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international competence center

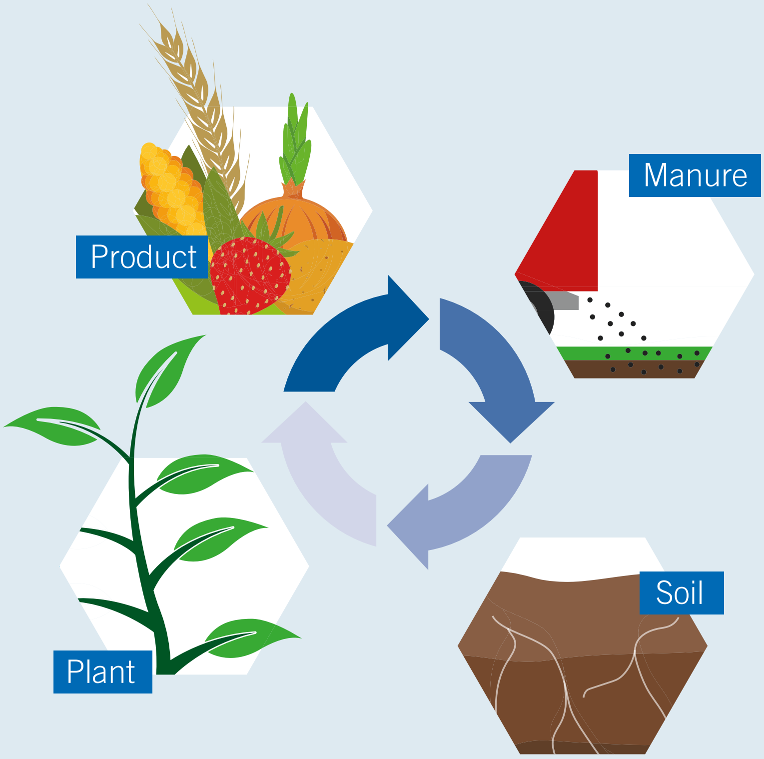
The science behind the tools

The Eurofins Agro international competence center is the global research and innovation organization of Eurofins Agro. It is the scientific backbone of the 600.000 annual analyses of soil, plant, food, feed, fertilizer, water, manures and wastes. Established in 1927 and currently located in Wageningen, the Netherlands.

Advances in analytical techniques and instruments for the analyses in agro & food have been fast in recent decades. The context and criteria of the analyses have also changed rapidly. Food quality and safety are much higher on the societal and political agenda's than 20 years ago. Food security has changed into nutritional security. Environmental sustainability has become a key issue in food and feed production, and farmers in an increasing number of countries have to comply with series of quality targets from both governments and food industries. As a result, the demand for rapid and accurate analyses of soil, plant, food, feed, fertilizer, water, manure and waste has increased tremendously. This requires constant innovation, that the Eurofins Agro competence center provides with setting standards in the international agro testing community.

System approach

Eurofins Agro embraces the system approach, acknowledging the inter-relationships and inter-dependency of the components of a system. By analyzing the agrofoodsystem and its components, we get a better understanding of the functioning of the agrofoodsystem, and of the factors that may influence and modify the systems. We believe that providing tools for the system approach contributes to improving crop productivity and quality, and to nutritional security and environmental sustainability.



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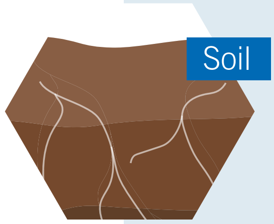


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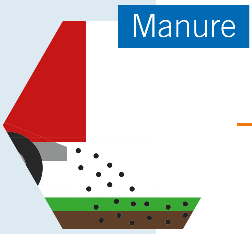
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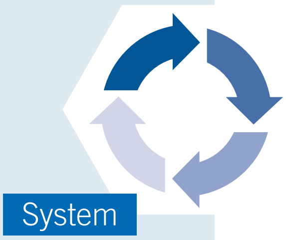
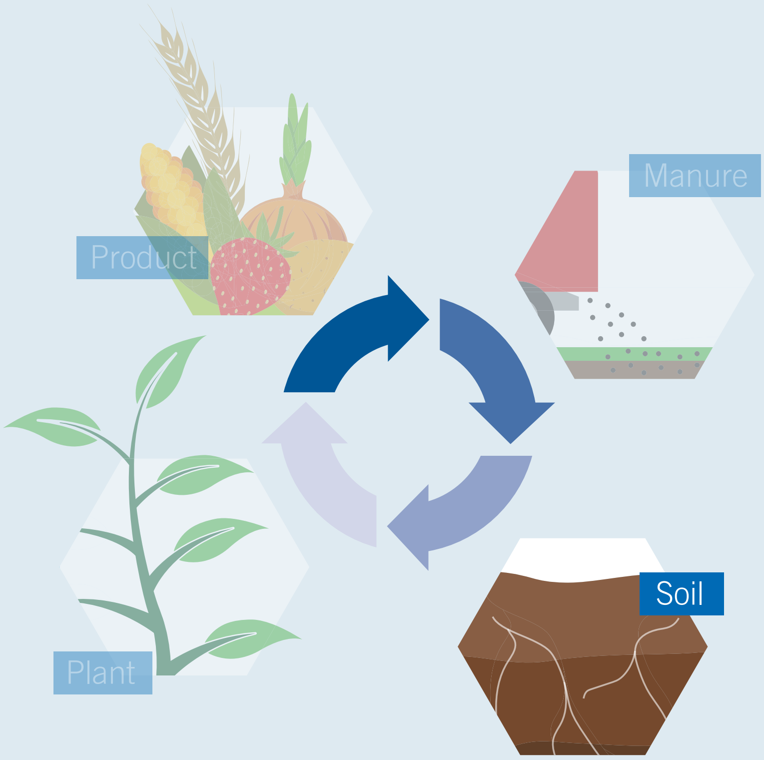

System

How we look at soil fertility



Chemical aspects

At the end of the 19th century soil testing started assessing the soil fertility status. At first only ‘total’ stocks of nitrogen (N), sulphur (S), phosphorus (P), potassium (K), calcium (Ca) and magnesium (Mg) were measured. An important discovery was that nutrients were present in the soil in several chemical forms, which vary in availability for uptake by plant roots. Since then, the search has started for suitable soil extractants and (much later) sensor techniques to relate soil fertility status to crop production potential and to the response of crops to nutrient inputs.

Physical aspects

Soil physical characteristics greatly influence crop yield potential and soil management options. Knowledge about assessing and improving soil physical conditions is therefore key to close yield gaps and increase resources use efficiency. There is a great need for tools and approaches to rapidly assess spatial variation in soil structure, subsoil compaction, water infiltration and holding capacity, soil workability, etc.

Biological aspects

Farmers all over the world increasingly realize the importance of the soil biological fertility for producing healthy crops in a sustainable manner. Soil harbours a huge biodiversity but many species are still unknown. Soil life is also involved in soil-borne diseases, which may negatively affect crop yield and resource use efficiency. In recent years a wide range of new approaches and techniques have been developed to assess soil biodiversity and the role of this biodiversity in sustainable crop production systems.

Farmers' perceptions of soil tests: case study in The Netherlands

J.A. Reijneveld[1] and **O. Oenema**[2]

Abstract

Soil tests are indispensable in farm management. Results of soil tests may help farmers in decision making geared to high crop yields in an economic profitable and environmental sustainable manner. Testing of farmers' fields and fertilization recommendations based on soil tests have a history of almost 100 years now in some European countries, although not all farmers in these countries make use of soil tests equally and regularly. Recently the importance of soil tests and science-based fertilization recommendations has been emphasized also for developing countries, to close yield gaps, to improve nutrient use efficiency and to decrease nutrient losses.

There is however surprisingly little information about farmers' perceptions towards these soil tests. Here, we report on a study investigating farmers' opinions about soil tests and fertilization recommendations in the Netherlands. The Netherlands is of interest since it has more than 90 years of experience with soil tests at farmers' request and with fertilization recommendations based on these soil tests. A written questionnaire was developed, tested among 25 farms, revised, and subsequently sent out to a random selection of 1000 arable and horticultural farmers in different regions in the Netherlands in 2010. By then, there were about 12 thousand arable farms with a total area of 0.5 million ha, and 10 thousand horticultural farms with a total area of 0.1 million ha. The questionnaire was structured in 5 parts. Part 1 contained general questions (type and acreage of farm, crop rotation, age, level of education). Part 2 sought information about the use of information from soil tests and its appreciation (goal of soil tests, which test are important or lacking, how are results used for fertilization plans). Part 3 dealt with the use of animal manures and fertilizers (types, reasons). Part 4 dealt with soil P test (what is the target soil P status, how useful are different soil P tests). Part 5 was about soil fertility and soil quality in general (what are the main concerns). The results of completed questionnaires were analyzed; mean values obtained in the different groups were compared by t-tests, and Chi-square test (X^2) statistics were generated for comparisons of frequencies of categorical data.

Results

The response rate of our written survey (20%) was relatively low but comparable with other studies (e.g. Hayman and Alston, 1999). Results of the questionnaires were intertwined with land use history and education level. Interest in soil tests and fertilization recommendations was high among respondents; soil tests are regarded as the most important factor in realizing a sound fertilization plan (Figure 1). Extension services play an important role in translating soil tests results into fertilization plans.

Soil P status was considered the most important characteristic of the soil test, followed by Ca status and soil organic matter (SOM). Despite the perceived importance of soil phosphorus (P) status, most farmers (70%) also expressed doubt about the soil P test and associated P fertilization recommendations. As a result, farmers strived for higher than recommended soil P values. This may also be a response to the introduction of governmental regulations; P application limits were introduced in 2006, and soil P test based P application limits in 2010. Application limits have step-wised decreased over time until balanced P fertilization was achieved in 2015 at national level.

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Main concerns of farmers regarding soil fertility were SOM content, soil P status, soil structure and soil life (Figure 2). The latter two are not part of regular soil tests, suggesting that soil testing programs should pay more attention to these issues. Several respondents indicated that information was missing about (i) soil life, (ii) soil structure, (iii) quality of soil organic matter and micronutrients. This information was used to develop, implement, and promote new soil tests for routine analyses e.g. Phospholipid-derived fatty acids-method (PLFA), analytical pyrolysis, and 0.01 M $CaCl_2$ for micro nutrients, for respectively soil life, organic matter quality and micro nutrients.

Conclusion

Our study shows that results of soil tests are appreciated by farmers; soil tests form an important ingredient for setting up a fertilization scheme and are therefore be a useful tool for prudent use of nutrients, as emphasized by Sutton et al. (2013).

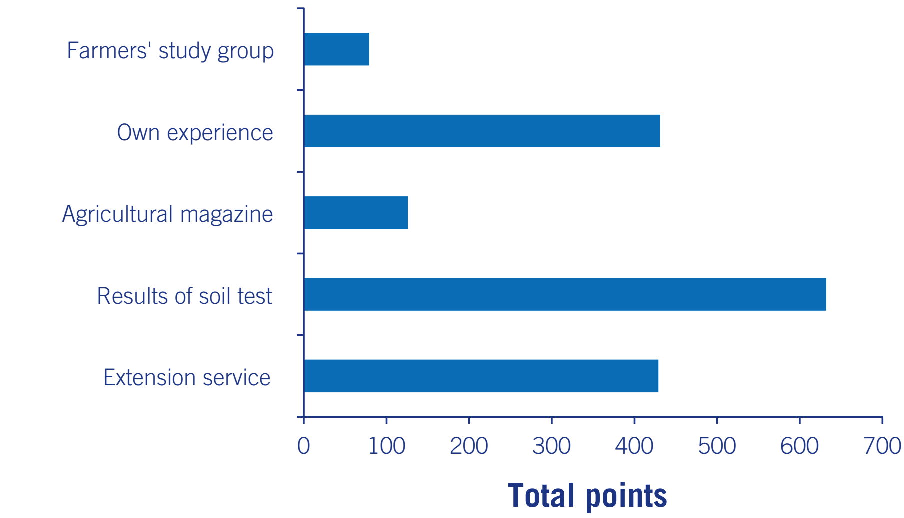

Figure 1
Responses to the question 'Who or what is most important when making a fertilization plan?'

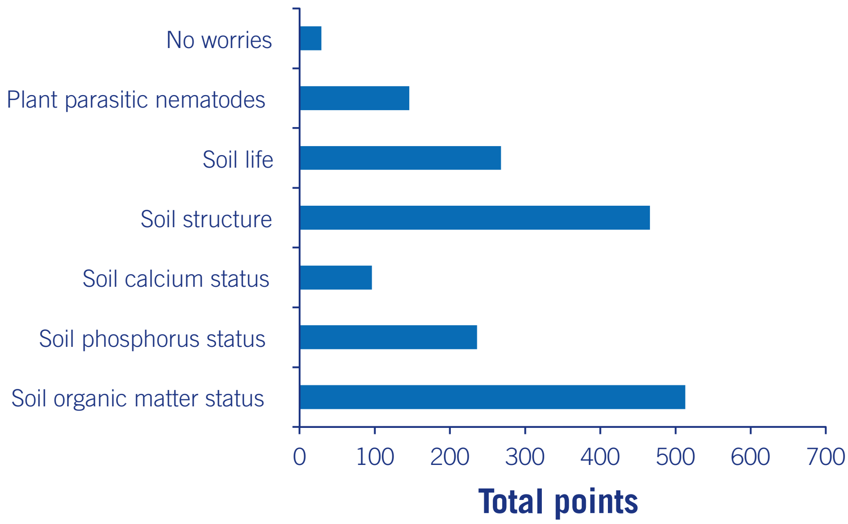

Figure 1
Responses to the question 'What is most worrisome regarding soil fertility in the future?'

