. No endorsement of named products is intended, nor is any criticism implied of other alternative, but unnamed, products.

HGCA is the cereals and oilseeds division of the Agriculture and Horticulture Development Board.

CONTENTS

1. **ABSTRACT**

This project set out to promote the sustainable recycling of organic manures in UK agriculture, through the development of Near Infrared Reflectance Spectroscopy (NIRS) as a robust and reliable technology for multi-nutrient manure analysis.

Within this research, NIRS has been successfully developed for estimation of the nutrient content of livestock manures and biosolids via multiple, rapid, scanning of fresh samples. The initial focus of the research was on the development of robust calibration models for estimation of dry matter, total N, NH_4-N, SO_3, P_2O_5, K_2O, MgO and pH, covering a range of manure types and treated biosolids. Performance of the calibrations for conventional analysis of manure and biosolids samples was as follows: *excellent* – DM, total N; *successful* – NH_4-N, P_2O_5; *moderately successful* – SO_3, K_2O; *moderately useful* – MgO. Performance for pH was unsatisfactory, which was not a surprise given the limited range in pH (almost all between pH 6.5 and 9.0), even within the very large range of samples scanned and analysed.

Manure and biosolids samples from the NIR spectral database were also selected for N mineralisation and N recovery studies to develop a calibration model for the estimation of N release from the organic component of manure N. Data for the calibration model were derived from a large experiment with ryegrass grown in large pots, tracking the release of N from manures applied to 3 different soil types (clay loam, sandy loam, loamy sand) at two sites for 3 years (180 manures in total). Correlations with derived variables were good, and best with the data expressed as g N/tonne/day, indicating a good estimate of manure N uptake over the season was possible via the NIRS scanning procedure. The improvement in the calibration of the NIRS model for predicting N recovery, compared to proportion of available N alone as a predictor, indicates that the NIRS model is able to account for the mineral N component and mineralisation of organic N in estimating manure N release.

In the final year of the project, the developed NIR calibration models were used to study nutrient content of intensively sampled manure storage and field spread manures. The results were used to evaluate a range of sampling strategies, the conclusions supporting current advice for a minimum of ten samples from well constructed solid manure storage. They also confirmed the benefits of a reliable analytical service for improved utilisation of manure nutrients.

The potential for NIRS-based characterisation of soils for nutrient content and soil microbial activity/community, was also assessed. The soil characterisation work showed potential for application with certain nutrients; organic matter, total C, pH, total P, organic P, total N; it appeared likely that the models could be improved by increasing the calibration sample base and by restricting calibration to a single soil type.

2. SUMMARY

This LINK Project set out to promote the sustainable recycling of organic manures in UK agriculture, through the development of Near Infrared Reflectance Spectroscopy (NIRS) as a robust and reliable technology for multi-nutrient manure analysis. Current wet chemistry methods of laboratory analysis for organic materials are slow, relatively expensive and can give inconsistent results for solid and semi-solid materials (due to the variability of the material and the small sample size used by current wet chemistry methods). Interest in manures and biosolids as nutrient sources has increased as a result of increased fertiliser prices and farmers are now more receptive to the use of practical aids for improved recycling of manures (i.e. lab analysis, nutrient management software etc.). A reduced-cost and reliable technology for manure analysis is thought likely to encourage greater uptake of manure analysis as a strategic aid to manure and nutrient management and, thereby, reduced environmental impacts of manure use and increased profitability of crop production.

Within this research, NIRS has been successfully developed for estimation of the nutrient content of livestock manures and biosolids via multiple, rapid, scanning of fresh samples. The initial focus of the research was on the development of robust calibration models for estimation of dry matter, total N, NH_4-N, SO_3, P_2O_5, K_2O, MgO and pH, covering a range of manure types and treated biosolids. This process required the collection of a large number of samples in order to cover the range of likely analyses, sample types and NIR spectral variability. In 2008-2009 almost 2200 samples were collected, from throughout the UK and Ireland, with the active involvement of the project industry partners. A detailed sampling protocol was produced to ensure a consistent approach for the collection, labelling, storage and submission of samples. All samples were logged on receipt and NIR scanned as soon as possible thereafter. Principal Component Analysis (PCA) was used to select a set of calibration samples (418 samples) that were representative of the spectral variability of the larger database. These samples were NIR scanned again (6x) and analysed by wet chemistry following a robust sample homogenisation procedure developed within earlier research.

NIRS scanning of the manures was performed using a FT-NIR instrument, the Matrix-I (Bruker UK, Ltd) and refinement of the calibration models was undertaken with expert chemometrics input from Bruker. From the statistics associated with the validation procedure (Appendix 2), performance of the calibrations for conventional analysis of manure and biosolids samples was as follows: *excellent* – DM, total N; *successful* – NH_4-N, P_2O_5; *moderately successful* – SO_3, K_2O; *moderately useful* – MgO. Performance for pH was unsatisfactory, which was not a surprise given the limited range in pH (almost all between pH 6.5 and 9.0), even within the very large range of samples scanned and analysed.

Manure and biosolids samples from the NIR spectral database were also selected for N mineralisation and N recovery studies in order to develop a calibration model for the estimation of N release from the organic component of manure N. In this case, data for the calibration model were derived from a large experiment with ryegrass grown in large pots (10 litre), tracking the release of N from manures applied to 3 different soil types (clay loam, sandy loam, loamy sand) at two sites: ADAS Boxworth, Cambs and North Wyke, Devon, for 3 years (180 manures in total). The pots were set in plastic tunnels, with overhead irrigation, to ensure that moisture was not limiting and dry matter yield and N offtake were recorded for each cut of grass. The experiment generated a large amount of data from the 180 manures, all of which were NIR scanned; with the 6x replicate scanning procedure this resulted in a total of 1080 scans. Regressions were run with the calibration models and grass N uptake data expressed in several ways:

- kg manure N per hectare;
- N uptake as percentage of manure N applied;
- N uptake as percentage of manure organic N applied;
- g N per tonne applied;
- g N per tonne of manure applied per day;
- g N uptake per 100 day degrees (thermal time).

Correlations with these derived variables were good, and best with the data expressed as g N/tonne/day, thus indicating a good estimate of manure N uptake over the season was possible via the NIRS scanning procedure. However, detailed analysis of the experimental N uptake results also showed a good correlation between manure N recovery and the mineral N content of the manures (both as NH_4-N and NH_4-N as % of total N), indicating that N uptake was dominated by mineral N content. The improvement in the calibration of the NIRS model (R^2= 83%, 86% and 91% for the sand, clay and loam soil, respectively) for predicting N recovery, compared to proportion of available N alone as a predictor (R^2=70%), indicates that the NIRS model is able to account for the mineral N component and mineralisation of organic N in estimating manure N release. Further development of this NIRS calibration model is necessary in order to separate out the effect of the mineral N from the organic N content of the manure and to develop this predictive capacity for use by farmers.

In the final year of the project, the developed NIR calibration models were used to study nutrient content of intensively sampled manure storage and field spread manures. The results of these variability studies were used to evaluate a range of sampling strategies, the conclusions supporting current advice for a minimum of ten samples from well constructed solid manure storage. Where visual examination suggest a more variable and uneven (e.g. shape, size and age) store, however, more intensive sampling is likely to be necessary. The results also confirmed the benefits of a reliable analytical service for improved utilisation of manure nutrients.

Within a separate phase of the work, the potential for NIRS-based characterisation of soils for nutrient content and soil microbial activity/community, was also assessed. The soil nutrient work required the collection of a large number of samples from across England and Wales, but the focus of NIRS assessment of microbial activity was restricted to a single soil type sampled from a number of sites across the North Wyke farm. The soil characterisation work showed potential for application with certain nutrients; organic matter, total C, pH, total P, organic P, total N; it appeared likely that the models could be improved by increasing the calibration sample base and by restricting calibration to a single soil type. Other research focused on the development of NIRS for phospholipid fatty acid (PLFA) and, thereby, an estimate of the soil microbial community and identification of more microbially 'active' soils which may influence the mineralisation potential of manures. Initial correlations with PLFA extraction data were encouraging when working with a single soil type. Limitations at present relate to the very small sample size for scanning and the low levels of PLFA present in the soils included in the study.

Highlights of the research, presented throughout the project within reports, press articles, conference papers and farming events (e.g. Grassland-MUCK2008 and 2011) have attracted significant industry interest. Key information and advice have been compiled within promotional material (posters, technical leaflets) used at events and in farmer leaflets (AHDB and Eurofins) produced in support of the recent launch of a NIRS-based commercial service for manure analysis. This service is to be provided initially by Eurofins Laboratories, under the terms of a two-year licence agreed by the consortium.

2.1. NIRS model development – manures

In order to develop a robust calibration model for analysis of manures by NIRS, large numbers of manure samples were required from across the UK, with the aim of covering the anticipated range in variability in the chemical and physical characteristics of manures and NIRS spectral variability. NIRS scanning of the manures was performed, throughout, using a FT-NIR instrument, the Matrix-I (Bruker UK, Ltd). A detailed sampling protocol was produced to ensure a consistent approach for the collection, labelling, storage and submission of samples. All samples were logged on receipt and NIR scanned as soon as possible thereafter. In the first two years of the project, a total of 2177 samples of manures (cattle and pig FYM and slurries) and biosolids (digested sludge cake, heat/lime stabilised, digested bioliquids and pelletized solids) were collected from across the UK with significant input and active cooperation of industry partners. Single NIR scans were undertaken on these samples to establish the initial spectral database. A further 2508 replicate (6x) scans were taken subsequently, to develop the calibration set comprising 418 samples. Refinement of the calibration models was undertaken with expert chemometrics input from Bruker; from the statistics associated with the validation procedure (Appendix 2), performance of the

calibrations for conventional analysis of manure and biosolids samples was as follows: *excellent* – DM, total N; *successful* – NH_4-N, P_2O_5; *moderately successful* – SO_3, K_2O; *moderately useful* – MgO. Performance for pH was unsatisfactory (r^2 0.6, RPD 1.63), which was not a surprise given the limited range in pH (almost all between pH 6.5 and 9.0), even within the very large range of samples scanned and analysed. Eurofins now offer NIRS analysis of manures as a commercial service using the calibration models produced in this project under licence to the project consortium.

2.2. NIRS model development - mineralization of manure organic N

Development of an NIRS calibration model for uptake of manure N was based on a large experiment with ryegrass grown in large pots (10 litre), tracking the uptake of N from manures applied to 3 different soil types (clay loam, sandy loam, loamy sand) at two sites: ADAS Boxworth, Cambs and North Wyke, Devon, for 3 years. The experiments were set in plastic tunnels, with overhead irrigation, to ensure that moisture was not limiting to grass growth and DM yield and N offtake were recorded for each cut of grass. The experiment was successfully completed, generating a large amount of data on N uptake from a total of 180 manures, all of which were NIR scanned and analysed by wet chemistry; with the 6x replicate scanning procedure this resulted in a total of 1080 scans. Spectral averaging gave 180 spectra for model development. Regressions were run with the grass N uptake data expressed in several ways: kg manure N per hectare; N uptake as % manure N applied; N uptake as % manure organic N applied; g N per tonne applied; g N per tonne of manure applied per day; g N uptake per 100 day degrees (thermal time). Correlations with these derived variables were good; best with the data expressed as g N/tonne/day, thus indicating a good estimate of manure N uptake over the season was possible via the NIRS scanning procedure. However, detailed analysis of the experimental N uptake results also showed a good correlation between manure N recovery and the mineral N content of the manures (both as NH_4-N and NH_4-N as % of total N), indicating that N uptake from the manure was dominated by mineral N content. The NIRS model is able to account for the mineral N component and mineralisation of organic N in estimating manure N release. Further development of this NIRS calibration model is necessary in order to separate out the effect of the mineral N from the organic N content of the manure and to develop this predictive capacity for use by farmers.

