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CICLO: XXXII

OPTICAL MEASUREMENTS FOR IMPROVING CROPS AGRONOMIC MANAGEMENT

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1. Introduction

Modern agriculture needs to front multiple challenges, including the growing needs of the increasing population, uncertainties due to climate change, shortage of water resources, rising energetic and environmental costs, and stringent environmental regulation, as well as global resources consumption, lower land availability and crop productivity growth. A sustainable intensification of crop production is needed to fulfil the future request while, at the same time, reducing the environmental impact of the agricultural sector (Foley *et al.*, 2011) and increasing the efficiency of invested resources. Ecosystem-based approaches have to be followed to improve the overall sustainability of cropping systems. Indeed, a sustainable crop production system not only recognise the need for an adequate food supply, but also provides profits for the farmers, preserves and enhances the natural resource base, positively contributes to the quality of life of individuals and communities, and ensures the nutritional value and safety of food (Pisante *et al.*, 2012). The need of improving sustainability is clearly highlighted in the 2030 Agenda for sustainable development, that includes 17 goals, with the aim of ending poverty, protecting the planet and improving the lives and prospects of everyone. Several goals are relevant for the agricultural sector, particularly goals 2 and 12. Goal 2 aims at ending hunger, achieving food security and improved nutrition, as well as promoting sustainable agriculture. In addition, Goal 12 promotes sustainable consumption and production patterns.

Nowadays, agriculture should be designed to increase the long-term efficiency, productivity, and profitability of the cropping systems, then minimising the negative externalities.

1.1 Precision agriculture as a tool for improving the agricultural system

Many innovative tools and techniques are nowadays available for improving crop production by enhancing input use efficiency, thus achieving high profitability and limiting adverse environmental cost. Among them, precision agriculture (PA) allows to take into account crop variability within a field, through location-specific management (Basso *et al.*, 2005). Then, PA is linked to the management of the variability, both in space and in time. The magnitude of this variability is a good indicator of the suitability of a variable management plan. There are many definitions of PA. According to the United States Department of Agriculture (USDA), PA is a management system that is information and technology based, is site specific and uses one or more of the following sources of data: soils, crops, nutrients, pests, moisture or yield, for optimum profitability, sustainability and protection of the environment (USDA, 2007). A simpler definition was proposed by Gebbers and Adamuck (2010), who assessed that PA is a way to apply the right treatment in the right place at the right time. In a modern concept, PA can be considered as the digital face of agriculture, that introduces computational and information technologies in farming systems. Site-specific crop management (SSCM) is a component of PA that can be defined as matching resource application and agronomic practices with soil and crop requirements as they vary in space and time within a field (Whelan and McBratney, 2000). This definition includes the idea that PA is an evolving management strategy, based on the integration of technologies that permit the collection of data on an appropriate time scale (Batte and Van Buren, 1999).

1.2 Research on PA and potential application of PA technologies

Up to now, various researches on different PA technologies have been conducted all over the world, showing a continuous increase starting from the middle 1990s (*Figure 1*).

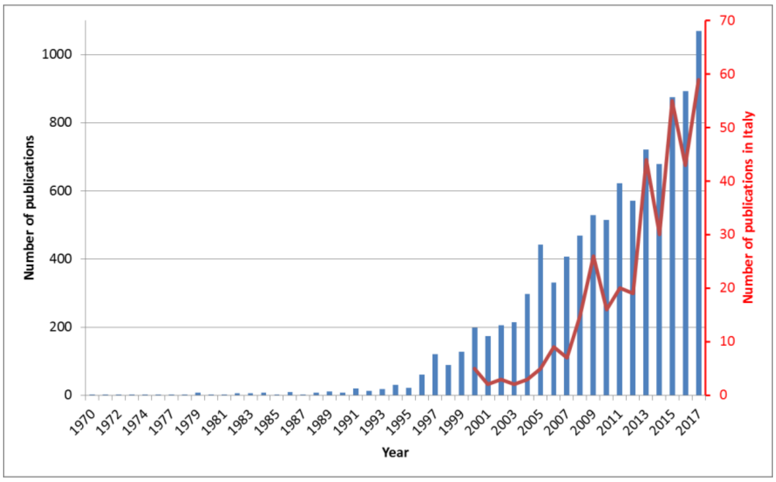

Figure 1: Number of publications on PA technique, including precision agriculture, precision farming, and site-specific crop management in title, keywords, or abstract (Source: Scopus database accessed 12 June 2018).

In Italy, the research interest on PA started some year later, rising especially from 2012.

The penetration of advanced digital technologies within the agricultural systems is rapidly increased in the last years, especially in more advanced economies. While digital technologies allow the practical application of PA, on the other hand only the interpretation of variability makes it feasible. The introduction of PA technologies requires a scientific understanding of the variability that can be detected in the cultivated soils, due to both the natural variability in soil properties and the agrotechniques (Bocchi *et al.,* 2000). Moreover, the correct management of spatial variability requires suitable data analysis procedures. Geostatistics allow describing and better understanding the spatial distribution of each variable detected in a crop field. Geostatistical tools (*e.g.* variograms and kriging) have been developed for investigating the spatial distribution of the observations taking into account their spatial dependence, then assessing their spatial variability (Bocchi and Castrignanò, 2007). Multivariate geostatistical techniques can be used to quantitatively measure complex interactions, then suggesting an improved management of spatial and temporal variability linked to several aspects of crop production.

Nevertheless, PA adoption is increasingly impacting also developing countries. However, several concerns associated with the diffusion into the agricultural system still exist. McBratney *et al.* (2005) proposed the use of both a spatial and environmental indices to determine the overall suitability of PA application in a specific area. The arable land (hectares per inhabitants) can be considered a useful spatial index, even if it does not consider the intrinsic variability within a given area.

Table 1: Spatial index of different areas (Source: FAO, 2018)

Area	Spatial index (ha of arable land per inhabitants)
Australia	1.93
North America	0.55
Europe and Central Asia	0.37
Central Europe and the Baltics	0.36
Latin America and Caribbeans	0.28
Sub-Saharian Africa	0.21
European Union	0.21
Middle East and North Africa	0.12
South Asia	0.12
East Asia and Pacific	0.11

In general, the larger the arable land is, the greater the spatial potential for PA adoption (McBratney *et al.*, 2005). Consequently, Australia and North America appear the most suitable for PA adoption (*Table 1*). Fertiliser consumption (kg per hectares of arable land) can be used as environmental index, indirectly evaluating the impact of agriculture on water quality and soil sustainability (*Table 2*). Those countries with high fertiliser consumption can better improve fertiliser management through the adoption of PA techniques, achieving more benefits in increasing fertiliser use efficiency (McBratney *et al.*, 2005).

Table 2: Fertiliser consumption in different areas (Source: FAO, 2018)

Area	Fertiliser consumption (kg of fertilisers for ha of arable land)
East Asia and Pacific	327.9
South Asia	164.5
European Union	157.2
North America	126.9
Central Europe and the Baltics	125.4
Latin America and Caribbeans	122.7
Middle East and North Africa	105.7
Europe and Central Asia	77.2
Australia	53.6
Sub-Saharian Africa	15.0

Moreover, a key factor for PA adoption is represented by the degree of variability. Indeed, the higher variability detected in a field leads to an easier implementation of PA techniques (Tekin, 2010).

1.3 Public investments on precision agriculture in Europe

All over the world, governments and public institutions encourage precision farming, to facilitate a digital evolution in the agro-industry. Farming systems are different, considering the wide range of climate, soil characteristics, cultivated crops, and management practices. Then, policy-makers need to define objectives and challenges, as well as needs and priorities to face specific political and social pressures, suggesting also instruments to profitably meet these specific goals. In Europe, the implementation of PA is stimulated through Common Agriculture Policy (CAP) instruments (European Union, 2014). Considering CAP 2014-2020, several goals are relevant for PA, among which enhancing farm income, improving agricultural competitiveness, fostering innovation, providing environmental public goods, and pursuing climate change mitigation and adaptation. In Italy, several Italian regions produced Rural Development Programs (RDP) to reach these objectives, helping farmers to become innovative through support and money funding.

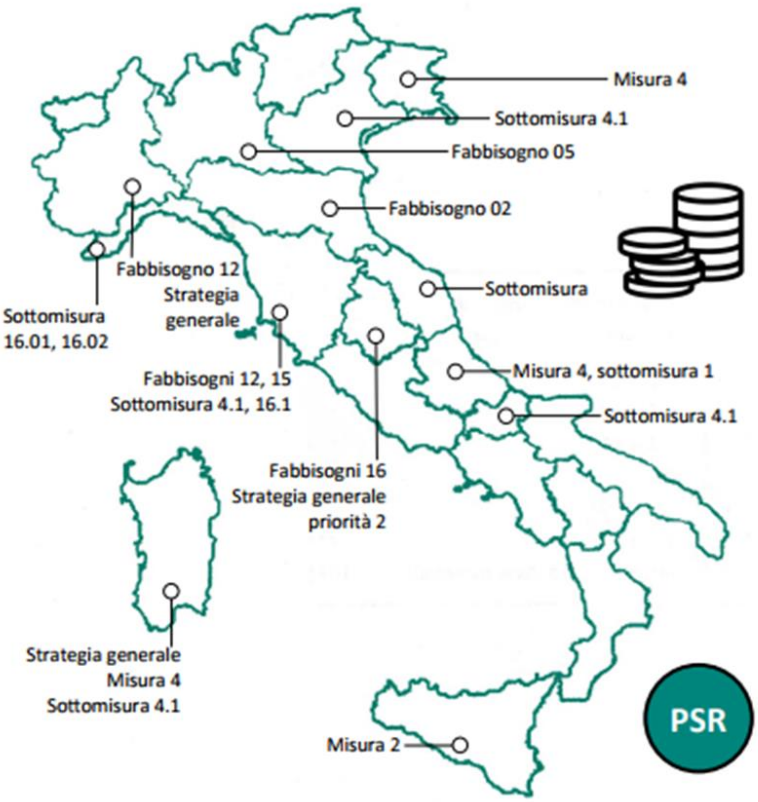

Figure 2: Possibility of money funding through Rural Development Programs in Italy

However, the widespread adoption of such improved technologies by farmer is still lacking, mainly because they require additional skills and knowledge, labour, or investments in new equipment and technologies (Van Evert *et al.*, 2017). Despite the enormous increase in the technology available to farmers, the practical adoption of PA has been less than expected, partially because the quantification of benefits compared to the costs of investment is still missing. In 2017, the Italian Ministry of Agricultural, Food and Forestry Policies (MiPAAF - Ministero delle Politiche Agricole, Alimentari e Forestali) issued the document Linee guida per lo sviluppo dell'agricoltura di precisione in Italia. The purpose was to get a picture of the adoption of PA techniques in Italy, then evaluating the tools to widespread their application. Nowadays, in Italy, PA techniques are adopted on approximately 1% of the Utilised Agricultural Area (UAA). The goal is to extend PA application on 10% of UAA by 2021.

1.4 Technologies available for precision agriculture and main barrier for adoption

Up to now, the advancement in research on PA, combined with the higher availability of innovative technologies, provides technical solutions that can support farmers in their decision-making process. To this end, these solutions have to be widely integrated in the farm management system, allowing their evaluation on commercial farms representing a wide range of different crops and geographic areas.

The development of accurate positioning systems allows managing spatial variability through appropriate farming practices. In particular, in arable farming, controlled traffic and auto-guiding systems represent the most successful applications of PA techniques, as well as variable rate application of agricultural inputs (*i. e*. seeds, water, fertilisers, and agrochemicals). However, PA adoption not only allows improving the ecological impact of the agricultural sector, optimising at the same time farming cost, but also contributes to the collection of further data useful for validating the current technologies and to produce advancements in research and developments.

However, currently, PA technologies are not used to their full potential by farmers, as a consequence of the trade-off between available technologies, agricultural industry, and farmers' decision making (Ondoua and Walsh, 2017). The main reasons for the low adoption of PA technologies at farm level are the high investment costs and farmers' perception of ease of use, as well as the lacking of technical support offered by technology dealers. Indeed, a participatory approach is needed to foster PA development, leading to a widespread adoption.

1.5 Precision Nitrogen management

Among the various components of the agricultural system that have been evaluated considering their potential for site-specific management, soil fertility has a crucial role (Pierce and Nowak, 1999). Indeed, PA allows delivering customised inputs on the basis of georeferenced crop information, or partitioning the field in zones that require homogeneous agronomic management.

Appropriate nitrogen (N) management is one of the main challenges of the agricultural systems worldwide. Nitrogen inputs are essential for stimulating crop growth, as they help maximising crop yields and contribute to crop quality. However, deficient or excessive N applications have a detrimental effect on economic and environmental aspects of crop production. Providing crop with deficient N supply results in lower crop yield, and then poor economic return. Conversely, excessive N amounts have been reported as a cause of environmental pollution (Tubaña *et al.*, 2011). Indeed, N is a very mobile and dynamic nutrient. Consequently, N is highly susceptible to losses through leaching, ammonia volatilisation, and N_2O emission that reduce soil fertility and create adverse impact on the environment (Cameron *et al.*, 2013). Ammonia volatilisation contributes to acid rains. N leaching contaminates water resources, increasing eutrophication and health risks. N_2O emission leads to the depletion of the ozone layer and heavily contributes to climate change. In Europe, agriculture is responsible of 80-90% of NH_3 emissions, 50-60% of N_2O emissions, and 40-60% of N loadings of surface water (Oenema *et al.*, 2007).

Nowadays, N management strategies commonly adopted by farmers are characterised by a low Nitrogen Use Efficiency (NUE) (Shanahan *et al.*, 2008). Ladha *et al.* (2005) reported that more than 50% of the applied N is not used by crops, then increasing the risk of environmental pollution. Farmers usually apply high uniform N rates to avoid yield losses, often exceeding crop needs. Since ecological challenges become more and more important, optimising N fertilisation is needed. Then, efficient N management strategies have to be adopted. Aiming at better defining crop needs, crop N requirements can be estimated by mean of a N balance, calculating the difference between N outputs and inputs at field scale (Grignani *et al.*, 2003). Total N amount that has to be supplied with fertilisers can be estimated according to *Equation 1*:

$$F = Y * b + S_i + Z - (B_{fx} + A_d + M_f + M_c + S_m + R_i) \qquad (1)$$

where F is N supplied with fertilisers, Y*b is crop N uptake determined as the product of expected yield and crop N concentration, S_i refers to N immobilisation due to crop residues, Z represents N losses due to volatilisation, leaching, and runoff, B_{fx} is N fixation due to leguminous crops, A_d represents N input due to atmospheric deposition, M_f is residual N from previous organic fertilisation, M_c and S_m is N deriving from crop residues or soil organic matter mineralisation respectively, and R_i is mineral N available at the beginning of the growing season.

However, uniform N application across the field discount the spatial variability in crop response to N fertilisation. Then, the careful management of the soil-plant system using newly developed technologies can increase the sustainability of the farming system, and reduce the impact of agriculture on the environment. Indeed, precision agriculture tools can be used to estimate site-specific crop N needs, considering spatial variation of N dynamics across the field (Shanahan *et al.*, 2008). Crop N demand varies within a field, as a consequence of the different crop yield potential of different sub-regions. Then, the need of tuning site-specific N recommendations on the basis of the expected yield appears evident.

Estimating crop yield potential prior to harvest is a pivotal element in precision fertilisation strategies (Moges *et al.*, 2007). Indeed, crop yield variability is determined by biotic and abiotic factors, the latter associated with both soil and crop properties (Hornung *et al.*, 2006, Bunselmeyer and Lauer, 2015). Consequently, in literature several approaches are suggested to drive N fertilisation, at the same time complying with field variability. They can be classified into two main categories: soil-based methods and crop-based methods.

Soil-based information can be used to define sub-field regions with similar yield limiting factors, known as management zones (MZ) (Doerge, 1999). The delineation of MZ allows identifying areas with similar yield potential and input use efficiency.

On the basis of the expert knowledge of their fields and their agronomic experience, farmers are able to identify specific areas of the field regularly providing low or high productivity. However, several tools are nowadays available for defining MZ with an objective approach. To this end, various crop and soil properties, used as single data or as combination of data layers can be used (Longchamps and Khosla, 2017). Topography, elevation, soil properties, bare-soil aerial imagery, soil apparent electrical conductivity, as well as canopy images and yield data can be used to delineate the boundaries of the MZ (Khosla *et al.*, 2002; Schepers *et al.*, 2004). Nowadays, MZ delineation is more and more oriented towards a multivariate approach for three main reasons. First of all, new advancements in sensing technologies (*e.g*. electromagnetic induction sensors, spectroscopy, satellites, optical proximal sensors) together with global positioning system allow to reduce both labour and cost of monitoring the variability in soil and crop properties at fine scale (Diacono *et al.,* 2013). The second reason is related to the improved knowledge in statistical and GIS software, that allows the use of complex geostatistical analysis for accurately delineating MZ (Nawar *et al.*, 2017). Last, crop monitoring and yield data represent all biotic and abiotic factors that affect crop production. Then, their integration with soil variables can profitably improve the explanation of field variability associated with both soil and crop properties (Hornung *et al.*, 2006; Bunselmeyer and Lauer, 2015). However, crop yield varies temporally across the field as a consequence of climate differences across the growing seasons (Schepers *et al.*, 2004). Then, several studies (e.g. Nawar *et al.*, 2017; Maestrini and Basso, 2018) suggest to consider yield history, including at least three-year data to identify more stable MZ.

On the other hand, remote and proximal sensors have been widely used to monitor crop N status during the growing season (Corti *et al.*, 2018). Indeed, crop variables associated with N management are highly correlated with chlorophyll content (Samborski *et al.*, 2009). Reflectance data measured at specific wavelengths can be used for the mathematical computation of several vegetation indices (VIs) (Bajwa *et al.*, 2010). However, converting VIs values into practical N recommendation is fundamental to integrate crop monitoring into the agricultural practices adopted by farmers.

Several studies evaluated different approaches to obtain prescription functions suggesting N amounts corresponding to VIs values.

One of the simplest methods consisted in an a priori definition of VIs thresholds that can be considered as representative of a good nutritional status for the crop. Upward or downward adjustments of predefined N topdressing is needed when VIs values are below or above the threshold, respectively. Peng *et al.* (2010) reviewed several studies conducted in China where site-specific N management (SSNM) was compared to traditional N management for topdressing fertilisation in rice. The authors identified the application of 30 kg N ha^{-1} at mid-tillering and 40 kg N ha^{-1} at panicle initiation stage as topdressing N rates conventionally adopted by farmers. These rates have been adjusted by ±10 kg N ha^{-1} on the basis of SPAD values. The SPAD thresholds of 35-37 were used for indica varieties, and they were increased by two units for japonica varieties, as suggested by a previous study by Huang *et al.* (2008). Overall, Peng *et al.* (2010) pointed out that SSNM reduced N supply by 32% on average, while increasing grain yield by 5% with respect to traditional farmers' managements.

Several studies proposed to exploit optical properties of leaf pigments to provide a fast, cost-effective, and accurate estimate of crop biomass or grain yield prior to harvest, as well as crop N concentration and uptake (Corti *et al.*, 2018). In the past years, the use of VIs for yield estimation has achieved growing importance. Raun *et al.* (2001) predicted the potential yield of winter wheat using NDVI. In-season estimated yield (INSEY) was calculated as the average NDVI acquired in two post-dormancy dates, divided by the cumulative growing degree days (GDD) for the period between the two sensing days. This procedure allowed expressing wheat growth in terms of NDVI, integrating both early season growing conditions and growth rate in the computation of INSEY (Teal *et al.*, 2006). Across a range of different agro-environments, the R^2 between wheat grain yield and INSEY was 83% (Raun *et al.*, 2001), then assessing their strong correlation. Moreover, GDD allowed taking into account a wide range of growing conditions (Raun *et al.*, 2001). Lukina *et al.* (2001) developed an algorithm for determining topdressing N requirements on the basis of grain yield prediction. The INSEY was computed according to Raun *et al.* (2001), just substituting GDD with the number of days from planting to sensing as also proposed by Raun *et al.* (2002). Then, INSEY was used to estimate grain N uptake. At the end, topdressing N fertilisation was calculated as the difference between grain N uptake and early season N uptake, divided by Nitrogen Use Efficiency (NUE). Nitrogen Use Efficiency varies with fertiliser type and fertilisation strategy, as well as the agro-environments. Typically, NUE is assumed equal to 0.7, but the value can range between 0.33 and 0.80 (Lukina *et al.*, 2001).

However, prescription functions that allow quantifying topdressing N requirements are often site, crop, or variety-specific. Consequently, further extension to other agro-environments, crops, and varieties are needed to promote a widespread application of PA techniques. A research need is to exploit the available data to create mathematical and decision models for driving the practical management of N fertilisation. Then, on the whole, the Ph. D. activity aimed at evaluating the potential advantages derived from the application of PA techniques on rice and maize cropping system considering their effects on crop yields, Nitrogen Use Efficiency, and profitability for farmers. The Ph. D. activity was supervised by Professor Dario Sacco and co-supervised by Prof. Carlo Grignani and Dr. Louis Longchamps. The latter, collaborating with Professor Raj Khosla of Colorado State University (Fort Collins, USA), led the research period at Agriculture and Agri-Food Canada, in Saint Jean sûr Richelieu research centre (Quebec, Canada).

1.6 Structure of the Ph. D. thesis

This Ph. D. thesis summarises the research activities conducted at the Department of Agriculture, Forest, and Food Sciences (DISAFA) and at Saint Jean sûr Richelieu research centre of Agriculture and Agri-Food Canada, showing the main obtained results. It is divided in three chapters, each one corresponding to scientific papers published or submitted in different peer-reviewed journals.

- Chapter 2: Cordero, E., Moretti, B., Miniotti, E. F., Tenni, D., Beltarre, G., Romani, M., Sacco, D. (2018). Fertilisation strategy and ground sensor measurements to optimise rice yield. European Journal of Agronomy 99: 177-185.
- Chapter 3: Cordero E., Moretti B., Miniotti E. F., Tenni D., Beltarre G., Romani M., Grignani, C., Sacco D. Statistical model to

overcome rice variety effect in precision nitrogen fertilisation (submitted)

- Chapter 4: Cordero, E., Longchamps, L., Khosla, R., Sacco, D. (2019). Spatial management strategies for nitrogen in maize production based on soil and crop data. Science of the Total Environment 697, 133854.

Chapter 2 describes the study conducted on Centauro rice variety, that led to optimise N application in terms of both total N amount and splitting during the growing season, as well as to the determination of prescription functions driving topdressing N application at panicle initiation stage on the basis of VIs measured with crop proximal sensors just before N application. Then, the statistical procedure was extended also to other rice varieties, with the aim of evaluating quantitative and qualitative tools for obtaining prescription function, then overcoming rice variety effect in the determination of N supply. The results, just submitted, are presented in Chapter 3. Chapter 4 refers to the research activity conducted during the research period abroad. The study compared different N management strategies (both uniform and variable N rate) on maize production, through a multi-site-year experiment conducted in Colorado. Their effect on maize grain yield, NUE and farmers' net return was evaluated, then suggesting a practical tool to choose the N fertilisation strategy that best applies in each agro-environments.

Further insights of the research activity have been published on an Italian specialised journal for the agricultural sector, but it was not included in the Ph. D. thesis. Cordero *et al.* (2017) reported the preliminary results of the experiment conducted on rice, showing to technician and Italian farmers the main advancements on PA in Italian rice cropping systems.

Moreover, during the Ph. D activity, preliminary results of the research activity have been shown in both italian and international conferences as posters or oral presentations, and then published in the specific book of abstract as follows:

- Cordero, E., Moretti, B., Miniotti, E., Tenni, D., Beltarre, G., Romani, M., Sacco, D. (2016). Optimizing topdressing fertilisation through ground sensing measurements in rice. Atti del XLV Convegno della Società Italiana di Agronomia La ricerca agronomica verso il 2030: gli obiettivi globali di sviluppo sostenibile, Sassari, 20-22 settembre 2016 (poster)
- Cordero, E., Moretti, B., Miniotti, E. F., Tenni, D., Beltarre, G., Romani, M., Grignani, C., Sacco, D. (2018). Deriving fertiliser VRA calibration based on ground sensing data from specific field experiments. Proceedings of the 14th International Conference on Precision Agriculture (unpaginated, online) (oral presentation)
- Cordero, E., Longchamps, L., Khosla, R., Sacco, D. (2018). Intergrating soil and crop-based methods for maize variable nitrogen fertilisation. Atti del XLVII Convegno della Società Italiana di Agronomia L'agronomia delle nuove agriculturae (biologica, conservativa, digitale e di precisione...), Marsala, 12-14 settembre 2018. (oral presentation)
- Damatirca, C., Cordero, E., Sacco, D. (2019). Analysing cover crop presence in Piedmont rice paddy area through satellite images. Atti del XLVIII Convegno della Società Italiana di Agronomia Evoluzione e adattamento dei sistemi colturali erbacei), Perugia, 18-20 settembre 2019. (oral presentation)

Last, Cordero *et al.* (2020) is an article showing the research data elaborated during the research period at Agriculture and Agri-Food Canada, with the aim of describing the data collection process.