Using NIRS, multi-nutrient 0.01 M $CaCl_2$ extractions and the intensity, buffering capacity and quantity concept as tools for fertilization recommendations

J.A. Reijneveld[1], **K.M. Brolsma**[1], **P.C.J. van Vliet**[1], **M.J. van Oostrum**[1] and **O. Oenema**[2]

Abstract

In most countries, fertilization recommendations are based on single soil tests of nutrients (either intensity or quantity assessments). It has been suggested repeatedly that two or more soil tests per nutrient may provide more insight in the temporal dynamics and availability of plant nutrients in soil and soil buffering, and crop response to fertilization. One may distinguish between at least two nutrient fractions in the soil: the quantity factor representing the potential soil stock and the intensity factor which is the easily (plant-) available fraction. The capacity of the soil (stock) to 'buffer' the plant available fraction is a third important factor. However, the introduction of the 'intensity-buffering-quantity' concept in practice has been delayed, in part because of the relatively high cost of analyzing multiple nutrient-availability fractions in soil.

The purpose of this paper is to describe the development, introduction and use of the 'intensity-buffering-quantity' concept in fertilization recommendations in the Netherlands. The development of the multi-nutrient 0.01 M $CaCl_2$ extractions (1:10 W/V) and the development of Near Infra-Red Spectroscopy (NIRS) for analyzing soil properties have been crucial for the introduction of 'intensity-buffering-quantity' concept in practice. The use of 0.01 M $CaCl_2$ as single soil nutrient extractant for farmers' fields was introduced in the 1990s. From 2004, it was introduced as multi-nutrient soil nutrient extractant in agricultural practice in the Netherlands. Nowadays 0.01 M $CaCl_2$ is used to measure Dissolved Organic Nitrogen (DON), NO_3, NH_4, S, P, K, Mg, Na, and Mn, Cu, Co, Se, B, Zn, Si, Mo, Fe and pH. The use of NIRS for analysing soil characteristics started in the 1990s and was introduced into agricultural practice from 2004 onwards. Currently more than 20 soil characteristics are assessed by NIRS including N-total, S-total, P-total, K-CEC, TOC, SOM, SOC, $CaCO_3$, clay, silt, sand, and CEC (Figure 1).

The concept

Due to development of the 0.01 M $CaCl_2$ multi-nutrient extractant for analysing easily extractable soil nutrients (intensity) and of the NIRS for analyzing mostly total nutrient contents (buffering and quantity), it was possible to stepwise develop and implement the intensity-buffering-quantity concept into agricultural practice. Validation experiments have been performed to validate the intensity-buffering-quantity concept using the results of 250 fertilization experiments and re-analysis of soil samples from the archive of Wageningen University TAGA.

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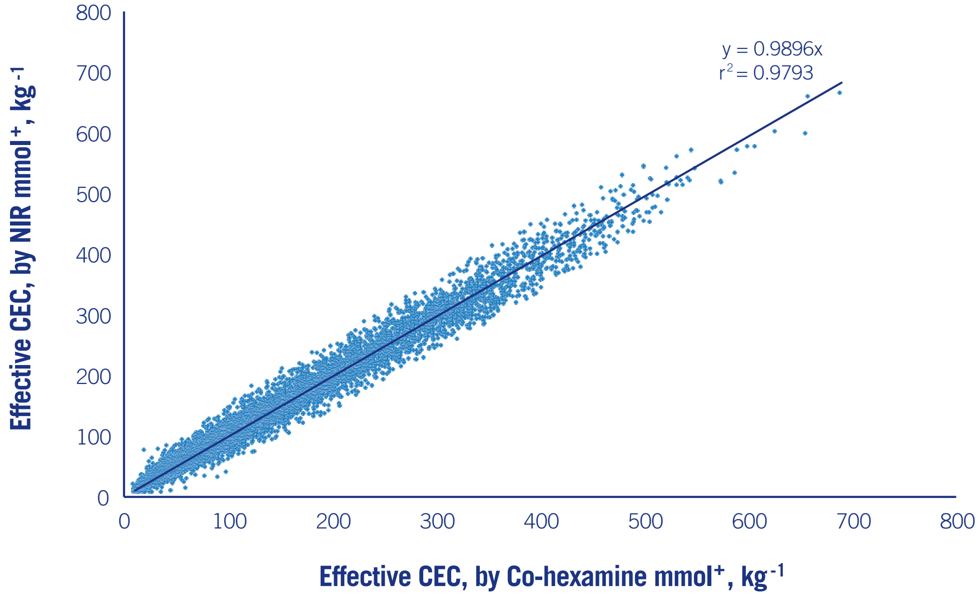

Figure 1
Comparison of effective cation exchange capacity (CEC), mmol+ kg^{-1}, as measured by Co-hexamine versus NIRS.

Conclusion

The accuracy of NIRS is very high for N-total, S-total, TOC, SOM, SOC, clay and CEC ($r^2 \geq 0.95$ & RPD ≥ 4.50), and high/good for P-total, K-CEC, $CaCO_2$ ($r^2 \geq 0.85$ & RPD ≥ 2.75). Median values of the differences were <2.5%. The advantages of only two basic soil tests ($CaCl_2$ and NIRS) over the classical methods are (i) the short analysis time and reduced costs, (ii) the introduction of the intensity-buffering-quantity concept in fertilization recommendations, (iii) the potential to introduce additional data and information on soil characteristics, including soil organic matter fractions and soil biological indices, on routine basis. Moreover, (iv) the 0.01 M $CaCl_2$ multi-nutrient extractant and NIRS can be introduced relatively easily elsewhere in the world, also in small laboratories, whereby the results can help to further optimize crop productivity and sustainability.

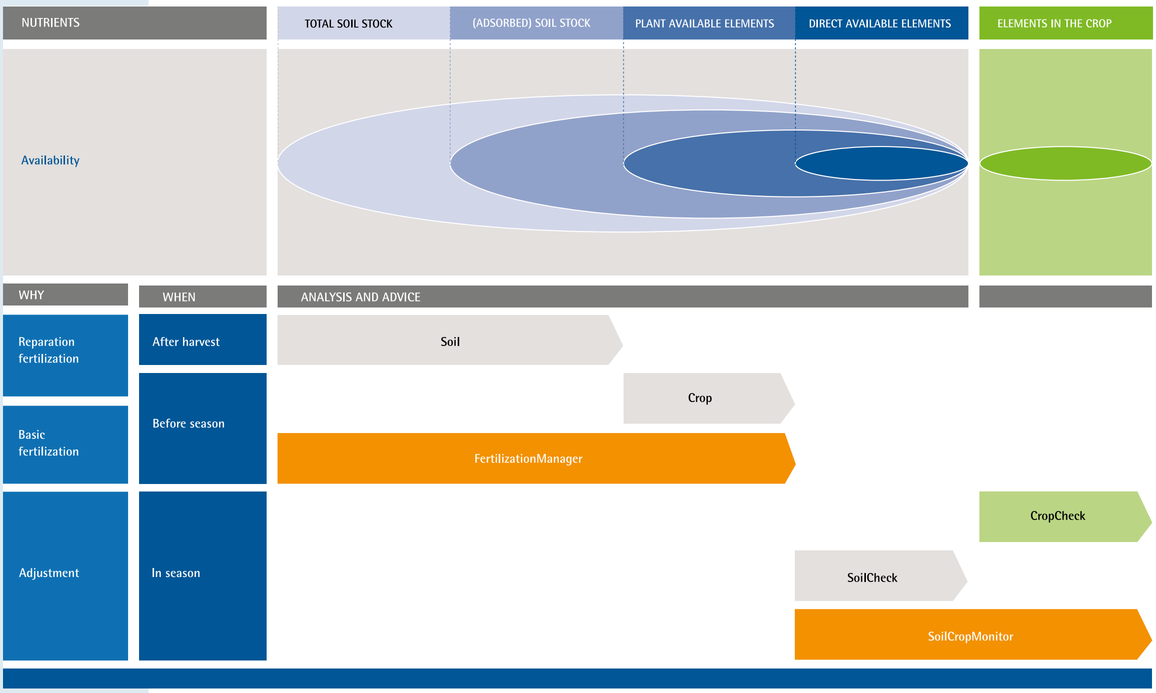

Figure 2
Eurofins Agro offers insight in the nutrient stock in the soil, the condition of the soil and the uptake by the crop. Fertilization Manager is the most complete analysis of the soil. Soil Crop Monitor provides insight in soil and crop during the season.

Potassium pool dynamics

J.G.L.M. Rongen, K. M. Brolsma and **J.A. Reijneveld**

Abstract

Potassium (K) is an essential nutrient for plant growth. K is supplied by soil, but when the soil supply is not sufficient, additional fertilizer should be applied in the correct amount as both too low and too high fertilizer applications will result in sub-optimal crop production and quality. Supraoptimal applications also lead to leaching of K. For optimal fertilization recommendations, it is therefore important to be able to understand and predict soil K supply potential. The distribution of K in the soil can be divided into K in the i) soil solution, ii) exchangeable K, iii) non-exchangeable K, and iv) structural K in minerals. Fertilization recommendation concepts are often based on one or more of these soil K-fractions. The intensity, buffering capacity and quantity fertilization concept uses both the readily plant available K in the soil (i; also referred to as intensity or K-$CaCl_2$) and K bound at the cation exchange exchange complex (CEC) (ii; referred to as quantity or K-CEC). There are however also concepts that get some renewed attention, which emphasize the importance of a specific K-CEC-saturation (ii), or ratio of saturations for each of the major cation nutrients (Ca, Mg, and K); the basic cation saturation ratio (BCSR) concept. An optimal saturation of 5% K-CEC is often promoted.

Several studies (e.g. Kopittke and Menzies, 2007; Van Rotterdam-Los, 2010) showed that the primary need is for an adequate amount of nutrient in the soil solution (i) combined with a buffering capacity of the soil (ii), regardless of the resulting percent saturation. Focusing strictly on theoretical ideal percentages and ratios can result in some nutrients still being below sufficient levels in the soil or in some cases in excess.

Evaluation K-intensity

This study aims to evaluate variation of K-intensity and its relationship with soil properties. We used a large database of routine soil tests (> 100,000) originating from agricultural land in the Netherlands. Soil intensity characteristics were measured with multi nutrient 0.01 M $CaCl_2$ (1:10 W/V), soil quantity with among others Co-hexamine for effective cation exchange complex (ECEC), (so measured at actual pH), and K-, Mg-, Ca-, and Na-CEC. The data set also included among others, soil properties like clay content, pH-$CaCl_2$, and sampling details like date, crop and sampling depth. For showing the range, we used the 2.5, and 97.5 percentile values; so excluding the more exceptional values.

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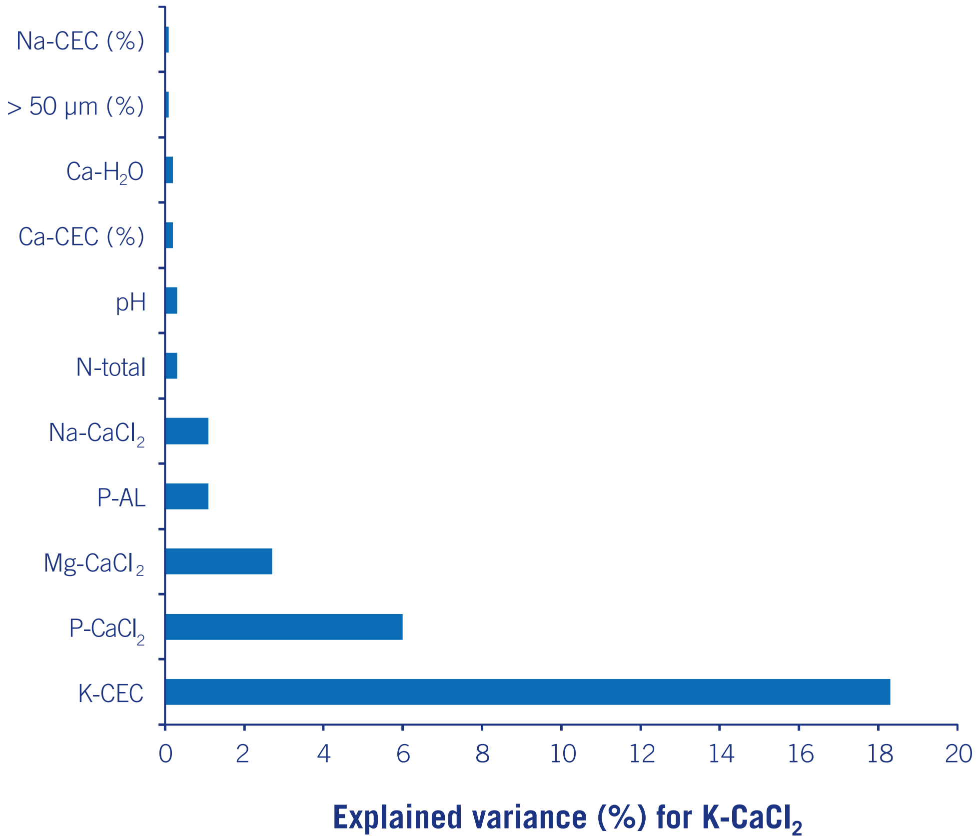

Figure 1
Explained variance per soil characteristic for K-$CaCl_2$, based on redundancy analysis.