2.3. NIRS model development – soils

Potential for NIRS-based characterisation of soils, for nutrient content also required a large number of samples from across England and Wales, but the focus of NIRS assessment of soil microbial activity/community was restricted to a single soil type sampled from a number of sites across the North Wyke farm. The NIRS-based characterisation of soils showed potential for application with certain nutrients; organic matter, total C, pH, total P, organic P, total N and that the models could be improved by more calibration samples and restricting to a single soil type. Research on soils was also directed towards the development of NIRS for phospholipid fatty acid (PLFA) and, thereby an estimate of the soil microbial community and identification of more microbially 'active' soils which may influence the mineralisation potential of manures. Initial correlations with PLFA extraction data were encouraging when working with a single soil type. Limitations at present relate to the very small sample size for scanning and the low levels of PLFA present in the soils included in the study. The difficulties of extending the use of NIRS across a range of soils has been reported by other authors and it was anticipated that ,at best, the current research might provide demonstration of 'proof of concept', rather than a useable calibration model.

2.4. Manure variability in storage and spreading

In the final year, the developed NIR calibration models for manures were used to undertake intensive sampling studies on the variability of manure storage heaps. The scan data were used to evaluate a range of sampling strategies from manure heaps; the results supported current advice for a minimum of ten samples from a well constructed and managed solid manure storage. However, where visual examination suggests a more variable and uneven (e.g. shape, size and age) store, more intensive sampling is likely to be necessary. The results also confirmed the benefits of a reliable analytical service for improved utilisation of manure nutrients, when combined with good sampling procedure.

2.5. Project outputs

Highlights of the research, presented throughout the project within reports, press articles (BGS, BPEX, Potato Council), conference papers and farming events (e.g. Grassland-MUCK2008-2011; Cereals; Dairy Event) have attracted significant industry interest. Key information and advice has been compiled within promotional material (posters and leaflets) used at events and in farmer leaflets (AHDB and Eurofins) produced in support of the recent launch of an NIRS-based commercial service for manure analysis.

2.6. Future R&D resulting from this project

The reduced analytical cost (initially perhaps 50% cost of conventional services available via commercial laboratories), speed, improved reliability and additional information provided by the commercial service for NIRS (currently offered by Eurofins) should encourage greater uptake of manure analysis and, thereby, improved nutrient management, reduced crop production costs and reduced potential for environmental emissions. However, one major gap in the current calibration models is the lack of capability for poultry manures; also the exclusion of uric acid-N analysis (not necessary other than for poultry manures).

At the commencement of the LINK project, support for the research was not available from the poultry sector and, hence, because of resource constraints, poultry manures could not be included within the programme. However, because of their high nutrient content and potential value, poultry manures are frequently analysed by farmers - more often than other manures but, sometimes, at the exclusion of uric acid analysis. The success of the initial work now provides further incentive to extend the NIRS capability to poultry manures. Moreover, the success of the initial research and the experience gained will provide a sound foundation for this further development; to now include layer manure, broiler and turkey litter-based manures and duck manures. The exclusion of uric acid analysis, possibly because of lack of information, limited availability of commercial analytical capability, or high cost, is a significant omission since uric acid is an important component (up to 20-30%) of the potentially “available” N content of poultry manures. Previous research on manure treatment, has demonstrated that drying stabilises the uric acid content of the manure, which reduces ammonia emissions in house and, following land application, results in a significant improvement in efficiency in utilisation of manure N by the crop (Smith et al., 2001). An early scoping study on use of NIRS for manure analysis (Defra project NT2508) also showed this technique to work well for uric acid analysis of poultry manures.

The NIRS model was very successful at predicting N uptake. Seasonal N uptake comprises mineral N and N released from organic N; and in-season N uptake is dominated by mineral N. If possible, further work to separate out N uptake from mineral and organic N may be useful in improving understanding of N fluxes following manure applications to land. However, amendment/adjustment of the current models may prove sufficient for practical application of the NIRS output; the best prediction may be a combination of NIRS N uptake prediction combined with MANNER algorithms for N loss.

Information and important outputs from the successful N release prediction capability should be incorporated within the key advisory systems/tools (e.g. MANNER, PLANET, RB209, Manure Booklets) at the earliest opportunity.

2.7. Industrial relevance and plans for future commercial exploitation

Plans for the commercial exploitation of an NIRS-based analytical service for manure analysis are now well advanced, with the encouragement and active involvement of most of the industry partners within the project consortium. This service is to be provided initially by Eurofins Laboratories, under a two-year licence, under terms agreed by the consortium. A preliminary, “commercial launch” of this service was undertaken by Eurofins at the Grassland-MUCK Event at NAC, Stoneleigh, in May, 2011. This involved running the Bruker Matrix-I instrument, on the AHDB Stand, with attendant, pre-event publicity and an opportunity for farmers to see the machine scanning a range of samples and generating reports; also to have some of their own manures analysed without charge, with results delivered after the event.

The research partners are particularly grateful for the input and guidance of AHDB (HGCA, BPEX, DairyCo and Potato Council), including that of their legal advisers during the drafting, consultation and agreement of the terms of the licence. Whilst the final details of the licence remain to be agreed and checked by the AHDB solicitor, it is proposed that the licence will allow Eurofins exclusivity over the initial two-year period, at which time (pending review by the project consortium) the technology, including calibration models, will be made available to others who may wish to run a similar service.

Both Eurofins and AHDB have been actively promoting this service; both have produced technical leaflets on improved manure analysis. Eurofins have included technical information on their website and plan to run workshops for farmers and consultants.

Whilst there has been interest in promotion and use of the new NIRS service by Natural England Catchment Officers, NE have enquired about NIRS capability for poultry manures, since these are often of significant interest within catchment campaigns for improved nutrient management planning. This confirms the significant gap in the current NIRS service and the importance of the proposed additional R&D.

Visitors from Teagasc, Johnstown Castle discussed opportunities for future research collaboration on manures-NIRS. Johnstown Castle Laboratories have a Perkin Elmer, spectrum 400 FT- NIR/IR.

APPENDIX 1: SAMPLING AND COLLECTION OF MANURES AND BIOSOLIDS FOR DEVELOPMENT OF NIRS CALIBRATION MODELS

Introduction

The development of a robust calibration model for analysis by NIRS requires reference to large numbers of samples; this is particularly the case with materials as heterogeneous and variable as manures, slurries or biosolids. Therefore an early objective of the project was the collection of a large number (the more the better) of manure samples representative of the range likely to be encountered across the UK. To ensure that manures with as wide a range of analyses and NIRS spectral variability were included within the models, samples covering a wide range of livestock and manure management systems were required; also from a wide a range of geographical locations. The sample set, for each of the manure categories was intended to be representative of those major factors impacting on manure quality – diet, bedding type and amount, stock and manure management, storage and geographical location.

Materials and Methods

In each of the first two years of the research, collection of a target of 1050 samples of manures and biosolids from across the UK was planned, with the aim of covering the anticipated range in variability in the manures, both in terms of chemical and physical characteristics, and NIRS spectral variability. Projected sample totals for individual manure types, in each of the first two project years were 250 of both pig and cattle FYM, 150 of both pig and cattle slurries and 250 biosolids, reflecting the anticipated variability of these different materials. Advice on the representative sampling of manures and slurries is available within published guidelines (Chambers *et al.,* 2001). A detailed sampling protocol, based partly on this published information, was produced to ensure a consistent approach for the collection, labelling, storage and submission of samples. Screw-top polythene bottles (*ca.* 1 litre), for slurries, and strong plastic bags for solids, together with card labels and information collection forms, were distributed (with separate forms for slurry, FYM and biosolids - Annex 1). Recommended equipment for sampling was as follows:

Slurry/liquid sludge sampling

- 8 – 10 litre bucket (for mixing) with spout;
- Sample collection bottle/pot (2- 5 litres) with handle or neck for fastening to line/cord; weighted if possible to make it sink;
- Rope or cord, long enough to retrieve sampling bottle/pot from slurry tank/lagoon;
- Stick to mix sample in bucket;
- Sample labels and permanent marker;
- Plastic bags and elastic bands to contain the sample bottle;
- Information collection form (Annex 1).

FYM and sludge biosolids sampling

- Plastic bags (not less than 250 gauge and approx 30cm x 50cm);
- Garden fork to dig into heap;
- Spade or gouge auger for sampling sludge cake;
- Tray or plastic bin large enough to mix 5kg of manure;
- Sample labels and permanent marker;
- Information collection form.

Sampling procedure

Rubber gloves and protective clothing was recommended for sample collection. The dangers of sampling enclosed slurry stores (pits or tanks) were emphasised, because of the risk of inhaling highly toxic gases.

Recommendations for sampling were as follows: The sample bottle to be labelled on the outside with site name (farm), sample i.d. (as shown on form) and date, with this information also recorded on the small card label. Lower the weighted bottle/pot tied to a rope into the slurry. Allow it to sink, then retrieve the pot and tip the contents into a clean bucket. Repeat the process from different positions if possible. Stir the bulked slurry samples in the bucket, filling the 1 litre sample bottle. Clean the outside of the bottle and place inside the 30cm x 50cm plastic bag. After completion, place the information proforma inside a thin gauge plastic bag and enclose, together with filled sample bottle, inside the large plastic bag. Fold the top after excluding air and close with elastic band and attached card label.

For solid manures, the process is essentially the same. Label the 30cm x 50cm sample bag with site name (farm), sample i.d. (as shown on form) and date. After mixing the bulk sample on the tray or in the plastic bin, place about 1 kg of the material inside the plastic bag. Fold the bag, excluding as much air as possible – this should be rolled into a cylinder shape[1], securing with elastic bands at either end. To one of the bands attach a card label showing the sample id information. Enclose the sample, together with the completed information proforma, inside a further large plastic bag. Fold the top, again excluding air and close with elastic band and attached card label.

The sampling protocol included guidance on rapid despatch via courier services to the laboratory. The collection procedure required that the material be well mixed, if possible, at sampling and thus, representative of the location of the store/handling system from which they were collected. For the purpose of NIRS calibration model development, material from different parts of a store (e.g. from outer layers, centre and base of a solid manure heap) could be sampled separately, since it is clear that such variability would affect both the nature and properties of the manure, the NIRS spectral output, as well as the analysis. Key information about each sample was requested via the sample collection proforma (Annex 1) completed by the sampler at the time of collection.

All samples were logged on receipt and scanned as soon as possible thereafter. After initial scanning, the samples were frozen and stored until selection of a subset for full chemical analysis by conventional laboratory methods. A combination of Principal Component Analysis (PCA) in the first year, and a combination of PCA and outlier analysis in the second year based on preliminary models, was used to select the samples for chemical analysis and use in the calibration model development (*ca.* 120 samples, i.e. 24 of each manure category in each of the two years). After thawing, the selected samples were multi-scanned (6x) and analysed by wet chemistry, the solids after rapid freezing in liquid nitrogen and homogenisation using a technique developed in previous research (Smith *et al*., 2005).

[1] Note – the cylinder shape is to facilitate freezer storage.

Results

Sourcing and collection of samples of manures and biosolids was facilitated, throughout, by the wide consortium membership and the active participation of project partners. In addition, demonstration of the developing technology at national farming events (Grassland-MUCK 2008; Dairy Event 2008 and 2009) provided opportunity for farmers/consultants to bring samples of manures for scanning and provision of results, with suitable samples contributing to the NIRS calibration model development. In consultation with project partners and technical contacts across Europe, provision was also made to receive samples from other countries; the importation of such samples requiring a Defra Animal Health Import Licence, for each of the planned, 6-month sample collection periods and certified arrangements for sample incineration, following scanning/laboratory analysis.