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2. Paper I: Fertilisation strategy and ground sensor measurements to optimise rice yield

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Fertilisation strategy and ground sensor measurements to optimise rice yield

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2.1 Abstract

Nitrogen (N) fertilisation is the main agronomic practice that affects rice yield and quality; similarly, its mismanagement can affect both economic and environmental aspects of crop production. Therefore, it is highly important to direct N fertilisation during the critical growth stages of rice development using vegetation indices (VIs). To this end, a two-year experiment was conducted in 2014 and 2015 in Castello d’ Agogna (PV), northwest Italy. The study had three aims: i) establish the best N fertilisation management in temperate rice cropping systems, in terms of total N supply and splitting, to maximise crop yield and N apparent recovery (AR); ii) evaluate the capability of crop N status indicators (CNSIs) measured at panicle initiation stage (PI) to determine grain yield; iii) derive Nfertiliser_rate_at_PI = f(CNSI) from a field trial to attain specific yield goals.

Results obtained for Centauro variety suggested that to maximise yield while avoiding AR reduction, a low dose of about 50 kg N ha^{-1} should be supplied during early growth, then increased at PI. In addition, the final topdressing fertilisation can compensate for any previous stage supply deficiency and can be determined from VI measurements. Findings also identified the normalised difference red edge (NDRE) index as the best VI to determine rice N status in specific agro-environmental conditions. SPAD and NDVI values measured with Rapid Scan can be used to determine N fertilisation at PI, although such measurements require correction through Sufficiency Indices (SIs) calculated as the ratio between VI measurements and VI values of a well-N fertilised plot. The trial also demonstrated that plots supplied with N amounts of 140 kg N ha^{-1} (pre-sowing and tillering stages combined) can serve as reference plots for SI calculation that allows to consider the effect of weather and soil variability on VI measurements. A notable exception to this finding was NDVI measured with GreenSeeker, which showed limited ability to assess rice N status under study environmental conditions. Indeed, both VI and the derived SI were influenced by seasonal and soil fertility conditions.

Finally, a specific statistical method to derive calibration functions for variable rate application fertiliser spreaders from a suitable experiment was defined. These functions will establish the N amount to be supplied at PI related to the CNSI measure. For each CNSI, a specific slope of the calibration function is determined while the intercept is varied depending on the grain yield goal. The higher the acceptable reduction relative to the maximum obtainable yield, the lower the N supply required at PI.

Keywords: Crop yield estimation; Crop N status; Site-specific N management; Vegetation Indices; Precision Agriculture; Variable rate fertilisation.

2.2 Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, being the staple food for three billion people (Barker *et al.*, 2007). On a world scale, rice is grown on an area of 157 million hectares (PROSPERA, 2012). In Italy, rice cultivation is mainly concentrated in the northwest, and it is cropped on about 227 000 ha (Ente Nazionale Risi, 2015).

Nitrogen (N) fertilisation is the main agronomic practice that affects yield and quality of rice crop. Mismanagement of N fertiliser may affect both the economic and environmental aspects of crop production (Tubaña *et al.*, 2011a). Nitrogen deficiency results in smaller leaf area, lower chlorophyll content, and biomass production, which lead to stunted crop growth and yield (Lin *et al.*, 2010). Excessive N input on the other hand, results in a dense canopy structure that facilitates pest and disease development, and leads to reduced plant resistance (Wu *et al.*, 2015, Hue *et al.*, 2016). Moreover, it can bring on lodging and extend growth periods and maturity achievement (Dong *et al.*, 2015; Liu *et al.*, 2015). Excessive N fertilisation has also been reported to pollute the environment through N leaching and both N2O and NH3 emission (Nguyen *et al.*, 2008).

Therefore, tools to calibrate the application of N fertilisers during critical growth stages are needed to improve both grain yield and nitrogen use efficiency (NUE) while avoiding N losses (Sathiya and Ramesh, 2009; Yoseftabar, 2013). In flooded systems, rice requires sufficient N input during the early and mid-tillering stages to maximise panicle number, and during the panicle initiation stage (PI) to optimise the number of spikelets per panicle and percentage of filled spikelets (Biloni and Bocchi, 2003, Bah *et al.*, 2009, Xue *et al.*, 2014). Nitrogen also increases sink size during late panicle formation (Manzoor *et al.*, 2006; Lee *et al.*, 2009; Tayefe *et al.*, 2014), which raises grain yield.

The rate of N fertilisation and the growth stages critical to optimising N application vary with rice cultivar (Bah *et al.*, 2009). Several destructive and non-destructive methods have been developed to establish optimum N fertilisation by monitoring crop N status (Tubaña *et al.*, 2011a). An ideal method to monitor N status has the following characteristics: non-destructive, fast, cost-effective, reliable, and obtains a value representative of the entire field (Xue *et al.*, 2004; Bajwa *et al.*, 2010). Optical properties of some leaf pigments, and in particular chlorophyll, have been shown to be reliable crop N status indicators that can be determined through vegetation indices (VIs) (Muñoz-Huerta *et al.*, 2013). Different instruments are able to measure light transmission through leaf (chlorophyll meters, e.g. SPAD-502) or canopy reflectance (e.g. GreenSeeker and Rapid Scan). These optical measurements are normally affected by growth stage, cultivar, soil water availability, and non-N nutrient deficiencies (Muñoz-Huerta *et al.*, 2013), as well as by sun angle, soil roughness, and soil colour.

Vegetation indices are calculated from sensor data, based on certain waveband combinations (Bajwa *et al.*, 2010). The most frequently used are NDVI (Normalized Difference Vegetation Index) and NDRE (Normalized Difference Red Edge) indices (Rouse *et al.*, 1974; Barnes *et al.*, 2000). NDVI has been reported to have low sensitivity at high chlorophyll content or abundant aboveground biomass, that induce saturation (Li *et al.*, 2010; Kanke *et al.*, 2012; Shi *et al.*, 2015). In rice, the index becomes saturated when aboveground biomass is about 4000 kg ha-1 and total N uptake reaches about 100kg ha^{-1} (Yao *et al.*, 2014). Therefore, the anticipation of VI measurements must consider that, in flooded rice systems, reflectance is influenced by the presence of water, especially during the early growth stages when canopy cover is limited (Yao *et al.*, 2014). Measurements taken at PI to guide the last topdressing N application fit with the optimal time for data acquisition.

Under dense green biomass, NDRE is a more suitable N status measure, as it is less susceptible than NDVI to saturation (Barnes *et al.*, 2000; Kanke *et al.*, 2016). Red edge wavelength (730 nm), corresponding to the

inflection point of the crop reflectance curve; combined with the NIR band has shown to be the most effective measure for estimating rice plant N uptake before the heading stage (Yu *et al.*, 2013).
Previous studies have shown that these VIs can be used to estimate rice yield based on the relation between crop spectral measurements and N status that allows to quantify N requirements (Chang *et al.*, 2005; Tubaña *et al.*, 2011b). Needed now is a quantitative estimate of the VIs and grain yield relationship to develop a precise rice N fertilisation management that is part of today's move to precision agriculture. Such a tool has the potential to improve crop N management and to mitigate negative environmental impacts of intensive rice production (Zhang *et al.*, 2011).

This study pursued the following goals:

- establishment of the best N fertilisation management, in terms of total N supply and splitting, to maximise crop yield and N apparent recovery (AR);
- evaluation of the capability of crop N status indicators (CNSIs) measured at PI to determine grain yield;
- derivation of Nfertiliser_rate_at_PI = f(CNSI) from a field trial to attain specific yield goals.

These tools will allow the development of a site-specific crop management strategy that can be adapted to different agro-environments reducing the effect of spatial variability, avoiding the negative impacts of N imbalances.

2.3 Materials and methods

2.3.1 Site description and soil properties

An experiment was designed to test a wide range of crop N statuses through different fertilisation managements expected to correlate with crop yield.

The study was carried out during two growing seasons (2014 and 2015) in an experimental field of the Rice Research Centre of Ente Nazionale Risi, located in Castello d'Agogna (PV) in northwest Italy (8° 41' 52'' E; 45° 14' 48'' N).

Climate of the area is temperate, with hot summers and two main rainy periods in spring and autumn. In *Figure* 1, the mean temperature and rainfall recorded during the experimental periods of March-October in 2014 and 2015 in Castello d'Agogna have been compared against 30-year (1984 to 2013) values. Site soil properties are summarised in *Table 1.*

Table 1: Soil properties of the experimental site.

Soil property	**Value**
Sand (%)	30.3
Silt (%)	55.7
Clay (%)	14.0
pH	6.1
Organic matter (%)	1.66
Total N (%)	0.089
C/N ratio	10.9
CEC (meq/100g)	9.3
Exchangeable K (ppm)	50
Olsen P (ppm)	20

The soil texture was silty loam. Other soil characteristics included low organic matter content, slight acidity, and low available N. The C/N ratio was well balanced with normal organic matter mineralisation. The Cation Exchange Capacity (CEC) was low as expected with low organic matter and clay contents. P Olsen content was high, while exchangeable K availability was very low. Further details of the soil at the site can be found in Miniotti *et al.*, 2016.

2.3.2 Experiment design and agronomic management

The experiment compared four rates of N supplied as the sum of N amount supplied at the pre-sowing and tillering stages ($N_{PRE+TILL}$, 0-60-100-140 kg N ha^{-1}) combined with four N rates supplied at PI (N_{PI}, 0-30-60-100 kg N ha^{-1}). A plot supplied with a total N amount of 300 kg ha^{-1} (200 + 100 kg N ha^{-1}) was added as an over-fertilised plot. In all plots, dry granular urea was used as fertiliser. Treatments were laid out using a split plot design with $N_{PRE+TILL}$ in the main plots and N_{PI} in the subplots. Each main plot measured 4.5*26.4 m and was divided into subplots measuring 4.5*6.6 m. Four replications for each treatment were established.

Both in 2014 and 2015 cultivar Centauro was planted, a round grain variety. The trial was ploughed in spring with conventional tillage equipment, after which it was laser levelled, and then harrowed. Phosphorus (56 kg ha^{-1} of P_2O_5) and potassium (112 kg ha^{-1} of K_2O) were uniformly applied in all plots before harrowing, using a 0-14-28 fertiliser. Water seeding was carried out on May 19, 2014 and May 18, 2015. Water was managed with continuous flooding for most of the growing season. The only exceptions were a “pin-point” period of 4-6 days to allow for root extension, and two other 4-6-day periods of drainage for mid-season fertiliser and herbicide application during the second half of June and July, as is the traditional management of the area. Final draining occurred on September 8, 2014 and August 29, 2015. Adequate measures to control diseases were taken throughout plant growth. Weed control was performed with oxadiazon, cyhalofop butyl, propanil, MCPA, and halosulfuron methyl. The crop was harvested between October, 21 and 29 and between October 12 and 21 in 2014 and 2015, respectively. The date difference merely reflected when the crop matured, as determined by N supply.

2.3.3 Field measurements

SPAD, NDVI, and NDRE were determined using suitable instruments to establish crop N status at PI (July 15, 2014 and July 13, 2015). The Soil-Plant Analysis Development (SPAD) index was determined using SPAD-502 (Konica Minolta, Japan). Readings were acquired from the fully expanded top leaf of plants, approximately one-third of the distance away from the tip, as described in Lin *et al.*, 2010. To obtain a representative value for the entire plot, 20 readings for each plot were taken from 20 leaves belonging to twenty different plants. For NDVI calculations, both GreenSeeker (Trimble©, Sunnyvale, California, USA) or Rapid Scan (Rapid Scan CS-45, Holland Scientific, USA) handheld active optical sensors were used. The first device detects canopy reflectance in the red (660 nm) and NIR spectral regions (770 nm), whereas the second incorporates three optical measurement channels (670, 730, and 780 nm), of which the first and third were used for NDVI calculations. The wavebands used to determine NDVI are different for the two instruments, so the two VIs are indicated as GS NDVI and RS NDVI for GreenSeeker- and Rapid Scan-read measurements, respectively. Finally, NDRE was measured and calculated using only Rapid Scan, considering canopy reflectance at 730 and 780 nm wavebands.

The measurements were collected by holding the instruments approximately 0.5 m above the rice canopy and walking at a constant speed along the entire length of the plot, as suggested in Xue *et al.*, 2014. Due to the technical characteristics of the instruments, sensor measurements width is approximately 0.3 m. Two measurements were taken from each of the length-wise sides of the plot in each treatment. The two values were then averaged to determine the mean value for each plot. Biometric measures at PI are usually well correlated to final grain yield (Bajwa *et al.*, 2010). To confirm this relationship, aboveground biomass, total N concentration, and total N uptake were also determined. Aboveground biomass was collected from three 0.25 m^2 areas, oven-dried at 40°C to a constant weight, and then analysed by the Dumas method to establish N concentration. Then, total N uptake at PI was also calculated by multiplying plant N concentration and the sampled biomass (Zavattaro *et al.*, 2012).

Grain (normalised to a moisture content of 14%) and straw yield were determined at harvest. In order to have three sub-samples, three 0.25 m^2 areas were harvested by hand in each plot. Only a bulk of the three sub-samples of both grain and straw was analysed for N concentration using the above-mentioned method. Finally, N AR was determined according to Zavattaro *et al.* (2012):

$$AR = \frac{N\ removal - N\ removal_{0N}}{N\ fertilizer} \tag{1}$$

where N removal is the amount of N removed as yield, N $removal_{0N}$ represents the amount of N removed by the unfertilised plot, and N fertiliser is the total amount of N supplied with fertiliser application.

2.3.4 Data analysis

Statistical analysis was performed using R software, version 3.3.0 (R Development Core Team, 2016).

General Linear Model (GLM) was used to explain yield and AR as a function of different N rates and splitting, year, block, and their interactions as follows:

$$\begin{gathered} x = \beta_1 * N_{PRE+TILL} + \beta_2 * {N_{PRE+TILL}}^2 + \beta_3 * N_{PI} + \beta_4 * {N_{PI}}^2 \\ +\beta_5 * N_{PRE+TILL} * N_{PI} + \beta_6 * N_{PRE+TILL} * YEAR + \beta_7 * N_{PI} * YEAR \\ + BLOCK + YEAR \end{gathered} \tag{2}$$

where x represents yield and AR, while β_1 to β_7 represent the slopes of the covariates. YEAR is the year effect related to the agro-climatic conditions and BLOCK is the block effect.

With an aim to determine the N_{PI} that maximises grain yield and AR for each N rate supplied at pre-sowing and tillering stage, the first order partial derivative was calculated with respect to N_{PI} and then set to zero.

The resulting equation is:

$$N_{PI} = \frac{-\beta_3 - \beta_5 * N_{PRE+TILL}}{2 * \beta_4} \qquad (3)$$

Equation 3 can be expressed as N_{PI} = f($N_{PRE+TILL}$) only when N_{PI} and $N_{PRE+TILL}$ show significant interaction. Otherwise, N_{PI} and $N_{PRE+TILL}$ contribute both to increases in yield and AR. In such an instance, no compensative effect exists and the equation cannot be calculated.
A correlation analysis was also applied to investigate the capability of different indicators to determine crop N status. The different indicators of crop N status (CNSI) here considered were both VIs (SPAD, GS NDVI, RS NDVI, NDRE), and biometric measures (aboveground biomass, its N concentration and total N uptake) detected at PI.
Next, grain yield was determined through the same GLM as mentioned above, only CNSIs took the place of $N_{PRE+TILL}$. The goal was to determine the relations between N_{PI} and CNSIs, under the larger aim of describing an equation to establish the best N_{PI} based on CNSIs. This statistical model was built as:

$$\begin{aligned} Yield = \gamma_1 * N_{PI} + \gamma_2 * {N_{PI}}^2 + \ \gamma_3 * CNSI + \gamma_4 * CNSI^2 + \\ + \gamma_5 * CNSI * N_{PI} + \gamma_6 * CNSI * YEAR + \\ + \gamma_7 * YEAR * N_{PI} + BLOCK + YEAR \end{aligned} \qquad (4)$$

where γ_1 to γ_7 represent the slopes of the covariates.

To obtain good indicators of crop N status in a given season and location, Sufficiency Indices (SI) and Response Indices (RI) were also calculated and used as CNSIs. Several studies on different cropping systems (Holland and Shepers, 2013; Muñoz-Huerta *et al.*, 2013; Xue *et al.*, 2014) suggested that a well-fertilised plot serve as a reference plot to calculate SIs, defined as the ratio of vegetation index obtained from a to-be-evaluated crop to VI of a well-N fertilised plot, integrating the confounding effect provoked by factors other than crop N status (Hussain *et al.*, 2000; Holland and Shepers, 2013). Hussain *et al.* (2000) state that reference plot establishment is suitable in irrigated rice conditions. Indeed, continuous flooding, common in temperate rice cropping systems, avoids water stress onset and consequent influence on rice spectral response. Moreover, Tremblay and Belec (2006) put forth that the reference plot might be considered as an internal standard against which measurements taken in other plots can be compared. Consequently, in order to standardise VIs measurements considering site-specific conditions, a reference plot has to be established in each location. RI is defined as the ratio of the vegetation index measured on the to-be-evaluated crop to the vegetation index measured in an unfertilised plot (Mullen *et al.*, 2003). Last, an equation to optimise fertiliser application, as a function of a measured CNSI value was determined for CNSIs that have shown a negligible effect of year and soil variability, originally or after transformation in SI or RI.

A statistical model was then built to determine yield from PI N supply and CNSI values as follow:

$$Yield = \gamma_8 * N_{PI} + \gamma_9 * {N_{PI}}^2 + \gamma_{10} * CNSI + \gamma_{11} * CNSI^2 + \\ + \gamma_{12} * N_{PI} * CNSI \qquad (5)$$

where γ_8 to γ_{12} represent the slopes of the covariates.

Year, block, and their interactions were not included in the statistical model, as after the results analysis, these parameters were shown not to be significant in determining yield.

To derive the most appropriate N fertilisation as a function of measured CNSI, the Maximum Grain Yield Approach was followed. Therefore, a first order partial derivative with respect to N_{PI} was calculated for *Equation 5* and then set to zero to determine the N amount that has to be supplied at PI to maximise rice grain yield.

First order partial derivative with respect to N_{PI} can be expressed as:

$$Yield' = \gamma_8 + 2\gamma_9 * N_{PI} + \gamma_{12} * CNSI \tag{6}$$

After rearranging the equation and setting the partial derivative to zero, N supply at PI can be determined as:

$$N_{PI} = \frac{-\gamma_8 - \gamma_{12} * CNSI}{2\gamma_9} \tag{7}$$

Again, *Equation 7* can be expressed as N_{PI} = f(CNSI) only when N_{PI} and CNSI show significant interaction. Otherwise, N_{PI} and CNSI both contribute to increase yield and AR. In such an instance, no compensative effect exists and the equation cannot be calculated.
Results analysis highlighted that a high N amount has to be supplied at PI to achieve the highest grain yield, as the function that describes maximum grain yield shows a smooth curvature close to the peak. Worthy of note is the considerable reduction in N rates that can be obtained with just a slight reduction in maximum grain yield. So, a method to determine NPI to achieve a reduced yield was studied, with the assumption that the reduced yield could be considered as a percentage of maximum grain yield (*Equation 8*).

$$Reduced\ yield = R * Maximum\ Yield \tag{8}$$

where R is the reduction coefficient, and assumed to be 0.90, 0.95, 0.99, 0.995, and 0.999 to analyse different reductions in maximum grain yield and the consequent reductions in N fertiliser applied. Moreover, this approach allows identification of the CNSI threshold over which no further N fertiliser must be added, depending on the grain yield goals.

2.4 Results

2.4.1 Climate

The two cropping seasons exhibited different climatic conditions. Rainfall was plentiful in the summer of 2014. Conversely, the 2015 summer saw reduced rainfall (704 mm) and higher temperature (13°C) compared to the 30-year means (*Figure 1*).

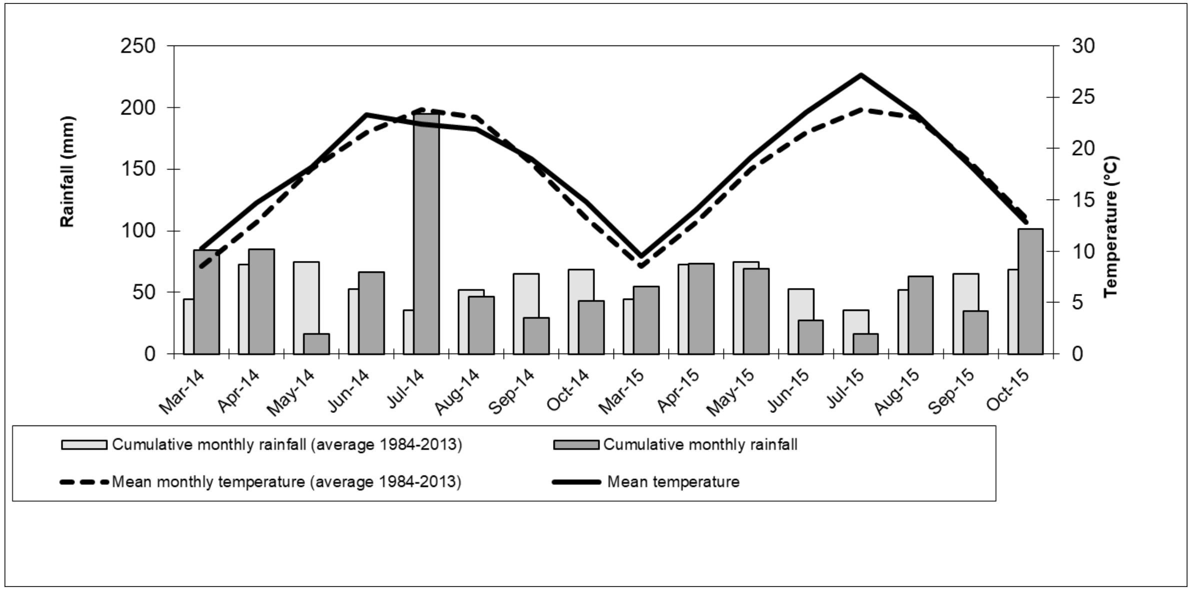

Figure 1: Average monthly temperature and rainfall over the 2014-2015 experimental period compared to the 30-year trend.

2.4.2 Response of rice yield to different N rates

Rice grain yield showed a parabolic increase with rising total N supply ($N_{PRE+TILL}$ plus N_{PI}) in both years (*Figure 2*). Maximum grain yield (11.1 Mg ha^{-1}) was achieved in 2014 when a total of 200 kg N ha^{-1} was applied, while in 2015 the maximum grain yield (11.2 Mg ha^{-1}) was reached with 120 kg N ha^{-1}. Additional N increases resulted in either a constant yield or yield reduction in both years.

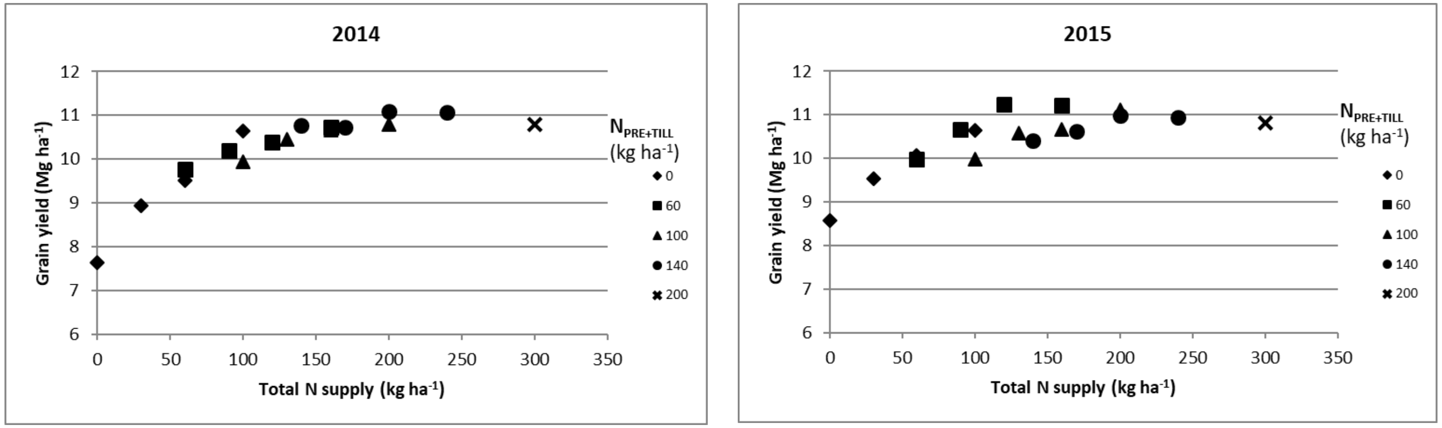

Figure 2: Grain yield response curve as average of four blocks at increasing levels of total N supply for year 2014 (left) and 2015 (right).

Total N uptake (grain + straw) behaved differently than did grain yield. The trend was almost linear across all explored N fertilisation levels in both years (*Figure 3*), despite different N uptake value ranges in each year (2014: 127 to 325 kg ha^{-1} and 2015: 129 to 223 kg ha^{-1}).