The soil organic matter content (SOM) ranged between 0.1 - 15.3%, pH between 3 - 7.1, % particles clay (<2µm) between 1 - 13, % particles silt (>2 and <50 µm) between 1 - 29 and % particles sand (>50 µm) between 3 - 94. K-intensity levels (K-$CaCl_2$) varied from 8 up to 249 mg K kg^{-1}. K-quantity (K-CEC) levels varied from 0.5 up to 6.6 mmol+ kg, or 0.03 K-CEC% up to 8.9 K-CEC%. Within the suggested optimum of 5% (± 0,5%) K-CEC we found a similar large range in K-intensity and absolute (so not percentage since the percentage was fixed at 5%) K-quantity (mmol+ kg^{-1}). We used redundancy analysis (Canoco 5.0) to test which of the measured soil variables affected K-intensity. K-intensity (within 5%-K-CEC) was explained by absolute K-CEC 18.3%, P-$CaCl_2$ 6%, Mg-$CaCl_2$ 2.7%. Other soil characteristics like soil texture and pH had little effect on K-intensity.

Conclusion

Our results indicate that there is a wide range of K-intensity within a suggested 'optimal' K-CEC percentage. The 10^{th} percentile is 37 and 90^{th} percentile is 145 mg K kg^{-1}. Since K-intensity is highly positively related (r^2 0.97) to the absolute content of K at the CEC, all management efforts improving the effective CEC (increasing organic matter content, or clay%, or increasing pH) will have a positive effect on K-intensity. The next step is to further verify the agronomic optimal range for (K-)intensity for a given (K-)quantity and buffering capacity.

Agronomic relevance of extractable organic nitrogen

G.H. Ros[1] and **J.A. Reijneveld**[2]

Abstract

The soil organic nitrogen (N) pool supplies N during the growing season supporting crop development. The extractable organic N fraction (EON) is considered to be the most dynamic and bioavailable one. Because of its dynamic nature, EON has been used as an estimate of the N supplying capacity of soils and as an indicator of changes in soil and fertilizer management as well an indicator of environmental N losses. This study aims to evaluate the spatial and temporal variation in EON and its relationship with land use, seasonality, soil properties and soil depth. We analyzed 14.500 soils with the 0.01M $CaCl_2$ extraction procedure. Basic soil properties like soil organic matter (SOM), clay, pH, CEC and sampling details like date, crop and depth are also monitored. The influence of these environmental factors was evaluated using Restricted Maximum Likelihood mixed models. Partial Least Squares (PLS) regression was additionally used to model relationships between EON, chemical and physical soil characteristics.

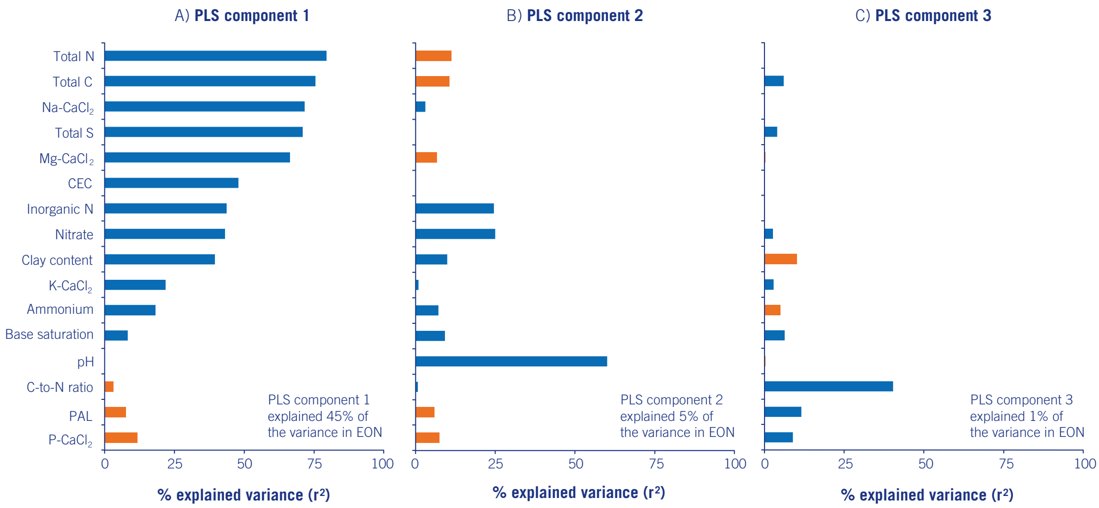

Figure 1
Explained variance for all variables in the model of EON, which explained 50% of the variation in EON. Bars with the same color are positively related to each other within the component (blue bars are positively correlated with mineralizable N, orange ones are negatively correlated with mineralizable N). Variables are sorted according to the % explained variance in the first PLS component.

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Averaged EON levels varied from 2 up to 50 mg/kg^{-1}, with higher levels in peat and clay soils than in sandy soils. Grassland fields had on average higher levels than maize and arable fields, whereas EON levels decreased with depth. We found that EON was strongly and positively related to factors associated with SOM: N-total, organic C, S-total and CEC. EON was negatively correlated to C-to-N ratio and pH whereas higher levels of phosphorus were associated with increased levels of EON. While accounting for multi-collinearity, soil pH had a small but positive impact on EON (Figure 1). Land use had a significant influence on EON, with the highest EON levels found in grassland soils. The differences among grassland, maize and arable soils might reflect differences in fertilizer history. Grassland fields are more frequently fertilized with dairy manure and are known for their organic carbon buildup within the rooting zone, both resulting in higher levels of labile organic matter. Texture had a stronger effect on EON than land use suggesting that the physical and chemical soil characteristics have more influence on EON than land use. The effect of sampling month varied between 3 and 34%. In addition, EON decreased over years 2006 to 2011 even when the effect of soil type, crop, depth and sampling month was taken into account. Since this behavior was not present for N-total, this suggests that EON is associated to changes in SOM quality.

A positive relationship among SOM, clay and EON can be explained by stabilization by clay particles. The amount of clay or silt will also affect porosity, aeration, water availability and the root development whereas all these factors affect processes controlling EON, in particular the biological ones. Consequently, soils with more EON contain more biodegradable C and available nutrients whereas an increase in clay content will decrease their availability for microbial uptake. EON had also a significant influence on the potential mineralization rate of N in agricultural soils (not shown) likely due to its association with the organic matter content ($P<0.001$). This co-linearity among EON, texture and SOM explains part of the variation found in other studies calibrating agronomic relevance of EON. Neglecting this co-linearity issue will hamper our mechanistic understanding. Due to its strong association with N-total and the low variability throughout the season, it can be questioned whether EON is more sensitive to short term changes in management or environmental conditions than total SOM.

Conclusion

Nevertheless, numerous studies have shown that within small boundaries in time or space EON might respond quicker to alterations in soil management than total soil N. Our study still confirms these finding because only 50% of the variation in EON could be contributed to soil properties. Long term changes in EON might reflect impact of decreasing manure inputs although this hypothesis is not confirmed yet. Whether changes in EON give an indication of changes in ecosystem services of SOM on the long term and at the landscape scale is still unknown, partly due to the fact that the nature and the contribution of these pools to mineralization, plant nutrition, sorption and leaching is less well understood. Overall, using EON as an indicator for processes controlling the turnover of soil organic matter seems possible.

Potential N mineralization rates in soil: an agronomic assessment

G.H. Ros[1] and **J.A. Reijneveld**[2]

Abstract

Sustainable nitrogen (N) management on arable and dairy farms requires a thorough assessment of the N supply from soil. Since 1900, soil N supply has been estimated using biological and chemical methods, including tests for available nitrate and (potentially) mineralizable N. Biological assays of N mineralization are usually applied to assess the potential of soils to supply N. However, no single approach has yet received broad acceptance across a wide range of soils since the availability from soil organic matter (SOM) during the growing season is a function of multiple biotic and abiotic factors, inducing weather conditions, management and cropping history. A widely adopted approach is the short-term anaerobic incubation quantifying the ammonium released from microbes killed by the anoxic condition in a soil-water mixture incubated for 7 up to 14 days under anaerobic conditions. Recent studies suggest that predictions of N availability improve when chemical soil extraction procedures (extracting a labile N pool from soil) are combined with other soil properties in multiple regression models. The potential of this combined approach to come up with a robust estimate of an N availability index across a wide range of soils has not been proven yet. This study aims therefore to evaluate the spatial and temporal variability in anaerobic N incubation pools using 6.640 samples incubated for 7 days. It also evaluates the potential of Near-Infrared Spectroscopy (NIR) to assess the N availability of soils directly using field based data from unfertilized grassland, corn and potato crops.

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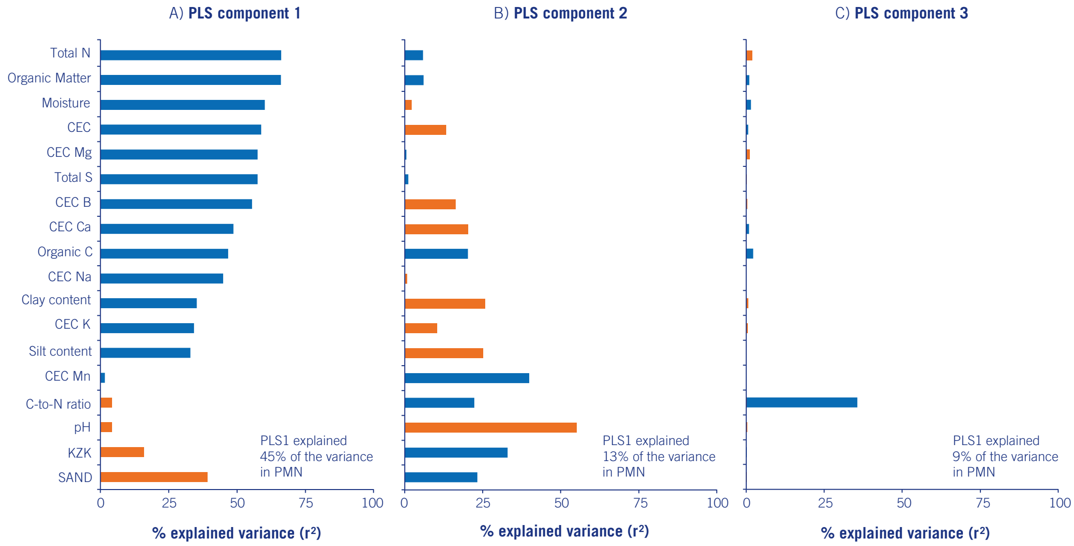

Figure 1
Explained variance for all soil variables in the model of Potential Mineralizable N (PMN), which explained 67% of the variation in PMN. Bars with the same color are positively related to each other within the component (blue bars are positively correlated with PMN, orange ones are negatively correlated PMN). Variables are sorted according to the % explained variance in the first PLS component.

Using Partial Least Squares (PLS) regression we showed three soil components controlling the size of the potential mineralizable N (PMN) pool in soils (Figure 1). The most important one is strongly positively correlated with SOM and organic N content in soils and explains almost 50% of the variation in PMN. The quality of the organic matter – as assessed by the C-to-N ratio and the SOM-to-SOC ratio – and the pH additionally explain another 26% of the variation. Cropping history, soil texture and land use affected PMN with higher levels found in grassland systems than arable systems. A combined approach of soil properties and SOM fractions can be useful for predicting potentially mineralizable N for a range of soils and climatic conditions. High uncertainty within replicates of the biological assay to analyze potential N mineralization in the lab however hampers accurate prediction for those soils with PMN values below 50 mg/kg^{-1}.

Conclusion

Environmental and economic concerns continue the need for routine methods of estimating N availability indices similar to methods for P, K and other macronutrients. We showed that NIR spectroscopy has considerable potential to predict soil N mineralization directly (data not shown), since it had comparable reproducibility and accuracy as the biological assays used. Quantification of N mineralization rates in biological assays usually differ from the actual plant N availability in the field. This questions the applicability of PMN in agronomic fertilizer recommendation systems. Validating PMN on multiple field and incubation studies, we showed that PMN was positively associated with higher N uptake in unfertilized fields. Correcting for temporal variation in weather conditions showed potential to quantify the natural N release of soils. So, combining the NIR-based prediction of potential N supply with actual measurements of (expected) precipitation and temperature resulted in a robust index supporting the N sustainability of agriculture.

Implementation of routine soil life analyses for agricultural practices

N. Poot, K. Brolsma, P.C.J. van Vliet, M. Oostrum and **J.A. Reijneveld**

Abstract

Farmers in affluent countries are increasingly aware of the importance of soil health. Soil health is important for soil ecosystem services, including organic matter decomposition, nutrient supply, and suppression of soil-borne diseases. Industries have responded to this increased awareness and an increasing diversity of soil amendments and biostimulants have become available on the market in recent years, with the aim to increase soil health. However, the way to achieve optimal soil health remains unclear. Also, the knowledge about which soil amendments are beneficial in which situations is very limited. In short, an accessible and affordable method is missing to characterize the microbial community, which allows to give practical insight to farmers.