In the first year, a total of 1019 samples were collected and scanned between January and June 2008; the total comprised 190 pig FYM, 134 pig slurry, 338 cattle FYM, 176 cattle slurry and 181 biosolids samples. Principal Component Analysis was used to select a total of 118 samples to represent the spectral variability and range of manure analysis, in order to assemble preliminary nutrient calibration models (these samples, comprising 14 pig FYM, 41 pig slurry, 20 cattle FYM, 22 cattle slurry and 21 biosolids).

In 2009, a total of 1158 samples were collected and scanned between January and July 2009; the total comprising 254 pig FYM, 135 pig slurry, 377 cattle FYM, 145 cattle slurry and 247 biosolids samples. PCA was again used in combination with outlier analysis to select a further subset of 120 samples (24 of each manure type) for chemical analysis, to expand the range of spectral variability and analyses and continue the development of robust nutrient calibration models. Details of samples collected for this task over the initial two project years are summarised in Table 1.

Table 1. Manure and biosolids sampling for NIRS calibration development; numbers of samples collected during first and second project years and selected for wet chemical analysis.

	FYM		Slurries		Biosolids
	Cattle	Pig	Cattle	Pig	
Winter 2007/08 (yr1)	338	190	176	134	181
Winter 2008/09 (yr2)	377	254	145	135	247
Selected for analysis (yr1)	20	14	22	41	21
Selected for analysis (yr2)	24	24	24	24	24

Discussion and conclusions

The collection of the large number of manure and biosolids samples (an annual target of >1100 samples) presented a difficult set of logistical and practical problems, not least of which was careful packaging of samples so that courier services were prepared to accept these materials for carriage. However, thanks to the active and substantial assistance of partners within the project consortium (especially AHDB extension officers within DairyCo, BPEX and EBLEX, GrowHow Technical staff and their distributors; and sludge managers within Severn Trent and Yorkshire Water and Agrivert Ltd., these problems were overcome and all targets were achieved, albeit with some difficulty in meeting the pressures for timely delivery (necessary to allow the early development and improvement of the calibration models).

In addition to the total of 238 samples selected for the calibration work (Table 1), the 180 samples scanned for inclusion in the pot experiments on N release (Appendix 3) were also analysed for the nutrient analytes and included in the model regression, resulting in a total calibration sample set of 418, significantly in excess of the original target in the project work-plan of 210 samples.

The possibility of increasing the catchment area for samples, to the inclusion of manure samples from other parts of Europe would have assisted the problem of achieving a timely delivery of the large sample target and may have allowed the potential benefit of wider applicability of the calibration models, once developed. Although appropriate bio-security precautions for handling and disposal of samples were made through Animal Health import licence and planned use of approved incineration facilities for disposal of imported samples, the logistical difficulties and high cost of sampling and shipment (with sample acceptance by courier services from other countries a significant issue), resulted in the importation of few samples from outside of the UK. Pig slurry samples, around 30 in total, were received from Ireland, with the kind cooperation of our Teagasc partners.

Overall, this difficult task was successfully completed and provided the basis for the development of a robust set of NIRS manure analysis calibration models and a useful additional nutrient analysis database for pig, cattle FYM and slurries and Water Industry biosolids (Appendix 2). These analyses data will contribute to national database of manure analysis, which is used when revising national standard values for different manure types for inclusion in the organic manures section of RB209 (Anon, 2010).

APPENDIX 2: DEVELOPMENT OF NUTRIENT NIRS MODELS FOR THE RAPID CHARACTERISATION OF IMPORTANT MANURE TYPES

Introduction

The research objective was to develop a technique for the rapid, low cost analysis of solid and semi solid manures based on Near Infrared Reflectance Spectroscopy (NIRS). Current methods of laboratory analysis are slow, relatively expensive and can give inconsistent results. NIRS is a technique involving measurement of the spectrum of infrared energy reflected from a sample illuminated by white light. The spectral properties of the sample are related to chemical composition and need to be calibrated against classical “wet chemistry” data in order to predict the composition of subsequent samples. Accurate calibration is dependent on reliable reference data and routine wet analysis of manures has proven problematic due to the heterogeneous nature of manure samples. Homogenisation using mechanical mixers at room temperature is ineffective due to material being extracted from rather than incorporated into the final mix. Sample homogenisation in this research was ensured by sample brittleness after rapid freezing in liquid nitrogen and subsequent smashing, rather than reliance upon a cutting action at room temperature. This technique would be too expensive to use on routine samples and can only be realistically used for the accurate calibration of a secondary rapid measurement technique. NIRS technology allows multiple rapid scans with spectral averaging to be recorded covering more of the ‘as is’ material than would normally be taken for a wet method, thereby further reducing the error involved in analysis. In order to cover the range of likely analyses, sample and NIR spectral variability, a very large number of samples was scanned and a representative calibration set selected using Principal Component Analysis (PCA), over two years. Additionally samples scanned for the mineralisable N models, over three years, were also included in the nutrient model development. The research involved the development of calibration models for estimation of the content of dry matter (DM), total nitrogen (N), ammonium-N (NH_4-N), sulphur (SO_3), phosphate (P_2O_5), potash (K_2O), and magnesium (MgO).

Materials and method

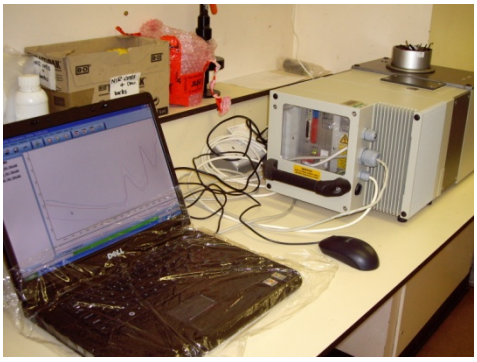
NIRS scanning

Within this research, the NIRS scanning was performed using a FT-NIR instrument, the Matrix-I (Bruker UK, Ltd). The samples are placed in a sample cup, with a clear quartz base which allows the sample to be irradiated from below and the reflected IR spectrum recorded, with the results of multiple scans integrated using the Opus software (version 6 © Bruker UK). The Matrix-I, which has been used previously for analysis of immature grain samples, was chosen due to its robustness, sensitivity and its potential for on-farm measurement. Samples are routinely scanned three times, though in the case of potentially heterogeneous manure samples, a 6x scanning

procedure has been used to develop the calibration set. To establish the initial spectral database for selection of calibrants at a later date, samples were single scanned and frozen until the target sample numbers had been reached. The target sample numbers were designed to cover as much of the spectral variation as possible. PCA selection of a representative subset of these then allows for efficient use of laboratory resources with redundancy in the calibration set kept to a minimum. Once the selection process was complete the selected samples were thawed and 6x re-scanned before homogenisation of the sample and submission to the laboratory for wet analysis.

In each of the first two years of the research, a target total of 1050 samples of manures and biosolids were to be collected from across the UK, with the aim of covering the anticipated range in variability in the manures, both in terms of chemical and physical characteristics, and NIRS spectral variability. The projected sample totals for individual manure types were 250 of both pig and cattle FYM, 150 of both pig and cattle slurries and 250 biosolids samples, reflecting the anticipated variability of these different materials. Robustness in NIRS models is achieved by adding to the database over time, thereby including seasonal and geographical sample changes in the model.

(a)



(b)

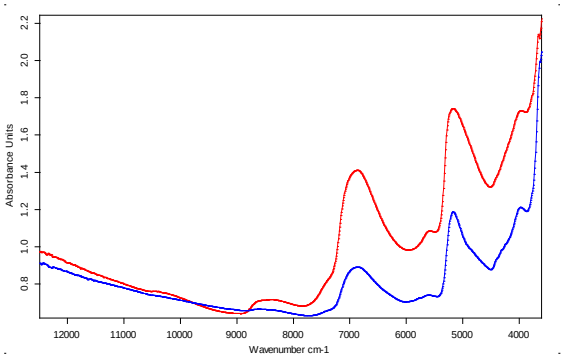


Figure 1. Scanning of manure samples for nutrient content: (a) using the Bruker Matrix-I NIRS and (b) NIR spectra of two cattle manure samples.

Homogenisation of samples

The homogenisation procedure prior to this research involved using a rotary blender at room temperature. The main problem observed with this was the tendency for the wet fibrous content to wrap around the cutting blades of all homogenisers trialled. This extracted material from the sample that could not be broken down. Trialled devices ranged from food mixers to specialist laboratory homogenisers such as the Ultra Turrax machine supplied by Carl Stuart limited. All failed to produce an acceptable homogenate which is essential for consistent wet analysis.

The procedure developed in a previous project, NT2508 (Smith et al., 2005), involved cooling the material to very low temperatures using liquid nitrogen. The resulting brittle state then allowed the blades of a powerful homogeniser to break the whole sample down. Preparing the sample involved suspension of a representative aliquot in a Dewar flask containing liquid nitrogen. A suitable sample basket was constructed in the laboratory. This consisted of a chrome steel wire basket, 13cm x 13cm x 13cm, with the addition of a bent wire loop to act as a handle. This was further lined with 1mm steel meshing to prevent sample material dropping through into the liquid nitrogen. A powerful homogeniser was used to break up the hard frozen material (FOSS 2096).

Initially 500g sub-samples were frozen at -18°C before treatment with liquid nitrogen. The homogeniser could not cope with a single frozen block of this size. This was overcome by creating disc shaped sub-samples of the fresh unfrozen material of approximate dimensions 50mm diameter by 6mm thick. The addition of this step also removed stones which can damage the blades. These pre-frozen discs, when added to the basket, created airspace between the bulk sample which allowed easier homogenisation. The resulting product, while at -190°C, resembled a powder. Attempts to homogenise material frozen at -18°C only resulted in an unacceptable coarse material and the lower temperature was required to achieve the needed brittleness. While using this procedure, experience showed that using material first frozen at -18°C reduced the amount of liquid nitrogen used per sample but still allowed the necessary break up of the sample bulk. Also a minimum amount of 400g was required, with amounts less than this producing an undesirable coarser particle size. Figure 1 illustrates the homogenisation procedure.

a) Sample as received

b) Loading the sample cage

c) Lowering the sample into liquid nitrogen

d) Transferring the sample to the homogeniser

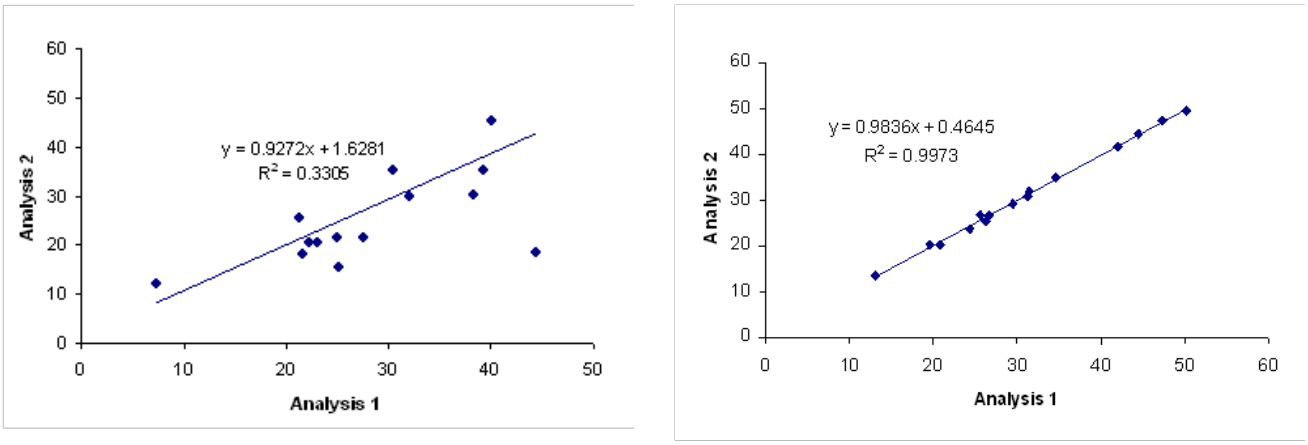
e) Typical sample after homogenisation, before thawing.