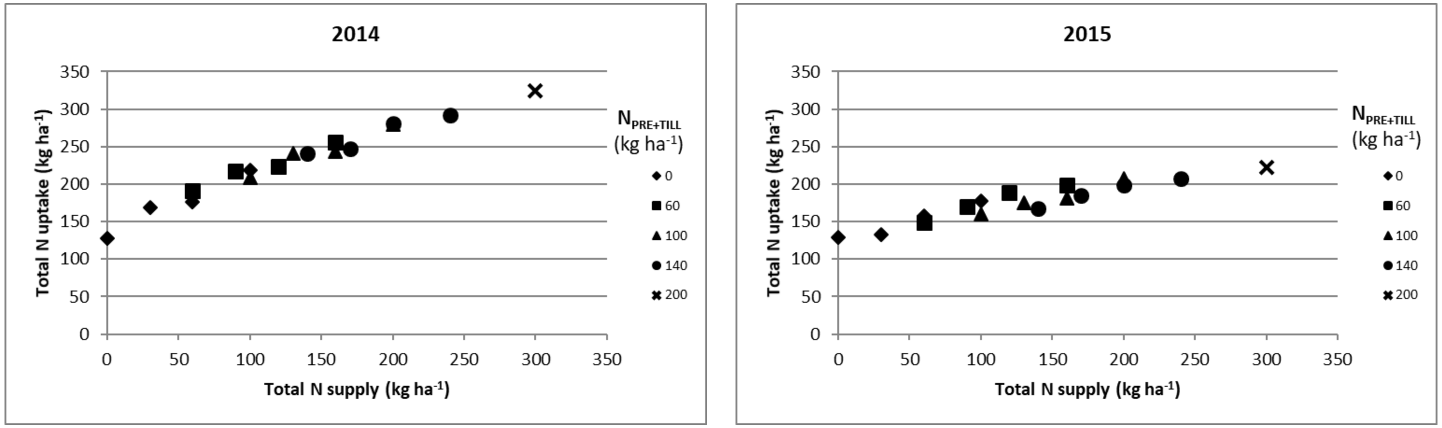

Figure 3: Total N uptake (grain + straw) obtained in 2014 (left) and 2015 (right) as an average of four blocks at increasing total N supply levels.

Consequently, AR values were different between the two growing seasons, even though N uptake in the unfertilised treatments was quite similar in both years (*Figure 4*).

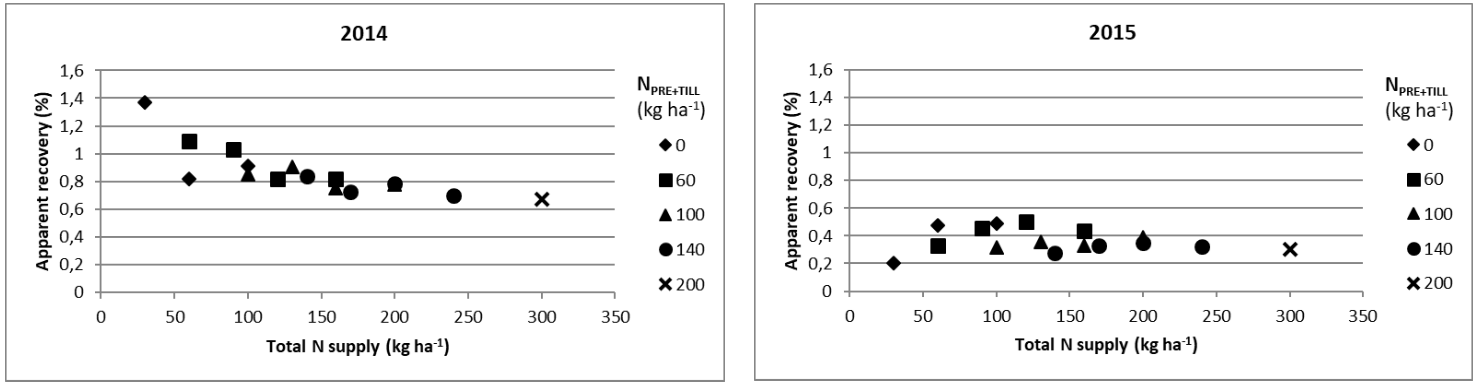

Figure 4: Apparent recovery (AR) as an average of four blocks, obtained in 2014 (left) and 2015 (right).

In both years AR decreased when N supplied increased, but with a slope more pronounced in 2014. In 2014, at lower N levels, mineral fertilisation promoted higher crop exploitation of soil N resources than observed in the unfertilised plot, and produced AR values above 1.0. On the contrary in 2015, AR values were lower and remained almost constant with rising N amount.

The GLM based on *Equation 2* was applied. Results are reported in *Table 2.*

Table 2: P(F) of the effects of N supply, YEAR and BLOCK on the different parameters recorded at harvest. The last row reports R^2 values.

Effect, covariate, or R^2	Grain yield	Total biomass	Total N uptake	Total N concentration	Apparent recovery
$N_{PRE+TILL}$	P<0.001	P<0.001	P<0.001	0.034	n. s.[a]
$N_{PRE+TILL}^2$	P<0.001	0.002	n. s.	n. s.	n. s.
N_{PI}	P<0.001	0.001	P<0.001	0.001	n. s.
N_{PI}^2	0.011	n. s.	n. s.	n. s.	n. s.
$N_{PRE+TILL}$*N_{PI}	P<0.001	0.028	n. s.	n. s.	n. s.
$N_{PRE+TILL}$*YEAR	0.001	P<0.001	P<0.001	n. s.	0.036
N_{PI}*YEAR	n. s.	n. s.	0.046	n. s.	0.004
BLOCK	n. s.	n. s.	n. s.	n. s.	n. s.
YEAR	0.001	n. s.	n. s.	P<0.001	P<0.001
R^2	0.779	0.786	0.871	0.840	0.712

[a]n. s. = not significant; total biomass: sum of grain and straw; Total N uptake at harvest: sum of grain and straw N uptake; Total N concentration: weighted average N concentration of grain and straw.

Grain yield and total biomass production were influenced by N applied at the pre-sowing plus tillering stages, N supplied at PI, and their interaction. Total N concentration and total N uptake were instead influenced by N fertilisation at the sum of pre-sowing and tillering, and PI stages, but not by their interaction. Differences among years were significant for yield, total N concentration, and AR. Interaction between year and N supply at pre-sowing and tillering stages or at PI was significant for most of the variables considered.

2.4.3 Crop yield maximisation and consequences on N apparent recovery

The N amount that must be supplied at PI to maximise yield as a function of $N_{PRE+TILL}$, can be calculated using *Equation 3* (represented in *Figure 5*).

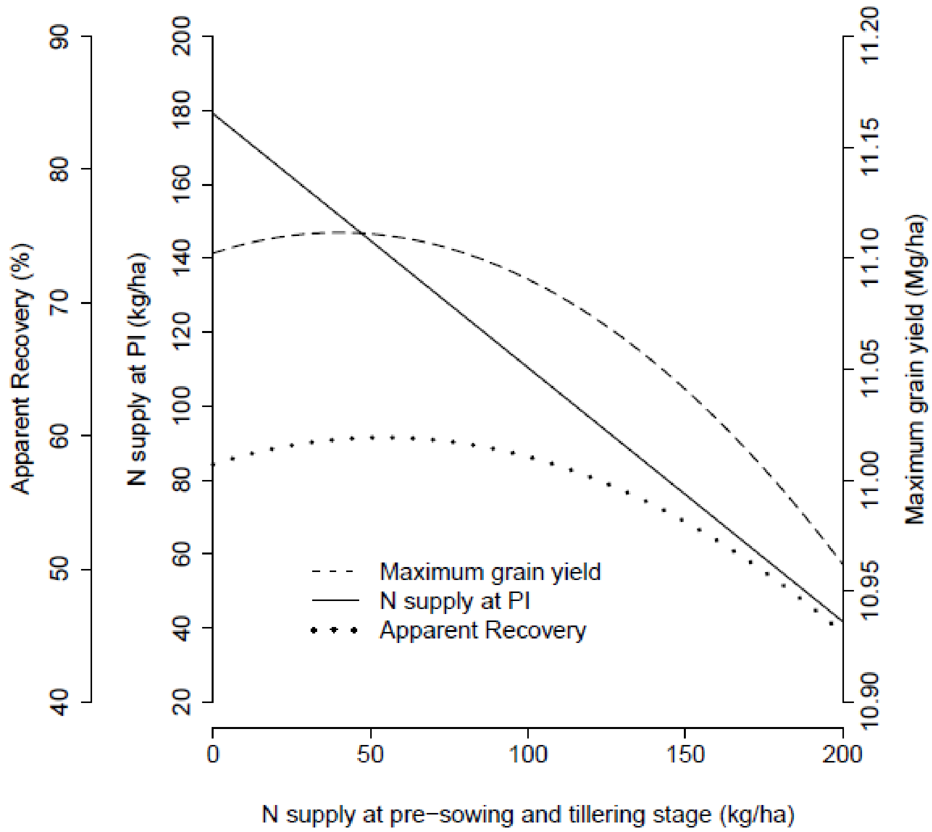

Figure 5: N rate that must be supplied at PI to maximise grain yield for each N amount supplied at the pre-sowing and tillering stages.

Maximum grain yield (11.1 Mg ha^{-1}) was achieved when about 42 kg N ha^{-1} was applied during pre-sowing and tillering; maximum AR (59.8%) was reached with a $N_{PRE+TILL}$ of about 53 kg N ha^{-1}. When 42 kg N ha^{-1} was applied at the pre-sowing and tillering stages, AR fell slightly (59.7%). As *Figure 5* shows, 150 kg N ha^{-1} must be supplied at PI to maximise yield, while just 140 kg N ha^{-1} is enough to maximise AR. Nonetheless, some uncertainties are associated with these early stage and PI fertilisation values.

2.4.4 Capability of different VIs to determine N status at PI stage

The capability of different VIs to determine rice N status at PI was verified by correlation analysis. Results are reported in *Table 3*.

Table 3: Pearson correlation coefficients between different VIs and crop N status at PI stage (N=124).

	Biomass	N concentration	N uptake	SPAD	GS NDVI	RS NDVI
SPAD	0.728**	0.714**	0.739**			
GS NDVI	0.657**	0.556**	0.618**	0.809**		
RS NDVI	0.695**	0.614**	0.654**	0.843**	0.833**	
NDRE	0.842**	0.766**	0.820**	0.854**	0.826**	0.833**

** = P(r)<0.010; aboveground biomass, its N concentration and N uptake were measured at PI.

Analysed VIs correlated highly with one another as their coefficients were above 0.8. They also correlated highly with biometric measures, with very high coefficients for NDRE and falling progressively for SPAD, RS NDVI, and GS NDVI. Moreover, VIs correlated better with crop aboveground biomass than with N uptake, except for SPAD. Alternatively, N concentration determination was the poorest. Differences recorded between GS and RS NDVI in the various correlations related to the differing wavelengths used by GreenSeeker and Rapid Scan.

2.4.5 Capability of different CNSIs to determine grain yield

The capability of different CNSIs, including both biometric measures and VIs measured at PI, to determine yield was investigated using GLM according to *Equation* 4. Results are shown in *Table* 4.

Table 4: P(F) of the different CNSIs in determining grain yield. Each column head title must be considered as a CNSI in the GLM. The last row reports R^2 values.

Effect, covariate, or R^2	CNSI						
	Biomass	N concentration	N uptake	SPAD	GS NDVI	RS NDVI	NDRE
N_{PI}	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
N_{PI}^2	0.004	0.014	0.003	0.010	P<0.001	0.003	0.001
CNSI	P<0.001	P<0.001	P<0.001	0.002	P<0.001	0.044	P<0.001
$CNSI^2$	0.002	P<0.001	P<0.001	0.021	0.009	n. s.	P<0.001
N_{PI}*CNSI	P<0.001	0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
YEAR*CNSI	n. s.[a]	n. s.	n. s.	n. s.	P<0.001	n. s.	n. s.
YEAR*N_{PI}	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.	n. s.
BLOCK	n. s.	n. s.	n. s.	0.039	n. s.	0.013	n. s.
YEAR	n. s.	n. s.	n. s.	n. s.	P<0.001	n. s.	n. s.
R^2	0.717	0.618	0.701	0.639	0.680	0.692	0.769

[a]n. s. = not significant; aboveground biomass, its N concentration and N uptake and VIs were measured at PI.

All CNSIs and most of their squares detected at PI were significant on determining crop yield. Interaction of the different CNSIs with N supply at PI was also found to be significant for all considered CNSIs.

According to *Table* 4, the biometric measures evaluated at PI were good at determining grain yield as demonstrated by high R^2 values; biomass was best, followed by N uptake, and N concentration alone led to a poorer estimate. All VIs performed better than N concentration. The NDRE R^2 was even better than that obtained by biomass. The two NDVIs were shown to be a little less able to determine grain yield; SPAD was the poorest. Moreover, VIs are essentially proxy measures of biometric variables as the correlation analysis demonstrated and can be extensively measured.

Except for NDRE, year or block effect was significant for all VIs. This makes quantification of N fertiliser needs based on VI measurements difficult because of the wide variation in agro-climatic and soil conditions. Consequently, Sufficiency Indices (SIs) and Response Indices (RIs) were calculated. Plots that received 60, 100, 140, or 200 kg N ha^{-1} as $N_{PRE+TILL}$, or 0 kg N ha^{-1} were considered reference plots to determine the SIs or RIs, respectively. Then, the *Equation* 4 was applied to determine rice grain yield at the best using SIs and RIs as CNSIs.

SIs and RIs calculated for each VI were also found to determine yield significantly (*Table* 5). Interaction between SI or RI and N supplied at PI was always significant too. Maximum R^2 values were obtained for all SIs except GS NDVI using as a reference plot those receiving 60 kg N ha^{-1} at pre-sowing and tillering. With these SIs, block effect was negligible, while year effect was significant for SIs calculated from SPAD and GS NDVI. If plots considered as reference received 140 kg N ha^{-1} at the pre-sowing and tillering stages, R^2 values were slightly lower (except for GS NDVI), but year and its interaction effects were not significant (except again for GS NDVI). As previously shown, year and block effects were originally negligible only for NDRE. SI calculation considering reference plots as those receiving $N_{PRE+TILL}$ = 140 kg N ha^{-1} allowed their effect to be eliminated from SPAD and RS NDVI measurements. Consequently, calibration functions were calculated for NDRE, SPAD SI, and RS NDVI SI only, as they were more suitable to assess rice N status in specific agro-environmental conditions.

Table 5: P(F) of the capability of response indices (RIs) (reference plot fertilised at 0 kg N ha^{-1}) and sufficiency indices (SIs) (reference plot fertilised at 60, 100, 140, 200 kg N ha^{-1}) to determine yield. The last column reports R^2 values.

Nref[a]	SI/RI	N_{PI}	N_{PI}^2	SI	SI^2	N_{PI} * SI	YEAR * SI	YEAR * PI	BLOCK	YEAR	R^2
0	SPAD	P<0.001	0.013	0.008	0.050	P<0.001	n. s. [b]	n. s.	n. s.	n. s.	0.615
	GS NDVI	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	0.002	n. s.	0.001	0.733
	RS NDVI	P<0.001	0.004	0.004	0.008	P<0.001	n. s.	n. s.	n. s.	n. s.	0.664
	RS NDRE	P<0.001	0.002	P<0.001	P<0.001	P<0.001	0.007	n. s.	n. s.	0.001	0.738
60	SPAD	P<0.001	0.007	P<0.001	0.001	P<0.001	n. s.	n. s.	n. s.	0.047	0.675
	GS NDVI	P<0.001	P<0.001	P<0.001	0.022	P<0.001	P<0.001	0.040	n. s.	P<0.001	0.736
	RS NDVI	P<0.001	0.002	0.003	0.008	P<0.001	n. s.	n. s.	n. s.	n. s.	0.729
	RS NDRE	P<0.001	0.001	P<0.001	P<0.001	P<0.001	n. s.	n. s.	n. s.	n. s.	0.784
100	SPAD	P<0.001	0.013	0.028	n. s.	P<0.001	n. s.	n. s.	0.048	n. s.	0.602
	GS NDVI	P<0.001	P<0.001	0.001	0.015	P<0.001	P<0.001	n. s.	0.005	P<0.001	0.724
	RS NDVI	P<0.001	0.002	0.001	0.004	P<0.001	n. s.	n. s.	0.013	n. s.	0.692
	RS NDRE	P<0.001	0.002	P<0.001	0.001	P<0.001	n. s.	n. s.	0.009	n. s.	0.726
140	SPAD	P<0.001	0.008	P<0.001	0.005	P<0.001	n. s.	n. s.	n. s.	n. s.	0.659
	GS NDVI	P<0.001	P<0.001	P<0.001	0.007	P<0.001	P<0.001	n. s.	n. s.	P<0.001	0.751
	RS NDVI	P<0.001	0.002	0.013	0.030	P<0.001	n. s.	n. s.	n. s.	n. s.	0.712

	RS NDRE	P<0.001	0.001	P<0.001	P<0.001	P<0.001	n. s.	n. s.	n. s.	n. s.	0.772
200	SPAD	P<0.001	0.009	P<0.001	0.004	P<0.001	n. s.	n. s.	0.024	n. s.	0.651
	GS NDVI	P<0.001	P<0.001	P<0.001	0.006	P<0.001	P<0.001	n. s.	0.041	P<0.001	0.734
	RS NDVI	P<0.001	0.002	0.046	n. s.	P<0.001	n. s.	n. s.	0.018	n. s.	0.699
	RS NDRE	P<0.001	0.001	P<0.001	0.001	P<0.001	n. s.	n. s.	n. s.	0.031	0.765

[a]Nref = N applied to the reference plot (kg ha^{-1}).

[b]n. s. = not significant; RI = ratio between VI of considered plot and VI of unfertilised plot; SI = ratio between VI of the considered plot and VI of reference plot receiving the amount of fertiliser reported in the first column.

2.4.6 Calibration functions aimed at reaching specific yield goals

Figure 6 shows the calibration functions obtained when considering different grain yield goals. The solid black line represents the first estimation of the calibration function that has to be used to achieve the maximum grain yield for the three different CNSIs values. The dashed black lines represent the calibration functions obtained when a reduction coefficient (R) is used with respect to maximum grain yield. R was assumed equal to 0.999, 0.995, 0.99, 0.95, and 0.90 in the various scenarios, respectively.

As expected, all calibration functions recommended lower N amounts when vigour increases. When NDRE, SPAD SI, and RS NDVI SI reached 0.5, 124, and 110, respectively, no additional N supply at PI was required to maximise yield. For each CNSI, the slope of the function remains almost constant, while the intercept varies depending on the R value being proportional to it. Therefore, yield reduction limits the N amount that has to be supplied at PI.

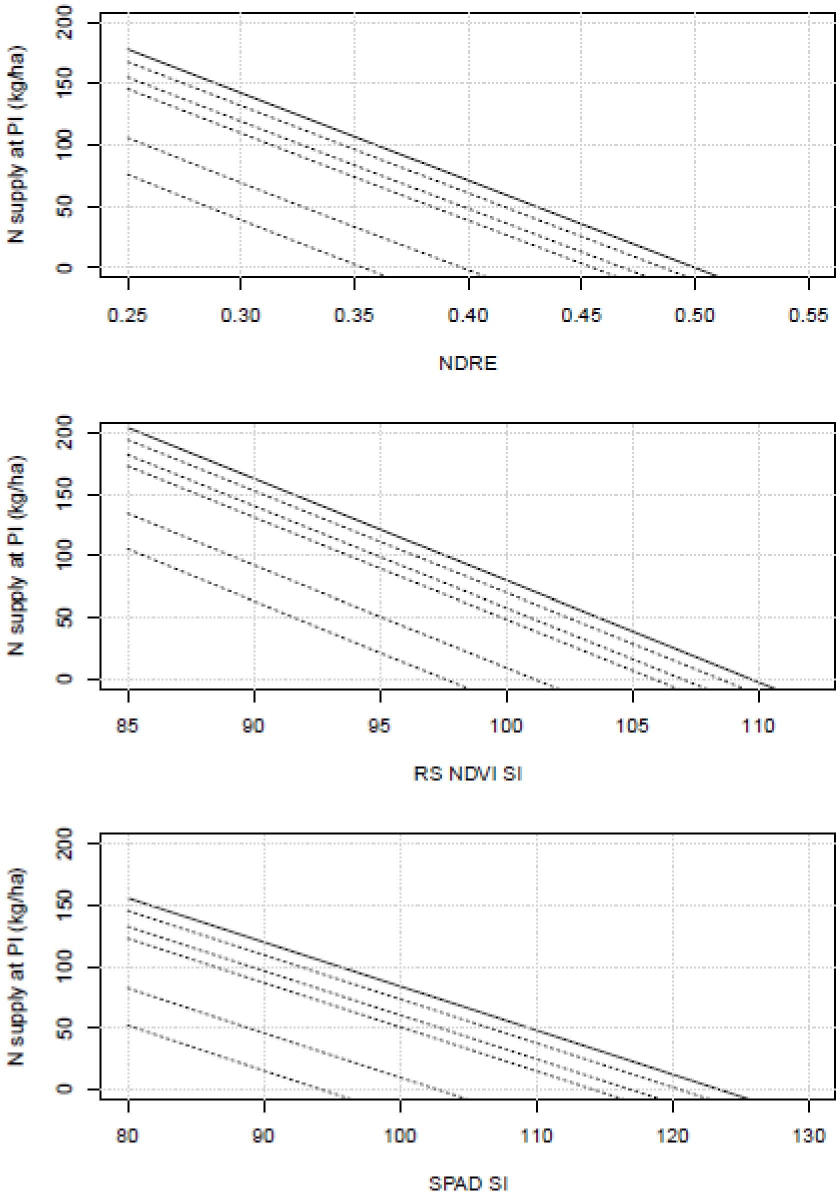

Figure 6: Calibration functions for VRA fertiliser spreader obtained while establishing progressively decreasing grain yield goals. The solid black line represents the calibration function for maximising grain yield. The dashed black lines represent the calibration functions for obtaining 0.999, 0.995, 0.99, 0.95, and 0.90 of maximum grain yield.

2.5 Discussion

2.5.1 Crop yield maximisation and consequences on N apparent recovery

In each year of the experiment, a similar (about 11.2 Mg ha^{-1}) maximum grain yield was reached, but it was accomplished with very different total N amounts associated with different crop densities in the two years. Maximum grain yield was reached with about 200 kg N ha^{-1} in 2014 and 120 kg N ha^{-1} in 2015. Rice grain yield stayed constant or decreased with additional N fertiliser, showing a parabolic trend. The absent or negative effect of further N doses after the peak yield confirmed the results of previous studies, in which yield declines were linked to lodging or disease (Dong *et al.*, 2015; Liu *et al.*, 2015).

Grain yield was affected by N fertilisation at the pre-sowing + tillering and at PI stages and by their interaction, which revealed the potential to compensate with an N topdressing fertilisation at PI for deficient N supplies during the initial stages. These results align with reports of previous studies by Manzoor *et al.* (2006) and Lee *et al.* (2009). Rice responded well to increased N supply at PI, especially when low N amounts had been applied during the early growth stages.

In general, increases in N supply reduced apparent recovery (AR) (Xue and Yang, 2008; Yesuf and Balcha, 2014). Differences between the two cropping seasons were evident. Even though N uptake of the unfertilised plots was similar in both seasons, 2015 AR values were lower than 2014. In 2014, reaching maximum grain yield resulted in an AR of nearly 0.8 versus the 2015 value of just below 0.5. The lower 2015 AR value is a consequence of reduced N uptake mainly from a lower N concentration in the aboveground biomass of rice at harvest (2.26 % and 0.95 % in 2014 and 2015, respectively). Indeed, the 2014 crop was denser than in 2015. In 2015, rice compensated for a low panicle density with an increase in spikelets per panicle and in 1000-grain weight, which produced the same grain yield. Consequently, AR values were very different, despite similar

N uptake in the unfertilised treatment in both years. *Equation* 3 established the N supply required at PI for each $N_{PRE+TILL}$ maximising grain yield or AR. The relationship between grain yield or AR and $N_{PRE+TILL}$ showed a clear parabolic trend. Maximum grain yield and maximum AR were obtained separately and they approximated the same total N amount (about 195 kg ha^{-1}), with about 50 kg N ha^{-1} supplied at the first two applications and the remaining dose at PI. The amount of N at the pre-sowing and tillering stages needed to maximise AR was slightly lower than that required to maximise grain yield.

2.5.2 Capability of different CNSIs to determine grain yield

Correlation analysis showed that VIs (SPAD, GS NDVI, RS NDVI, and NDRE) were highly correlated with biometric measures at PI. In particular, NDRE had the highest correlation coefficients. All VIs correlated most with total aboveground biomass, except as expected for SPAD, which correlated better with total N uptake. NDVI, measured with both GreenSeeker and Rapid Scan instruments at PI, correlated less well with crop N status than other Vis did.