Several methods have been proposed for the characterization of the soil microbial community. Phospholipid fatty acid analysis (PLFA) is considered to be a robust method with the ability to determine microbial biomass and different groups of microbial taxa. PLFA's are present in the cell membranes in living cells of all organisms. PLFA's degrade rapidly after cell death, thereby providing an accurate indicator of the living microbial community. Many literature studies have shown that PLFA analysis is able to determine shifts in microbial communities due to different soil management strategies, degradation and other environmental influences. Thereby giving great potential as an accessible and affordable method to improve insight in microbial communities and develop practical bio-indicators.

To validate the PLFA method, an experiment has been carried out to determine the effect of different storage methods on the fatty acid composition. A soil sample of 1,5 kg was collected, sieved, homogenized and divided into 20 subsamples. Five subsamples were pre-treated and stored per storage method. A PLFA analysis has been performed directly after treatment and after 1, 2, 4 and 8 weeks of storage time. The different treatments were cooling (4°C), freezing (-20°C), freeze-drying (stored in the freezer at -20°C) and drying at 40°C (stored at room temperature). The storage method had a significant effect on almost all individual PLFA parameters including microbial, bacterial and fungal biomass ($P=<0.001$).

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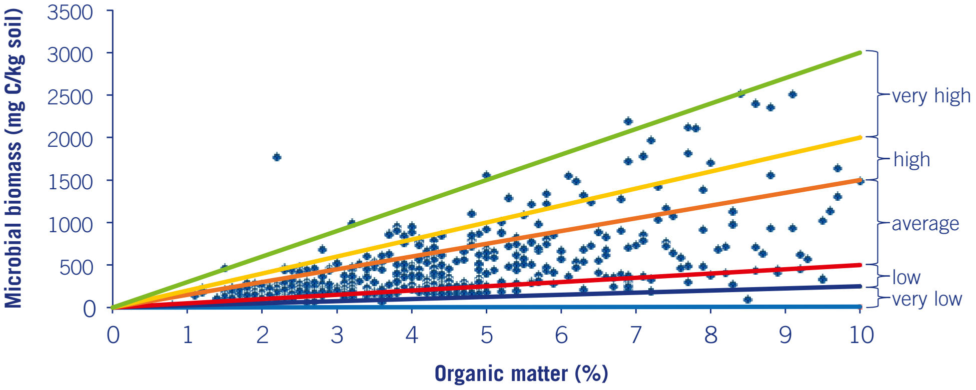

Figure 1
Relationship between soil organic matter and microbial biomass The lines indicate the percentiles on which the threshold values are based (min-10% = very low, 10-25% = low, 25-75% = average, 75-90% = high, 90%-max = very high).

For most parameters, storing the sample in the fridge resulted in the lowest amount of PLFA recovery over time. No significant effect of the storage time was found on the total microbial biomass. There was no significant difference between freezing and freeze-drying. For practical reasons, we have chosen to sieve, homogenize and store (sub)samples in the freezer until PLFA analysis.

To create threshold values and gain insight in correlations, a dataset has been created with 900 soil samples throughout the Netherlands including chemical, physical and biological parameters. The samples were derived from grasslands and arable fields from sandy, clay and peat soils. Based on these data, a near infrared spectroscopy (NIRS) calibration curve has been developed for three PLFA parameters (total microbial-, fungal- and bacterial biomass). The amount of total microbial biomass is positively correlated to the organic matter content of the soil ($P=<0.001$). Therefore, the threshold value of the microbial biomass is corrected for organic matter for that specific sample. In Figure 1, the microbial biomass in relation to the soil organic matter content is shown, including the percentiles that indicate the threshold values. The threshold values for the fungi/bacteria ratio are based on the percentiles per soil type.

Conclusion

The biological parameters "total microbial biomass" and "fungi/bacteria ratio" have been implemented on routine soil analyses. For all parameters a NIRS calibration curve have been developed. Hereby trends in e.g. microbial biomass can be examined. Currently, PLFA analysis is performed in several field trials with different management strategies and addition of biostimulants. This allows to seek for correlations between soil microbial community characteristics and relevant outcomes like yield, quality and disease suppression. Eurofins Agro aims to link the results to relevant bio-indicators and practical advice and to implement increased knowledge about soil health into agricultural practice.

Routine nematode screening through qPCR analysis

N. Poot and **C.A.M. Sibbel-Wagemakers**

Abstract

Every single farm that cultivates crops has plant-parasitic nematodes. Nematodes may cause severe economic damage, through yield loss, quality damage and export restrictions. Initially, the build-up of plant-parasitic nematode populations in soil can go unnoticed. Once nematode-induced crop damage is visible, it is usually too late and (costly) measures are needed to control the population. Periodic screening of fields gives timely insight in the build-up of plant-parasitic nematodes and can minimize economic losses.

Eurofins Agro has developed quantitative polymerase chain reaction (qPCR) tests to analyze more than 25 relevant plant-parasitic nematode species. The technique multiplies a small amount of target DNA of a specific nematode many times. With qPCR, a single target nematode can be detected in a sample containing around 10,000 different, unknown nematodes. In Table 1 the Limit of Detection (LoD) is shown for different genera. The qPCR analysis is done at the entire suspension, while with microscopic analysis, this is limited to 20% of the suspension. In general for microscopic analysis, when a high number of a certain genus is present, 20 adult nematodes are randomly selected and determined at species level under a microscope with a higher magnification. Taxonomic identification may be limited by age or sex of the nematodes. The results will be extrapolated to the entire suspension. With qPCR, all nematodes in the suspension are determined at species level. Low numbers and suppressed populations have therefore a higher detection chance with qPCR compared to microscopic analysis (Figure 1). When a low number of highly damaging nematodes are present in a mixed population with less to no damaging nematodes of the same genera, the higher detection chance of qPCR can be a big advantage. The nematodes are quantified through a fluorescence which is incorporated into the DNA and calculated back with a calibration curve.

Genus	Limit of detection
Ditylenchus spp.	1 nematode
Globodera spp.	1 larvae
Meloidogyne spp.	1 nematode
Paratrichodorus spp.	1 nematode
Paratylenchus spp.	1 nematode
Pratylenchus spp.	3 nematodes
Rotylenchus spp.	1 nematode
Trichodorus spp.	1 nematode

Table 1
Limit of detection of the qPCR tests for different genera. For each genera, qPCR tests have been developed for different species.

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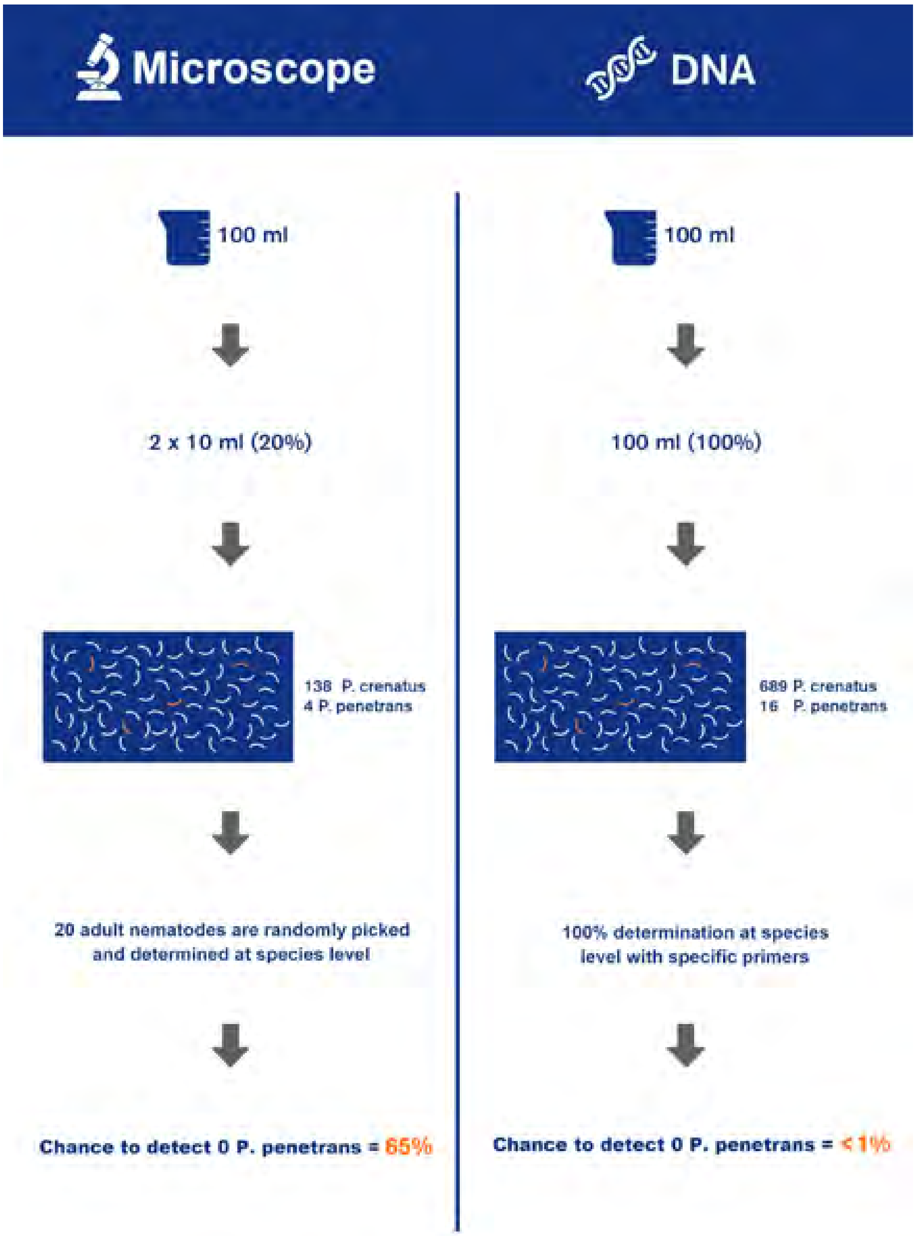
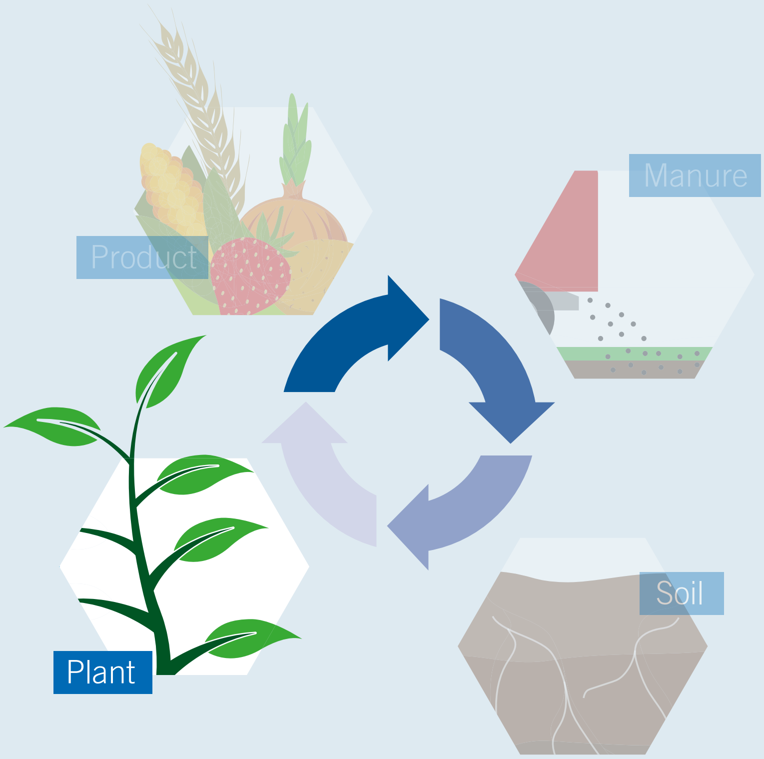


Figure 1
The detection chance of suppressed populations with microscopic and DNA analysis. The suspension in this example contained 690 *Pratylenchus crenatus* (P.c.) and 20 *Pratylenchus penetrans* (P.p.). For most crops P.c. will not cause a problem but P.p. can cause severe yield and quality damage. It is therefore important to detect P.p. at low numbers.

Conclusion

qPCR analysis is a reliable and specific method to screen for plant-parasitic nematodes. Small numbers and suppressed populations are better detected compared to microscopic analysis. Knowing which nematode species are present, also when occurring in low numbers or mixed populations, can be crucial information for the farmer. When needed, adequate control measurements can be taken to prevent economic losses. Routine nematode screening should be part of every farm's management.

How we look at plant growth and nutrition



Optimal plant growth is needed to get sufficient product of high quality with low impact on the environment. Cutting edge tools are necessary to obtain insight in plant available nutrients, soil nutrient buffering capacity, actual uptake of nutrients. Tools can also include plant growth information by satellites, drones or sensor measurements.

Dry leaf tissue and plant sap analyses compared

J. Hardeman and **P.C.J. van Vliet**

Abstract

Adequate nutrient supply, from the soil or from applied nutrients, is vital to crop production. A correct supply of nutrients is therefore needed. Insight into how much and which nutrientsa crop has absorbed allows growers to better adapt the fertilization to the needs of the cropand respond appropriately to growth disruptions.

An analysis of the crop provides the right information, enabling adjustment of the fertilization to the crop's needs. There are two common methods of obtaining insight into the nutrient uptake by the crop and the nutrient levels in the crop:

- Dry matter analysis (material dried at 70°C; extracted using HNO_3) determines both the bound and dissolved nutrients in oven-dried plant material. It therefore provides information on all the nutrients present in the plant.
- Plant sap analysis (material frozen at -20°C; extracted sap analyzed) determines the nutrient composition of pressed plant material after it has been frozen and thawed. This analysis determines the nutrients in the sap and the cytoplasm and provides a picture of the recent uptake of nutrients by the plant.