Figure1. Homogenisation procedure

Results and discussion

An earlier research project involved the development of a homogenisation procedure and a homogeneity study was carried out to demonstrate the effectiveness of the new technique. Samples of manures from various animal types were submitted for analysis as blind duplicates both before and after homogenisation.

The results demonstrate an improvement in the homogeneity of the sample using the liquid nitrogen process developed. An improvement in repeatability here benefits interpretation of data for field use and leads to an improved correlation when matching to spectral data for generating NIRS prediction models. The use of this technique for routine use however would prove prohibitively expensive and is only used here to generate reliable NIRS models. The plots below demonstrate the improvement the technique offers, in this case for N content of manures (poultry, pig and cattle solid manures).



a) before sample homogenisation b) after sample homogenisation

Figure 2. Sample homogenisation procedure for analysis of manures – total N (g/kg DM) (Poultry, Pig & Dairy manures)

A total of 1019 samples were collected and scanned between January and June 2008, with the active support of the project partners. The total comprised 190 pig FYM, 134 pig slurry, 338 cattle FYM, 176 cattle slurry and 181 biosolids samples. Principal Component Analysis was used to select a total of 118 samples to represent the spectral variability and range of manure analysis, in order to assemble preliminary nutrient calibration models. In addition the 58 samples selected for the first year mineralisable N pot experiments were included in the nutrient model development. These samples, comprising 25 pig FYM, 49 pig slurry, 37 cattle FYM, 28 cattle slurry and 37 biosolids, were multi-scanned (6x) and analysed by wet chemistry, the solids following homogenisation. The multiple scans were spectrally averaged before performing model regression. In 2009, a total of 1158 samples were collected and scanned between January and July 2009; the total comprising 254 pig FYM, 135 pig slurry, 377 cattle FYM, 145 cattle slurry and 247 biosolids

samples. PCA was again used in combination with outlier analysis from the preliminary models to select a further subset of 120 samples (24 of each manure type) for chemical analysis. The 60 samples selected for the second year mineralisable N pot experiments were again included. These samples, comprising 36 pig FYM, 26 pig slurry, 46 cattle FYM, 33 cattle slurry and 39 biosolids, were multi-scanned (6x) and analysed by wet chemistry, the solids following homogenisation. The final 62 samples of the third year mineralisable pot experiments were multi-scanned (6x) and analysed by wet chemistry, the solids following homogenisation, to expand the range of spectral variability and analyses and continue the development of robust nutrient calibration models. In total this gave 418 samples for the final nutrient model calibration set comprising 70 pig FYM, 80 pig slurry, 101 cattle FYM, 75 cattle slurry and 92 biosolids samples.

A summary of the scanning activity:

- 2177 single scans to establish initial spectral database;
- 2508 replicate (x6) scans taken to develop calibration set comprising 418 samples.
- A summary of the wet chemistry analysis results is presented in Table 1.

Table 1. Summary of wet analysis results (averages of 418 duplicate analyses).

ID	DM (g/Kg)	Total N (g/Kg)	P_2O_5 (mg/Kg)	SO_3 (mg/Kg)	K_2O (mg/Kg)	MgO (mg/Kg)	NH_4N (mg/Kg)
Bio-solids							
Min	169.00	3.05	1742	1360	253	648	7
Max	890.00	46.50	56299	26334	6005	12360	4353
Mean	331.52	12.65	14206	8277	1021	2296	1841
Pig slurry							
min	2.71	0.50	41	44	466	13	156
max	147.00	9.22	6652	3319	6652	2620	4839
mean	33.81	3.22	1083	807	2434	376	2195
Pig FYM							
min	164.50	1.65	771	690	992	421	9
max	857.00	20.70	22050	18457	16158	7891	5032
mean	259.57	7.98	5912	4061	6700	2058	1999
Cattle slurry							
min	1.77	0.14	33	29	299	27	105
max	157.50	17.80	2460	2650	6842	1633	2729
mean	62.73	2.51	904	753	2546	608	953
Cattle FYM							
min	162.74	2.63	885	917	1054	339	41
max	846.00	28.15	32147	49499	29025	6832	4808
mean	273.78	7.25	3571	3577	8359	1824	755

Upon completion of spectra collection and analysis, refinement of the calibration models was undertaken with expert chemometrics input from Bruker. Calibration optimization was performed with 50% of the data first used as a test set. The test and calibration sets were exchanged after the initial regression to test the robustness of the data to form a model. Regressions were run with this test set selected by both analyte value and by PCA. To create the final working versions regression models using cross validation were created as these use all the available data. The regression runs were limited to the bandwidth 9500cm^{-1} to 3800cm^{-1} in order to reduce interference due to noise. The removal of some samples from the calibration set was necessary and included effluent samples of very low solids content, in fact “dirty water” samples, and also samples with very low nutrient content (at p.p.m. level). Some of these samples were almost translucent and, hence, unsuitable for reflectance-based analysis.

From the statistics associated with the validation procedure, Malley *et al.* (2005) proposed the following guideline for describing the performance of calibration models for environmental samples, based on the r^2 (correlation coefficient) and RPD statistic (standard deviation of the analyte set divided by standard error of prediction): r^2>0.95, RPD>4 - *excellent*; r^2= 0.9-0.95, RPD 3-4 – *successful*; r^2= 0.8-0.9, RPD 2.25-3 – *moderately successful*; r^2= 0.7-0.8, RPD 1.75-2.25 – *moderately useful*. It was considered that some calibrations with r^2<0.7 may be useful for screening purposes.

In selecting the final models, as well as the above, consideration was given to the number of ranks (terms) selected to describe the analyte and the magnitude of the model loadings. As a general rule-of-thumb, loadings greater than 10,000 indicate that noise within the spectra rather than analyte signal may be being modelled. Also the model with the lowest number of ranks is preferred so as to avoid over fitting of the model to the calibration set, however having said this some complex systems such as manures will require more ranks.

Model statistics obtained on test set validation and final cross validation model are presented below, with calibration models in Figure 3 (a-g):

Dry matter

- Model based on test set selection by lab value
- Set A R^2 94.94 RPD 4.44
- Set B R^2 96.66 RPD 5.48
- Model based on test set selection by PCA
- Set A R^2 95.10 RPD 4.52
- Set B R^2 96.19 RPD 5.12

Cross validation model - R^2 96.07 RPD 5.05 13 ranks, loadings ~ 7000

Total nitrogen

- Model based on test set selection by lab value
- Set A R^2 96.84 RPD 5.62
- Set B R^2 92.60 RPD 3.68
- Model based on test set selection by PCA
- Set A R^2 92.05 RPD 3.55
- Set B R^2 94.38 RPD 4.22

Cross validation model - R^2 94.78 RPD 4.38 14 ranks, loadings ~ 20

Ammonium nitrogen

- Model based on test set selection by lab value
- Set A R^2 93.74 RPD 4.00
- Set B R^2 94.99 RPD 4.47
- Model based on test set selection by PCA
- Set A R^2 91.18 RPD 3.37
- Set B R^2 93.33 RPD 3.87

Cross validation model - R^2 90.75 RPD 3.29 11 ranks, loadings ~15000 (reworked originally 80,000)

Phosphate as P_2O_5

- Model based on test set selection by lab value
- Set A R^2 93.50 RPD 3.92
- Set B R^2 93.61 RPD 3.96
- Model based on test set selection by PCA
- Set A R^2 91.92 RPD 3.52
- Set B R^2 90.96 RPD 3.33

Cross validation model - R^2 90.85 RPD 3.31 8 ranks, loadings ~20,000 (reworked originally 100,000)

Sulphate as SO_3

- Model based on test set selection by lab value
- Set A R^2 86.96 RPD 2.77
- Set B R^2 85.38 RPD 2.62
- Model based on test set selection by PCA
- Set A R^2 87.30 RPD 2.81
- Set B R^2 87.78 RPD 2.86

Cross validation model - R^2 88.11 RPD 2.90 7 ranks, loadings ~ 15,000

Potassium as K_2O

- Model based on test set selection by lab value
- Set A R^2 82.86 RPD 2.42
- Set B R^2 82.62 RPD 2.40
- Model based on test set selection by PCA
- Set A R^2 74.62 RPD 1.99
- Set B R^2 77.97 RPD 2.13

Cross validation model - R^2 83.54 RPD 2.46 12 ranks, loadings ~ 30,000 (reworked originally 6,000000)

Magnesium as MgO

- Model based on test set selection by lab value
- Set A R^2 76.82 RPD 2.08
- Set B R^2 73.77 RPD 1.95
- Model based on test set selection by PCA
- Set A R^2 67.60 RPD 1.76
- Set B R^2 81.48 RPD 2.32

Cross validation model - R^2 69.94 RPD 1.82 4 ranks*, loadings ~ 800

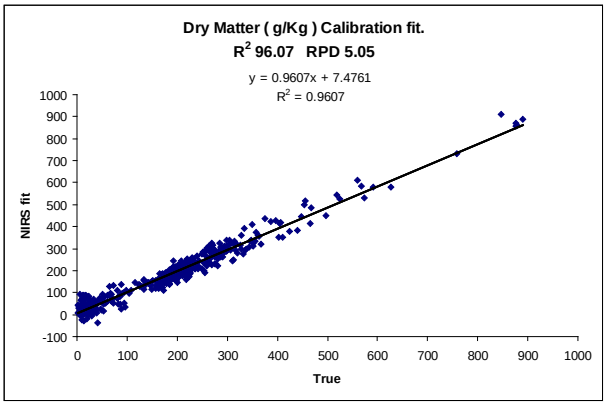
* rank low may be correlating to some grand physical property

The pH models gave poor correlations, below 0.70, so were abandoned.

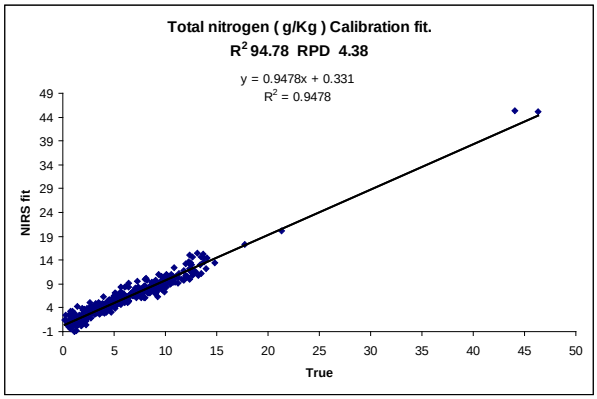
On this basis, performance of the calibrations for conventional analysis of manure and biosolids samples was as follows: *excellent* – DM, total N; *successful* – NH_4-N, P_2O_5; *moderately successful* – SO_3, K_2O; *moderately useful* – MgO.

Near infrared radiation (9000cm^{-1} – 4000cm^{-1}) is absorbed by various chemical bonds, mainly C-H, N-H, O-H, all of which are characteristic of organic matter. It is therefore no surprise that the calibrations have been particularly successful for dry matter (moisture), nitrogen, ammonium-N and phosphate content, but rather less so for minerals, potassium and magnesium (Figure 3 a-g). Performance for pH was unsatisfactory (r^2 0.6, RPD 1.63), which was not a surprise given the limited range in pH (almost all between pH 6.5 and 9.0), even within the very large range of samples scanned and analysed.