This result may arise from the presence of an abundant biomass at which saturation starts to reduce index sensitivity (Kanke *et al.*, 2012; Muñoz-Huerta *et al.*, 2013; Novotna *et al.*, 2013; Cao *et al.*, 2016).

In this study, NDVI saturated at a biomass production of about 7600 kg ha^{-1}, or an N uptake of 180 kg ha^{-1}.

The correlation between NDRE and biometric measures at PI was also strong under dense biomass. Indeed, the red-edge wavelengths utilised by Rapid Scan are more sensitive at higher levels of chlorophyll content, as is illustrated by its strongest correlation with crop N concentration at PI, and consequently with rice N uptake. In this study, NDRE saturation effects were first noted at biomass production levels of about 8000 kg ha^{-1} or 190 kg N ha^{-1} of total N uptake.

When CNSIs replaced $N_{PRE+TILL}$ in the statistical model (i.e., using *Equation* 4 instead of *Equation* 2), all CNSIs and most of their squares resulted significant on determining grain yield. Moreover, interaction with N rate supplied at PI was also significant on determining yield for all CNSIs considered. This confirms that topdressing N fertilisation at PI can compensate for low CNSI values, showing again that N supplied at PI can balance low $N_{PRE+TILL}$ amounts. All CNSIs demonstrated themselves to be good at determining grain yield, with R^2 values near or above 0.60. NDRE was the best.

Nonetheless, as year or block differences were detected for all VIs except NDRE, SIs were calculated from other corresponding VIs to overcome the influence of year and block on VIs values. Year and block can be assumed to represent the effect due to climate and soil variability in the direction of blocks. Sufficiency Indices calculated using reference plots that received 140 kg N ha^{-1} supplied during pre-sowing and tillering combined removed agro-climatic and soil variability effects best. In addition, statistical models applied to SIs improved yield determination, as R^2 reached values higher than 70%. Consequently, the results of this study not only confirmed the benefit of establishing a well-fertilised reference plot to obtain better indicators of in-season rice N status (Hussain *et al.*, 2000), but also estimated that plots receiving 140 kg N ha^{-1} as the sum of the pre-sowing and tillering stage N supplies, can serve as a reference plot for Centauro variety. Of course, the reference plot must be relocated each year to avoid long term effects of differentiated N fertilisation (Holland and Shepers, 2013).

2.5.3 Calibration functions aimed at reaching specific yield goals

Calibration functions are improved when obtained from CNSIs not influenced by agro-climatic conditions and soil variability. In this study, NDRE alone demonstrated these features. However, through SI calculations, block and year effects were also made negligible for SPAD and RS NDVI, which made it possible to determine the calibration functions for these CNSIs in addition. It is recommended that NDRE be determined at PI, but SPAD and RS NDVI can work, so long as SIs referred to these VIs are calculated from a well-N fertilised reference plot. Obtained calibrations associate required N supplies at PI to field-measured CNSIs, and the calibration Equation relates to a specified grain yield goal. The highest yield goal requires the highest supply of N at PI. Alternatively, a lower yield goal permits a reduced N_{PI} amount, as the slope of the function remains almost constant while the intercept is proportional to the grain yield goal.

One method to determine an acceptable maximum yield reduction is selection of a CNSI threshold over which N fertilisation at PI is ineffective. Suitability of the threshold should be based on the field and potential yield in specific situations.

2.6 Conclusions

Results reported in this study suggest that yield and apparent recovery maximisation are not conflicting goals. The statistical models developed here indicate that Centauro variety grain yield is optimised most effectively when N fertiliser supply is reduced in the early growth stages and concentrated at PI (about 70% of total N). Moreover, a topdressing N fertilisation amount can be determined from measured CNSI values to avoid N imbalances. This study confirmed that VIs measured at PI act as biometric proxy measures, and help avoiding time-consuming and destructive analyses. NDRE was demonstrated to be the best at determining grain yield variability specific agro-environmental situations. It can be used to determine Centauro variety calibration functions that improve N fertilisation.

Sufficiency index (SI) calculations that consider as reference those plots receiving 140 kg N ha^{-1} N supply (sum of the pre-sowing and tillering stages) can correct SPAD and RS NDVI measurements, making possible to calculate the calibration function for these CNSIs as well. NDVI measured with GreenSeeker was less suitable for making N fertilisation determinations at PI for Centauro variety in the environmental conditions presented in this study, as both the index and derived SI were influenced by ago-climatic conditions and soil variability.

The determined calibration functions allow a site-specific rice N fertilisation management that accounts for year and spatial variability, and avoids consequent negative environmental impacts. It must be noted that the calibration functions were derived only for Centauro variety under the specific environmental conditions presented. Therefore, extension to other rice varieties and environments can be obtained following the same method presented in this work.

2.7 Acknowledgements

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3. Paper II: Statistical model to overcome rice variety effect in precision nitrogen fertilisation

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3.1 Abstract

Nitrogen (N) fertilisation determines rice yield and quality. Rice grain yield can be estimated as the product of plant density, tillering capacity, spikelets number per panicle, grain weight, and reduced by sterility percentage. Different rice varieties have different response to N fertilisation, due to the different role of yield components in determining grain yield. Normalised Difference Red Edge (NDRE) can be used to quantitatively estimate rice grain yield from spectral measurements at panicle initiation stage (PI), then driving topdressing N application. Prescription functions converting NDRE values into N supply at PI have to be adapted to the different rice varieties. To this end, a multiple year experiment was conducted in Castello d’Agogna (PV), northwest Italy, between 2011 and 2018, involving four different rice varieties belonging to three different grain types. Then, the study aimed at i) adapting the statistical procedure to obtain prescription functions for different rice varieties; ii) defining strategies to adapt prescription functions to new rice varieties through qualitative and quantitative tools.

Results highlighted different growth patterns among the rice varieties, leading to different response to both N fertilisation and NDRE interpretation. Indeed, depending on both timing and amount of N application, N supply implies a different development of grain yield components. Consequently, the integration of Principle Component Analysis (PCA) and path analysis is a promising strategy for using a qualitative and quantitative tool for choosing the best prescription function for each rice variety, on the basis of their agronomical traits.

Keywords: precision N fertilisation, optimal N splitting, grain yield components, PCA, path analysis, topdressing

3.2 Introduction

Rice (*Oryza sativa* L.) is the most important cereal crop for human consumption in the world, cultivated in different agro-ecosystems. Nowadays, Italy is the leading rice producer in the European Union, accounting for 41% of European rice production (FAOSTAT, 2008-2017 average).

Nitrogen (N) is a key element in rice production, involved in many biochemical and physiological activities (Djaman *et al.*, 2016). Then, N is closely associated to both grain yield and quality (Zhu *et al.*, 2016). Deficient soil N availability hampers growth and development of rice plants. Conversely, excessive N supply increases vegetative development, favouring the incidence diseases, *e.g.* blast, (Shaiful Islam *et al.*, 2009) as well as rice quality decline and lodging of rice plants (Youseftabar *et al.*, 2012). Moreover, unbalanced N fertilisation in paddy fields heavily contributes to environmental pollution and water eutrophication, through ammonia volatilisation, nitrous oxide emission, N runoff, and N leaching (Yang *et al.*, 2013). In flooded rice systems, leaching is the main loss pathway, followed by ammonia volatilisation (Linquist *et al.*, 2013). Ladha *et al.* (2005) reported that fertiliser N recovery in rice is quite low, approximately 46%. Aiming at enhancing the overall sustainability of rice cropping system, it is important to synchronise N supply with crop requirements both in time and space. Splitting N application into pre-plant and topdressing during both early tillering and mid-season is suggested as an efficient method for rice cultivation, allowing for a more efficient N use during the growing season (Russo, 1996; Fageria and Baligar, 1999; Ghaley, 2012). According to Zhang *et al.* (2013), rice grain yield can be defined as the product of yield sink capacity and filling efficiency. Consequently, it is closely related to the number of fertile spikelets per unit area, that is the product of panicle density and spikelets number per panicle (Yoshida *et al.*, 2006). Then, rice grain yield can be estimated as the product of plant density, tillering capacity, spikelets number per panicle, grain weight, and reduced by sterility percentage. The N application strategy, considering both N rates and the number and timing of application, affects crop response to applied fertilisers (Hirzel *et al.*, 2011). Hashim *et al.* (2015) reported that rice N uptake increases until reproductive stage, with maximum N uptake from tillering to flowering (Gebremariam and Baraki, 2016). Conversely, during the ripening stage, rice re-translocates N from the vegetative parts to the developing grains, then using N stored in plant tissues. Nitrogen application in different rice growth stages triggers a different crop response. Therefore, N supplied at pre-planting and early growth stages promotes rice growth and tillering, determining the number of panicles (Bah *et al.*, 2009; Hirzel *et al.*, 2011; Moe *et al.*, 2014; Tayefe *et al.*, 2014). During the mid-season, N application contributes to promote the number of differentiated spikelets, to reduce the degeneration of differentiated spikelets, and to increase the percentage of filled grains, when N is supplied at panicle initiation stage (PI), at the beginning of spikelets differentiation and at heading time, respectively (Zhang *et al.*, 2013). Several studies (Inamura *et al.*, 2003; Bah *et al.*, 2009; Lee *et al.*, 2009; Sathya and Ramesh, 2009) suggest that, among topdressing applications, N supply at PI is the most effective for improving yield attributes. Moreover, Cordero *et al.* (2018) stated that larger N rates at PI can potentially compensate for deficient N supply at early growth stages.

Generally, N is applied at a uniform rate across the entire field, based on grain yield goals (Teal *et al.*, 2006), but not accounting for the spatial variability of grain yield potential across a field, that leads to different crop N requirements (Thompson *et al.*, 2015) as well as soil N availability.

In addition, different rice varieties have different response to N fertilisation especially for time of fertiliser application, depending on their agronomic traits (Bah *et al.*, 2009). Consequently, grain yield estimation considering both spatial variability and different requirements between rice cultivars is fundamental in precision N fertilisation.

Crop sensing is an effective tool for the identification of in-field variability, allowing for variable rate application (Tagarakis and Ketterings, 2017). Indeed, optical sensors that rely on canopy reflectance in the visible and NIR regions of the electromagnetic spectrum can be profitably used to estimate crop variables associated with N management, such as grain yield, as well as crop N concentration and uptake (Corti *et al.*, 2018). A quantitative estimation of grain yield from mid-season spectral measurements is needed to drive topdressing variable rate N fertilisation using crop sensing (Tagarakis and Ketterings, 2017; Cordero *et al.*, 2018). Cordero *et al.* (2018), after comparing different vegetation indices (VIs), concluded that NDRE was the most effective in determining rice grain yield at PI in the specific agro-environment. These results confirmed previous studies by Mutanga and Skidmore (2004) that indicated that, at high canopy density, crop biomass is better estimated by VIs based on wavelengths located in the red edge region of the electromagnetic spectrum.

In Italy, traditional N fertilisation management consists in a pre-sowing application, followed by two successive N supplies at tillering and PI (Biloni and Bocchi, 2003, Zavattaro *et al.*, 2008). Variable rate N application based on rice proximal sensing can be profitably used to optimise topdressing fertilisation at PI, to avoid N imbalances. Cordero *et al.* (2018) developed a statistical procedure to obtain prescription functions converting VIs values into practical N recommendations for Centauro variety. However, the extension to other rice varieties is needed to promote a widespread application of precision fertilisation in rice. Then, the study has different aims:

- the adaptation of the statistical procedure to obtain a prescription function linking N_{PI} and NDRE measured at PI for different rice varieties;
- the definition of strategies to adapt prescription functions to new rice varieties through qualitative or quantitative tools.

3.3 Materials and methods

A multiple year experiment took place at the Rice Research Centre of Ente Nazionale Risi in Castello d'Agogna (PV), north-west Italy (8° 41' 52'' E; 45° 14' 48'' N), between 2011 and 2018. Climate of the area is temperate, characterised by cold winters and warm summers. Rainfalls occur mainly in spring and autumn, corresponding to the first stages of rice development and the harvesting period, respectively. According to Köppen-Geiger climate classification, it is defined as Cfa (Köppen, 1936). Miniotti *et al.* (2016) and Cordero *et al.* (2018) have previously described the soil of the experimental site. Relevant traits include silty-loam texture, low soil organic matter content, and well-balanced C/N ratio.

The main aim of the trial was to create vigour variability just before the last topdressing N application, in order to measure the effect of different amount of N supplied at PI.

The field trial involved four different rice varieties (Gladio, Centauro, Carnaroli, and Ronaldo). These varieties belong to three grain types defined according to EU standards and based upon physical parameters as grain length, width, and length to width ratio: Gladio is classified as long B, Centauro is a round grain variety, while both Carnaroli and Ronaldo belong to long A grain type, all related to the Japonica genotype. Rice varieties have been chosen because of their widespread cultivation in the north-west area, as well as the genetic differences among them, which implies a different agronomic response to increasing N rates. The experiment was carried out during two growing seasons for each variety, with the only exception of Ronaldo. In particular, Gladio was grown in 2011 and 2013, Centauro in 2014 and 2015, Carnaroli in 2016 and 2017, and Ronaldo in 2018. Since 2011, a specific experimental setup was studied to induce different crop vigour before the last topdressing fertilisation, by diversifying N supplied as sum of pre-sowing and tillering stage applications ($N_{PRE+TILL}$), then evaluating the effect of increasing N rates supplied at PI (N_{PI}). Treatments were arranged in a split plot design, with $N_{PRE+TILL}$ in the main plots (4.5*26.4 m) and N_{PI} in the subplots (4.5*6.6 m). For each treatment, four replicates were established. Nitrogen levels have been varied across the years of the experiment, to adapt N fertilisation taking into account the specific N requirements of the different rice varieties. Treatments resulted from a factorial combination of N fertilisation levels at $N_{PRE+TILL}$ and N_{PI}. Relevant details about N management are shown in *Table* 1.

Table 2: Nitrogen levels used in factorial combination in each year of the experiment for each rice variety.

Rice variety	**Grain type**	**Year**	**$N_{PRE+TILL}$ (kg ha^{-1})**	**N_P (kg ha^{-1})**
Gladio	Long B	2011	60-100-140-180	0-30-60-100
		2013	0-60-100-140	0-30-60-100
Centauro	Round	2014	0-60-100-140-200	0-30-60-100
		2015	0-60-100-140-200	0-30-60-100
Carnaroli	Long A	2016	0-30-60-90	0-30-60-90
		2017	0-30-60-75-90	0-30-60-90
Ronaldo	Long A	2018	0-60-100-140	0-30-60-100

N fertiliser was supplied as dry granular urea.

The typical agronomic practices of the region were adopted. All plots were spring ploughed, laser-levelled and rotary harrowed to have an optimal seedbed preparation. Phosphorus (P) and potassium (K) were uniformly supplied before harrowing distributing 300 kg ha^{-1} of a 0-14-28 mineral fertiliser, to prevent the direct or indirect effect of P and K deficiency on rice response to N fertilisation.

Rice was broadcasting water seeded. Then, water management involved pin-point flooded method (Hardke and Scott, 2013; Miniotti *et al.*, 2016). Flooding conditions were gradually stopped during the seedling stage to allow for root extension. Then, flooding was re-established, maintaining a permanent ponding water depth of 5-10 cm until the final drainage that occurred approximately one month prior to harvest. The only exception were short drainage periods for the application of adequate post-emergence herbicides and fungicide if needed, as well as topdressing N application at tillering and PI.

Rice N status at PI was monitored using Rapid Scan (Rapid Scan CS-45, Holland Scientific, USA). The instrument has three spectral measurements channels: 670, 730, and 780 nm. Canopy reflectance detected at 730 and 780 nm was used for the arithmetical computation of NDRE. Measurements were collected walking at a constant speed along the whole length of the plot, by holding the instrument about 0.5 m above the rice canopy. This method was previously followed by Cordero *et al.* (2018) and suggested by the manufacturer's instruction manual.

Harvest was carried out at crop maturity, between the end of September and the end of October, depending on the climate, the different maturity duration of the rice varieties, as well as crop maturation due to N management. At the end of the growing season, crop yield was measured on a 3.3*5.4 m area with a combine harvester. After collection, rice grains were dried and rice yield was expressed at 14% moisture content. Moreover, three areas of 0.25 m^2 were hand-harvested in each plot. Yield components (*i.e.* plant density, tillering capacity, number of spikelets per panicle, 1000-grain weight, and sterility percentage) were determined. Plant density was calculated on three 0.25 m^2 sampling areas within each plot, as well as panicle density at the end of the growing season. Tillering capacity was calculated as the ratio between panicle density and plant density. The number of spikelets per panicle and sterility percentage were detected on a sample of 20 panicles for each plot, while 1000-grain weight was determined on two replicates for plots.

3.3.1. Data analysis

Statistical analysis was performed using R software, version 3.5.1 (R Development Core Team, 2018). The statistical procedure described by Cordero *et al.* (2018) was applied to each rice variety, to determine the optimal fertilisation strategy accounting for their specific agronomic traits. Rice grain yield was considered as the sum of the contribution due to early-growth stage and topdressing N application, as well as their interaction. Then, looking at the average effect over the growing seasons, the statistical procedure previously proposed in Cordero *et al.* (2018) was applied to each rice variety, as shown by *Equation 1*.

$$\begin{aligned} Grain\ yield = {} & \beta_1 * N_{PRE+TILL} + \beta_2 * {N_{PRE+TILL}}^2 + \beta_3 * N_{PI} \\ & + \beta_4 * {N_{PI}}^2 + \beta_5 * N_{PRE+TILL} * N_{PI} \end{aligned} \tag{1}$$

where β_1 to β_5 are the slopes of the covariates.

Then, N_{PI} that maximise grain yield corresponding to each N rate supplied as sum of pre-sowing and tillering stage application was determined, through first order partial derivative calculation and setting to zero (*Equation 2*).

$$N_{PI} = \frac{-\beta_3 - \beta_5 * N_{PRE+TILL}}{2 * \beta_4} \tag{2}$$

After that, N_{PI} determined through *Equation 2* was substituted into *Equation 1*, obtaining the function that describes the grain yield potential corresponding to each $N_{PRE+TILL}$ and N_{PI} amount. Again, first order partial derivative was calculated and set to zero, calculating $N_{PRE+TILL}$ that allows obtaining maximum grain yield potential for each rice variety. This value was then substituted in *Equation 2*, to determine N_{PI} that maximise grain yield potential. Consequently, this procedure allowed determining the optimal N splitting during the growing season for each rice variety, that, substituted in *Equation 1*, determined the maximum grain yield potential for each rice variety.

Following this data analysis, the same statistical procedure was applied with NDRE replacing $N_{PRE+TILL}$ in the GLM previously mentioned.

Consequently, prescription functions that convert NDRE values in practical topdressing N recommendations for each rice variety were determined using *Equation 3*:

$$N_{PI} = \frac{-\gamma_3 - \gamma_5 * NDRE}{2 * \gamma_4} \tag{3}$$

where γ_n are the slopes of the covariates obtained from the fitting of the model explaining grain yield as function of NDRE and N_{PI}.

Results highlighted large differences among the rice varieties, clearly showing the influence of varietal traits on optimal N management. Hence, the present research studied a statistical procedure to define the best prescription curve, taking into account the peculiar characteristics of each rice variety.

Rice grain yield (GY) is determined by grain yield components. Then, it can be estimated by the product of plant density, tillering capacity, spikelets number per panicle, 1000-grain weight, and percentage of filled spikelets, as reported in *Equation 4*.

$$GY = D * T * N_S * W * (1 - S) \tag{4}$$

where D is plant density, T is tillering capacity, Ns is spikelets number per panicle, W is 1000-grain weight, and S is sterility percentage.
After that, with the aim of building an additive model that considers grain yield as the sum of the contribution of each component, natural logarithm was applied to both members of *Equation 4*, as follows:

$$\ln GY = \ln D + \ln T + \ln N_S + \ln W + \ln(1 - S) \tag{5}$$

A Principal Component Analysis (PCA) were applied to the logarithmic values of grain yield components in order to reduce the number of explanatory variables, by converting strongly correlated variables into uncorrelated principal components (PCs) (Gómez-Limón and Riesgo, 2009). The PCA was applied by means of the library FactoMineR (Le *et al.*, 2008). The extracted PCs indicated the analytical parameters that mostly contributed to differentiate rice varieties. Before applying PCA, data were standardised by subtracting the average value, then dividing the difference by standard deviation. Communalities were calculated to determine the proportion of variable variance explained by PCs, then identifying the variables that better explained the variability among the rice varieties (Gaudino *et al.*, 2014).
Path analysis (Wright, 1921) was used to investigate the relationships among all response variable, partitioning the correlation between yield, yield components, and N fertilisation and NDRE into direct and indirect effects. Path analysis was conducted using R *lavaan* package (Rosseel, 2012). Path analysis was performed on log-transformed grain yield components, to meet the general assumptions of linear, additive relationships among the variables of interest (Land, 1969). Moreover, variables were standardised, by subtracting the mean and dividing for the standard deviation. This procedure allows to express the relationships

among the variables as variations in standard deviation units (Youngerman *et al.*, 2018). Two different path models were applied to each rice variety, as shown in *Figure 1*¸ representing path diagrams that graphically illustrate the cause-effect relationships (represented by arrows) among the variables (represented by rectangles).

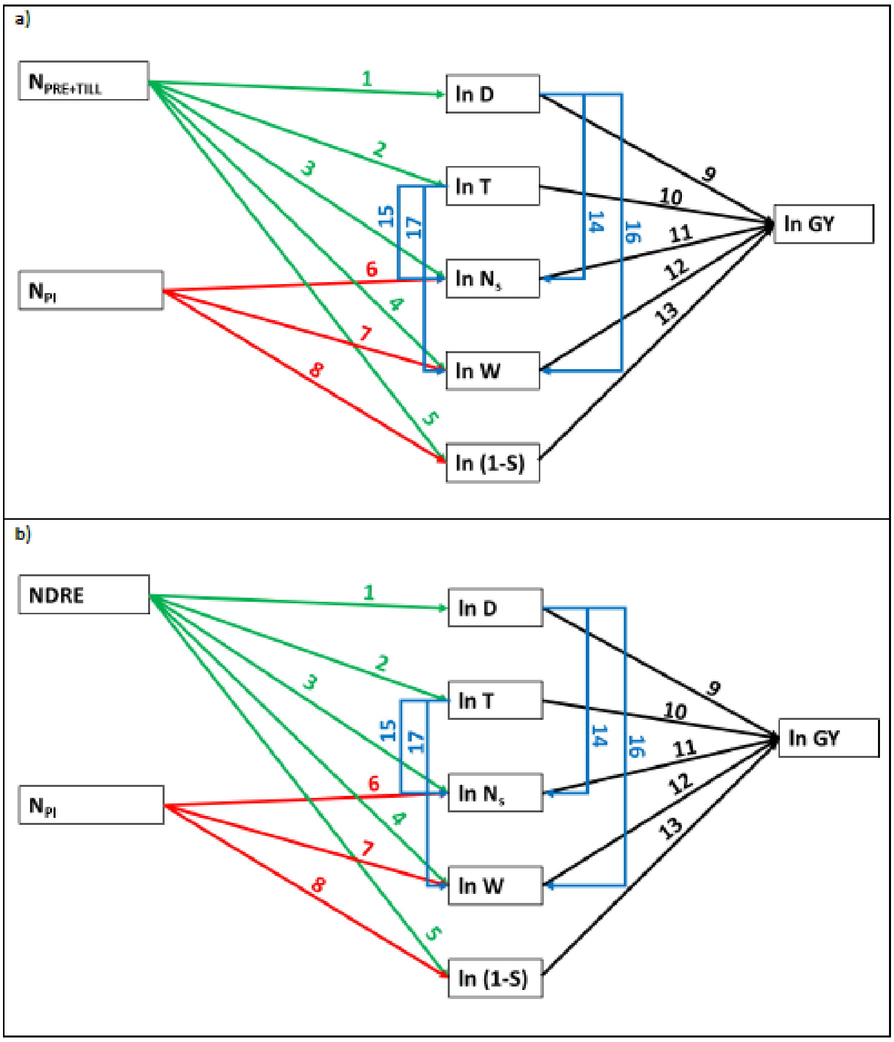

Figure 1. Path diagrams used to graphically illustrate the cause-effect relationships (arrows) among the variables (rectangles). Numbers are used in tables 8 and 10 to report the different coefficients.

The first path model (*Figure 1a*) was applied to each rice variety, to analyse the effect of grain yield components on grain yield, and to verify if the effect of N fertilisation on grain yield was mediated through yield components. Variables were arranged in the initial path model on the basis of general knowledge about rice response to N fertilisation. Indeed, several studies (Bah *et al.*, 2009; Hirzel *et al.*, 2011; Moe *et al.*, 2014; Tayefe *et al.*, 2014) suggested that early growth stage N application promotes tillering and rice growth, while topdressing N supply positively affects the number of differentiated spikelets and increases the percentage of filled grains (Inamura *et al.*, 2003; Bah *et al.*, 2009; Lee *et al.*, 2009; Sathya and Ramesh, 2009; Zhang *et al.*, 2013).