Both methods have their pro's and cons. Plant sap analysis is a fast analysis (next day result) while the dry matter analysis is a robust method with a longer turnover time (2 to 3 days). As is reported in literature, the results of the plantsap analyses are influenced by a range of factors like time of sampling, sampled part of the plant or the growing stage of the plant.

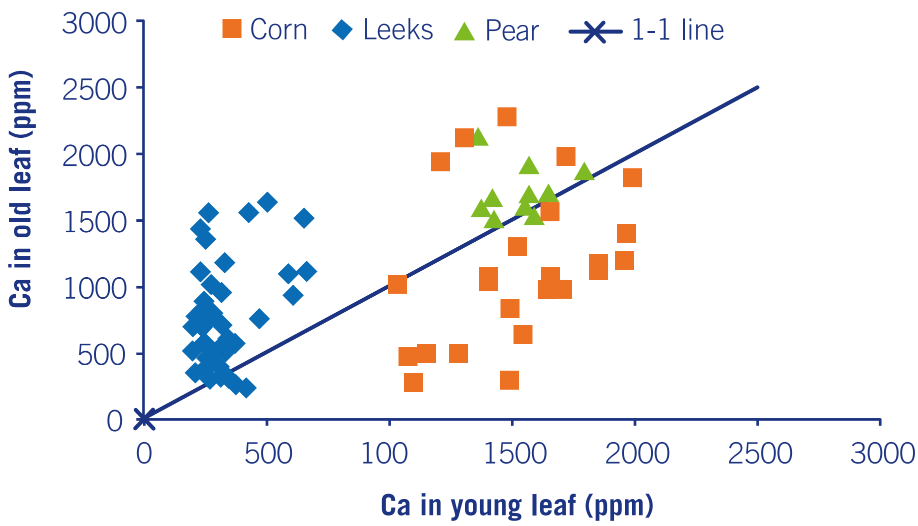


Figure 1
Results of plant sap analysis for calcium (Ca) in old and young leaves of corn, leeks, and pear.

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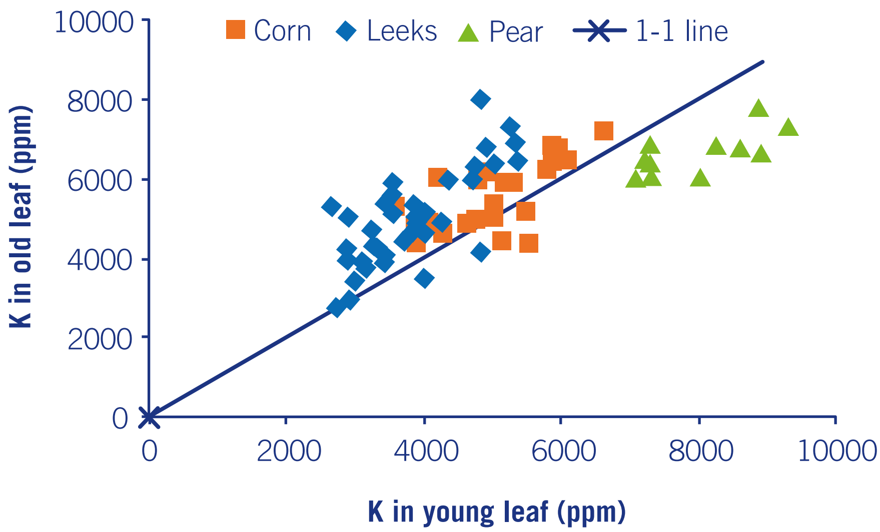

Figure 2
Results of plant sap analysis for potassium (K) in old and young leaves of corn, leeks, and pear.

Here we studied the differences and similarities in plant sap and dry matter analysis. We used a data set from agricultural trials in the Netherlands in corn, leek and apples. Besides the nutrient content of the leaves/crop, the soil was analyzed both at the start of the growing season as well as during the growing season. We carried out regression analysis comparing dry matter with plant sap, examining temporal effects, and effect of soil status and fertilization treatments on nutrients in tissue. The relation between dry matter analyses and plant sap was puzzling. Molybdenum (Mo) for example showed a high positive relation (r^2 >0.85.; $p<0.05$: $n=77$) for as well older, as younger leaves, throughout the entire growing season. On the contrary, plant sap Calcium (Ca) in the younger leaves showed a negative correlation with dry matter analyses, while no relation was found between dry matter analyses and plant sap Ca in the older leaves.

The ease with which nutrients are transported through the plant is likely to explain some of the differences. Readily soluble elements such as potassium (K), sodium (Na), chlorine (Cl) and nitrate (N) are mainly present in the sap and the cytoplasm, whereas calcium (Ca), magnesium (Mg) and phosphorus (P) are largely bound in the plant cell structures. Consequently, the results from a dry matter analysis and a plant sap analysis are likely to differ for certain nutrients. In addition, the life cycle of the plant consists of various stages (such as the vegetative stage, the generative stage and the reproductive stage) which may influence the chemical composition of the sampled. When interpreting the results of the two methods it is, therefore, important to take the plant's development stage into account. In the Figures 1 and 2 differences for K and Ca (measured via plant sap analysis) for old and young leaves of corn, leeks and pears are shown.

Conclusion

These figures show that the analysis patterns are different for different crops. In corn leaves twice as much potassium is found than calcium. Also plant sap calcium can be three times higher in older corn leaves compared to young corn leaves. So crop type and growing stage of the sampled part of the plant are influencing the plant sap results.

Phosphorus fertilization recommendations for potato based on a combined soil test indicator

P. Ehlert[1], **J.A. Reijneveld**[2] and **O. Oenema**[1]

Abstract

The current phosphate (P) fertilization advice for arable crops and field vegetables in the Netherlands uses a soil test based on an extraction with water (Pw-value) and is thus an intensity parameter. The parameter does not take into account the capacity of soil to supply phosphorus to the crops that will become plant available on the longer term. By neglecting this capacity to supply soil phosphorus on the longer term the phosphorus requirement of crop can be overestimated.

In the Netherlands a change in phosphorus recommendations based on soil testing has started about ten years ago. Focus on a single phosphorus soil test parameter which determines crop available soil phosphorus on the short term (intensity parameter) is changed to a combination of the determination of both the intensity and the capacity of a soil to supply phosphorus also on the short and the longer term. For this the term combined soil phosphorus indicator has been introduced. As a measure for the intensity parameter 0.01 M $CaCl_2$ (1:10 w/v) is used and for the capacity parameter an extraction of 1:20 (w/v) with ammonium lactate-acetic acid.

The aim of this research is to develop a new advisory system for potato that is based on the combined soil phosphorus indicator. The method for developing the new advisory system for potato made use of the measurements of the same phosphorus field experiments on which the calibration on Pw-value for potato was based. These field experiments are conditioned by the selection criterion that intensity and capacity parameters show poor correlation. Air dried soil samples of the field experiments were archived and could be used for the new calibration of the response of potato on phosphorus fertilization on the combined soil phosphorus indicator. Preliminary tests showed that storage did not exerted an change in soil P test values. These data were complemented with data from other phosphorus field experiments with potato. Ultimately 179 field experiments were selected which were conducted in the period 1958-2008. Yield maxima of these fields experiments did not change in this period taking annual temporal variation into account.

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Soil samples were analyzed on a soil phosphorus intensity parameter P-$CaCl_2$ and a soil phosphorus capacity parameter based on P-AL-value. Also the soil parameters pH, organic matter, texture and other nutrients were determined either by extraction with 0.01 M $CaCl_2$ (1:10 w/v) or by near infrared spectrophotometry (NIRS).

A general statistical soil fertility model was chosen to integrate phosphate availability soil parameters on the short and longer term and controllable cultivation measures. Controllable cultivation measures in this study included fertilization application rates of nitrogen and potassium. Parameters were estimated with the Restricted Maximum Likelihood (REML) procedure.

Taking into account the environmental factors (location, experimental year) including the number of soil samples per field experiment, the nonlinear model explains 90% of the variance. Phosphate intensity (P-$CaCl_2$) and phosphate capacity (P-AL-value) without taking environmental factors into account explain around 10% of the variance. Nitrogen (N) fertilization had no significant effect on yields due to the small range in application rates. Potassium (P) fertilization was not included in the statistical model although an significant but not logical effect was found.

Conclusion

Based on this nonlinear model three phosphorus fertilizer recommendation were derived:

1. A crop-based recommendation that, for given values for the combined phosphorus indicator, recommends a phosphate application rate for achieving the optimum economic return.
2. A soil-based recommendation to maintain target values for the combined phosphorus indicator to achieve an optimum for crop production.
3. A soil-based recommendation for reparation of a soil phosphorus that is too low for crop production.

Water quality and fertilization in high intensity horticulture

T. Aanhane

Abstract

The world population is expected to reach 9.8 billion in 2050 and 11.2 billion people in 2100, according to the United Nations' most recent forecast. To be able to feed the world in the next 30 years, the world must produce as much food as has been produced in human history so far. This requires more efficient food production systems compared to today's systems.

High intensity horticulture can make an important contribution to higher yields and a sustainable production. Greenhouses (i.e. closed systems) provide a protected environment to grow crops. In addition, innovative systems of climate control and fertilization management offer the possibility to optimize growing conditions. For instance, hydroponics enable us to grow produce without soil and thus allows food production in any place, anywhere. However, adequate nutrient supply is vital to crop production. A correct supply of nutrients in the water source is therefore needed. But does the grower know how much of the various nutrients the crop has absorbed and how much extra nutrients are required? Regular water analysis is needed, to provide the right information, enabling the grower to adjust the fertilization in the water to the crop's needs. High salt (like sodium (Na) and chloride (Cl)) concentrations in the water can harm plant growth and plant development. A suboptimal pH can have a negative effect on the uptake of essential nutrients. Too high Fe-values can result in clogging of drippers and sprinklers due to the formation of an insoluble iron salt. In the Netherlands a lot of greenhouse growers are used to weekly analyses of their fertigation water.

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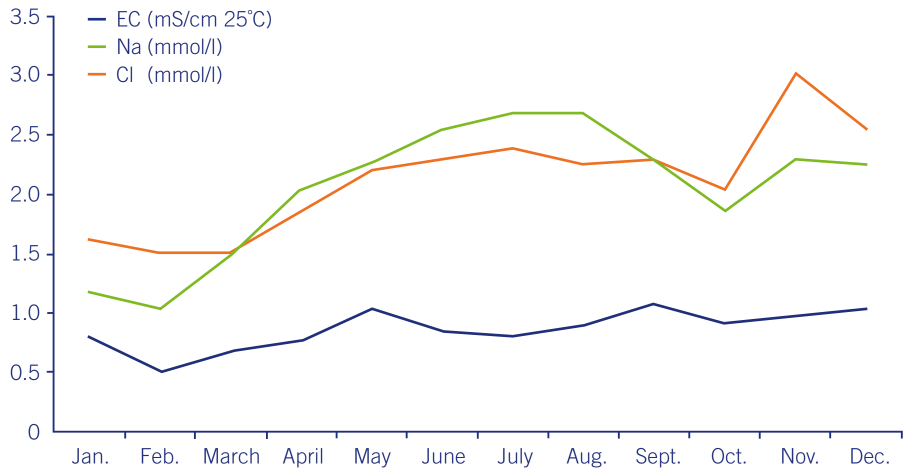
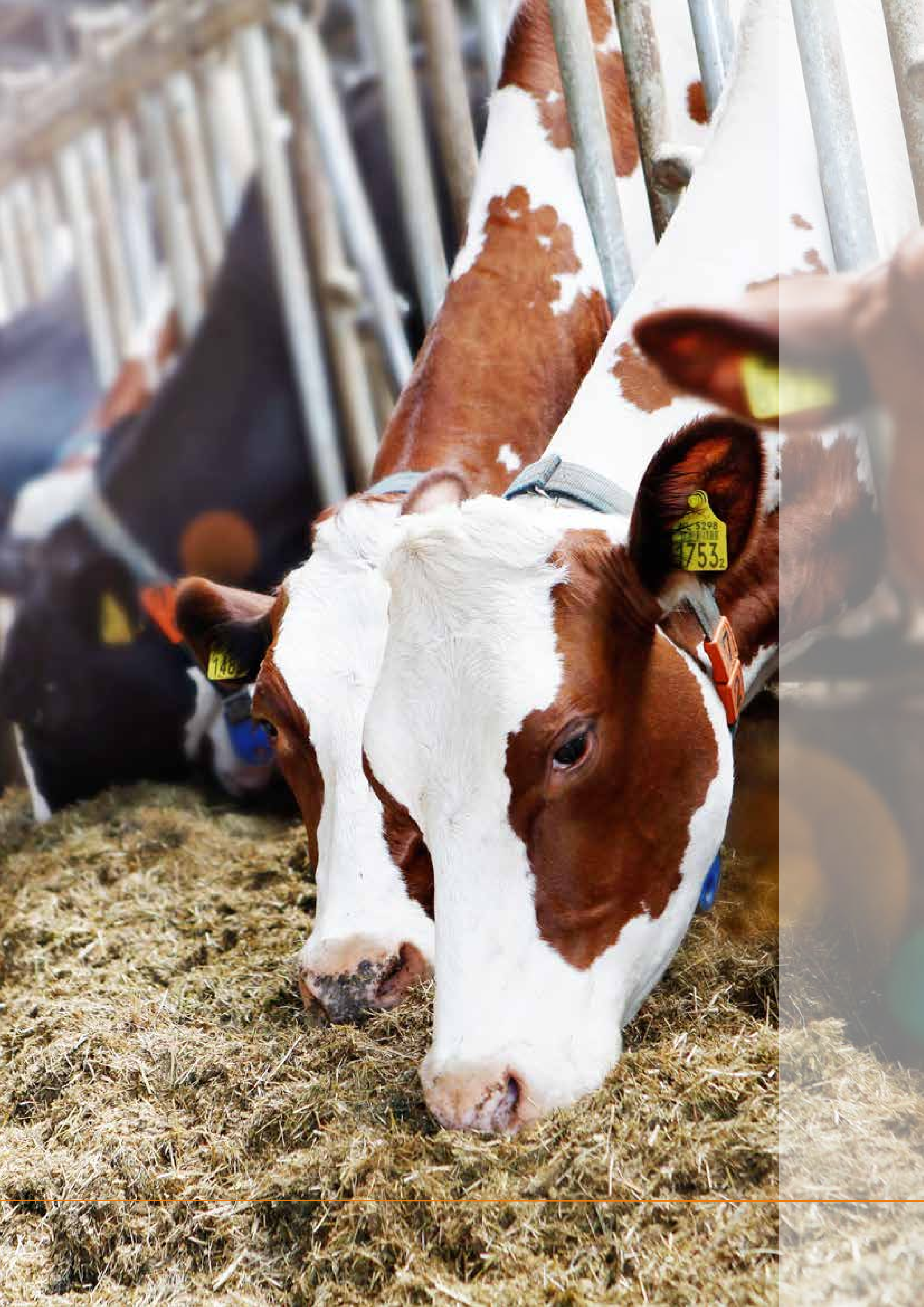