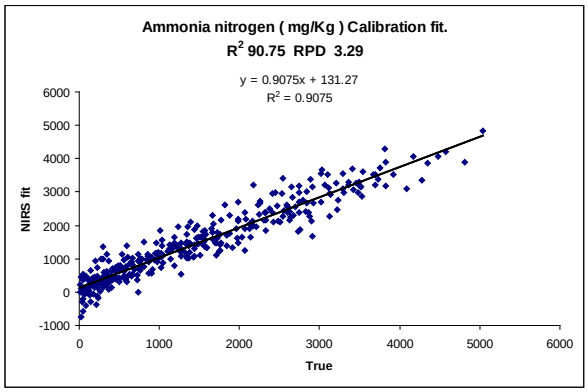
(a)



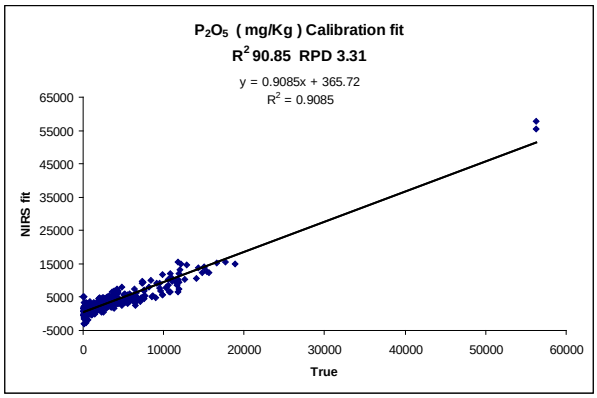
(b)



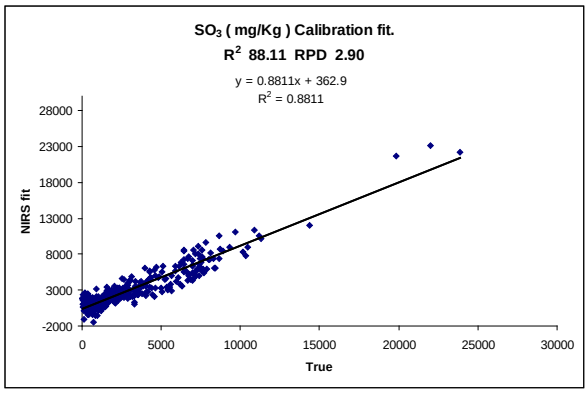
(c)



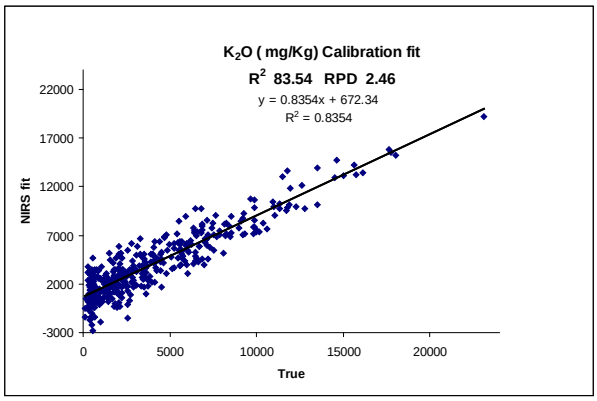
(d)



(e)



(f)



(g)

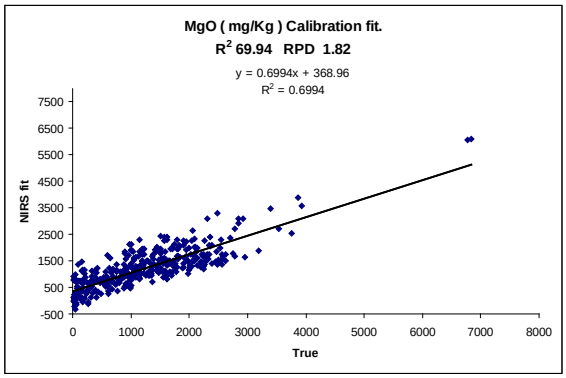


Figure 3. Calibration models for manures: (a) DM content; (b) total N content; (c) ammonium N content; (d) P_2O_5 content; (e) SO_3 content; (f) K_2O content; (g) MgO content.

Conclusions

The research has confirmed the potential of NIRS to provide a rapid, reliable and reduced cost analysis capability for farm manures, slurries and biosolids. The calibration models for conventional analysis parameters were improved by the exclusion of very dilute effluents, where the low solids content and very low nutrient content appears to reduce the reliability of the reflectance assessment. It is therefore suggested that a threshold limit of between 2% and 5% solids content be set, below which NIRS should not be used for sample analysis. It is envisaged that the further development of a predictive capability for mineralization of manure organic N (Appendix 2) will enhance interest in NIRS as an analytical tool.

Highlights of the research, presented throughout the project within reports, press articles, conference papers and farming events (e.g. Grassland-MUCK2008 and 2011) have attracted significant industry interest. Key information and advice have been compiled within promotional material (posters, technical leaflets) used at events and in farmer leaflets (AHDB and Eurofins) produced in support of the recent launch of a NIRS-based commercial service for manure analysis. This service is to be provided initially by the research partner, Eurofins Laboratories, under the terms of a two-year licence agreed by the consortium.

APPENDIX 3: STUDY OF N RELEASE FROM SELECTED MANURES AND DEVELOPMENT OF AN NIRS MODEL FOR PREDICTION OF MANURE ORGANIC N MINERALIZATION

Introduction

The aim of this work was to produce a NIRS model capable of predicting the N release from organic manures. NIRS technology has demonstrated the potential for predicting 'in-direct' measurements. As well as predicting analytes that have a direct relationship to the degree of bond stretching and bending that is recorded in a NIRS spectrum, the spectrum can demonstrate correlation to properties that are not immediately apparent. An example of this is the NIRS prediciton of *in vivo* digestibility in cattle, which has been an established standard in the dairy feed consultancy for over 20 years.

Pot trials growing perennial rye grass were carried out for 3 years (2008, 2009 and 2010) at ADAS Boxworth (Cambridgeshire) and North Wyke Research (Devon). At each site in each year 30 manure types were applied to 3 different soil types (180 manures in total). Grass N offtake was used to track N release from the different manures.

Materials and methods

Sourcing soils

Soil for the pot experiments were sourced at the start of the project from three locations;

- ADAS Gleadthorpe, Retford, Notts. Loamy sand soil of the Cuckey association. Referred to in this report as the 'Sand soil'.
- Crediton, Devon. Sandy clay loam soil of the Crediton association. Referred to in this report as the 'Loam soil'.
- ADAS Boxworth, Cambridge, Cambs. Clay soil of the Hanslope association. Referred to in this report as the 'Clay soil'.

The 3 soils were selected based on their contrasting texture, to assess the potential impact of soil type on N release. The soil from each location was taken from an arable field that had not received any recent manure applications.

Table 1. Characteristics of the soils used in the pot experiment

Soil type	**pH**	**Mineral N NH_4-N+NO_3-N**	**Total N g/100g DM**	**P_2O_5 mg/l**	**K_2O mg/l**	**MgO mg/l**
Loamy sand	7.0	11	0.09	39 Index 3	127 Index 2-	60 Index 2
Sandy clay loam	6.2	18	0.20	73 Index 5	177 Index 2-	163 Index 3
Clay	6.1	25	0.26	20 Index 2	215 Index 2+	158 Index 3

Sourcing manures

As part of WP1 (development of a NIRS model for the rapid characterisation of manures; Appendix 2), a total of 2177 manure samples were collected in the first 2 years of the project. The manures for use in the pot experiments (60 per year) were selected from this database of manure samples, based on a combination of PCA analysis to ensure inclusion of spectrally diverse samples, and expert judgement regarding the likely N release characteristics of the manures and to ensure inclusion of different manure types, i.e. pig/cattle and FYM/slurries/biosolids.

Set up of growing trials

Pots were 28cm diameter, 10 L pots and were filled with *c.*10 kg of soil. Prior to the start of the experiment, samples of the selected manures were scanned to give a total N content based on the preliminary NIRS model. Manure application rate was calculated to apply a target rate equivalent to 500 kg/ha (3.08 g/pot) total N for solids manures and 250 kg/ha (1.54 g/pot) total N for liquid manures (slurries and liquid biosolids). Solids manures were thoroughly mixed with the soil prior to filling the pots. Liquid manures were applied to the soil in the pot and then mixed into the soil surface with a hand fork. The incorporation of manures into the soil would have minimised ammonia volatilisation losses. A representative sample of each manure was taken at the time the experiment was started and analysed for dry matter (DM), pH, total N (Kjeldahl method), NH_4-N and NO_3-N, and total P_2O_5, K_2O, SO_3 and MgO.

At both sites, pots were kept in polytunnels with automatic overhead irrigation (Fig 1). Irrigation rate was monitored to ensure sufficient water to all pots. The pots were in individual saucers, and any water draining through the soil was collected and allowed to be reabsorbed. Air temperature within the polytunnel was recorded using a TinyTalk (Gemini Data Loggers).

There were 3 replicates of each of the 30 manure types and 9 replicate 'control' (no manure) treatments on each soil type, giving a total of 297 pots at each site in each year. The pots were arranged within the polytunnel in a randomised block design. In order to avoid possible edge effects, a row of discard pots were included as the outside row around the trial. Fertiliser P, K and S was applied to all pots to ensure these nutrients were not limiting to grass growth.

The pots were sown with Perennial rye grass in April/May and the grass was cut 4 or 5 times over the *c*.180 day season (with the last cut in late Oct/Nov). Harvest dates were dependant on growth, with the earlier cuts (when the grass was growing vigorously) closer together than the later season cuts. At each harvest, the grass was cut to a height of 2 cm. Harvested grass was dried at 80^{o}C, weighed and then analysed for total N (Dumas method).

Figure 1. Pots in the polytunnel at ADAS Boxworth

Data analysis

Total grass N offtake was calculated for each cut. Manure N offtake in the grass was calculated by subtracting the N offtake from the control treatments for each soil type. Manure N offtake was expressed in the following units;

- kg manure N/ ha,
- N uptake as % manure N applied,
- N uptake as % manure organic N (total N minus mineral N) applied,
- g N uptake/tonne of manure applied,
- g N uptake/tonne of manure applied/ day,
- g N uptake/100 day degrees (thermal time).

Development of the NIRS model

Samples of the 180 manures used in the pot experiments were scanned using a FT-NIR instrument, the Matrix-I (Bruker UK, Ltd). The samples are placed in a sample cup, with a clear quartz base which allows the sample to be irradiated from below and the reflected IR spectrum recorded, with the results of multiple scans integrated using the Opus software (version 6 © Bruker UK). The NIRS scans of the pot trial manures and N uptake data generated from the pot trial for these manures were then put through the NIRS software to generate predictive models.

Residual N release

At the end of the 2008 and 2009 growing seasons, 6 manure treatments were selected from each site, plus the control treatment, to be kept on for a further year to assess manure N release in the second season following application (residual N release)[2]. The manures were selected to include those showing a slower N release rate, and with a higher proportion of total N in the organic fraction. The grass from these treatments was cut 2 or 3 times during the second season (multiple cuts to avoid the development of seed heads), dried at 80^{o}C and weighed. Grass from each cut was analysed for total N and manure N offtake calculated for the whole of the second season and expressed in the units listed above.