In the second path model (*Figure 1b*) NDRE replaced $N_{PRE+TILL}$, with the aim of evaluating if the different rice varieties have a different NDRE response, due to their different growth development, then requiring different N_{PI} management.

3.4 Results

3.4.1 Description of the four rice varieties and consequences on the optimal N splitting

The GLM in *Equation 1* described grain yield as a function of different N rates and splitting during the growing season. Results of the statistical analysis are reported in *Table 2*.

Table 3: P values of $N_{PRE+TILL}$ and N_{PI} rates on grain yield of the different rice varieties. The last row shows R^2 values.

Effects, covariates and R^2	**Gladio**	**Centauro**	**Carnaroli**	**Ronaldo**
$N_{PRE+TILL}$	P<0.001	P<0.001	n. s.*	P<0.001
$N_{PRE+TILL}^2$	P<0.001	P<0.001	P<0.001	0.004
N_{PI}	P<0.001	P<0.001	P<0.001	P<0.001
N_{PI}^2	P<0.001	0.004	0.032	P<0.001
$N_{PRE+TILL}$*N_{PI}	P<0.001	P<0.001	0.039	n. s.
R^2	0.798	0.779	0.473	0.959

* not significant

The GLM well described grain yield of the different rice varieties, as exhibited by the high R^2 values, especially for Ronaldo, Gladio and Centauro. Excepting for Carnaroli, grain yield was affected by total N rate supply as sum of pre-sowing and tillering stage applications. Conversely, N supply at PI always affect grain yield, regardless of rice varieties. Also, the interaction between early growth stage and mid-season N application resulted significant, with the only exception represented by Ronaldo.

Maximum obtainable grain yield, calculated according to Cordero *et al.* (2018), was the highest for Ronaldo, followed by Gladio and Centauro (*Table 3*). In Carnaroli, maximum grain yield was about 25% lower.

Table 3: Optimal N splitting during the growing season for Gladio, Centauro, Carnaroli, and Ronaldo varieties.

Rice variety	**Maximum obtainable yield ($Mg\ ha^{-1}$)**	**Total N supply ($kg\ ha^{-1}$)**	**$N_{PRE+TILL}$ ($kg\ ha^{-1}$)**	**N_{PI} ($kg\ ha^{-1}$)**	**Replacement value***
Gladio	11.1	199	103	96	0.313
Centauro	11.1	192	40	152	0.687
Carnaroli	8.3	120	42	80	0.332
Ronaldo	11.2	280	185	95	0.090

*It represents the amount of $\mathbf{N_{PI}}$ to be supplied for any unit of N missed at pre-sowing and tillering stage applications.

Total N supply ($N_{PRE+TILL}$ + N_{PI}) needed to maximise grain yield showed a similar trend. Ronaldo required the highest total N supply (280 kg ha^{-1})

during the whole growing season to reach the maximum obtainable yield. Gladio and Centauro achieved the maximum grain yield with 30% less total N. Moreover, Carnaroli, further reduced total N requirements by approximately 40% with respect to both Gladio and Centauro.

The differences among the rice varieties appeared evident also considering the optimal N splitting strategy during the growing season. Aiming at grain yield maximisation, Gladio took advantage in equally splitting N supply between early growth stages and topdressing application. Both Centauro and Carnaroli needed less N supplied as sum of pre-sowing and tillering stage application (20% and 33% of total N supply, respectively), then increasing N application at PI (80% and 67% of the total N amount, respectively). Conversely, Ronaldo variety required 66% of total N applied as $N_{PRE+TILL}$, then supplying the remaining 34% at PI.

Subsequently, the application of the GLM provided the coefficients that allowed determining the slope of the function linking N_{PI} with $N_{PRE+TILL}$, that corresponds to the replacement value of N_{PI} to $N_{PRE+TILL}$. In all rice varieties higher N supply at pre-sowing or tillering implied reduction at PI. The replacement value, that is the amount of N to be supplied at PI for any unit missed as sum of pre-sowing and tillering stage application, increased with the following order: Ronaldo, Gladio, Carnaroli and Centauro.

Table 4 shows the results of the GLM, when NDRE took the place of $N_{PRE+TILL}$.

Table 4: P values of NDRE and N_{PI} on grain yield of the different rice varieties. The last row shows R^2 values.

Effects, covariates and R^2	**Gladio**	**Centauro**	**Carnaroli**	**Ronaldo**
NDRE	P<0.001	P<0.001	0.005	P<0.001
$NDRE^2$	P<0.001	P<0.001	P<0.001	P<0.001
N_{PI}	P<0.001	P<0.001	P<0.001	P<0.001
N_{PI}^2	P<0.001	P<0.001	0.025	P<0.001
NDRE*N_{PI}	P<0.001	P<0.001	0.017	0.003
R^2	0.933	0.769	0.563	0.957

Grain yield can be accurately predicted through NDRE determination at PI stage. Indeed, R^2 values were quite high, especially for Ronaldo, Gladio and Centauro, that achieved R^2 values higher than 0.75. In all rice varieties, NDRE, N_{PI}, and their squares resulted significant on predicting grain yield. The interaction between NDRE and NPI resulted significant, too, showing models including NDRE more capable to drive NPI than models including $N_{PRE+TILL}$.

Prescription functions were determined according to *Equation 3*, that allowed adapting N supply based on NDRE readings to each rice variety, with the aim of obtaining maximum grain yield (*Figure 2*).

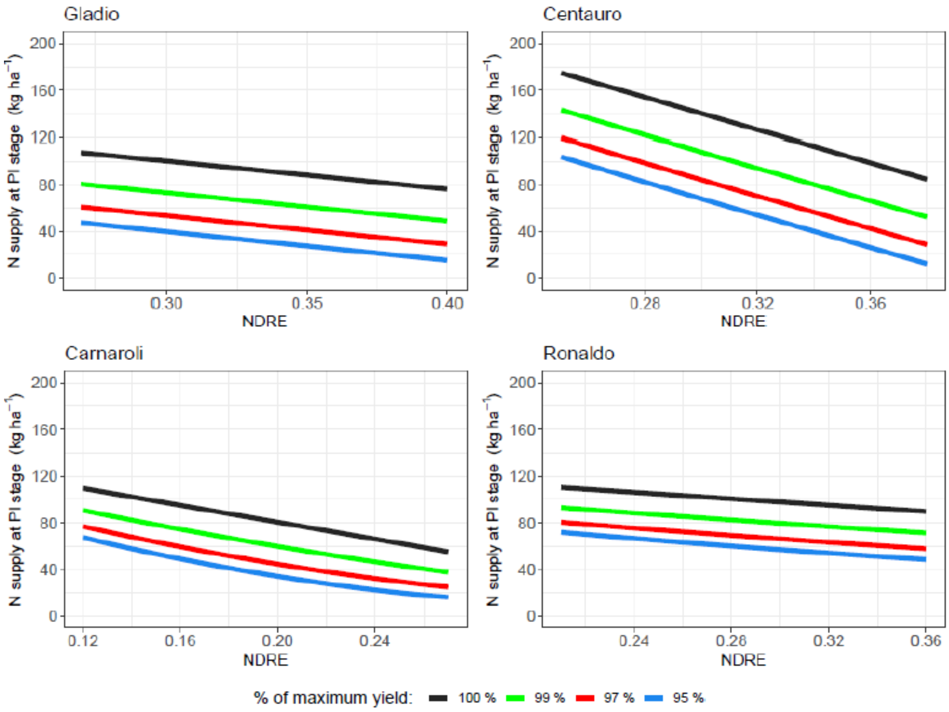

Figure 2: Prescription functions linking NDRE values to N supply at PI stage to achieve different grain yield goals for Gladio, Centauro, Carnaroli, and Ronaldo variety, respectively. Values are based on GLM application.

Despite the differences among the rice varieties, all prescription functions had a negative slope, as expected. The negative slope suggests to reduce N_{PI} for higher NDRE values, corresponding to higher crop vigour. Moreover, in the same variety, the slope remained constant when applying a reduction coefficient as a percentage of maximum grain yield, allowing the determination of NPI needed to obtain the reduced yield.

As a consequence, the best topdressing N fertilisation management based on NDRE determination at PI stage varied among the rice varieties. Maximum obtainable grain yields estimated though the model based on NDRE (*Table 5*) pair very well with those obtained from the model based on $N_{PRE+TILL}$ (*Table 3*).

Table 5: NDRE values, N supply at PI stage that allow obtaining maximum grain yield, and replacement value for Gladio, Centauro, Carnaroli, and Ronaldo variety.

Rice variety	**Maximum obtainable yield ($Mg\ ha^{-1}$)**	**NDRE**	**N supply at PI stage ($kg\ ha^{-1}$)**	**Replacement value***
Gladio	11.2	0.398	77	2.39
Centauro	11.1	0.349	106	6.93
Carnaroli	8.6	0.186	83	3.73
Ronaldo	11.0	0.345	92	1.36

*It represents the amount of $\mathbf{N_{PI}}$ to be supplied for any reduction of a centesimal unit (0.01) of NDRE.

However, such grain yield potential can be achieved corresponding to different NDRE values, that resulted higher in Gladio than in Centauro and Ronaldo. Carnaroli achieved maximum grain yield with to the lowest NDRE values. Topdressing N requirements at PI were different among the rice varieties, with the highest values recorded for Centauro, then decreasing progressively for Ronaldo, Carnaroli, and Gladio. Again, the replacement value indicates the marginal effect of N_{PI} for any centesimal reduction of NDRE.

The replacement value of N_{PI} in relation to NDRE is directly connected to the share of N_{PI} to total N estimated to achieve the maximum obtainable yield.

3.4.2 Principal Component Analysis as a qualitative tool for determining prescription functions

The application of PCA to log-transformed values of grain yield components allowed to obtain a set of uncorrelated PCs. According to Kaiser’s rule (Kaiser, 1960), the first two PCs were retained, as they recorded eigenvalues higher than 1. Overall, the extraction of the two main components explained 80,4% of the total variance. The PC1 explained 46.9% of the total variability while the PC2 33.5% of the total variability. The PC1 had the largest positive correlation with both 1000-

grain weight and sterility, contributing for 38.4% and 26.7%, respectively (*Table 6*).

Table 6: Variable loadings and communalities determined on log-transformed data. Bold values highlighted in which PC the variable had the highest loading.

Variable	PC1	PC2	Communalities
Plant density	-0.450	**-0.514**	0.467
Tillering capacity	-0.333	**0.564**	0.429
Spikelets number per panicle	-0.189	**0.639**	0.444
1000-grain weight	**0.619**	0.062	0.387
Sterility	**0.517**	0.075	0.273

On the other hand, PC2 resulted positively correlated with both spikelets number per panicle and tillering capacity, while negatively correlated with plant density, that contributed for 40.8%, 31.8%, and 26.4%, respectively. The highest communality was recorded by plant density, followed by spikelets number per panicle and tillering capacity. Conversely, the lowest value was recorded by sterility.

The PCA biplot was used to graphically depict the relationship between the rice varieties and grain yield components (*Figure 3*).

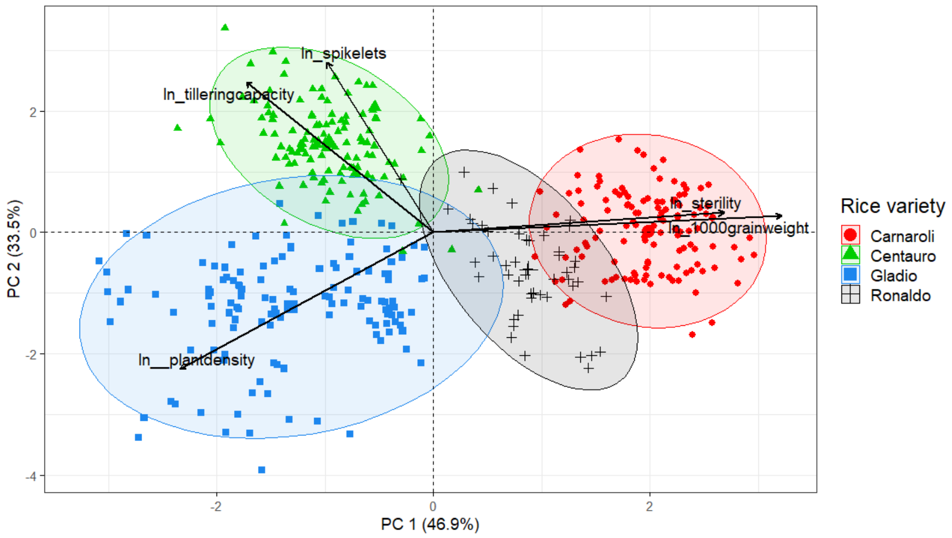

Figure 3: Biplot graph based on log-transformed data of grain yield components.

The graph clearly highlighted the presence of four distinct groups in the original dataset, that well fit the four different rice varieties considered in the present study, characterised by different agronomical traits.

Gladio and Centauro, Ronaldo and finally Carnaroli were mainly separated on PC1, mostly depending on morphological characteristics mainly promoted by N_{PI}, while Gladio, Ronaldo and Carnaroli and finally Centauro, were mostly separated on PC2, mostly depending on morphological characteristics mainly promoted by $N_{PRE+TILL}$.

However, PCA allowed only qualitatively evaluating the differences among the rice varieties.

3.4.3 Path analysis as a quantitative tool for determining prescription functions

Path analysis was used to quantify the different behaviour of the rice varieties. In the first proposed path model, N fertilisation showed a significant direct effect on grain yield components in Gladio, Centauro, and Ronaldo variety (*Table 7*).

Table 7: P value of the path type for the different rice varieties. Results are referred to the first path model.

Path type	**Gladio**	**Centauro**	**Carnaroli**	**Ronaldo**
Direct (fertilisation→yield components)	0.044	0.001	n. s.	P<0.001
Direct (yield components→yield)	P<0.001	n. s.	P<0.001	n. s.
Total indirect effect	P<0.001	P<0.001	0.009	P<0.001
Total effect	P<0.001	n. s.	P<0.001	n. s.

Yield components revealed a significant direct effect on rice grain yield only in Gladio and Carnaroli. Conversely, indirect effect, meaning the effect of N fertilisation on grain yield as mediated by yield components, was significant for all rice varieties.

Moreover, path analysis provided information about the development of grain yield components in the different rice varieties, as well as their influence in determining rice grain yield. Results are reported in *Table 8*.

Table 8: Path coefficients expressing the effect of cause variable on effect variable. Results are referred to the first path model. Arrow numbers represents the arrows reported in Figure 1a.

Arrow	Cause	Effect	Gladio	Centauro	Carnaroli	Ronaldo
1	$N_{PRE+TILL}$	Plant density	0.290	-	-	-
2	$N_{PRE+TILL}$	Tillering capacity	0.223	0.259	0.247	0.557
3	$N_{PRE+TILL}$	Spikelets number per panicle	0.138	0.228	-	-
4	$N_{PRE+TILL}$	1000-grain weight	-0.614	-0.533	-0.340	-0.660
5	$N_{PRE+TILL}$	Sterility percentage	-0.514	-0.372	-	-0.434
6	N_{PI}	Spikelets number per panicle	0.367	0.495	0.255	0.697
7	N_{PI}	1000-grain weight	0.124	-	-	-0.329
8	N_{PI}	Sterility percentage	-0.187	-	-0.296	-0.258
9	Plant density	Grain yield	0.788	0.284	0.293	0.561
10	Tillering capacity	Grain yield	0.712	0.473	0.236	0.716
11	Spikelets number per panicle	Grain yield	0.445	0.446	0.170	0.487
12	1000-grain weight	Grain yield	0.166	-	0.259	-
13	Sterility percentage	Grain yield	-	-0.223	0.165	-
14	Plant density	Spikelets number per panicle	-0.657	-0.218	0.219	-0.397
15	Tillering capacity	Spikelets number per panicle	-0.389	-0.295	0.176	-0.382
16	Plant density	1000-grain weight	-0.246	-	-0.238	-
17	Tillering capacity	1000-grain weight	-	-0.325	-0.175	-

Plant density was affected by N supplied as sum of pre-sowing and tillering stage applications only in Gladio variety. Early growth stage N application positively affected tillering capacity in all rice varieties, with the highest effect in Ronaldo. In both Gladio and Centauro, early growth stage N application, as well as topdressing N fertilisation at PI, positively affected spikelets number per panicle, with N_{PI} showing the highest effect in both varieties. Moreover, the effect was higher in Centauro than in Gladio. In Carnaroli and Ronaldo, only N_{PI} had a large positive effect on spikelets number per panicle. The 1000-grain weight resulted negatively affected by early growth stage N application in all rice varieties. In all rice varieties, ln (1-S) was negatively affected by N supply. In particular, in Gladio and Ronaldo both $N_{PRE+TILL}$ and N_{PI} showed a negative effect, while in Centauro and Carnaroli the parameter was affected only by $N_{PRE+TILL}$ and N_{PI}, respectively.
As excepted, yield components highly affected grain yield but differently between the considered varieties. In Gladio, plant density, tillering capacity, spikelets number per panicle, and 1000-grain weight had a significant positive direct effect on grain yield, with plant density having the greatest effect, followed by tillering capacity. In Centauro, tillering capacity recorded the highest positive effect, followed by spikelets number per panicle. Conversely, sterility percentage showed a negative effect. In Carnaroli, all grain yield components had a positive direct effect on grain yield, with plant density showing the highest effect and sterility pointing out the lowest. In Ronaldo, only plant density, tillering capacity, and spikelets number per panicle recorded a positive direct effect on grain yield, with tillering capacity showing the highest effect.
Significant effects were highlighted also among grain yield components each other. In Gladio, Carnaroli, and Ronaldo plant density and tillering capacity showed a negative effect on spikelets number per panicle. Conversely, Carnaroli had an opposite behaviour. In Gladio, plant density had a negative effect on 1000-grain weight. In Centauro, tillering capacity

negatively affected 1000-grain weight. In Carnaroli, both high plant density and high tillering capacity resulted in low 1000-grain weight. Conversely, in Ronaldo, neither plant density nor tillering capacity affected 1000-grain weight.

In the second path model, only indirect effect, *i.e*. the effect of NDRE on grain yield as mediated by yield components, resulted significant in determining grain yield for all rice varieties (*Table 9*).

Table 9: P value of the path type for the different rice varieties. Results are referred to the second path model.

Path type	**Gladio**	**Centauro**	**Carnaroli**	**Ronaldo**
Direct (NDRE→yield components)	P<0.001	n. s.	n. s.	0.002
Direct (yield components→yield)	P<0.001	n. s.	P<0.001	n. s.
Total indirect effect	P<0.001	P<0.001	P<0.001	P<0.001
Total effect	P<0.001	P<0.001	P<0.001	0.006

Considering the second path model, in all rice varieties, high NDRE values had a detrimental effect on both 1000-grain weight and sterility, with the highest values recorded in Ronaldo (*Table 10*).

Table 10: Path coefficients expressing the effect of cause variable on effect variable. Results are referred to the second path model. Arrow numbers represents the arrows reported in Figure 1b.

Arrow	Cause	Effect	Gladio	Centauro	Carnaroli	Ronaldo
1	NDRE	Plant density	-	-	0.281	-
2	NDRE	Tillering capacity	0.540	0.249	-	0.584
3	NDRE	Spikelets number per panicle	-	0.272	-	-
4	NDRE	1000-grain weight	-0.668	-0.439	-0.336	-0.780
5	NDRE	Sterility percentage	-0.297	-0.459	-0.301	-0.503
6	N_{PI}	Spikelets number per panicle	0.468	0.490	0.261	0.697
7	N_{PI}	1000-grain weight	0.376	-	-	-0.322
8	N_{PI}	Sterility percentage	-0.316	-	-0.298	-0.239
9	Plant density	Grain yield	0.677	0.284	0.292	0.564
10	Tillering capacity	Grain yield	0.846	0.473	0.235	0.721
11	Spikelets number per panicle	Grain yield	0.380	0.454	0.168	0.494
12	1000-grain weight	Grain yield	-	-	0.253	-
13	Sterility percentage	Grain yield	-	-0.223	-	-
14	Plant density	Spikelets number per panicle	-0.463	-0.246	0.173	-0.411
15	Tillering capacity	Spikelets number per panicle	-0.535	-0.326	-	-0.417
16	Plant density	1000-grain weight	-	-	-	-
17	Tillering capacity	1000-grain weight	-	-0.376	-0.172	-

As previously observed in the first path model, rice grain yield was positively affected by plant density, tillering capacity, and spikelets number per panicle in all rice varieties. Moreover the 1000-grain weight effect on grain yield disappeared in Gladio and was confirmed in Carnaroli. Sterility percentage reduced grain yield in Centauro. On the contrary, as for the first model, sterility had no effect for Carnaroli.
Huge differences were highlighted among the rice varieties. Indeed, in Gladio, Centauro, and Ronaldo grain yield was mostly affected by tillering capacity. Conversely, plant density showed the highest effect in Carnaroli, where it was positively affected by NDRE. Moreover, high NDRE values are related to high tillering capacity, with Ronaldo showing the highest effect. Again, significant compensative effects arose among grain yield components. In Gladio, Centauro, and Ronaldo varieties plant density, as well as tillering capacity reduced spikelets number per panicle. Moreover, in Centauro, high tillering capacity decreased 1000-grain weight, too. Conversely, in Carnaroli, plant density positively affected spikelets number per panicle, while tillering had a detrimental effect on 1000-grain weight.

3.5 Discussion

3.5.1 Description of the four rice varieties and consequences on the optimal N splitting

All rice varieties showed a significant yield response to N fertiliser application. Nitrogen supply both at early growth stage and at PI were effective in determining rice grain yield, as well as their interaction. These findings are consistent with previous results on Centauro variety reported by Cordero *et al.* (2018). Consequently, also for Gladio, Carnaroli, and Ronaldo varieties a possible compensation of deficient N supply at early growth stages can be achieved through huge application at PI. Then, this study agrees with previous results by Xiong *et al.* (2018), that indicated young panicle differentiation stage as an effective compensation period for N deficiency in super hybrid late rice.

Clearly, different rice varieties required different N management strategies, in terms of total N supply as well as splitting, considering both N rates and period of supply during the growing season. Ronaldo reached the highest grain yield, showing also the highest total N supply over the whole growing season. Gladio and Centauro showed similar N requirements, coupled with similar maximum obtainable yield. Total N supply resulted lower in Carnaroli, as a consequence of the reduced grain yield potential. Moreover, since Carnaroli is characterised by a higher plant height with respect to both Gladio and Centauro, the lower total N supply restricts yield losses due to lodging, generally promoted by larger N supply.

In all rice varieties, replacement value of N_{PI} with respect to $N_{PRE+TILL}$ express the capability of compensating deficient N supply at early growth stages with larger applications at PI. That is, for any N unit not taken up in the early growth phase a further amount of N equal to the replacement value should be applied at PI stage in order to reach the maximum obtainable yield.

Considering their absolute values, replacement values decreased progressively for Centauro, Gladio, Carnaroli, and Ronaldo pointing out the same capability of rice varieties to efficiently use N at PI stage. Therefore, the share of total N amount that has to be supplied at PI to achieve the maximum potential grain yield showed the same trend. This finding states that deficient early N applications can be easily compensated with an amount of N at PI that is almost proportional to the share of N distributed at PI with respect to total N supply, to optimise crop yield.

The differences in optimal N fertilisation splitting are related to the agronomic traits of the rice varieties. Gladio requires more N at early growth stages, as a consequence of the highest panicle density, mainly determined by the highest plant density (data not shown). Similarly, Ronaldo benefits more from N supply during early stages than at PI stage but this is more related to the increase in tillering capacity. Indeed, limited plant density can be normally controlled by increasing N application (Bah *et al.*, 2009; Hirzel *et al.*, 2011; Moe *et al.*, 2014; Tayefe *et al.*, 2014).

However, Huang *et al.* (2013) suggest to compensate the low plant density increasing N application late in the growing season. Results obtained in the present study agree with this finding for Carnaroli and Gladio, both characterised by lower plant density. For these varieties optimal N splitting suggests to reduce N supply as sum of pre-sowing and tillering stage application, then increasing N at panicle initiation stage. Also Centauro, recording the highest tillering capacity, as well as the highest spikelets number per panicle, benefit from N supplied at PI stage. In the different varieties, yield components were differently affected by N fertilisation at PI. Therefore, since Centauro had lower panicle density than Gladio, N supplied at PI stage increased spikelets number per panicle and reduced panicle size, calculated as the product between the number of fertile spikelets per panicle and single grain weight (Fageria, 2007). On the contrary N supplied at PI, considering the highest panicle density for Gladio, induced the highest sterility percentage (according to both model of path analysis, negatively correlated to N_{PI}) coupled with an increase of single grain weight. Overall, rice grain yield can be adequately estimated through NDRE determination at PI stage. However, NDRE values recorded differences in rice canopy reflectance that can attributed not only to rice N status, but also to agro-climatic conditions of the growing seasons, as well as soil fertility variability. Then, NDRE values can be profitably used to drive N fertilisation taking into account specific agro-environmental conditions. The significant interaction between NDRE and NPI recorded for Gladio, Carnaroli, and Ronaldo, as well, strengthened the hypothesis of compensating low NDRE values with topdressing N fertilisation, previously suggested by Cordero *et al.* (2018) on Centauro variety. Then, this study further confirmed that deficient N supply at early growth stage, responsible for low NDRE values, can be balanced through N application at PI, without compromising rice grain yield. Moreover, among topdressing applications, N supply at PI most improves yield attributes (Inamura *et al.*, 2003; Bah *et al.*, 2009; Lee *et al.*, 2009; Sathya and Ramesh, 2009)

Prescription functions can suggest topdressing N supply at PI stage on the basis of NDRE values measured just before N application, with the aim of achieving specific grain yield goals. The share of N to be supplied in PI, compared to the total, provides advice on the slope of the prescription function relating N at PI based on N at pre-sowing and tillering.