Figure 1
Average sodium (Na) and chloride (Cl) values and electric conductivity (EC) from water sources in Dutch greenhouses in the Westland in 2018.

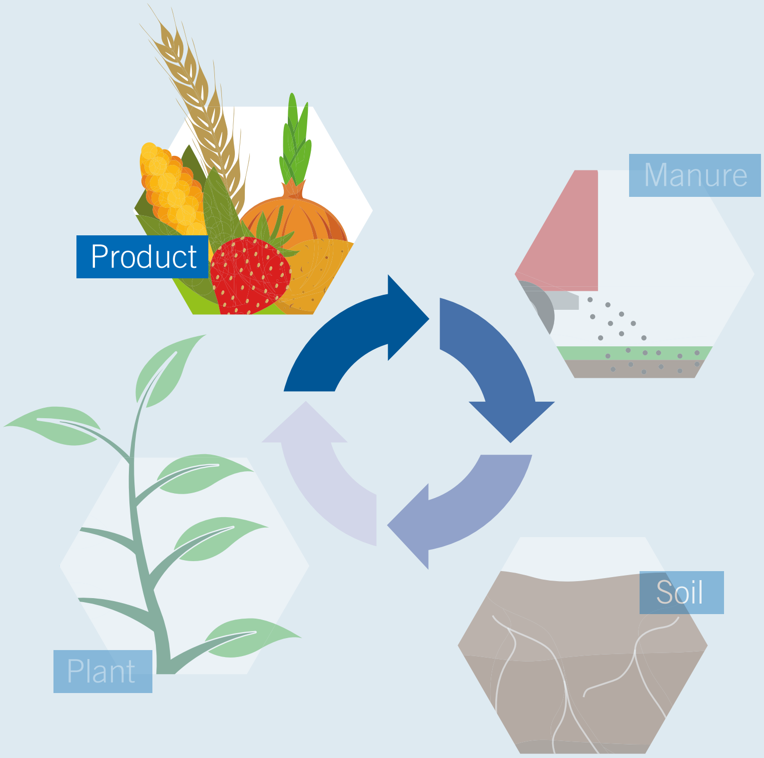
The importance of the analysis of the water source and fertigation water became very clear in the warm, dry summer of 2018. Due to drought, growers were forced to replace their commonly used water sources (rain water basins) with other water sources, such as ground water or surface water. Eurofins Agro collected water from over 100 greenhouse growers in Westland (The Netherlands). These solutions were analyzed using Inductively coupled plasma atomic emission spectroscopy (for sodium), discrete analysis spectroscopy and continuous flow spectroscopy (for chloride). The data showed that as a result of the shortage of rainwater, the sodium (Na) and chloride (Cl)-values of the starting water increased seriously (Figure 1). During the growing season, average values permanently exceeded the maximum levels of 1,5 mmol/liter with a maximum of 5 mmol/liter (references 1,2 and 3). Growers were forced to use expensive municipal tap water or decided to take (expensive) technical measures to remove sodium from their water.

Conclusion

A closed system (like horticulture) needs to be controlled carefully, because the system is very sensitive to mismatches in fertilization, bad water quality and diseases. Frequently monitoring water quality (esp. nutrient content) of the water source and the drain and fertigation water are the first steps to ensure a higher production.



How we look at product quantity and quality



Soil fertility is linked to food quality. It has been argued that long-term single N, P, K fertilzers applications and the growth of high yielding crops have decreased the amounts of secondary nutrients (e.g. calcium) and micro nutrients in the soil. This may have resulted in lowered levels of (micro)nutrients in vegetables or forage and as a consequence in a lower intake of micronutrients by humans or animals, respectively. Unbalanced inputs of nutrients may cause quality problems in potato (internal brown spot), lettuce (tipburn), and forages (milk fever).

Comparison of NIRS and wet chemistry methods for the nutritional analysis of haylages for horses

F.B. Fabri, S.C. Podesta, M.J. van Oostrum and **K.M. Brolsma**

Abstract

During the growing season, horses and ponies are – at least partially – being fed with fresh grass. However, health issues may appear following excessive uptake of fresh grass, depending also on its composition. Analysis of the grass quality, especially carbohydrates content, may provide insight into the risks of health issues. Grass consists of two general categories of carbohydrates: non-structural and structural. Starches and sugars are known as non-structural carbohydrates (NSC). The sugars can be divided in mono- and di-saccharides (simple sugar, detected by Luff Schoorl analysis), starch and non-starch poly saccharides (NSP) (not detected by Luff Schoorl analysis). NSC should not exceed 120 g/kg^{-1} DM in order to keep laminitis (inflammation of the laminae of the foot) under control (e.g George, 2013). Studies show that NSC can accumulate to > 400 g/kg^{-1} dry matter (DM), and may cause a serious health risk for horses. Within NSC, the NSP fraction is often considered as most harmful, and within NSP, fructans are an important fraction (up to 75%). Fructan concentration is influenced by genotype of the grass and environmental conditions (lower temperatures and insufficient moist will increase fructan concentrations). Some literature suggest an effect of nutrient status of soil and crop on the content of fructans.

We studied the effects of soil status and plant composition on the content of fructans, as well as the possibilities of analyzing fructans by Near Infrared Spectroscopy (NIRS) instead of the traditional method (Metodbeskrivning 22, SLL; Larsson, 1983). There is some debate about the supposed negative effect of fructans on laminitis, and whether fructans can be measured on routine basis. Insight in the relationships between fructans and potential health problems make grassland management for and nutrition of horses easier. NIRS analysis is cheaper and less time-consuming than traditional methods; therefore we explored whether fructans can be determined accurately in routine grass analyses via NIRS. In addition, NIRS enables quick measurement of multiple grass (quality) characteristics at once, whereas traditional methods can take up to several days for just one analysis.

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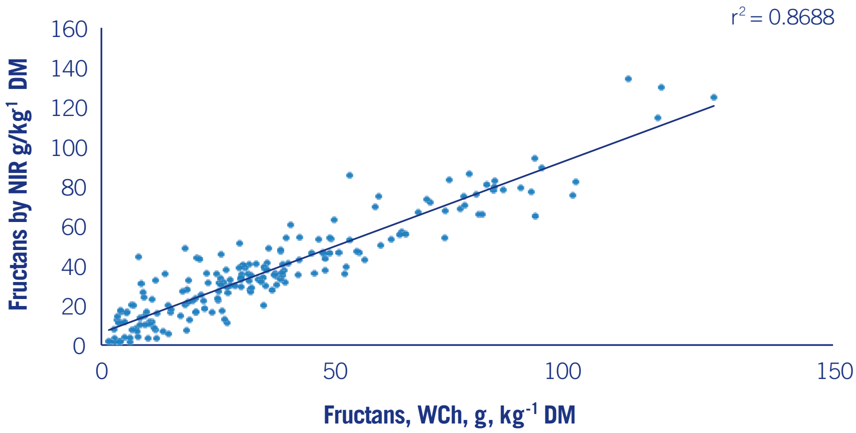

Figure 1
Comparison of fructans, g/kg^{-1} DM as measured by wet chemical method (WCh) versus NIRS.

For our research, 200 hay samples from several on-field trials were collected in the Netherlands and have been sent to the Sveriges Lantbruksuniversitet (SLU) in Uppsala, Sweden to be analyzed with the traditional method. At Eurofins Agro in Wageningen soil and grass was analyzed; including NIRS analyses (in dried material). Dry matter contents of the hay and grass were all above 500 g, kg^{-1} with a median of 862 g, kg^{-1} (sd. 117). Sugar ranged from 0.8 g, kg^{-1} up to 330 g, kg^{-1} (sd. 53.2). Fructans ranged between 0 and 11 g/kg^{-1} (sd. 2.23). Statistical analyses of the results of the field trial yielded a significant correlation between soil potassium status (K-$CaCl_2$) and fructans ($r^2 = 0.33$, $p < 0.05$), but no significant correlation was found between potassium in grass and fructans. Another significant correlation was between fructans and zinc (Zn, $r^2 = -0.63$, $p < 0.05$). This might be explained by the role of Zn in carbohydrate metabolism. Studies show a decrease of involved enzymes during Zn deficiency conditions.

Conclusion

A strong correlation between NIRS and wet chemical analysis ($r^2 > 0.85$, $p < 0.01$) was found (Figure 1). Median values of the differences were < 1%. The coefficient of variance was large (%cv: NIRS 69; wet chemical (WCh) 77) meaning that the variance in the dataset was sufficiently large to use NIRS as a replacement method for the wet chemical analysis method. Fructans can be reliably analyzed through the NIRS method. In order to manage the amount of fructans in grass and hay, it is important to understand how to change the fructan value. Through NIRS, fructans contents in grass and hay can be analyzed quickly, and relationships with other soil and plant characteristics may be established, including those observed during this study. This may contribute to establishing recommendations to for example lower potassium in soil and increase Zn in the grass/hay. We conclude that NIRS is a promising tool for analysis of the quality of grass and hay, and that more insight is needed into the relations between grass quality characteristics and fructans, and between fructans and laminitis.

The relationship between N:S ratio, protein yield and protein quality of grass grown on sandy soils

G.W. Abbink[1] and **S. Podesta**[2]

Abstract

On grassland-based dairy farms in the Netherlands, high protein yield from grassland combined with a high protein quality can save on imports of high protein feed as Soybean meal in concentrates. In addition, a high protein yield of grassland contributes to a high nitrogen (N) use-efficiency and may contribute to low N losses to the environment. Numerous studies have also shown the importance of sulphur (S) in N use-efficiency and the quality of protein in grass (e.g., Gierus, 2005). Application of sulphur fertilizers in spring time has become quite common in commercial dairy farming on sandy soils, especially since atmospheric S deposition and soil S contents have declined in the Netherlands. There are however doubts about the current recommendations for S fertilization. The goal of this study was to examine if the N:S ratio in grass can be used as a practical tool for the evaluation of the S status of the crop.