Results and discussion

Manure characteristics

The 180 manures collected for the pot experiments can be grouped into 7 main types:

- Cattle FYM (56 treatments)
- Cattle slurry (29 treatments)
- Pig FYM (31 treatments)
- Pig slurry (14 treatments)
- Liquid digested sludge from Severn Trent Water (3 treatments)
- Biosolids (digested cake) sourced from Severn Trent Water (19 samples)
- Biosolids (digested & conditioned) sourced from Yorkshire Water (21 treatments)
- Biosolids (dried & pelleted) sourced from Yorkshire Water (1 treatment)

In addition, there were 6 other manure treatments which don't fit into the categories above, including 3 biosolids from other sources, 2 pig slurry separated solids fractions and 1 cattle slurry separated solids fraction.

[2] Unfortunately, at North Wyke the 2009 pots were disposed off before selection of treatments for residual N release could take place, and there is only 1 year of data for residual N release from this site.

The mean and range of DM and nutrient concentrations for each of the manure types are shown in Table 2. Mean values for the livestock manures (pig/cattle manure/slurry) are similar to standard values quoted in the RB209 Fertiliser Manual (Anon, 2010), however there was a significant range in DM and nutrient concentrations within the manure types.

The proportion of total N in mineral form (NH_4-N plus NO_3-N) is greatest for the slurries (41% and 69% of total N for cattle and pig slurry, respectively) and liquid digested biosolids (55% total N). There was a wide range in the proportion of total N in mineral form within the livestock slurries (17-64% for cattle slurry, and 50-83% for pig slurry), which reflects the wide variation in DM content of the collected slurries (from <1% to *c*.20%). There was a significant relationship between dry matter content and proportion of total N in mineral form for the slurries (P<0.001, R^2=44%)

The proportion of total N in mineral form was lower for the solid manures (10% and 29% of total N for cattle and pig FYM, respectively) and biosolids (23% and 8% of total N for biosolids from Severn Trent Water and Yorkshire Water, respectively). The variation in the proportion of mineral N in the livestock manures may partly reflect the length of storage, with less mineral N in the older materials; however this is not a consistent relationship and there is no information on length of storage for some of the manures. The difference in mineral N content of the Severn Trent and Yorkshire Water biosolids reflects differences in biosolids treatments. The biosolids from both companies were digested and dewatered, however those from Yorkshire Water were further conditioned by composting with woodwaste or green compost, resulting in a slightly drier material with a lower available N content than the Severn Trent biosolids.

Table 2. Chemical characteristics of the different manure types used in the pot experiments.

Manure type	**Number of treatments**	**Dry matter %**	**Total N kg/t FW**	**Mineral N kg/t FW**	**Mineral N (% total N)**	**Total P_2O_5 kg/t FW**	**Total K_2O kg/t FW**	**Total SO_3 kg/t FW**	**Total MgO kg/t FW**
Cattle FYM	56	23 *(15-45)*	5.8 *(2.6-11.6)*	0.5 *(0.1-1.8)*	10 *(1-28)*	2.8 *(0.9-6.5)*	7.8 *(2.3-19.1)*	2.4 *(0.9-5.3)*	1.6 *(0.6-3.4)*
Cattle slurry	29	7 *(0.8-21)*	2.5 *(0.5-4.8)*	0.9 *(0.2-2.0)*	41 *(17-64)*	1.0 *(0.1-2.3)*	2.9 *(0.7-7.5)*	0.8 *(0.1-2.2)*	0.7 *(0.1-1.6)*
Pig FYM	31	20 *(12-26)*	7.7 *(4.6-10.2)*	2.3 *(0.1-4.6)*	29 *(2-65)*	4.7 *(1.3-9.8)*	5.8 *(1.4-11.9)*	3.4 *(1.1-11.9)*	1.7 *(0.5-3.0)*
Pig slurry	14	3 *(0.9-8)*	3.9 *(1.9-7.5)*	2.7 *(1.5-4.8)*	72 *(50-83)*	1.5 *(0.2-4.5)*	2.5 *(0.5-5.7)*	0.7 *(0.2-1.7)*	0.5 *(0.1-2.0)*
Bioliquids STW	3	3 *(2-3)*	1.9 *(1.2-2.8)*	0.9 *(0.6-1.1)*	55 *(31-96)*	1.7 *(0.9-2.3)*	0.1 *(0.1-0.2)*	0.8 *(0.5-1.0)*	0.2 *(0.2-0.2)*
Biosolids STW Digested cake	19	24 *(19-41)*	11.5 *(5.1-14.0)*	2.7 *(0.1-4.4)*	23 *(3-33)*	13.6 *(8.2-17.7)*	0.6 *(0.3-0.9)*	7.6 *(5.9-11.2)*	1.5 *(0.6-2.7)*
Biosolids YW Digested/conditioned	21	33 *(24-61)*	9.3 *(5.2-14.3)*	0.8 *(0.1-2.1)*	8 *(1-24)*	8.7 *(3.5-15.6)*	1.1 *(0.3-2.8)*	5.9 *(2.5-11.3)*	2.2 *(0.9-12.4)*
Biosolids YW Dried & pelleted	1	89	44.1	2.7	6	56.3	3.0	26.3	9.8

Values are averages for each manure type, with the range of values shown in parentheses.

There were 6 miscellaneous manure types which do not fit into these manure categories here and which are not included in this table. These include 3 biosolids from other sources, 1 separated cattle slurry (solid fraction) and 2 separated pig slurries (solid fractions).

Effect of soil type

The overall mean N recovery across all 3 soil types at each site and for each year (excluding North Wyke in 2009) varied between 20 and 25% of total N applied (Table 3). It is not possible to statistically assess the effect of year or site on N recovery as different manure types were used at each site and in each year. The N recovery in 2009 at North Wyke (8.5% of total N applied), was notably lower than in the other site years. This lower N recovery can be explained by poorer grass establishment due to problems with the overhead watering at the start of the 2009 season.

There was a significant effect of soil type on N recovery (P<0.05) at each site and for each year (excluding North Wyke in 2009, where poor establishment affected N offtake) (Table 3). Nitrogen recovery was greatest from the loam soil; N recovery from the sand and clay soils were similar in 3 site years, and greater from the clay than sand in 2 site years.

The difference in mean N recovery between soil types within each site year varied between 2.5 and 6.1% of total N applied. For a manure applied at a rate of 250 kg/ha total N, this indicates a potential difference in N recovery of between 6 and 15 kg/ha of N due to soil type. This represents a small but potentially significant difference in N release from manures between soil type, that may not be accounted for in estimates of crop available N.

Table 3. Grass manure N offtake (% total N applied) – effect of soil type

	ADAS Boxworth				**North Wyke**			
	Sand	**Loam**	**Clay**	**Overall mean**	**Sand**	**Loam**	**Clay**	**Overall mean**
2008	19.3 (a)	24.7 (b)	23.7 (b)	22.5	18.0 (a)	23.1 (b)	19.5 (a)	20.2
2009	24.4 (ab)	25.7 (b)	23.2 (a)	24.4	9.7	7.8	7.9	8.5
2010	19.5 (a)	*Not included*	23.8 (b)	21.6	17.9 (a)	24.0 (b)	18.0 (a)	20.0

Values in parentheses indicate significant differences (P<0.05; Duncan's multiple range test) between soil types within each site year.

Data from the loam soil from ADAS Boxworth in 2010 was excluded.[3]

[3] In 2010 at ADAS Boxworth, there was insufficient of the loam soil to fill all the pots, therefore more soil had to be collected. Care was taken to source the additional soil from the same place in the same field. However, N recovery from the control treatments for the loam soil were often greater than for the manure treatments, and the only possible explanation being that the newer soil was different to the original soil. Therefore, data from the loam soil from ADAS Boxworth in 2010 was excluded.

The differences in N recovery between the 3 soils may be explained by differences in soil texture and/or soil microbial populations. Nitrogen mineralisation of manures/crop residues has been shown to be greater in coarse than fine textured soils, as fine textured soils can protect organic matter from breakdown (Harris and Piha, 1991; Côte *et al*., 2000). This may help to explain the greater N recovery from the loam than clay soil, however, does not explain the lower N recovery from the sand than loam soil. Nitrogen mineralisation is a microbially mediated process and differences in N recovery may also result from differences in the microbial populations and activity in the 3 soils.

Effect of manure type

The amount of manure N recovered in the grass for the different manures (means) is shown in Table 4 and Figure 2. The proportion of the total manure N applied which was recovered in the grass varied between the manure types (Fig. 2). Nitrogen uptake was greatest from the high available N pig slurry (49% of N applied) and liquid digested biosolids (46% of N applied), and intermediate from the cattle slurry (25% of N applied). Nitrogen recovery was lower from the lower available N FYM and biosolids (10-23% of N applied).

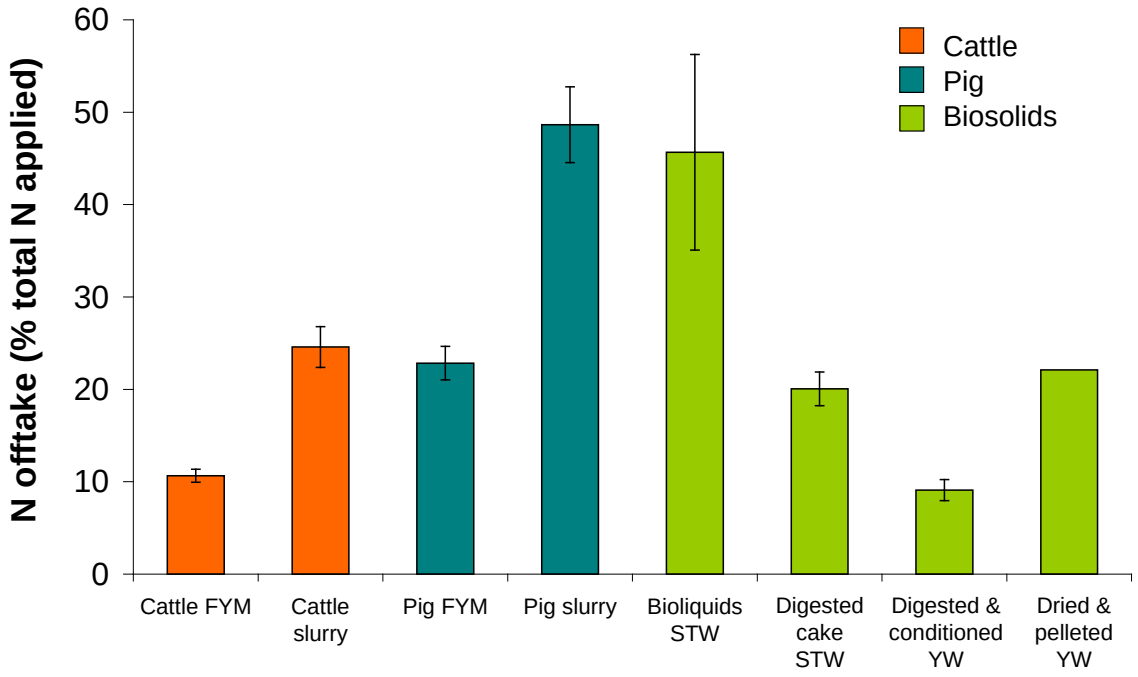


Figure 2. Manure N recovery as a proportion of total N applied for the different manure types

There was a strong relationship between N recovery in the grass and the available N content of the manure. Variation in the proportion of manure total N in the mineral form accounted for 70% of the variation in manure N recovery expressed as a proportion of total N applied (Fig. 3).