Indeed, the slope of the prescription functions, even if specific for each rice variety, remained almost constant because of the smooth curvature of the function that describes maximum grain yield close to the vertex. Consequently, the intercept, being related to the grain yield goal, can be chosen on the basis of the grain yield potential of the field. Hence, it is possible to adapt N supply to the specific characteristics of each agro-environment.

Despite the similar grain yield potential, NDRE value corresponding to maximum grain yield was higher in Centauro than in Gladio and Ronaldo. Consequently, Centauro requires larger application at PI stage for compensating the lower crop vigour detected before topdressing N application. This finding is further confirmed by the highest absolute value of the replacement value showed by Centauro variety, assessing that the effect of N_{PI} fertilisation in higher than for the other varieties.

3.5.2 Principal Component Analysis as a qualitative tool for determining prescription functions

The two PCs built from the original dataset well described the differences among the rice varieties, as well as the agronomic traits responsible for the differences among them. The PC1 clearly separated rice varieties in the left and right quadrant, clearly distinguishing Carnaroli variety from both Gladio and Centauro, with Ronaldo having an intermediate positioning. Both 1000-grain weight and sterility mostly contributed to PC1, indicating that Carnaroli is characterised by high 1000-grain weight, as well as lower sterility. Gladio and Centauro showed instead an opposite behavior. However, PC1 did not allow separating Gladio and Centauro, assessing their higher degree of similarity. The PC2 isolated the rice varieties in the upper and lower quadrants, detecting differences between Gladio and Centauro. Indeed, while Centauro is characterised by the highest tillering capacity and spikelets number per panicle, Gladio showed the highest plant density. Communalities analysis allowed the identification of spikelets number per panicle, plant density, and tillering capacity as variables more representative of the total variability. Conversely, sterility was found to be not representative of the variability among the rice varieties. These results agree with previous studies by Ortega Blu (2007) and De Souza *et al.* (2017), that indicated the low temperature during pollen formation and flowering as the main reason for sterility.

3.5.3 Path analysis as a quantitative tool for determining prescription functions

Path analysis showed that indirect effect resulted significant for all rice varieties, confirming the hypothesis that N fertilisation affects grain yield through the influence on grain yield components. Moreover, N fertilisation showed a significant direct effect on grain yield components in all rice varieties, with the only exception of Carnaroli. This finding agrees with the higher R^2 value recorded by the GLM used to explain grain yield as function of N amount and splitting during the growing season, in Gladio, Centauro, and Ronaldo. Moreover, path analysis allowed pointing out the different response of rice varieties to N fertilisation. Plant density showed the highest direct effect in determining rice grain yield in Gladio and Carnaroli, while in Centauro and Ronaldo tillering capacity was the most influent yield component. These results agree with previous studies by Samonte *et al.* (1998) and Artacho *et al.* (2009), that indicated panicle density as one of grain yield components having the greater direct effect on rice grain yield. In all rice varieties sterility percentage was negatively affected by N fertilisation. Indeed, according with previous studies (Hirzel *et al.*, 2011; Hirzel and Rodriguez, 2017), grain sterility increases corresponding to high soil N availability, due to both N fertilisation and natural N supply. In all rice varieties N supply as sum of pre-sowing and tillering stage applications promoted tillering capacity, showing the highest direct effect in Ronaldo variety. Then, deficient $N_{PRE+TILL}$ reduced tillering capacity, especially in Ronaldo variety. Examining the significant effects among the grain yield components, rice varieties showed different compensation strategies. As previously mentioned, both Gladio and Ronaldo compensated the reduced tillering by increasing spikelets number per panicle, while Centauro increased 1000-grain weight, as well. Conversely, Carnaroli reduced spikelets number per panicle, then increasing 1000-grain weight. Hence, path analysis allowed to better understand the optimal fertilisation strategy. Among the considered varieties, Ronaldo required the highest N amount as sum of pre-sowing and tillering stage application, as tillering was the main factor that influenced grain yield. Gladio required high early growth stage N application as well, as it was effective on both plant density and tillering capacity. In Centauro, considering the highest path coefficient, a smaller $N_{PRE+TILL}$ is needed. Moreover, in Centauro, spikelets number per panicle had a contribution to grain yield slightly lower than tillering capacity. Spikelets number per panicle is promoted more by larger topdressing amount at PI stage than by N application as sum of pre-sowing and tillering stage applications. Consequently, in Centauro variety, a little N amount has to be supplied at early growth stages, then increasing topdressing N applications. In Carnaroli, with the aim of promoting 1000-grain weight, $N_{PRE+TILL}$ has to be reduced because of its negative effect, then increasing N_{PI}.

Different crop vigour recorded just before N_{PI} application led to differences in grain yield for all rice varieties, because of the influence on the development of grain yield components. Then, NDRE can be conveniently used to drive N topdressing application.

3.6 Conclusions

The optimal fertilisation strategy has to be adapted considering the agronomical traits of the rice varieties, as they induce large differences in both N requirements and spectral response. Then, prescription functions determining N_{PI} as function of NDRE measured just before N application has to be adapted to the different rice varieties. This study pointed out that rice varieties showed different growth patterns, leading to wide differences in response to N fertilisation and NDRE interpretation. The role of the yield components determining the final grain yields is different in the rice varieties. Moreover, N fertilisation applications bring to a different development of grain yield components in each rice varieties depending on the interaction of timing and amount. Integration of Principal Component Analysis and path analysis helps in choosing the best prescription function on the basis of the agronomic traits of each rice variety, being a qualitative and quantitative tool, respectively. Then, the statistical procedure proposed in the present study can be extended to results of other experiments investigating the contribution of grain yield components to final yield, with the aim of extending prescription functions to other rice varieties.

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4. Paper 3: Spatial management strategies for nitrogen in maize production based on soil and crop data

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Spatial management strategies for nitrogen in maize production based on soil and crop data

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4.1 Abstract

Nitrogen (N) fertilisation determines maize grain yield (MGY). Precision agriculture (PA) allows matching crop N requirements in both space and time. Two approaches have been suggested for precision N management, i.e. management zones (MZ) delineation and crop remote and proximal sensing (PS). Several studies have demonstrated separately the advantages of these approaches for precision N application. This study evaluated their convenient integration, considering the influence of different PA techniques on MGY, N use efficiency (NUE), and farmer's net return, then providing a practical tool for choosing the fertilisation strategy that best applies in each agro-environment. A multi-site-year experiment was conducted between 2014 and 2016 in Colorado, USA. The trial compared four N management practices: uniform N rate, variable N rate based on MZ (VR-MZ), variable N rate based on PS (VR-PS), and variable N rate based on both PS and MZ (VR-PSMZ), based on their effect on MGY, partial factor productivity (PFP_N), and net return above N fertiliser cost (RANC). Maize grain yield and PFP_N maximisation conflicted in several situations. Hence, a compromise between obtaining high yield and increasing NUE is needed to enhance the overall sustainability of maize cropping systems. Maximisation of RANC allowed defining the best N fertilisation practice in terms of profitability. The spatial range in MGY is a practical tool for identifying the best N management practice. Uniform N supply was suitable where no spatial pattern was detected. If a high spatial range (>100 m) existed, VR-MZ was the best approach. Conversely, VR-PS performed better when a shorter spatial range (<16 m) was detected, and when maximum variability in crop vigour was observed across the field (range of variation=0.597) leading to a larger difference in MGY (range of variation=13.9 Mg ha^{-1}). Results indicated that VR-PSMZ can further improve maize fertilisation for intermediate spatial structures (43 m).

Keywords: precision fertilisation, variable rate N application, proximal crop sensing, management zones, data fusion

4.2 Introduction

Sustainable intensification of crop production is required to fulfil the growing consumption needs of humanity while reducing the environmental impact of agriculture (Cassman, 1999; Foley *et al.*, 2011). Sustainable cultivation requires a more efficient resource use, including fertiliser applications. Nitrogen (N) is among the most important nutrients supplied to maize for obtaining the full yield potential, as it affects both grain yield and quality (Miao *et al.*, 2007). A proper N management should aim to meet maize N needs, to avoid exceeding crop requirements. An optimally tailored N fertilisation could increase maize production and maintain soil fertility, while limiting environmental concerns through the reduction of N imbalances and inefficiencies (Ma and Biswas, 2015). Excess N is subjected to losses in the environment, through leaching, surface run-off, denitrification and ammonia emissions (Cai *et al.*, 2002; Ma and Biswas, 2015). Several studies reported that N losses in maize cultivation could range between 10 and 70% of the applied N, considering different environmental conditions and fertilisation management (Cai *et al.*, 2002; Delgado *et al.*, 2005; Wang *et al.*, 2014; Prasad and Hochnut, 2016). Crop N demand varies spatially and temporally within a field, due to the inherent variations in soil N availability, soil properties and crop growing conditions (e.g. edge effect) across the field (Khosla *et al.*, 2002; Nawar *et al.*, 2017). Two main approaches have been proposed in literature to adapt N fertilisation to the spatial variability: soil-based methods and plant-based methods. The former includes the concept of homogeneous management zones (Khosla and Shaver, 2001), while the latter relies on crop N status monitoring with crop canopy sensors during the growing season (Roberts *et al.*, 2012). Few studies have compared these two approaches or assessed the possibility of using them in combination.

The identification of management zones (MZ) represents a cost-effective method to manage field variability, through field classification into areas of broad similarities (Khosla *et al.*, 2002; Nawar *et al.*, 2017). Management zones approach was originally suggested to overcome the limitation of intensive grid soil sampling for mapping the variance of soil properties, due to high cost and labour (Fleming *et al.*, 2000). Therefore, it can be suggested as an alternative method to produce prescription maps for site-specific crop management, by identifying areas of similar productivity potential within a field (Hornung *et al.*, 2006). Indeed, in the location of a field where yield potential is low, added N fertiliser profitability can be reduced (Ma and Biswas, 2015). Doerge (1999) defined MZ as sub-regions of a field that express a homogeneous combination of yield limiting factors. Therefore, MZ can be considered as homogeneous areas within a field that show similar characteristics in landscape and soil conditions, that should lead to a similar yield potential and input use efficiency (Schepers *et al.*, 2004). However, the delineation of uniform sub-field regions may be challenging as different physical, biological and chemical processes acting simultaneously with different intensities and with complex interactions can affect crop yield potential (Moral *et al.*, 2010). Several techniques have been proposed in literature to delineate MZ, using various soil and crop properties individually or in combination (Longchamps and Khosla, 2017). Topography, bare soil aerial imagery, apparent electrical conductivity (ECa), farmers' management experiences together with yield maps have been extensively used to define the boundaries of MZ (Khosla *et al.*, 2002; Schepers *et al.*, 2004). Indeed, grain yield data, being a total reflection of all biotic and abiotic factors that can affect crop production, can be combined with other soil variables in order to explain field variability associated with both crop and soil properties (Hornung *et al.*, 2006, Bunselmeyer and Lauer, 2015). However different weights should be attributed to the different data layers, on the basis of their contribution to crop production variability (Hornung *et* *al.*, 2006). Moreover, yield patterns are often inconsistent across growing seasons (Hornung *et al.*, 2006). Therefore, it is important to also consider temporal variation of crop yield, which reflects climate variability across the growing seasons (Schepers *et al.*, 2004) and is not necessarily correlated to soil properties variations (Nawar *et al.*, 2017). The knowledge of yield history could improve MZ delineation through the identification of yield patterns at sub-field levels (Bunselmeyer and Lauer, 2015). Indeed, Maestrini and Basso (2018) built a spatial indicator that combines the processes that regulates yield by averaging the normalised values of each pixel over the yearly map, using the previous three-year data. Considering the complex interactions involved in yield variability, at least five or more years of yield data should be used to identify s*Table* MZ (Nawar *et al.*, 2017). Typically, traits such as low-lying topography, dark colour, and historic high yields were designated as zones of potentially high productivity, or high zones (Khosla *et al.*, 2002). Soil-based information used alone to manage maize N fertilisation may not always lead to improvement in Nitrogen Use Efficiency (NUE, defined as the grain yield obtained at a certain level of N supplied with fertilisers). Such an approach fails to account for in-season micro-variability (i.e., variability that occurs at shorter range) associated with crop N status, since the crop response in uns*Table* zones has been demonstrated to be strictly dependent on weather (Maestrini and Basso, 2018). Consequently, the delineation of MZ alone does not characterise the entire representation for variable N applications (Shanahan *et al.*, 2008). Crop monitoring, which exploits optical properties of leaf pigments, allows integrating soil, climate, agronomic management, and other environmental factors on crop N status (Shanahan *et al.*, 2008; Muñoz-Huerta *et al.*, 2013). Ground-based reflectance measurements have been proposed as promising tools to assess crop N status during the growing season (Roberts *et al.*, 2012). Several vegetation indices can be determined combining reflectance data recorded at specific wavelengths (Bajwa *et al.*, 2010). Among these, the most widely used is Normalised Difference Vegetation Index (NDVI), calculated as the difference between the NIR and red reflectance divided by the sum of these two values (Shanahan *et al.*, 2008. NDVI values are positively correlated with leaf area index (LAI), green biomass and leaf N (Shaver *et al.*, 2010). Consequently, they provide a measure of canopy chlorophyll content in the field-of-view of the sensor. Maize growth stage at the moment of spectral data acquisition heavily affects NDVI values. Teal *et al.* (2006) demonstrated that NDVI readings acquired at V8 (8-leaf) maize growth stage showed the highest ability to distinguish in-field N variability. Shaver *et al.* (2010) found out that the best time for maize N status monitoring is between V10 and V12 growth stages. This is in line with the optimal sensing period reported in the Trimble's Greenseeker manufacturer's manual (https://www.manualslib.com/download/1485318/Trimble-Greenseeker-Rt-200.html), a sensor widely used for NDVI determination at field scale.

Several studies have demonstrated separately the potential advantages of soil-based and plant-based methods of driving variable N fertilisation in maize, while very few tried to investigate the possibility of integrating them (e.g. Longchamps and Khosla, 2015). The information from MZ delineation is potentially complementary to ground-based active sensors for crop N status monitoring, and could further improve NUE, economics and overall sustainability of maize cropping systems (Khosla *et al.*, 2010; Roberts *et al.*, 2012). The integration of the two approaches may allow tailoring N rate algorithms for each MZ independently, through the detection of both soil and crop properties correlated with crop productivity, then demonstrating the advantages derived by this data fusion, considering different information layers.

This study aimed at verifying the hypothesis that uniform N management practices can be improved through PA techniques, taking advantage of a) proximal crop sensing and b) MZ delineation, and overall c) combination of the two strategies. The specific objectives of this study were to assess the influence of precision N management practices on i) maize grain yield, (ii) NUE, and (iii) farmer’s net return.

4.3 Materials and methods

4.3.1 Site and soil characteristics

The experiment was carried out over three crop growing seasons (2014, 2015 and 2016) in four different experimental sites in north-eastern Colorado (USA), located in Fort Collins, Ault Iliff, and Atwood (*Figure* 1).

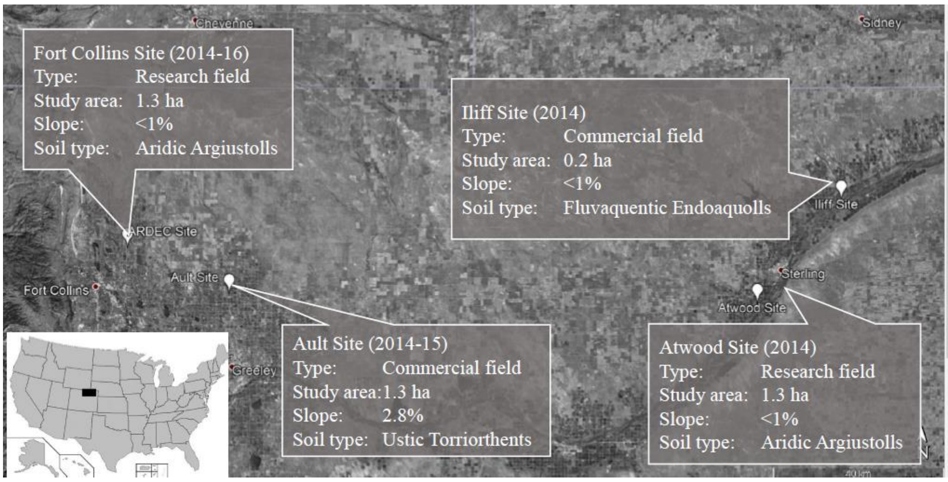

Figure 1: Map showing the location of each experimental site along with information about the farm type, the size of the study area within the field, the slope and the soil type according to USDA National Resources Conservation Services’ Web Soil Survey (www.websoilsurvey.sc.egov.usda.gov).

The climate of the area is classified as semi-arid (Moshia *et al.*, 2014), with a mean annual temperature of 10.1 °C and a mean annual rainfall of 408 mm (U. S. Climate Data, 2018).

Mean monthly temperature and cumulative monthly rainfall over the experimental period are shown in *Table* 1.

Table 1: Description of average climatic data for each site and each year for the crop growing seasons (May 1st to September 30th) of 2014-16. Table includes NOAA's normal weather conditions for the crop growing season from 1981 to 2010 for each location.

Site	Year	Average temperature (°C)	Total precipitations (mm)
Fort Collins	2014	17.4	236
	2015	17.9	254
	2016	18.2	89
	1981-2010	*17.9*	*227*
Ault	2014	17.7	259
	2015	18.1	227
	1981-2010	*19.8*	*215*
Iliff	2014	18.9	369
	1981-2010	*19.9*	*303*
Atwood	2014	18.2	435
	1981-2010	*19.0*	*290*

Prior to the start of the experiment, maize was continuously cultivated on all experimental sites for a period of at least three years.

The main soil properties of the experimental fields are summarised in *Table* 2.

Table 2: Main soil properties of the four experimental sites Mean values are reported. Sampling design consisted of random-within-grid inside the study area on a square grid of 40 m. [the extended version of the table is reported in Cordero et al., 2019]

Soil properties	**Fort Collins (n=82)**	**Ault (n=6)**	**Iliff (n=13)**	**Atwood (n=12)**
Sand (%)[a]	539	64	39	56
Silt (%)[a]	14	14	23	21
Clay (%)[a]	33	22	38	23
Organic matter (%)[b]	2	1	2	1
pH[c]	8	8	8	8
Nitrate N (mg kg^{-1})[d]	14	8	16	10
CEC (meq /100 g)[e]	31	25	33	21
Available P (mg kg^{-1})[f]	19	46	26	55
Exchangeable K (mg kg^{-1})[g]	318	255	695	320

Superscript indicates the method of measurement: [a]: hydrometer, [b]: loss-on-ignition, [c]: 1:1 water-soil, [d]: Cd reduction, [e]: Summation of exchangeable K, Ca, Mg and neutralisable acidity, [f]: Olsen method, [g]: ammonium acetate

Soil samples were collected at 0-20 cm depth prior to planting within each field, following a random-grid (40 m) spatial survey sampling design within the study area (Heltshe and Ritchley, 1984). Soil samples were then dried and analysed at a commercial laboratory (Ag Harris, Lincoln, NE).

4.3.2 Management Zones delineation

Management zones were used to characterise in-field variability, identifying areas of high, medium, and low productivity potential within the experimental sites. At Ault, Atwood, and Iliff sites, the delineation of MZ boundaries was accomplished through the Management Zone Analyst (MZA) free software, developed by Fridgen *et al.* (2004). The MZA uses a fuzzy k-means clustering algorithm to delineate MZ from geo-referenced field information, that showed effective results for zone delineation in previous studies by Odeh *et al.* (1992). Different clustering variables were used in the delineation process, notably: elevation, bare-soil aerial imagery of the field, and soil apparent electrical conductivity (ECa). Bare-soil imagery was acquired after field preparation and before sowing, using Google Earth Pro (Google LLC, Mountain View, CA) to select dates when there was no canopy cover in the selected field. The images exported from Google Earth Pro were georectified with at least six ground control points using the ArcMap software (ESRI, Redlands, CA). Soil ECa was measured on each field prior to planting in spring through EM38 (Geonics Ltd., Mississauga, Ontario, Canada), an electrical conductivity meter that measures ECa on the basis of the principle of electromagnetic induction at two depths. Data were collected in vertical dipole orientation. Sensor was combined with a GPS and data loggers, mounted on an all-terrain vehicle travelling in parallel transects. High-resolution soil ECa readings were acquired when the soil was at field capacity. The ECa data was overlaid with the satellite imagery from Google Earth Pro in the ArcMap software. The rough field topography was extracted from ECa survey data using the elevation data recorded by a Trimble Ag114 DGPS (Trimble

Navigation, Sunnyvale, CA) corrected by a VBS Omnistar (Omnistar, Houston, TX) signal providing a vertical resolution of about 2 m. Despite the low resolution for absolute topography measurements, the relative topography values were accurate enough to detect the overall spatial pattern of topography in each field. A grid of points was laid on the entire surface of the study area using the Fishnet tool from ArcMap on a 2 m by 2 m cell. Using a raster sampling tool from ArcMap, each point was attributed to the corresponding information: the Red, Green and Blue pixel value from the geotiff extracted from Google Earth Pro (raster sampling), the deep and shallow ECa value as well as the elevation value (nearest point algorithm) from the ECa survey dataset. The point feature file was then converted into a *Table* to be uploaded in the MZA software. The MZA software performed a fuzzy k-means clustering of the soil information used as input and provided simultaneously a range of cluster number. Mahalanobis distance was chosen as measure of similarity for allocating each individual observation to a particular cluster, as it is reported to be the most appropriate when correlation exists among variables (Fridgen *et al.*, 2004). Other option settings were defined, considering fuzziness exponent of 1.5, maximum number of iterations of 300 and convergence criterion of $P<0.0011$ according to Fridgen *et al.* (2004). The minimum and maximum number of zones was set to 2 and 6 respectively, in order to allow a sufficient differentiation avoiding at the same time excessive fragmentation of zones' sub-areas. Moreover, after performing the clustering procedure, the software calculated two performance indices, *i.e*. Fuzziness Performance Index – FPI and Normalised Classification Entropy – NCE, that allowed the decision of the most appropriate number of MZ for each field. The FPI measures the degree of separation between the zones, while NCE indicates the amount of disorganisation of each partitioning (Fridgen *et al.*, 2004). Consequently, the best number of MZ is achieved when both indices have the minimum value, leading to the least membership sharing and the greatest amount of organisation as a result of the clustering process. Therefore, by evaluating both FPI and NCE values, the optimal number of MZ was chosen. Finally, each geo-referenced soil measurement point was assigned to a specific management zone. The vector containing MZ values was transferred to the ArcMap software and converted into polygon features representing the MZ. The attribution of low, medium or high productivity potential of each management zone was reflective of the historical yield performances according to farmers' knowledge of the field. In Fort Collins, MZ had already been defined prior to the project using bare soil imagery, coarse elevation, and yield and management history as layers for delineation. The Rapid Eye satellite imagery platform was used to acquire bare soil imagery of the field. It deploys the Jena-Optronik multi-spectral imager (Jena, Germany), in five distinct bands of the electromagnetic spectrum: Blue (440-510 nm), Green (520-590 nm), Red (630-690 nm), Red-Edge (690-730 nm) and Near-Infrared (760-880 nm). Zone clustering was done using the AgriTrak Professional software (Agritrak L.L.C, Fort Morgan, CO, USA) described by Fleming *et al.* (1999). This method consisted of enhancing the contrast of the bare soil image into various strata or zones using the AgriTrak Professional software. Following which, the actual farmer of that field designated the zones with low, medium, or high productivity potential. The designation of zones was based on the historical knowledge of management practices and yield performance of that field. The delineated MZ in each experimental site are shown in *Figure 2*.