Group		Crop yield	Mean contents in fresh grass				Applied amounts of N and S kg/ha			
N:S ratio	number	relative N yield (index)	N:S crop	CP g/kg DM	Sol CP %	S g/kg DM	N-manure	N-fertilizer	S-fertilizer	N total: S artificial
< 8	69	102	7,0	199	26 *	4,6*	66*	63	17	8,8*
8 - 10	104	100	9,0	198	29	3,5*	79*	68	16	10,3*
10 - 12	49	99	10,9	207*	29	3,1*	95	72	16	11,9
>12	13	93*	14,4	196	35*	2,3*	94	73	8	14,8
Total	235	100	8,6	200	28	3,7	80	68	16	10,7

Table 1
Results of data-analysis. CP is crude protein and Sol CP is soluble crude protein (* significant $p < 0.05$)

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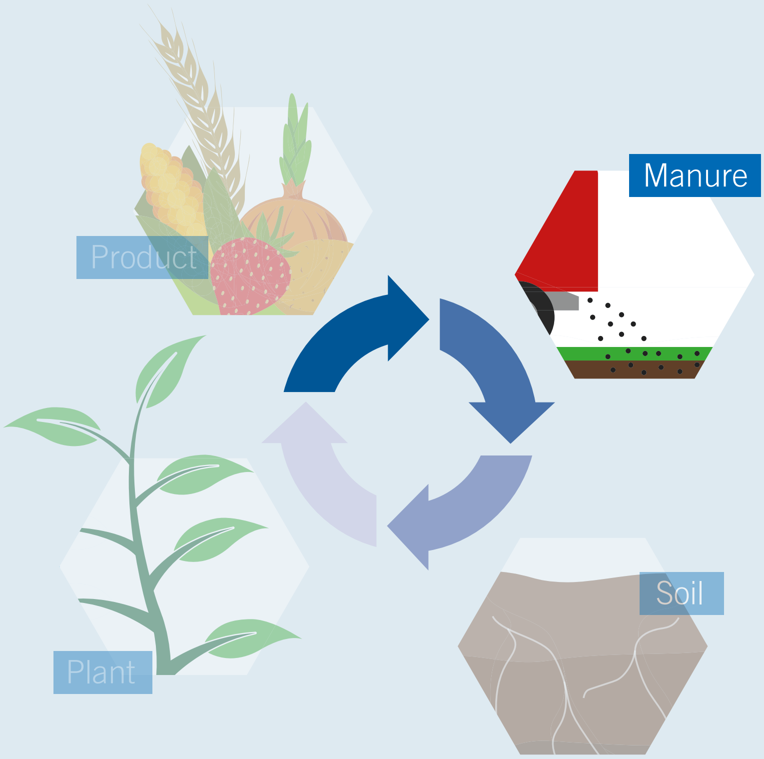
Results from 57 fertilization trials were collected in a database and used for statistical analysis. The field trials were conducted in seven different locations in the Netherlands, and examined the responses to cattle slurry and N fertilizer applications in both the first (April/May) and second harvests (June) in 2017 and 2018. The soil type of these locations was predominantly sandy soil (2-5% clay). The first cut was fertilized with on average 29 tons of dairy slurry and 80 kg of N from calcium ammonium nitrate (CAN) and or ammonium sulphate (AS). The second cut was on average fertilized with 14 tons of manure and an additional 58 kg N from CAN and/or AS. In addition, between 0 to 50 kg of S was applied via AS. The grass-quality was analysed by Eurofins Agro.

Conclusion

The results indicate that herbage protein yield was significantly lower when the N:S ratio was more than 12 (Table 1). The protein quality, indicated by the buffer soluble crude protein content (Sol. CP %) (Aufrère, 1991) was also significantly correlated to the N:S ratio; Sol. CP % was significantly higher (35%) for N:S ratios >12, and lower (26%) for N:S ratio < 8. However, grass with N:S < 8 has a high S-contents, which can be a risk in animal feeding (Kandylis, 1984). For an optimal protein yield and quality the target should be an N:S ratio of between 8 and 12.

Regarding the fertilization recommendations it can be concluded that also the amount of nitrogen fertilization should be taken into account for the right amount of sulphur application. The balance of total N (including manure) and artificial S was more important for an optimal N:S ratio in the crop than the amounts of N and S applied with artificial fertilizers alone. This suggests that the amount of plant-available S in dairy-slurry has little or no practical significance. This confirms previous research (e.g., Lloyd, 1994; Eriksen, 1995). So when a large portion of the nitrogen is applied through slurry, the S contents in the artificial fertilizer should be higher to optimize the N:S ratio of the fertilization. In conclusion, the N:S ratio in grass-analyses can be used as a practical tool for evaluating and optimizing the sulphur fertilization level of the crop on farm level.

How we look at manure, compost and fertilizers



Animal manures, composts and organic wastes are valuable sources of organic matter and nutrient elements, and serve to improve soil fertility, feed soil life, and nourish crops. However, manures, composts and organic wastes may also contain unwanted substances that may compromise soil quality and crop quality. Therefore, it is necessary to analyse these manures, composts and organic wastes, to be able to optimize nutrient supply to nutrient demand and to be able to guarantee food safety and quality.

Application of NIRS to determine the chemical composition of solid animal manure

R.P.J.J. Rietra[1]*, **J.A. Reijneveld**[2], **M. van Oostrum**[2] and **O. Oenema**[1]

Abstract

Animal manure is a source of organic matter (C), nitrogen (N), phosphorus (P) and other nutrients, and is used to amend soil fertility and to fertilize crops. However, the composition of animal manures is highly variable. For transporting manures between farms or to manure processing plants, the concentration of N and P in manures have to be analysed by accredited laboratories in the Netherlands. The sampling and analysis procedures for manure analyses (accreditation standards and reference methods) are laid down in the Fertilizers Regulation (LNV, 2005). These methods are relatively laborious and hence costly. However, non-standard 'in-house' methods may be used, provided these methods give similar or better analytical results than the reference method.

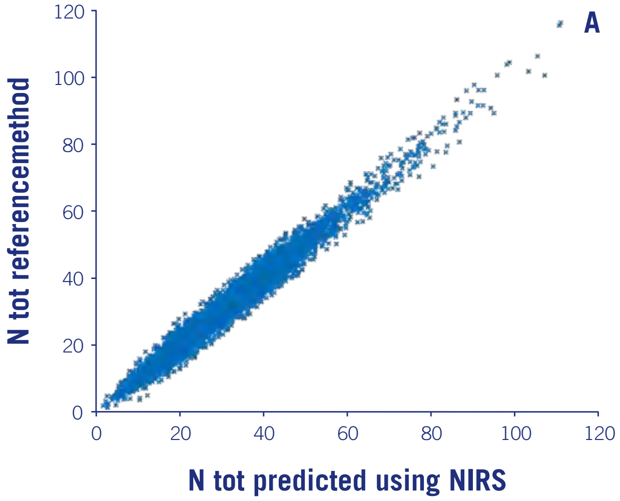


Figure 1A
Scatter plot of total nitrogen (N tot) determined using the reference method and the prediction using NIRS (mg/kg^{-1}).

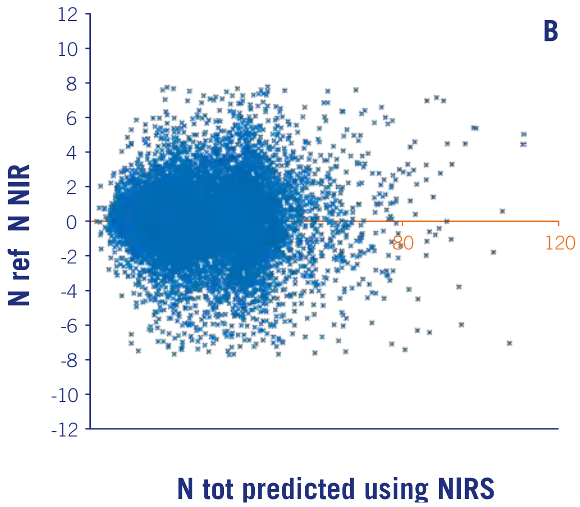


Figure 1B
Scatter plot of residuals versus predicted values.

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This study examines whether the N and P contents of different solid manures can be determined accurately via Near Infrared Spectroscopy (NIRS) instead of the wet-chemical reference methods. The advantage of NIRS over the classical methods is the much shorter analysis time. Also, NIRS offers the possibility to determine additional parameters rather easily, including organic matter, K, Ca and Mg. We evaluated the appropriateness of NIRS to determine the chemical composition of solid manure on the basis of three sets of performance criteria, namely 1) those of the Fertilizers Regulation, 2) NEN-EN-ISO criteria, and 3) criteria used in scientific literature. We present the results of paired validation measurements for N and P of 12.644 manure samples, using the classical wet acid digestion and analyses methods and NIRS. According to the Fertilizer Regulation, the median of the differences between two methods should be less than 2.5% for N and P. A second duplicate analysis is needed if the limit is not met, and if both duplicates do not meet the limit, the median should be reported. The median difference for N was 0.2% and that for P 0.7%. The repeatability of the NIRS method for N and P was 4.3% and 7.2%, respectively. The repeatability of the reference method for N and P was 7.2% and 7.4%, respectively.

The NEN-EN-ISO (2010) methods have been used to determine whether the results of the NIRS method are comparable to those of the reference methods (Figure 1). The systematic error of the NIRS method for N and P was very low, and not significant. In the validation step, the slope of the regression line between NIRS measurements and the reference measurements for N did not differ significantly from 1. The slope differed slightly but significantly from 1 for P. The standard deviation in the validation step was less than the standard deviation in the calibration step for both N and P.

Conclusion
Hence, the NEN-EN ISO indicates that the NIRS method is suitable for the determination of the N and P contents of solid manure, and that the calibration for P should be improved further. The assessment of the NIRS method in the scientific literature (e.g., Chen et al., 2013) has been done on basis of the ratio of prediction to deviation (RPD). An RPD of 3 or higher is often held as successful. The RPDs in the present study were > 5 for N, P and organic matter. For potassium (K) and magnesium (Mg), the RPDs were 3.6 and 2.7, respectively. The RPDs found in the present study were high compared to those in other studies (Chen et al., 2013), likely because of the much greater number of samples and the improved calibration procedure.

Innovative laboratory techniques for added value manure

R. Wolf[1], K.M. Brolsma[1], H.C. de Boer[2], P.C.J. van Vliet[1] and **K. Oltmer[1]**

Abstract

Ever since the negative effects of intensified agriculture on the environment have emerged on the political agenda, the reputation of animal manure has been rapidly decreasing. Nitrogen leaching to groundwater resources, ammonia emission and unwanted odour emissions – these are frequently used arguments with which animal manure has gained a negative reputation. However, animal manure is an important supplier of organic matter and hence of vital importance for keeping up the productivity of agricultural land. It is therefore necessary that we start pointing out the positive aspects of animal manure.

In order to improve the knowledge of organic matter in animal manure we wan to address farmers' awareness, parameters characterizing organic matter in animal manure on a routine basis and innovative techniques for estimating breakdown organic matter.

Farmers' awareness

Farmers become increasingly aware that manure is an important source of organic matter in their fields. At our laboratory, the number of requested manure analyses including organic matter content has slightly increased over the last years. However, the percentages that are found in soils remain rather low, fluctuating around 4,5%. Most of the liquid and solid manure is applied without exact knowledge about the organic matter content and other relevant characteristics.

Parameters characterizing organic matter

For sustainable farming practices, aimed at closed nutrient and carbon cycles, it is questionable whether the use of average values of manure parameters is valid. Nowadays several parameters characterizing manure and its organic matter (OM) content can be analysed on a routine basis (organic matter content, total nitrogen (N-total), N-organic, N-NH_3, and the C/N-ratio. Cattle slurry in 2018 showed a wide range of variation in these parameters (Table 1). The costs of analysing more parameters than just the mandatory nitrogen and phosphate is an important reason for farmers to avoid asking for larger analysis packages. Future applications of new technologies to measure e.g. OM parameters will lower these costs.

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	Dry matter (g/kg)	Organic matter (g/kg)	N-Org (g/kg)	$N\text{-}NH_3$ (g/kg)	N-tot (g/kg)	C/N
Median	74.00	55.00	2.00	1.90	3.99	6.00
Average	73.74	54.78	2.02	1.89	3.90	6.29
Perc 0.1	48	33	1.4	1.3	2.88	5
Perc 0.9	96	73	2.7	2.3	4.77	8

Table 1
Results of cattle slurry analyses in 2018, sample size: 1000 (Perc = percentile).

Innovative techniques

Manure is an important source of organic matter in the soil. Relatively fast decomposing organic matter mainly serves as a food source for soil life, thus affecting e.g. the availability of mineral nitrogen to crops. Whereas the slowly decomposing organic matter supports soil fertility, e.g. improving soil organic matter status.

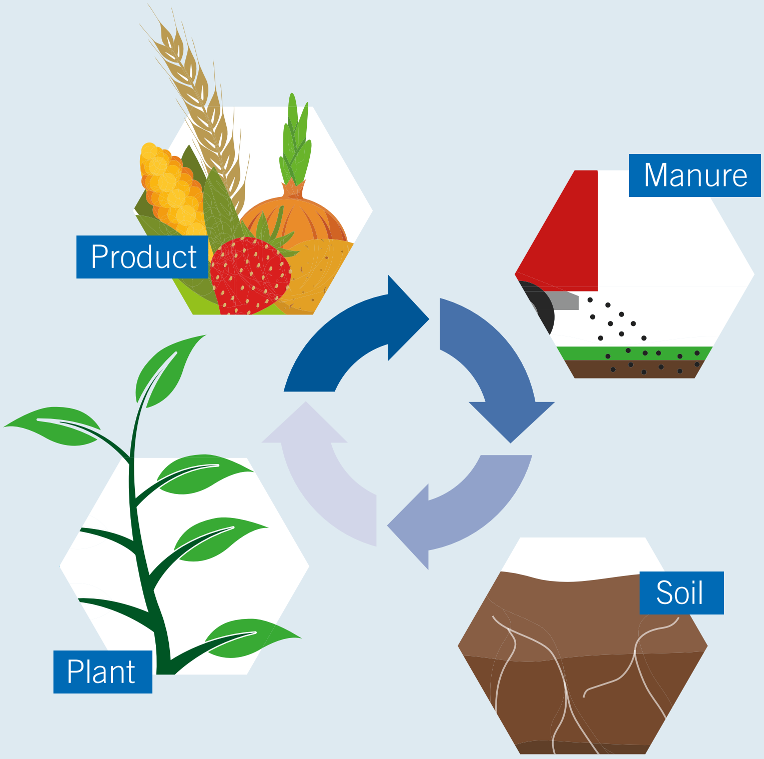
We used respiration measurements and pyrolysis GC-MS to investigate the usability of the pyrolysis technique in determining the effect of manure on soil organic matter quality. We used six types of manure and each type was collected from five different sources. The thirty manures were mixed with a sandy soil (89% sand, 7% silt, 4% clay, 6.4% organic matter). In the manures, the pH ranged from 6.6 to 9.5, organic matter from 3.4 to 33.1% and the carbon (C) to nitrogen (N) ratio varied between 2.6 and 23.9. Carbon and nitrogen mineralization was measured over time and the mixtures of manure and soil were measured via pyrolysis GC-MS. In addition, PLFA was measured to investigate the effect of microorganisms on mineralization.