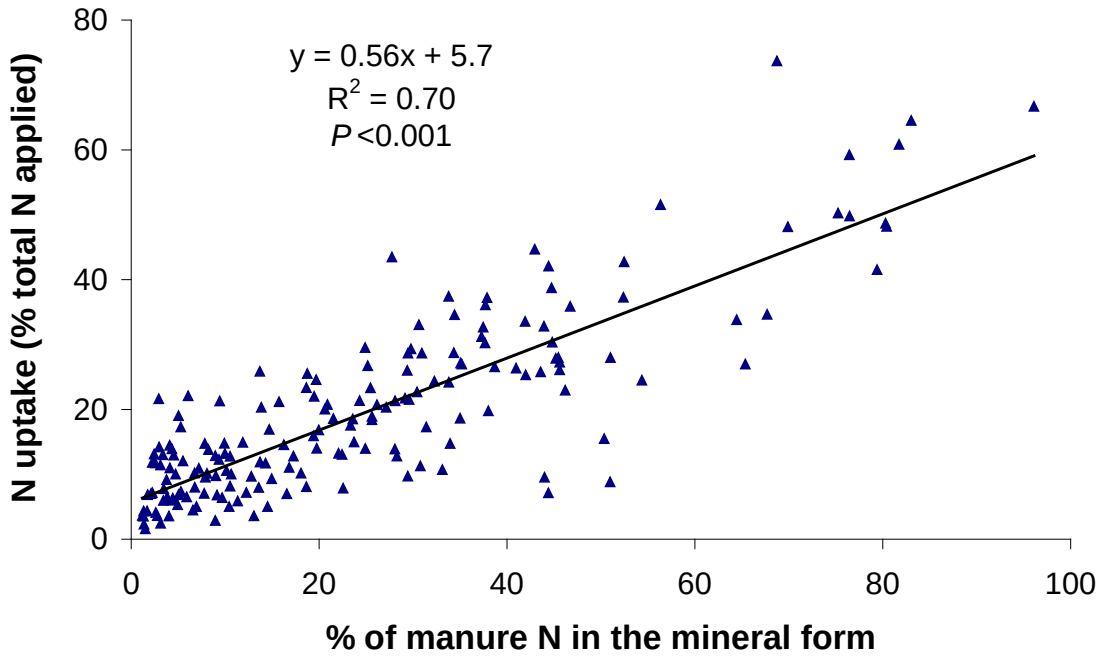


Figure 3. Relationship between manure N recovery and proportion of total N in the mineral form

The grass N recovery is the sum of uptake of the mineral N component and uptake of any organic N mineralised. It is clear from the results that the majority of the N uptake is attributable to the mineral N content of the manures. It is not possible within these data, to separate grass N uptake arising from manure mineral N and from mineralised organic N. Furthermore, it is possible that there may also have been some initial immobilisation of mineral N before/alongside mineralisation of organic N.

Table 4. Manure N recovery from the different manure types

Manure type	Number of treatments	Application rate (m^3 or t /ha)	Total N applied (kg/ha)	Mineral N applied (kg/ha)	Manure N uptake (kg/ha)	Manure N uptake (% total N applied)	Manure N uptake (% mineral N applied)	Manure N uptake (g/t manure applied)	Manure N uptake (g N/tonne manure/day
Cattle FYM	56	82	469	44	50 (4.0)	11 (0.7)	150 (13.7)	613 (48.7)	3.9 (0.3)
Cattle slurry	29	95	207	81	48 (6.3)	25 (2.2)	62 (5.4)	611 (82.6)	3.9 (0.6)
Pig FYM	32	70	532	160	124 (11.0)	23 (1.8)	114 (19.0)	1796 (159.0)	11.2 (1.0)
Pig slurry	13	68	253	185	113 (17.3)	49 (4.1)	66 (4.9)	1701 (136.0)	10.9 (1.1)
Bioliquids STW	3	120	227	107	95 (10.1)	46 (10.6)	92 (11.6)	801 (120.1)	5.1 (0.9)
Biosolids STW Digested cake	19	44	516	124	100 (8.5)	20 (1.8)	121 (35.5)	2277 (209.5)	15.2 (1.2)
Biosolids YW Digested/conditioned	21	59	521	41	47 (5.6)	9.1 (1.1)	191 (30.4)	852 (114.3)	5.0 (0.7)
Biosolids YW Dried & pelleted	1	12	549	33	121	22.1	365	9740	81.2

Values in parentheses are 1 standard error of the mean.

Pattern of N release over the season

In addition to determining the total manure N offtake from different manure types over the whole growing season, by cutting the grass either 4 or 5 times during the season, we are also able to look at the pattern of N release over the growing season. The pattern of N release over time for the different manure types was similar across site years. Figure 4 shows N offtake (as % total N applied) at each cut at ADAS Boxworth in 2009.

For all manure types, N offtake was greatest for the first grass cuts and declined over subsequent grass cuts. The difference in N offtake between the manure types was also greatest in the first 2 or 3 cuts, whilst towards the end of the growing season N offtake from all manure types declined to similar low levels. At ADAS Boxworth in 2009 (Figure 4) the majority of the difference in total N offtake for the whole season was the result of differences in N offtake in the first 2 cuts (first 52 days following manure application and grass sowing). Towards the end of the growing season, grass N offtake from the different manure types had fallen to similar low levels, with a mean N offtake of 2.1% of total N applied (range 1.5-2.6%) at the 4th cut and 1.3% of total N applied (range 1.1-1.5%) at the 5th cut. There was a trend across the site years for N uptake from pig manures (FYM and slurry) to be maintained at higher levels into the middle of the season (i.e. harvest 3) than the other manure types.

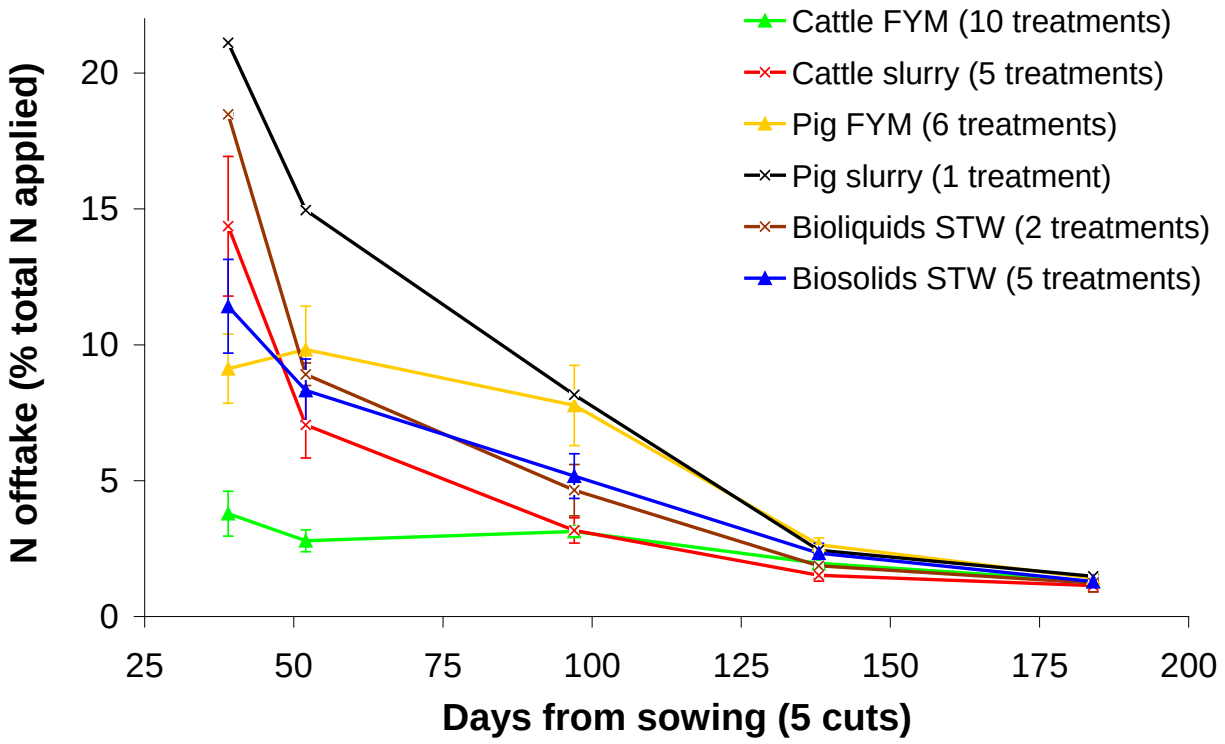


Figure 4. Nitrogen offtake (% total N applied) at each cut for manure types in 2009 at ADAS Boxworth

Second year N release

As part of the pot trial, 18 different manures were selected to be kept on for a further year to assess manure N release in the second season following manure application (residual N release). These 18 manures included 7 cattle FYMs, 4 pig FYMs and 7 biosolids (3 from Severn Trent Water and 4 from Yorkshire Water).

There was no effect of soil type (*P*>0.05) on N recovery in any of the 3 site years. This is in contrast to N recovery in the season of application, where N recovery was greatest on the loam soil (*P<0.05*) at each site and for each year (excluding North Wyke in 2009, where poor establishment affected N offtake). It is not clear why soil type would affect N uptake in the season of application, but not in the second year following application. It is possible that soil type was influencing factors controlling uptake of the manure available N (i.e. gaseous loss), and not affecting mineralisation of organic N, and therefore an effect of soil type was apparent in the first year (where N uptake was dominated by manure available N), but not the second year (where N uptake was from mineralisation of organic N).

There was no effect of manure type (*P*>0.05) on second year N recovery (Fig. 5). The overall mean second year N recovery across the 18 manures was small at only 4.2% of total manure N applied (equivalent to 5.0% of organic N applied, or 422 g N/tonne of manure applied). Therefore, against the likely background mineralisation of soil organic N, it was probably not surprising that no statistically significant differences in residual N release from manure types were observed.

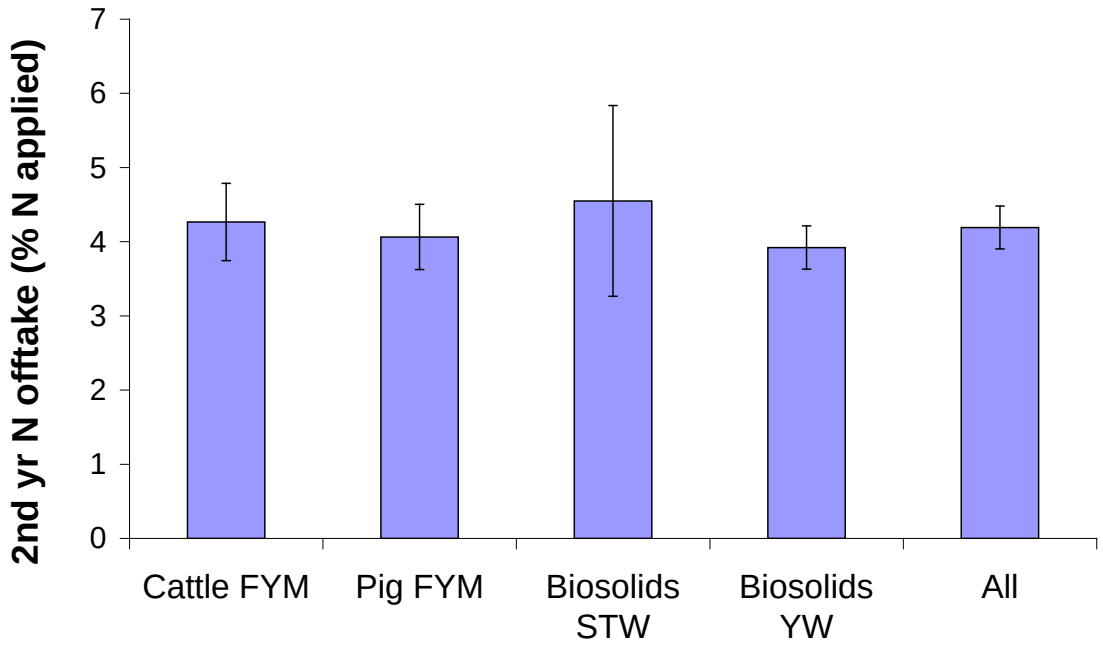


Figure 5. Second year 'residual' manure N recovery as a proportion of total N applied for the different manure types

Development of the NIRS predictable model for manure N release

A total of 180 manure samples were scanned over the three years and evaluated within the pot trial. With a 6x replicate scanning procedure this resulted in a total of 1080 scans. Spectral averaging gave 180 spectra for model development. Regressions were run using cross validation as there were only 180 samples available. Cross validation maximises the use of small amounts of data by using the data as both calibrators and validators. Larger sets would be validated against a separate test set as in the nutrient models. Regressions were run with the data expressed in the following units:

kg manure N/ha
N uptake as % manure N applied
N uptake as % manure organic N (total N minus mineral N) applied
g N uptake/tonne of manure applied
g N uptake/tonne of manure applied/day
g N uptake/100 day degrees (thermal time).

