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**DEVELOPMENT OF METHODOLOGIES AND COMPARATIVE AGRONOMIC
EVALUATION OF PRECISION NITROGEN FERTILIZATION DRIVEN BY REMOTE
SENSING DATA: A CASE STUDY ON DURUM WHEAT AND MAIZE**

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i. Riassunto

La tecnologia applicata all'agricoltura rappresenta il miglior strumento per conoscere e interpretare i processi che si sviluppano durante il ciclo delle colture e poterli di conseguenza gestire. Questa conoscenza è lo strumento che ci permette di poter prendere decisioni nel miglior modo possibile. L'agricoltura di precisione è un approccio che sfrutta informazioni e sistemi intelligenti per gestire le coltivazioni in maniera oculata e razionale. Il telerilevamento è una tecnologia che ci permette di avere informazioni georiferite e spazializzate in maniera ricorsiva e senza contatto per il monitoraggio delle colture. Insieme, oggi, sono in grado di offrire una gamma di soluzioni molto ampia per diverse applicazioni in campo agricolo soprattutto per colture estensive come i cereali. Per questo motivo stiamo assistendo ad uno sviluppo molto repentino di queste tecnologie che sta spingendo la ricerca a trovare soluzioni adeguate per un utilizzo operativo di questi strumenti da parte degli agricoltori.

È risaputo che se vogliamo ridurre l'impatto ambientale dell'agricoltura è necessario migliorare le nostre strategie di gestione dei processi produttivi mantenendo o migliorando le produzioni e contestualmente riducendo input ed esternalità negative. Considerando i volumi impiegati e i notevoli costi economico ambientali, la gestione ponderata della fertilizzazione è un fattore cruciale per perseguire obiettivi economici, ambientali e di efficienza d'uso. Lo sviluppo di una fertilizzazione effettuata secondo le reali esigenze della coltura, in maniera sito-specifica e con dosi variabili a seconda di necessità spesso mutevoli nello spazio e nel tempo, è un fattore chiave che sta trovando un ampio e rapido sviluppo.

Questo lavoro di ricerca nasce dall'esigenza di definire metodologie operative che possano beneficiare di tecniche di agricoltura di precisione per la gestione della fertilizzazione azotata a tasso variabile sfruttando informazioni sullo stato nutrizionale delle colture derivate da remoto tramite immagini satellitari. Le colture selezionate sono due dei più importanti cereali coltivati a livello nazionale, il mais ed il frumento duro, entrambe interessate da essenziali interventi di fertilizzazione per garantire produzioni adeguate. Le attività sono state sviluppate in un contesto agricolo a vocazione fortemente tecnologica e di ricerca applicata come quello dell'azienda Bonifiche Ferraresi Spa e gli obiettivi sono nati da reali esigenze di miglioramento delle tecniche produttive.

Sono diverse e molteplici le tecniche e le strategie disponibili per adeguare l'apporto di azoto (N) alle reali esigenze delle colture ma molti dei metodi disponibili si sono evoluti partendo dall'uso di misure dirette di parametri colturali tramite campionamenti, strumenti portatili o sensori prossimali. Una attenzione sempre più crescente viene invece data a sistemi di gestione delle tecniche agronomiche basate su dati acquisiti da remoto (satelliti, droni ecc) molti dei quali comprendono la

valutazione dello stato nutrizionale delle colture. L'attività di sperimentazione è stata incentrata in questo ambito e sull'uso di dati satellitari ottici e nel corso del suo sviluppo è stata suddivisa in due fasi principali (I e II) ripartite in tre anni 2018-2020 con obiettivi connessi tra loro.

Nella Fase I (F1) l'attenzione è stata posta sullo studio della capacità di monitoraggio dello stato nutrizionale del mais e del frumento duro da parte del satellite Sentinel-2 (S2). Le caratteristiche di questo satellite ottico multispettrale dell'Agenzia Spaziale Europea (ESA) sono ideali per lo sviluppo di applicazioni in ambito di AP e il tempo di rivisitazione di 5 giorni di qualsivoglia area studio e la risoluzione spaziale decametrica, ne fanno un prezioso strumento per avere informazioni dettagliate e costantemente aggiornate. Nello specifico è stata osservata l'affidabilità della valutazione dello stato nutrizionale azotato delle colture attraverso la stima del Nitrogen Nutrition Index (NNI) da immagini S2, concentrandosi sull'impatto della variabilità delle proprietà del suolo. Sono stati utilizzati indici di vegetazione (Vis) e variabili biofisiche (Bvs) derivate da S2 come il Green Area Index (GAI_S2), il contenuto di clorofilla fogliare (Cab_S2) e il contenuto di clorofilla fogliare (CCC_S2) per derivare l'indicatore NNI in prospettiva di un suo utilizzo per fertilizzazioni sito-specifiche.