Cumulative carbon mineralization after 168 days of incubation, corrected for the amount of soil carbon added, ranged between 17 340 to 460 809 µg C/g C. Pyrolysis GC-MS, using redundancy analysis, could explain 52% of the variance in cumulative C mineralization. Adding PLFA-results to the model increased the explained variance to 60% So, the turnover of organic matter from manure could be successfully linked to manure product quality, here measured using pyrolysis GC MS, and the active microorganisms, here characterized using PLFA.

Conclusion

It is important to improve the knowledge about the behaviour of organic matter in the soil and its contribution to soil fertility and plant growth. And, above all, finding a way to measure this behaviour on a routine basis in the laboratory.

How we look at the system approach



Consumers, policy makers, retail and processing industries increasingly demand certified products with guaranteed quality, which are produced in a sustainable manner. Such demands require system thinking and new approaches, combining arable and animal husbandry systems and avoiding losses in the field, in the processing chain and after consumption focussing on recirculating nutrients where possible. This requires better utilization of organic manures and optimization of nutrient supply and demand in time and space following the 4R principles (right place, right type, right amount and at the right time).

FieldScout: a tool that shows soil heterogeneity

P.C.J. van Vliet and **J.G.L.M. Rongen**

Abstract

Nowadays, regulations regarding fertilizer usage, crop protection and environmental issues make it more challenging for farmers to produce enough, healthy food using sustainable plant production systems which are also economically feasible. One of the major constrains farmers have to deal with is the heterogeneity of soil. Differences in chemical, physical, and biological characteristics within fields will have an effect on crop growth and yield.

Information on the spatial distribution of soil variability is vital for understanding crop yield variation. Such information allows implementing improved management of all agricultural inputs. One of the limiting factors is the cost of mapping soil variability. To gain site-specific information in a field, several options are available: scanning of soil is most often used, whereas intensive soil sampling is also possible; however, both options are expensive. Much cheaper is FieldScout, a tool developed by Eurofins Agro, that uses satellite images to show soil heterogeneity.

At the time of recording, satellite images provide information about the crop status in fields. The crop status in a field not only depends on soil conditions. Abiotic (weather) conditions and management also affect the crop status. By combining nine years of satellite images, seasonal effects, crop type, abiotic and management effects are largely cancelled out while effects of long lasting soil characteristics on crop status appear via (repetitive) biomass anomalies.

For each year and for each 10 x 10-meter pixel in every agricultural field in the Netherlands we have determined the deviation of the average crop status (using the WDVI) in that field. Combining the deviations in each field for nine years (2007 till 2015) resulted in locations in the field that are always performing better or worse than the average. We have classified the deviations into seven scores: 1 (always much worse) to 7 (always much better). In a preliminary study using eleven fields, we concluded that deviating locations required different management practices. An example of the FieldScout of a field is shown in Figure 1.

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FieldScout is a tool that shows structural variation present in fields and can be used to create awareness about spatial variability by farmers. The tool does not know what caused the variation: it may be due to differences in texture, soil compaction, acidity, soil biology, something else or a combination of factors. Several soil analyses and tools are available for farmers to assess the causes of the structural variation. Precision farming using location specific information on soil and crop status can be applied, thereby optimizing crop growth conditions and reducing negative side effects (as leaching of nutrients) on the environment.

Conclusion

FieldScout can contribute to a more precise management, providing nutrients at the correct amount at the correct location at the correct time, thereby contributing to a better usage of fertilizers and/or pesticides/herbicides, resulting in a more sustainable agriculture. In an earlier study good-producing areas were compared with poor-producing ones: in more than 50% of the fields the differences between the areas could be allocated to differences in the content of macronutrients. In other fields physical differences where present between good and poor producing areas. The tool is currently being applied to several fields located all over the Netherlands to determine which soil parameters mostly relate to soil heterogeneity. In this study we correlate several soil fertility parameters measured at different locations to the FieldScout scores.

Figure 1
FieldScout scores for a field in the Netherlands whereby the blue areas perform better than average while the red areas perform worse than the average in this field.

Potassium in soil fertility, nutrient uptake and product quality, a case study in onions and potatoes

K.M. Brolsma, J.G.L.M. Rongen and **J.A. Reijneveld**

Abstract

Potassium (K) is essential in crop production systems. Improving K use in crop production is key, since it is a non-renewable resource. Here, we show the importance of soil tests that include the intensity, buffering capacity and quantity concept for potassium in relation with crop uptake and quality characteristics.

In this study we combined the results of field trials including onions and potatoes. Soil K status was determined in all plots based on the intensity (0.01 M $CaCl_2$), buffering capacity and quantity concept (CEC-Co-hexamine). For all crops, yield and nutrient status of the harvested produce were determined at harvest (in dried material, HNO_3 extraction). For the onions, potassium in soil and crop was also measured during the growing season; with 1:2 0.01 M $CaCl_2$ in moisture soil, and HNO_3 extraction respectively. In the K fertilizer trial for onions (0, 100, 200 and 400 kg K_2O ha^{-1}), disease infestation was included in the growing season and used as an indicator for crop resilience. For the potatoes, a fertilizer trial was included using various organic products. As such 300 kg K_2O ha^{-1} was fertilized from mineral fertilizer only or combinations of organic and mineral fertilizers were used. At harvest of both crops, quality characteristics such as hardness of onion bulbs and discoloration of the potatoes were included.

Conclusion

Crop uptake and the crop level of potassium in onions could be explained by soil potassium (K-intensity), mineral K fertilizer use and crop K levels. Besides that, leaf potassium level was correlated with the infestation of Stemphylium leaf blight (*Stemphylium vesicarium*) (Figure 1). Onion and potato production and quality characteristics were related to soil potassium status when no potassium was applied. Furthermore, we show the effect of organic matter amendment on K uptake and level in potatoes. These results of this study will help to optimize K fertilization, based on the potassium level of the soil measured via the intensity, buffering capacity and quantity concept.

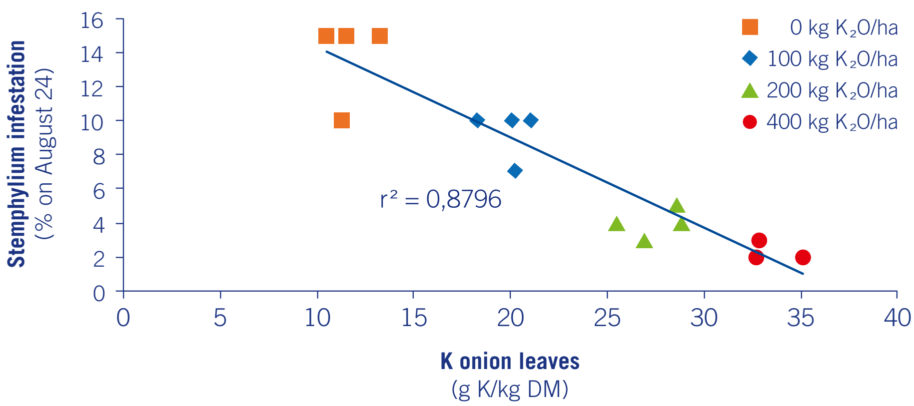

Figure 1
Stemphylium vesicarium infestation and the level of potassium in onion leaves.

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Using pyrolysis GC-MS, POXC and PLFA to determine organic matter dynamics in brassica seed production

A. Schoonbergen, J.A. Reijneveld, N. Poot., B. Fleurkens, P.C.J. van Vliet and **K.M. Brolsma**

Abstract

Organic matter is key for fertile soils and directly linked to soil chemical, physical and biological characteristics. However, a global decrease in organic matter is observed in agricultural soils. Even though, the soil organic matter level seems stable in the Netherlands, it´s quality is decreasing. Soil organic matter consists of carbon (C), nitrogen (N), phosphorus (P) and sulphur (S). We see for example lower soil organic S levels, which will reduce sulphur mineralization and affect crop growth. Therefore, maintaining or improving organic matter (quality) is of great importance.

In this study soil organic matter characteristics are measured with pyrolysis GC-MS, POXC and PLFA. Pyrolysis GC-MS is able to measure total soil organic matter and identify various signature pyrolytic products for protein, carbohydrate, and humic fractions. In contrast, POXC is used to extract the available carbon fraction from soil samples. Pyrolysis GC-MS and POXC together are able to measure a range of organic matter fractions in soil. In addition, PLFA analysis is used to determine microbial biomass, activity and diversity in soils, such as fungal/bacterial ratios that are linked with humification of organic matter. Microorganisms are important for the mineralization of sulphur, so PLFA, pyrolysis GC-MS and POXC can be used to explain soil sulphur dynamics. Since S supply is very important in the growth and development of Brassicaceae, we link S uptake here with measured soil organic carbon fractions.

Soil was sampled at four locations with different soil types on which brassica were grown, each location was sampled four times in 2018 and two times in 2019. Soil fertility was analysed following extraction with Cohex (1:20 m/v), $CaCl_2$ (1:10 m/v) and water (1:2 v/v). Brassica growth was measured over time, at harvest yield, germination capacity and seed mineral content were measured following extraction with HNO_3.

Conclusion

Preliminary results show that there is a correlation between seed S level and germination capacity of the seeds ($r^2 = 0.23$, $P < 0.05$). Furthermore soil cation exchange capacity was affecting seed yield ($r^2 = 0.32$, $P < 0.05$) and seed germination capacity (r^2 0.62, $P < 0.01$). Soil sulphur, S-H_2O and S-total, were related to pyrolysis GC-MS measurements (81% explained variance), adding PLFA or POXC did not result in an improvement of the model. Besides that, pyrolysis GC-MS measurements were associated to both POXC (92% explained variance) and PLFA (80% explained variance). So based on these results, pyrolysis GC-MS seems to be a promising tool to get more insight in sulphur dynamics and as such more insight in soil fertility.

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Improving the quality of tropical fruit by an integrated system approach

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Abstract

Dragon Fruit (Pitahaya; Family Hylocereus) is a fruit crop that is suitable for the humid tropics in Vietnam. Since the fruit has relatively high revenues compared to for example rice, the acreage increased very fast over the last years. The area dragon fruit is now estimated at 46,000 hectare in Vietnam. Main production areas are the provinces Binh Thuan, Tien Giang and Long An. Export to 'far away markets' like Europe, USA and Japan is preferred due to more stable prices. However, importers require a high fruit quality standard including a long shelf life. Knowledge on how to improve fruit quality is limited. Assessment reports mention over-use of water and fertilizers and expect increasing risks of pests and diseases, and in the end, a risk of decreased production and fruit quality (OECD, 2015). Since employment in the large production areas is becoming more dependent on only one crop, it also makes these regions vulnerable to changes in crop yield and quality and to changes in market prices.

Here, we report on the results of a project initiated by several public and private partners to optimize dragon fruit production and quality covering the entire value chain of this fruit. From Vietnamese side cooperatives and knowledge institutes are involved. The main focus of the project is on innovative methods to test soil fertility using sensor technologies like Near Infra-Red (NIR) soil analyses. With NIR, there is less need to set up a fully equipped laboratory since multiple soil characteristics can be measured after soil is dried and sieved, provided that appropriate calibration data sets are available.

Our results show the importance of nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), organic matter and pH, in addition soil physical properties. The results are used by farmers and their advisors (Vietnamese cooperatives) to optimize fruit production and quality. Results show a large variation in soil nutrient status and adjoining management practices. For example, P-intensity (P-$CaCl_2$), and P-quantity (P-AL) were limiting on some fields with pH-values of 6.4 or higher. Yet, liming was promoted on these fields, enhancing P-Ca precipitation and further decreasing the P-status. Decreased P-status will impede root development making the fruit among others more sensible to drought. Other fields showed very low pH-values (pH-$CaCl_2$ < 4.5), increasing the risk of aluminum toxicity, also impeding root development and consequently make the dragon fruit more drought sensible and less able to take up nutrients.

Conclusion

Regressions were made between soil characteristics and dragon fruit quality. First results showed a significant ($p<0.05$) relation between Ca-status (Ca-intensity and Ca-quantity), K-soil intensity, and shelf life. This relationship is found in literature as well since Ca enhances cell wall strength. The project aims at an integrated approach on water and soil management at farm level. In the presentation the concept of this project will be explained in more detail and also results of the first two years of the project.

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Colofon

In this publication the abstracts are bundled, which are part of the presentations by the experts of Eurofins Agro during the International Symposium of Soil and Plant Analysis (ISSPA) in Wageningen, The Netherlands, June 17 to 20, 2019.

ISSPA is devoted to the discipline of analysis of soils and plants, plant nutrition and nutrient management, and environmental quality. Since 1989, there have been 15 ISSPA in different parts of the world, including Australia, Canada, Greece, Hungary, New Zealand, Mexico, South Africa, The Netherlands, and the USA.

The ISSPA 2019 organization was in the hands of Eurofins Agro and Wageningen UR. ISSPA provides an opportunity for international scientists and professionals interested in agricultural testing and results interpretations to exchange knowledge, foster collaborations and learn advanced technologies. ISSPA 2021 will be held in Vancouver, Canada.

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