The regression runs were limited to the bandwidth 9500cm^{-1} to 3800cm^{-1} in order to reduce interference due to noise. The criteria mentioned in Appendix 2 were also relevant here for selection of the best models.

Regressions with the data expressed as g N/tonne/day gave the best correlation with spectra. Ranks for the selected models were between 5 and 7 and the loadings were no greater than 80.[4]

Calibration statistics (NIRS model for N release expressed as g N/tonne/day)

Clay – heavy type soil;

Cross validation model - R^2 91.19 RPD 3.37 5 ranks, loadings ~ 70

Loam – medium type soil;

Cross validation model - R^2 85.85 RPD 2.66 5 ranks, loadings ~ 80

Sand – light type soil;

Cross validation model - R^2 82.66 RPD 2.40 6 ranks, loadings ~ 55

[4] NIRS model development and model statistics are fully explained in Appendix 2.

Validation statistics

Clay – heavy type soil;

Cross validation model - R^2 87.44 RPD 2.82

Loam – medium type soil;

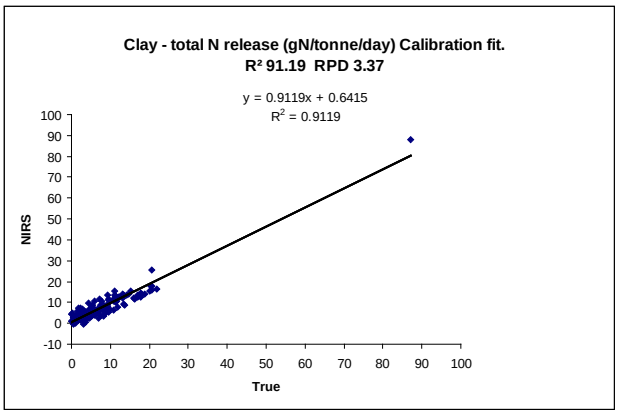
Cross validation model - R^2 84.21 RPD 2.52

Sand – light type soil;

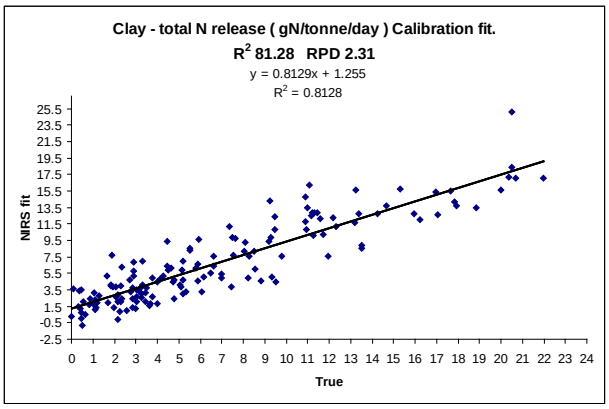
Cross validation model - R^2 80.23 RPD 2.25

Figure 6 presents the NIRS calibration plots. Calibration R^2 s are good and RPDs would indicate the models fall into the Malley classification of 'moderately successful'[5] (Malley *et al*, 2005). Another fact to consider when assessing performance of models of this type is that correlation to an 'in-direct' measurement would not be expected to be as good as those for wet analytes. As a comparison the model statistics of the *in vivo* digestibility in cattle is R^2 0.82 and RPD 2.33. It can be seen from Figure 5 plots a, c, and e that there is an influential sample (a biosolid) that also appears as the highest value in the total N nutrient model. However, good correlation with this sample removed is still maintained as demonstrated in Figure 5 plots b,d, and f. As this sample value is high in both models, this is a good indication that it is a valid sample to retain in the organic N working model, though ideally in NIRS models the distribution of values would be even between the extremes. If further funding can be secured to add poultry manures to the NIRS nutrient models (a recommendation for future research), there will be opportunity to add to these models and fill in deficiencies within the model assessment range.

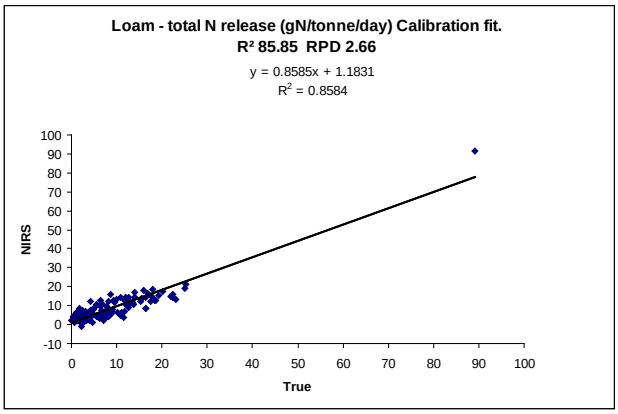
[5] The Malley classification is explained in Appendix 2.



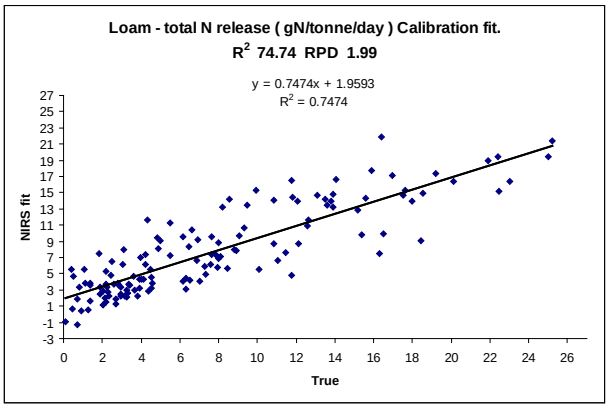
(a) N release in clay soil



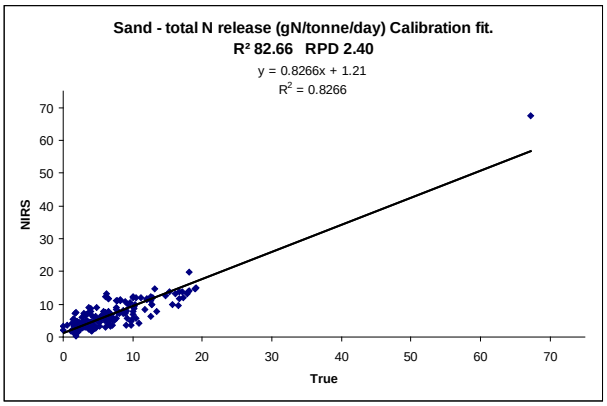
(b) N release in clay soil (high sample removed)



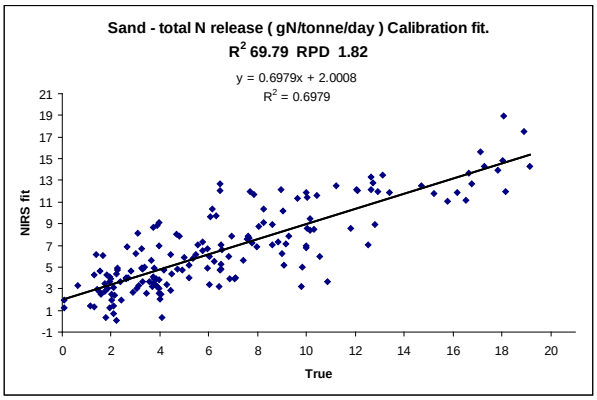
(c) N release in loam soil



(d) N release in loam soil (high sample removed)



(e) N release in sand soil



(f) N release in sand soil (high sample removed)

Figure 6. Calibration models for manures

Conclusions

This trial has generated a large amount of data on N uptake from a range of contrasting manure types. The amount of manure N that can be taken up by a crop is the sum of uptake from the mineral N component and uptake of any organic N mineralised. As expected, it is clear that the majority of the N uptake in the season of application is attributable to the mineral N content of the manures. It has not been possible within these data, to separate grass N uptake arising from the

manure mineral N and that from mineralised organic N. Furthermore, it is possible that there may have been some initial immobilisation of mineral N before/alongside mineralisation of organic N.

The improvement in the calibration of the NIRS model (R^2= 83%, 86% and 91% for the sand, clay and loam soil, respectively) for predicting N recovery, compared to proportion of available N alone as a predictor (R^2=70%), indicates that the NIRS model is able to account for the mineral N component and mineralisation of organic N in estimating manure N release. However, refinement of the calibration model will be necessary in order to separate out the prediction of organic N mineralisation, the component which is lacking within current advice.

The improved fit of the NIRS model when taking each soil separately rather than considering the 3 soil types together, indicates the capacity of the technique to account for other site specific factors controlling N uptake – either by affecting the availability of the manure available N content, and/or affecting mineralisation of manure organic N. This project has demonstrated the potential of the NIRS technique to provide farmers with an improved estimate of the fertiliser N value of their manure.

Further research

This project has demonstrated the ability of NIRS to predict N uptake from manures. However, further work is required to develop this predictive capacity for use by farmers.

The current NIRS model gives a prediction of N release in units of g N/tonne of manure/day. Further work is needed on how best to present/interpret these results for manure applications to crops with different length growing seasons, and also for manure applications at different times of the year.

Further work is also needed to confirm the controlling effect of soil type, and determine if this can be incorporated into a model that is applicable for all soil types. Additionally, the NIRS predictions of crop N uptake should be validated under field conditions with small plots.

REFERENCES

Anon. (2010) RB209 Fertiliser Manual (RB209). The Stationery Office.

Chambers, B. J., Nicholson, R.J., Smith, K. A., Pain, B. F., Cumby, T.R. and Scotford, I. M. (2001). Spreading systems for slurries and solid manures. Booklet 3, “Managing Livestock Manures”. Published by IGER, ADAS and Silsoe Research Institute (Revised edition, 2007).

Côté, L., Brown, S., Paré, D, Fyles, J. Bauhus, J. (2000). Dynamics of carbon and nitrogen mineralization in relation to stand type, stand age and soil texture in the boreal mixedwood, Soil Biology and Biochemistry, 32 (8-9), 1079-1090,

Harris, M. and Riha, S. (1991). Carbon and nitrogen dynamics in forest floor during short-term laboratory incubations. Soil Biology & Biochemistry, 23. 1035–1041.

Malley, DF, C McClure, P D Martin, K Buckley and W P McCaughey 2005. Compositional analysis of cattle manure during composting using a field-portable NIRS. Commun. Soil Sci Plant Anal, 36: 455-475.

Smith, K A, D Farrington, S Shelley, S Lister and P J Hobbs 2005. Rapid, reduced cost analysis of manures by near-infrared spectroscopy. CSG15 Final Project Report, Defra Research Contract NT2508 (available via Defra website: http://www.randd.defra.gsi.gov.uk/)