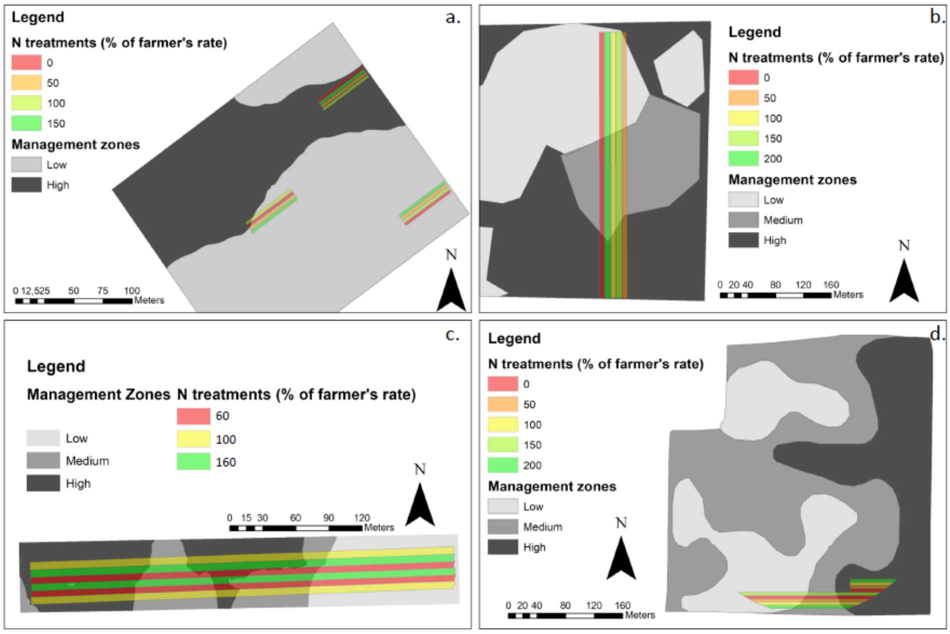

Figure 2. Maps of the four experimental sites (a. Iliff; b. Ault; c. Atwood and d. Fort Collins) showing the management zones and the N treatments.

Afterwards, QGIS open source software (http://qgis.org) was used to assign each yield point from the yield map obtained during the experiment to the corresponding MZ, through Voronoi polygons delineation. Subsequently, the information about the MZ corresponding to each yield point was added to the original dataset using QGIS. This procedure aimed to link each yield value to the productivity potential of the yield sampling point, expressed by the MZ.

4.3.3 Experimental design and treatments

This experimental setup at each site-year aimed at comparing four fertilisation practices, characterised by different N management in maize production:

- traditional farmers' management, with a uniform N rate (UR);
- variable rate N management based on crop proximal sensing (VR-PS);
- variable rate N management based on MZ delineation (VR-MZ);

- variable rate N management based on both crop sensing and MZ delineation (VR-PSMZ).

In each site-year, several N rates were tested, as shown in *Table* 3.

Table 3: Width of N strips and N rates (kg ha^{-1}) considered in the different locations and year of the experiment. Values in bold represent standard dose used by farmers.

Site-year	Location	Year	Width of N strips (m)	N rates (kg ha^{-1})
1	Fort Collins	2014	4.6	0 -85 – **170** – 255
2	Fort Collins	2015	4.6	0 – 60 – 120 – **180**- 240 - 300
3	Fort Collins	2016	4.6	0 – 60 – 120 – **180**- 240
4	Ault	2014	7.5	40 – **80** - 120
5	Ault	2015	7.5	0 – 40 – **80** – 120
6	Iliff	2014	2.3	0 – 75 – **150** – 225
7	Atwood	2014	6.9	100 – **170** – 275

For each site, during the first year of experiment a standard N dose (in bold in *Table* 3) was selected based on farmer's business as usual. During the second and third years of the experiment, the reference dose was slightly adjusted, if needed, in order to cope with crop needs.

Moreover, in each site, other N rates were tested in order to fit with higher or lower productive MZs or NDVI responses. The respective rates were chosen according to expected levels of productivity based on expert knowledge derived from farm managers. Unfertilised treatments were added in site-years 1, 2, 3, 5, and 6. In the other site-years, farmers preferred to add a minimal N fertilizer of 50% of their usual N rate to avoid further yield loss. Nitrogen treatment strips were imposed at each site-year, however, the size of the treatment strips varied across the site-years (*Table* 3). The width of the strip corresponded to the width of the fertiliser sprayer used by the farmer and the length corresponded to the entire length of the field when possible. When not possible, the strips were long enough to contain at least 15 yield data points (based on the assumption that a commercial combine harvester generates about one yield data point at every 2.5 m length) for each zone by treatment section. Nitrogen treatment strips were randomly distributed (randomised using the Sample function in R without replacement and with the seed of the number generator set to 123) within the field.

The comparison among the different fertilisation approaches was realised by selecting observations that fulfil specific conditions, then simulating the different fertilisation strategies. At each site-year the UR received various N rates distributed uniformly, without taking into account neither MZ, nor NDVI values obtained from PS.

The VR-PS was analysed selecting observations where increasing N rates were coupled with lower NDVI values and vice-versa, without accounting for MZ. Consequently, with the aim of identifying classes reflecting homogeneous crop vigour, NDVI values were clustered using k-means clustering to obtain NDVI classes. For each site-year, the number of NDVI classes was equal to the number of N levels. During data analysis, N rates were paired to NDVI classes, considering pairs where the highest N amount was coupled with the lowest crop vigour, then progressively considering lower N application at increasing crop vigour. The VR-MZ considered the observations where reduced N supply was coupled with lower productivity and increased N supply was coupled with higher productivity. Then, zones characterised by intermediate productivity received the standard N rate, while in high and low zones N rates was increased or reduced, respectively. The VR-PSMZ accounts for both soil productivity potential (through MZ) as well as crop N status (through in-season PS measurements). Three N rates were selected based on three NDVI classes (e.g. low NDVI received a high N rate), and these three selected N rates were modulated depending on which zone they were located in (e.g. very low N, low N and medium N for the low productivity zone). Depending on the number of N treatments available, not all site-years allowed a complete set of combinations.

4.3.4 Crop agronomic management

In all site-years, maize hybrids belonging to FAO maturity class 300 were grown. Standard agronomic techniques were adopted for all the crop growing seasons at each location. All field sites were conventionally tilled for planting, as presented in *Table* 4.

Table 4: Date and type of tillage operation for each site-year.

Site-year	Date	Type of tillage operation
1	20th November 2013	Disk harrow
	28th March 2014	Brillion mulcher
	1st April 2014	Brillion mulcher
2	30th April 2015	Spring-tooth harrow
3	25th November 2015	Disk harrow
	25th April 2015	Brillion mulcher
4	15th April 2014	Field cultivator
5	20th April 2015	Field cultivator
6	11th April 2014	Strip tillage
7	18th November 2013	Disk harrow
	15th April 2014	Brillion mulcher

Likewise, details of the agronomic management are reported in *Table* 5. In each site-year, the total amount of N fertiliser was localised in strips close to plant rows, at the 6th leaf crop stage development of maize (V6, according to Reitsma *et al.*, 2009). All N was supplied using urea ammonium nitrate (UAN), a 32% N fertiliser. In order to prevent drought stress, irrigation was carried out by means of a centre - Pivot system in site-years 1, 2, and 3 (*Table* 3); and a surface furrow irrigation system in site-years 4, 5, and 7, and a lateral move irrigation system in site-year 6. Water was applied uniformly across the entire experimental area, until the end of the crop dough stage (R4). The irrigation scheduling was performed by collaborating with farmers, primarily on the basis of soil moisture measurements, previous occurrence of precipitation, and related weather data as well as visual assessment of the field. Adequate pesticide treatments were undertaken throughout the maize growth, enabling an optimal control of diseases and pests. Fields were treated with chemical herbicides to control weed development.

Table 5: Details of the agronomic management.

Site-year	Maize hybrid	Relative days to maturity	Seeding date	Seed rate (seed ha^{-1})	Fertiliser application	Harvesting date
1	Dekalb DKC46-20RIB	96	29th April 2014	84 000	11th June 2014	30th October 2014
2	Dekalb DKC46-20RIB	96	27th May 2015	84 000	30th June 2015	19th November 2015
3	Dekalb DKC46-20RIB	96	6th May 2016	93 900	21st June 2016	21st October 2016
4	Pioneer P0474	104	5th May 2014	84 000	17th June 2014	24th October 2014
5	Pioneer 35F48AM1	105	2nd May 2015	93 900	23rd June 2015	12th November 2015
6	Pioneer P0157AM	101	19th May 2014	84 000	24th June 2014	23rd October 2014
7	Pioneer P0474	104	7th May 2014	84 000	17th June 2014	26th November 2014

4.3.5 Field measurements

Ground-based crop reflectance measurements were performed on different dates, corresponding to maize growth stage between the development of the 2nd and the 12th leaf (V2 to V12) (*Table* 6).

Table 6: Dates of NDVI measurements in the different years and locations.

Site-year	Dates of NDVI readings	Maize growth stage
1	June, 26th	V6-V7[a]
	July, 10th	V8-V10
	July, 14th	V10
	July, 17th2	V10-V11
	July, 21st	V11-V12
	June, 27th	V9
3	July, 5th	V12
	July, 8th	V14
4	June, 26th	V6-V7
	June, 17th	V8-V10
	June, 23th	V10
5	July, 1st	V10-V11
	July, 7th	V11-V12
6	July, 23rd	V2
7	June, 17th	V3-V4

[a]: Vn stage: development of the *n* leaf

The Greenseeker (Trimble, Sunnyvale, California, USA) handheld active optical sensor was used to determine NDVI, detecting canopy reflectance in the visible red (wavelength 660 nm) and in the NIR (wavelength 770 nm) spectral regions. The measurements were taken by holding the instrument at a distance of about 0.8 m above the maize canopy, as suggested by the manufacturer's instruction manual and reported in Solari *et al.* (2008). Reflectance measurements were acquired around noon, even though Padilla *et al.* (2019) demonstrated that radiation conditions did not alter NDVI values measured with active sensors. Being an active sensor not influenced by the sunlight (Solari *et al.*, 2008; Schmidt *et al.*, 2009), reflectance data was acquired walking at a constant speed alternatively along the crop rows. NDVI readings were acquired continuously on one of the central rows of each strip. Each NDVI measurement was georeferenced.

Grain yield, adjusted to a moisture content of 15.5%, was determined at harvest. At physiological maturity maize was harvested with a combine harvester equipped with a GPS receiver and a yield monitor, ensuring that all grain yield sampling points are geo-referenced. Experimental plots were located on commercial fields, then a different combine harvester was used at each location except for the Atwood site, where data was collected by hand. In Fort Collins, the grain was harvested using a 6-row Case combine harvester model Case IH 1660 (Case Corporation, Racine, WI) equipped with an AgLeader (AgLeader Technology, Ames, IA) yield-monitoring system. In Ault, the grain was harvested using an 8-row John Deere 9670 STS (Deere and Company, Moline, IL) combine harvester model equipped with a GreenStar yield-monitoring system. In Iliff, the grain was harvested using a 2-row John Deere 3300 (Deere and Company, Moline, IL) combine harvester model equipped with an AgLeader yield-monitoring system. Yield data was then cleaned following the procedures described in Khosla and Flynn (2008). In Atwood, a combine harvester equipped with a yield-monitoring system was not available and therefore, the yield values were harvested by hand on a 3 m length of maize row at 75 locations regularly distributed throughout the study area and evenly distributed across N treatments. Hand harvested maize ears were then transported to a facility where kernels were separated from the maize ears, weighted and analysed for moisture content using a Dickey-John GAC 2100b (Dickey-John Corp., Auburn, IL) grain analysis computer.

4.3.6 Data analysis

A database was built for each site-year. The databases reported the list of geo-referenced observations, each one referred to an area of 2*4 m^2.

For each area, N rate, belonging to a specific MZ, NDVI value and grain yield were provided.

Then, partial factor productivity (PFP_N) was determined for each area, as an indicator of maize NUE, according to Cassman *et al.* (1996):

$$PFP_N = \frac{Y}{N_T} \tag{1}$$

where Y represents grain yield and NT is the total amount of N applied, both expressed in kg ha^{-1}. Consequently, it was not possible to calculate PFP_N where no fertiliser was applied. Considering the agronomic output that can be obtained at a certain level of all N resources in the cropping system, PFP_N could be considered a useful integrative NUE index. Indeed, PFP_N takes into account total available N derived from both soil and N applied fertiliser (Cassman *et al.*, 1996; Ladha *et al.*, 2005).

Additional data columns containing NDVI classes were added to the original dataset, with the aim of identifying classes reflecting homogenous crop vigour. The NDVI classes were created using *k-means* clustering with the *k-means* function in the R stats package (R Core Team, 2018). For each site-year, the number of NDVI classes was equal to the number of N levels established for the experimental site.

A statistical procedure was applied in order to check the significance of the difference in grain yield among precision fertilisation practices and uniform practices. As grain yield depends mostly on N rate, the check of the significance was performed based on the same N rate for both practices. Average field grain yield and N rate for each precision fertilisation practices were calculated as the total grain yield or supplied N divided by the corresponding strip area. Corresponding values of grain yield in uniform practice were derived from interpolation of a linear model applied to the different site-years. The linear model was applied only to uniform N application data and expressed grain yield as a function of N rate accounting for an additive component due to site-year effects (*Equation 2*).

$$Yield = \mu + (site\_year) + \beta * \ Nrate \qquad (2)$$

where μ is the grand mean of all data, site_year is the fixed effect representing the shift from the grand mean of each site-year, Nrate is the covariate representing the N rate uniformly supplied, while β is its coefficient.

The statistical assumptions of homogeneity of variances and normality hypothesis of the residuals were graphically checked, as suggested by Zuur *et al.* (2010). Moreover, Laara (2009) stated that for large datasets the central limit theorem implies approximate validity of the statistical methods that require normality. Therefore, with the aim of comparing precision N fertilisation practices with uniform application of the same N amount, t tests were calculated for each PA approach against the corresponding value fitted on the LM model using the following *Equation 3*:

$$t = \frac{\bar{x} - fitted\ value}{\sqrt{SE_{uniform} + SE_{PA\ approach}}} \qquad (3)$$

Where $\bar{x}$ is the average grain yield of a given PA approach, fitted values are the grain yields for uniform N application predicted by the LM for the same N rate, and $SE_{uniform}$ and $SE_{PA\ approaches}$ are the standard errors of uniform and precision agriculture approaches, respectively.

In order to underline the Nrate effect, both grain yields represented by □ and fitted values where shifted by site-year to be represented on a single equation, according to *Equation 4*.

$$grain\ yield_{shifted} = grain\ yield - (site\_year) \qquad (4)$$

The same procedure was applied to PFP_N values, but including also the reciprocal of N rate as covariate, with the aim of introducing the hyperbolical components into the model (*Equation 5*).

$$PFP_N = \mu + (site\_year) + \gamma_1 * Nrate + \gamma_2 * \frac{1}{Nrate} \quad (5)$$

where again μ is the grand mean, site_year is the effect related to the site-year, while Nrate represents the N rate uniformly supplied, and γ_n are its coefficients.

Finally, an economic evaluation was conducted, with the aim of assessing the influence of precision N management practices on farmers' net return. Net return above N fertiliser cost (RANC) was calculated as the difference between grain yield market value and N fertiliser cost (Bachmaier and Gandorfer, 2009). The calculation was computed as previously reported in Casa *et al.* (2011). Maize grain prices were based on Agricultural Statistics (2017) published by USDA. The values employed were 0.15 \$ kg^{-1}, 0.14 \$ kg^{-1}, and 0.13 \$ kg^{-1}, for 2014, 2015, and 2016, respectively. The price of UAN fertiliser was obtained from a fertiliser retail dealer in Colorado which was equal to approximately 16 000 \$ metric ton^{-1} (15.70 \$ kg^{-1}). Then, aiming at assessing the influence of precision N management practices on PFP_N, grain yield, and RANC, radar charts were created for each location and year of the experiment. The considered variables (*i.e*. PFP_N, grain yield, and RANC) were standardised by centring on zero (by subtracting the mean) and further scaling them dividing by the standard deviation, so that they have a standard deviation equal to 1. This procedure allowed incorporating the different variables on a comparable scale.

Lastly, the presence or absence of a spatial pattern in grain yield data was investigated through Moran's I test (Moran, 1950); following which, the spatial structure was described with a semivariogram, which is a plot of semivariances as a function of distances between the observations.

Geostatistical methods implemented in the library GeoR (Ribeiro and Diggle, 2016) were used for the estimation of the empirical semivariogram. After that, standard theoretical variogram models (exponential, Gaussian, and spherical) were fitted to the empirical semivariogram. With the aim of assessing the theoretical model that best fitted the empirical semivariogram, the goodness of fit was evaluated through the Akaike's Information Criterion (AIC), then taking into account also the complexity of the given model. For each year and location, the model that showed the lowest AIC value was considered the most appropriate to represent the experimental semivariogram, according to McBratney and Webster (1986). Semivariograms were described using range (*i.e*. the distance at which observations are no longer spatially autocorrelated), sill (representing the maximum variance of the field relative to grain yield, disregarding the spatial structure), and nugget (*i.e*. the microscale variation or measurement error). Statistical analysis was performed using R software version 3.4.3 (R Core Team, 2018) and R Studio version 1.1.183 (RStudio Team, 2016).

4.4 Results

Mean temperature during the growing season correlates with the obtained grain yield, with higher values in site-year 3 and lower in site-years 1, 4, 5 (*Table* 1). Also, annual total precipitation highlighted a different amount among the site-years (*Table* 1).

Figure 3 shows the overall yield response to N rates, expressed as the average N application at field scale, across the site-years and the N management strategies. Site-year effect was removed according to *Equation 4*.

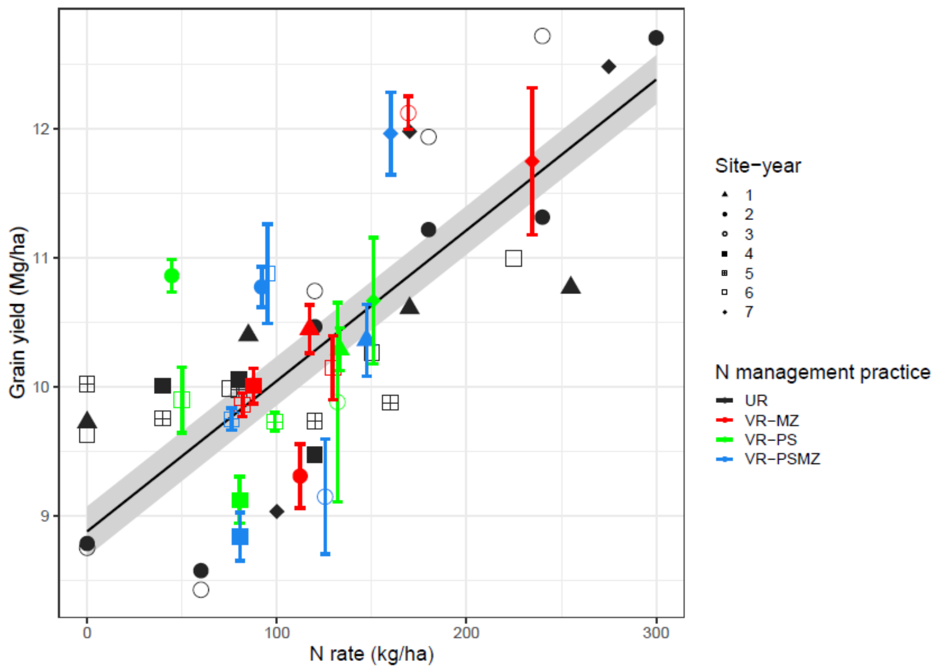

Figure 3: Grain yield response to N rate for uniform (black symbols) and precision fertilisation practices (coloured symbols). For all N management practices, grain yield shifted is represented according to equation 4. Nitrogen rates for precision fertilisation practices are the average values supplied at field scale. For precision N management practices, error bars represent standard errors. Grey area represents the confidence interval calculated for uniform N management practice.

The linear model used in the study was suitable at fitting the experimental data (R^2=0.61). Nrate was significant (P(F) = P<0.001, df numerator = 1, df denominator = 4139); site-year was significant as well (P(F) = P<0.001, df numerator = 6, df denominator = 4139).

In general, for uniform N management practices maize grain yield increased with increasing N rates. The application of the linear model to uniform treatments allowed to parametrise the crop response function to increasing N rates. Precision N management yields were then compared with uniform application, considering the average amount of N applied on the whole treatment. A general trend cannot be highlighted. In particular, VR-PS and VR-PSMZ maintained grain yield with respect to the uniform application of the same N amount in five site-years, while VR-MZ did in six. Moreover, in three site-years, VR-PSMZ improved grain yield, while VR-PS and VR-MZ did in other one site-year.

Figure 4 shows PFP_N values obtained through the different N management practices in each site-year, corresponding to each N supply after removing site-year effect.

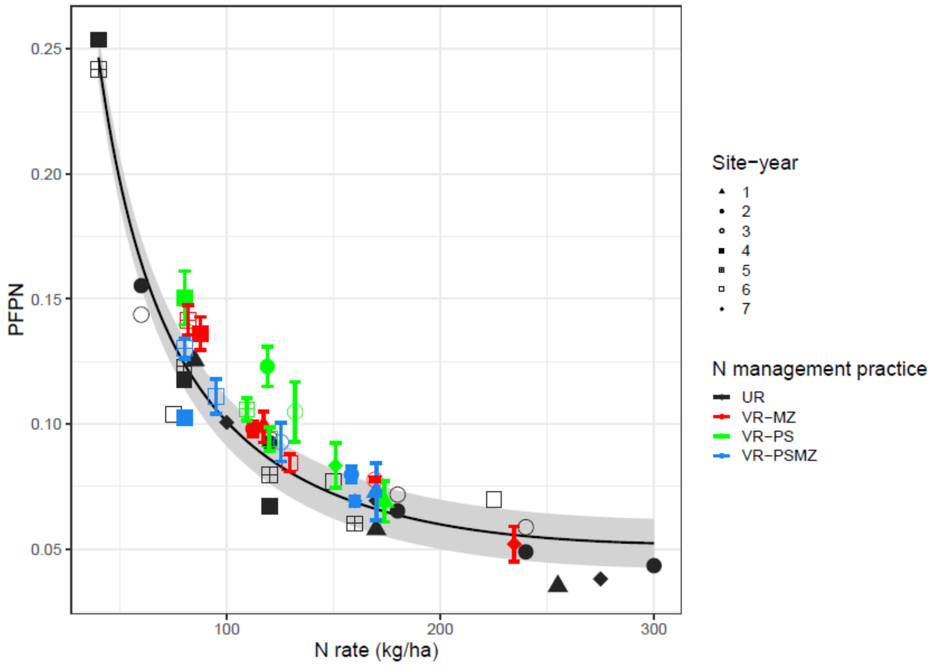

Figure 4: Partial factor productivity (PFP_N) as function of N rate for uniform (black symbols) and precision N fertilisation practices (coloured symbols). For all N management practices, $PFP_{N\ shifted}$ is represented according to equation 4, introducing PFP_N instead of grain yield. Nitrogen rates for precision fertilisation practices are the average values supplied at field scale. For precision N management practices, error bars represent standard error. Grey area represents the confidence interval calculated for uniform N management practice.

The linear model referred to uniform N application and used to express PFP_N as a function of N rate properly fitted PFP_N values obtained in the present experiment (R^2=0.92). Nrate and 1/Nrate were significant (P(F) = P<0.001, df numerator = 1, df denominator = 3390); site-year was significant as well (P(F) = P<0.001, df numerator = 6, df denominator = 3390).

Overall, PFP_N values decreased with increasing N rates. As expected, in all site-years the lowest PFP_N was obtained with the highest uniform N supply. Figure 4 clearly highlights the potential of precision fertilisation techniques to increase PFP_N. Hence, in most site-years, PFP_N values obtained through precision fertilisation practices lay over the curve fitted on uniform N rates.

Table 7 shows grain yield and PFP_N values obtained with precision fertilisation practices, compared to uniform supply of the same N amount through Student's t test, as described in *Equation 3*.

Table 7: Grain yield and PFP_N values obtained with uniform or variable rate application of the same N amount, compared through Student's t test.

Site -year	PNMP[a]	N rate (Kg ha^{-1})	Grain yield (Mg ha^{-1})			PFP_N[b]		
			Uniform	PNMP	P(t)	Uniform	PNMP	P(t)
1	VR-MZ	117	10.2	10.4	n. s. [c]	0.082	0.099	n. s.
	VR-PS	174	10.4	10.3	n. s.	0.06	0.069	n. s.
	VR-PSMZ	170	10.6	10.4	n. s.	0.061	0.073	n. s.
2	VR-MZ	112	**10.2**	9.3	0.002	0.086	0.098	n. s.
	VR-PS	119	9.4	**10.9**	P<0.001	0.081	**0.123**	0.007
	VR-PSMZ	158	10	**10.8**	0.001	0.064	0.08	n. s.
3	VR-MZ	169	10.9	**12.1**	P<0.001	0.061	0.078	n. s.
	VR-PS	132	10.4	9.9	n. s.	0.074	0.105	n. s.
	VR-PSMZ	125	**10.3**	9.1	0.007	0.078	0.093	n. s.
4	VR-MZ	88	9.9	10	n. s.	0.109	**0.136**	0.047
	VR-PS	80	**9.8**	9.1	0.004	0.118	**0.15**	0.042
	VR-PSMZ	81	**9.8**	8.8	P<0.001	0.118	0.102	n. s.
5	VR-MZ	82	9.8	9.9	n. s.	0.116	0.142	n. s.
	VR-PS	109	10	9.7	n. s.	0.088	0.106	n. s.
	VR-PSMZ	80	9.8	9.7	n. s.	0.119	0.13	n. s.
6	VR-MZ	130	10.4	10.1	n. s.	0.075	0.084	n. s.
	VR-PS	120	9.5	9.9	n. s.	0.081	0.094	n. s.
	VR-PSMZ	95	10	**10.9**	0.038	0.101	0.111	n. s.
7	VR-MZ	235	11.6	11.7	n. s.	0.050	0.052	n. s.
	VR-PS	151	10.6	10.7	n. s.	0.066	0.083	n. s.
	VR-PSMZ	160	10.7	**12**	0.001	0.063	0.069	n. s.

[a]PNMP= precision N management practice; [b]PFP_N = partial factor productivity; [c]n.s. = not significant; bold underlined values highlight the highest values when comparing uniform and precision N management practices considering the same N supply.

In site-years 1 and 5, precision fertilisation practices did not positively affect grain yield, it resulted in similar grain yield as compared to the uniform application of the same N rate. In the other site-years, the impact on grain yield was different, depending on both site-year and the precision N management practice. In particular, in site-year 2, VR-MZ reduced grain yield by approximately 9% compared to uniform supply of the same N amount. Conversely, both VR-PS and VR-PSMZ raised grain yield, by 16% and 8%, respectively. In site-year 3, VR-MZ increased grain yield by 11%, while VR-PSMZ led to a grain yield reduction (-12%). Moreover, N supply based on proximal sensing did not affect grain yield. In site-year 4, VR-MZ obtained a grain yield value similar to that of the same uniform N supply, while both VR-PS and VR-PSMZ led to a moderate reduction, approximately equal to 7% and 10%, respectively. In site-year 6, VR-PSMZ improved grain yield by 9%, while the other precision N fertilisation practices did not affect grain yield. Lastly, in site-year 7, VR-PSMZ increased grain yield with respect to uniform application of the same N amount (+12%), while both N supply based on proximal sensing or MZ delineation obtained similar grain yield levels. Then, despite differences among the site-years, maize grain yield improvement seems to not to be the main outcome of precision fertilisation practices.

In general, precision N fertilisation practices increased PFP_N compared to uniform supply of the same N amount (*Table* 7). However, only in site-years 2 and 4, PFP_N improvement resulted to be significant. In particular, in site-year 2, VR-PS increased PFP_N by approximately 52%. In site-year 4 VR-MZ and VR-PS improved PFP_N by 25% and 27% respectively.

Radar charts were used to represent the positioning of each N fertilisation practices according to their respective contribution to PFP_N, grain yield, and RANC for each year and location (*Figure 5*).

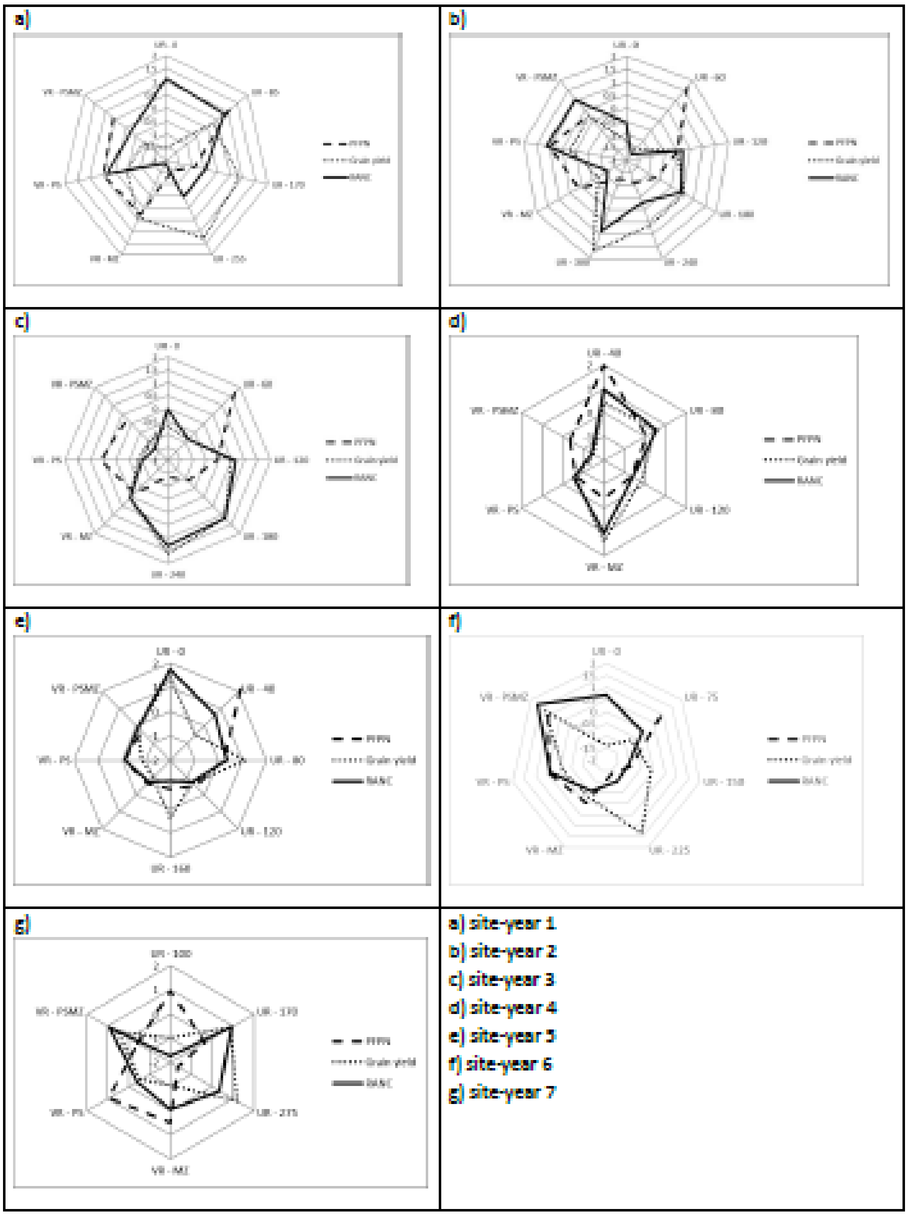

Figure 5: Radar graph showing the positioning of each N fertilisation practice according to their respective contribution to PFP_N (PFPN), grain yield, and net return above N fertiliser cost (RANC) for all site-years. UR – x: uniform N application of x kg ha^{-1}; VR-PS: variable rate N management based on crop sensing; VR-MZ: variable rate N management on MZ; VR-PSMZ: variable rate N management based on both crop sensing and MZ delineation.

A rational N management would lead to reductions in N losses and improvement in crop yield and PFP_N. In each year and location of this study, the N fertilisation management that allowed to obtain the highest RANC was considered the best N fertilisation practice. Indeed, RANC value is a useful indicator that takes into account the effect of N management both on grain yield and PFP_N. The highest RANC coupled with the highest PFP_N values were observed in site-years 1, 5, and 6. In the other site-years, RANC value was shown to be mostly related to the grain yield levels, achieving the highest value corresponding to higher grain yield levels. Moreover, in site-year 6, VR-PSMZ resulted the most profitable N fertilisation practice, leading to the highest PFP_N coupled with a negligible, but significant, grain yield decrease (2%).

Finally, theoretical semivariogram models were used to analyse the spatial patterns in grain yield data in each site-year, with the aim of linking the presence of a spatial structure in grain yield data with the application of precision N fertilisation strategies.

Table 8: Moran I p value, best theoretical variogram model, partial sill, range of spatial dependency, and nugget recorded in each location and year of the experiment.

Site-year	**Moran *I* p value**	**Model**	**Partial sill**	**Range (m)**	**Nugget**	**Best N management practice [a]**
1	-	-	-	-	-	UR-85
2	<0.01	gaussian	5.7	11.3	0.2	VR-PS
3	<0.01	exponential	4.3	16.2	0.2	UR-240
4	<0.01	exponential	3.0	101.9	0.5	VR-MZ
5	<0.01	exponential	0.2	42.6	0.6	UR-40
6	<0.01	exponential	2.4	8.8	0.0	VR-PSMZ
7	-	-	-	-	-	UR-170

[a]: Nitrogen management practice that maximises Net return above N fertiliser cost (RANC), according to *Figure 5*

Results reported in *Table* 8 showed the presence of spatial structure in most (5 out of 7 site-years) of the site--years in this study, with the exception of site-year 1 and 7. Exponential model was the best fit for the experimental semivariogram on the basis of AIC, apart from grain yield data acquired in site-year 2 that were best described through a Gaussian model. The range of spatial structure, setting the limit of the autocorrelation and beyond which spatial structure does not exist anymore, varied among the different site-years. In particular, the range of spatial dependency was 9 m in site-year 6 while in site-year 2 and 3 it was 11 m and 16 m, respectively. In site-year 4 and 5, spatial range values were higher and estimated to be 102 and 43 m, respectively. The range of spatial autocorrelation indicated the scale of spatial variability detected in the field. Higher range values are related to large scale variability, and vice versa. Semivariograms of grain yield, together with their approximate theoretical models, are reported in *Figure 6*.

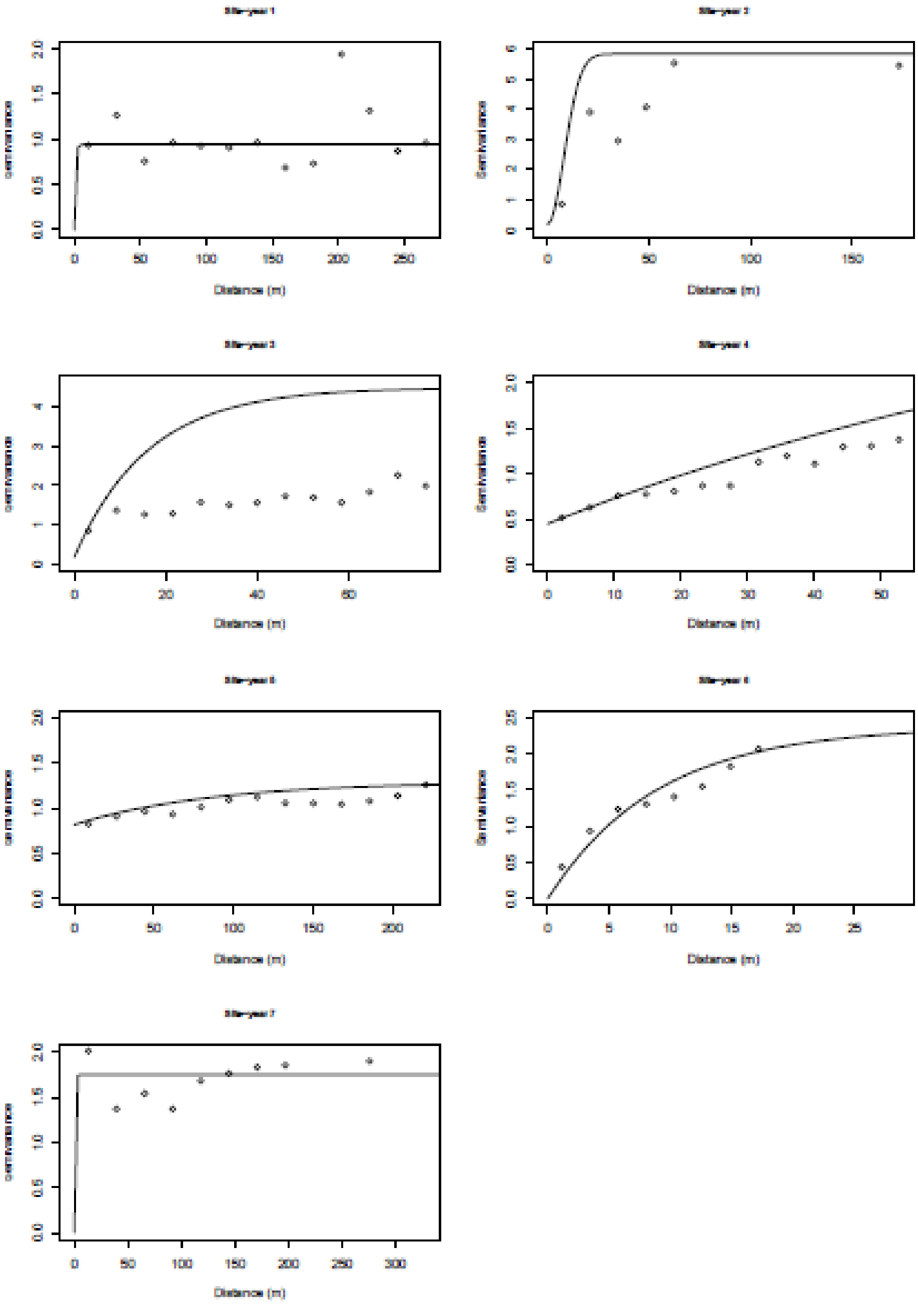

Figure 6: Semivariograms of grain yield data obtained in each site-year.

4.5 Discussion

In traditional maize cropping system in Colorado, N fertiliser is usually applied uniformly and at high rates (around 225 kg ha^{-1}), as farmers want to ensure that N is not the limiting factor in their maize production system. Mean N fertilisation in the region varied between 100 and 250 kg ha^{-1} depending on the different locations, with a mean annual uptake of about 215 kg N ha^{-1} (Inman *et al.*, 2005). Although N requirements become larger with increasing grain yield, crop production and N application are not linearly correlated. In general, the results obtained in this study highlighted a trend where the highest N supply is combined with the lower PFP_N. This was anticipated as in theory, a field where no nitrogen is added would result with an infinite PFP_N (i.e. divided by zero) even though yield could be very low. In order to appeal farmers, N application should be conveniently reduced in order to maintain grain yield, thus increasing PFP_N. This finding was particularly evident in the experiment conducted in site-years 1, 2, and 3 where, by reducing uniform N supply to 180 kg ha^{-1}, PFP_N significantly improves by 25 to 49%, against a grain yield reduction varying approximately from 1.5 to 11% with respect to the value recorded supplying the highest N rate.

In general, PFP_N decreased with increasing N rates, confirming previous results reported by Barbieri *et al.* (2008) and Ma and Biswas (2016). This may indicate that maize was unable to absorb or utilise N at higher N rates. Another possible explanation is that higher N amount increased also N losses in the environment, which exceeded crop N uptake (Delgado *et al.*, 2005). In this study, the total amount of N fertiliser was applied in experimental strips around the 6th leaf crop growth stage development. Splitting N application so that N supply is synchronised with maize uptake may improve nitrogen use by the crop, as suggested by Sharma and Bali (2018).

Overall, variable rate N management did not increase grain yield with respect to uniform N application when the same total N amount was used. This finding agrees with previous results by Ma *et al.* (2014). Indeed, where statistical differences in grain yield were detected, precision fertilisation practices increased or reduced maize grain yield of approximately 10%, corresponding to about 1 Mg ha^{-1}. However, crop yield and N efficiency should both be considered for agroecosystem improvement (Jin *et al.*, 2012). Results from this study clearly demonstrated the potential of precision fertilisation techniques for increasing PFP_N.

The economic evaluation suggests that the optimisation of N management not only improved the environmental sustainability of the agricultural system, but also positively affected farmers' economic return above N fertiliser cost. Improving PFP_N is a promising tool to also increase the profitability for the farmers. Farmers choose the best fertilisation practice on the basis of RANC maximisation. However, it appears evident that RANC is largely affected by maize grain yield, due to the large influence of fertiliser application on maize production value. Consequently, the results strengthened the hypothesis that a compromise between achieving high yield and increasing PFP_N is essential.

Variable rate input application requires to quantitatively assess spatial variability of grain yield at a field scale (Kravchenko *et al.*, 2005). The analysis of semivariogram models determined the range of spatial dependency, allowing the link between the spatial structure of grain yield and the performance of the different N management practices. Indeed, range determination allowed choosing the best fertilisation practice, that can maximise RANC.

On the whole, N application based on crop proximal sensing during the growing season was shown to be the best precision N management practice when the range of spatial variability is lower than 16 m. Conversely, for higher range, up to 102 m, N supply based on MZ delineation performed better. These results agree with previous findings by Schepers *et al.* (2004), that have reported that MZs are a promising tool to identify spatial variability in grain yield for spatial range higher than 16 m, leading to the identification of distinct spatial patterns. Uniform N application was the best approach where no spatial dependency was detected. As shown in site-year 5, for intermediate range value (43 m), the integration of crop proximal sensing and MZ delineation improved both PFP_N and grain yield with respect to PS alone, but negatively affected RANC.

In general, the high level of spatial structure corresponds to a high potential for variable rate N application to increase the profitability for the farmer. The only exception was represented by site-year 3, where the most profitable N management was uniform N application of 240 kg ha^{-1}, despite the presence of spatial autocorrelation. Therefore, in these situations where spatial patterns were not highlighted or the variability in crop vigour across the field led to a moderate difference in grain yield, site-specific management is not suitable. Indeed, in site-year 5, despite a range equal to 43 m, the best fertilisation practice was uniform application of 40 kg N ha^{-1}.

Furthermore, N application based on crop proximal sensing during the growing season was shown to be suitable especially when maximum grain yield difference among the NDVI classes was substantial (CV>20%). In this experiment, such high value has been recorded only in site-year 2 and 5 (data not shown), where the best N management were VR-PS and VR-PSMZ respectively, confirming that crop N status monitoring can be used to more efficiently apply N inputs. Both in site year 4 and 7, as well as in site-year 3, grain yield difference among the NDVI classes showed CV values varying between 10 and 15%. In these situations, VR-PS could not potentially be a promising tool to manage in-field micro-variability. However, in site-year 3, VR-PS has shown to be a promising fertilisation practice to increase PFP_N. But considering the moderate variation of grain yield among NDVI classes, the increment of N rate used in the present study should have been fairly large to compensate for small differences in crop vigour, leading to low yield level. Hence, this approach needs to be further tested with finer levels of N supply. Indeed, Kitchen *et al.* (2010) and Roberts *et al.* (2010) have stated that crop sensing can be used to more efficiently tune N inputs. However, they have considered N increments of 34 kg ha^{-1} while in the present study the experimental setup established 60 kg N ha^{-1} increments. In site-year 1 and 5, grain yield did not vary among the NDVI classes, showing a high uniformity across the field. Consequently, N supply based on crop proximal sensing is not a suitable approach. Moreover, in site-year 4 and 5, the factor that induced grain yield variability may have a range longer than the range that can be optimal for using proximal crop sensing to drive N fertilisation.

The delineation of management zone defines sub-field regions with similar yield-limiting factors, for which a single rate of a specific crop input is appropriate (Schepers *et al.*, 2004; Vrindts *et al.*, 2005).

However, in site-year 7, grain yield did not vary across the management zone (data not shown), assessing that, despite different yield-limiting factors, the yield potential is similar across the field. In such a situation, uniform N supply was proven to be the most profitable practice.

In site-year 4, N supply on the basis of MZ delineation achieved the best compromise between high grain yield and PFP_N values, evaluated on the basis of RANC. This can be mainly attributed to the reduction of N supply in the low productivity areas, according to a previous study by Koch *et al.* (2004). In site-years 4 and 5, furrow irrigation method was adopted over multiple years. Furrow irrigation transports soil particles and subsequently nutrients, inducing an important soil macro-variability that creates areas with different fertility within the field. This large-scale variability is confirmed by the presence of a spatial range of 102 m. Consequently, N management on the basis of the different MZ is able to better consider soil macro-variability. However, in site-year 5, the uniform application of

40 kg N ha^{-1} led to the highest PFP_N, combined with a negligible grain yield loss. Interestingly, the synergic use of MZ delineation and PS for driving N application improved both PFP_N and grain yield with respect to PS alone. A possible explanation is that crop proximal sensing during the growing season can well asses crop micro-variability, but is less effective in evaluating field macro-variability. Conversely, VR-MZ is an optimal N management practice when the field exhibit a strong macro-variability, with areas with similar yield limiting factors. Consequently, the combination of proximal sensing and MZ delineation can be a promising tool to consider both large and small scale sources of variability. Therefore, the integration of soil-based and plant-based methods to drive fertiliser applications can be considered a promising tool for N use efficiency without impacting grain yield, strengthening the hypothesis that supported the present study. Then, the present study confirmed the potential of precision fertilisation to improve maize cultivation sustainability, but also highlighted that the choice of the optimal N fertilisation strategy needs to be related to the range of spatial variability detected in the field.

4.6 Conclusions

The achievement of both high yield and high NUE is needed to increase sustainability without negatively impacting crop productivity.

Precision fertilisation practices have been shown to be promising tools for improving PFP_N without negatively impacting maize grain yield, thus increasing farmers' profitability. However, adaptation to specific agro-environments is needed.

The quantitative evaluation of the spatial patterns in grain yield has been demonstrated to be an important tool to guide precision agriculture application. Variable rate N management based on MZ delineation is the best practice when large-scale variability is detected. Conversely, variable rate N management based on crop proximal sensing is more suitable

when the yield-limiting factors are related to a small-scale variability. Their integration can be helpful to manage both macro and micro-variability that may exist in a crop field, further improving maize fertilisation, and enhancing the overall sustainability of the cropping system.
However, the need of considering whether the higher economic revenue can compensate for added cost for services or technologies required for variable rate N supply appears evident.

4.7 Acknowledgments

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5. Conclusions

The main purpose of this Ph. D. thesis was to extend the current understanding on precision fertilisation, by analysing different fertilisation strategies on different crops and agro-environments.

Particularly, Paper I and Paper III confirmed the positive environmental impact of PA techniques. In Paper I, maximising rice grain yield did not compromise N apparent recovery. Similar outcomes have been reported in Paper III, where precision fertilisation practices have been shown to be promising tools for increasing Nitrogen Use Efficiency without negatively affecting maize grain yield.

Paper I developed a statistical procedure to optimise rice N fertilisation, that can be adapted to different rice varieties grown in different agro-environments. Moreover, Paper I provided a statistical methodology for obtaining prescription functions linking crop vigour measured through vegetation indices and topdressing N supply. Then, prescription function based on the spectral response of rice canopy provided an objective tool to quantify last topdressing N application that can be applied in different agro-environment, then overcoming previous research gaps. Indeed, up to now, N recommendation relying on crop vigour were suggested on the basis of empiric regressions, estimated considering local agro-climatic conditions, cultivars, *etc*.

Moreover, the experimental method reported in Paper I was realised in an experimental site. However, with the aim of promoting a widespread adoption of PA techniques, similar trials for obtaining prescription functions can be realised at farm level. The main advantage in realising such experiments is that farmers can adapt the prescription functions to the peculiar characteristics of their fields, then evaluating the effect of PA strategies on their cropping systems.

The statistical procedure for obtaining prescription functions proposed in Paper I has been extended to different rice varieties, highlighting a variety-specific relationship between crop vigour and N requirements. Paper II allowed understanding the differences among them in terms of both spectral behaviour and response to N fertilisation is mainly due to a different development of grain yield components, as well as to their different contribution in determining the final yield. Integrating qualitative and quantitative statistical tools (such as Principal Component Analysis and Path Analysis) can investigate the specific contribution of grain yield components to the final yield, allowing the extension of prescription functions to other rice varieties. Then, this study can profitably contribute for obtaining a widespread application of precision fertilisation in rice. Further researches are needed to evaluate if the different spectral response of the rice varieties could be ascribed to a different crop canopy development. To this end, Leaf Area Index (LAI) measurement could be a useful tool. Moreover, LAI measurement is a promising tool for highlining changes in canopy geometry that can be ascribed to stress conditions due to other key elements in crop production (*e.g.* water stress). Consequently, LAI measurement can be used to improve the estimation of N requirements based on crop vigour in irrigated crop. Indeed, combining vegetation indices and LAI measurements could provide useful information for better managing the simultaneous presence of both N and water stress. This occurrence is common in our agro-environment, in which N is not the unique yield limiting factor in most crops. Therefore, integrating VIs and LAI measurements could allow the extension of the proposed statistical procedure for determining prescription functions to other crops, different from rice.

Paper III suggested the integration of management zones delineation and crop vigour monitoring as helpful tool for managing both large and small-scale variability that can exist in a crop field. This finding strengthens the hypothesis that optical crop monitoring should be conceived as a part of an integrated system that combines additional information related to soil variability.

Moreover, Paper III reported the quantitative evaluation of spatial patterns in grain yield as a practical tool for choosing the best fertilisation strategy in a specific agro-environment. However, it appeared evident the need of evaluating the effect of precision fertilisation techniques on farmers' profitability, for choosing the fertilisation practice that best applies in each agro-environment. Then, further studies are required to better understand if the higher economic revenue obtained through the optimisation of the fertilisation strategies can compensate the added costs for precision farming technologies and services. A quantified information of the farm profit augmentation and an accurate estimation of investment cost for precision agriculture technology purchase is needed. Therefore, more research should be focused on the contribution of precision agriculture to the increase of production efficiency, considering both yield and economics.

On the whole, the Ph. D. activity provided a complete evaluation the potential advantages derived from the application of precision fertilisation techniques on different cropping system, considering their effects on both crops yield and resources use efficiency.

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