Due prove sperimentali in pieno campo, rispettivamente su maize e frumento duro, per la generazione di variabilità nutrizionale artificialmente indotta tramite diversi trattamenti di N, sono state condotte manipolando gruppi di parcelle in aree con caratteristiche del suolo differenti. La raccolta dei dati in campo ha evidenziato una diversa risposta delle colture al tasso di N e al tipo di suolo in termini di NNI, biomassa (W) e concentrazione di azoto (Na%). Per entrambe le colture, le parcelle di una classe di suolo (FOR1) hanno evidenziato valori di Bvs inferiori e condizioni di stress notevolmente superiori rispetto ad altre classi di suolo anche per tassi di N elevati. Le analisi dei campioni di suolo hanno mostrato per la classe FOR1 differenze statisticamente significative per il pH, rispetto alle altre classi di suolo, indicando che questa proprietà potrebbe essere un fattore limitante per l'assorbimento dei nutrienti e quindi la crescita delle colture, indipendentemente dalla quantità di N distribuita alla coltura. L'analisi di correlazione tra Bvs misurate e prodotti basati su satelliti (Vis e S2_bvs) ha mostrato che è possibile: (i) ricavare direttamente l'NNI da CCC_S2 ($R^2=0.76$) e dal Normalized Difference Red Edge index (NDRE) per il maize ($R^2=0.79$) o da Transformed Chlorophyll Absorption Ratio Index (TCARI) per il grano duro ($R^2=0.61$); (ii) indirettamente stimare NNI dal rapporto tra la quantità di azoto assorbito dalla coltura (Plant Nitrogen Uptake actual - PNUa) e la l'assorbimento critico di azoto (Critical Plant Nitrogen Uptake - PNUc) derivato utilizzando CCC_S2 ($R^2=0.77$) e GAI_S2 ($R^2=0.68$) rispettivamente.

Grazie ai risultati ottenuti nella F1 sono stati messi a punto le metodologie e gli algoritmi per l'applicazione di fertilizzazioni a tasso variabile di N in un contesto di confronto agronomico tra

metodi di distribuzione spazialmente distribuiti (VR1-VR2) e non (UR). La Fase II (2019-2020) dello studio ha perciò previsto la creazione di due ulteriori campi sperimentali, rispettivamente su mais e frumento duro, suddivisi in parcelle per la distribuzione, in pieno campo e in tempo reale, di quantità differenziate di azoto in base alla variabilità nutrizionale riscontrata da immagini satellitari acquisite a ridosso del periodo delle fertilizzazioni e all'uso di indicatori come la differenza tra l' N uptake assorbito e critico (ΔPNU) per VR1, l'indice di sufficienza (SI) per VR2 e il fabbisogno di azoto definito da un bilancio agronomico semplificato (ASB) per UR.

La valutazione della performance dei diversi metodi di definizione dei fabbisogni azotati da remoto (VR1 – VR2) in confronto a quello standard aziendale (UR), è avvenuta attraverso l'analisi dei dati di produzione (mappe di resa) ed il confronto tra 3 indicatori: i) la resa media espressa in $t\ ha^{-1}$; ii) l'efficienza d'uso dell'azoto Partial Factor Productivity dell'azoto (PFPN $kg\ kg\ N\ ha^{-1}$); iii) il ritorno netto (RANC $\text{€}\ t$) in base ai costi sostenuti. I valori puntuali delle mappe di resa, sono stati utilizzati per un'analisi della distribuzione spaziale della resa nelle parcelle e nelle classi di suolo attraverso tecniche di geostatistica uni o multivariata (ordinary e Pseudo co-Kriging). Questa analisi geostatistica più approfondita non ha però fornito risultati soddisfacenti anche a causa di ingenti danni alle due colture accorsi in alcune aree dei due campi sperimentali. In generale, la produzione è stata inferiore della media aziendale del 24% per il frumento duro e del 18% per il mais. Nonostante ciò, i quantitativi di azoto totale utilizzati sono stati inferiori rispetto alla media aziendale dal 22 al 48% nel frumento duro e attorno al 40% nel mais. L'indicatore di efficienza d'uso PFPN ha evidenziato che sia per il grano che per il mais il metodo migliore è stato quello del bilancio agronomico (UR) con $38.2\ kg\ kg\ di\ N\ ha^{-1}$ per il grano e $88.2\ kg\ kg\ di\ N\ ha^{-1}$ per il mais, seguito dal metodo VR1 (ΔPNU) con $35.2\ kg\ kg\ di\ N\ ha^{-1}$ per il grano e $84.4\ kg\ kg\ di\ N\ ha^{-1}$ per il mais. Da un punto di vista economico (RANC) il metodo ΔPNU ha generato un maggior utile netto in entrambe le colture pari a $647\ \text{€}\ t$ (+20% rispetto a UR) per il grano e $2198\ \text{€}\ t$ (+ 6% rispetto a UR) per il mais. I metodi supportati da dati satellitari hanno quindi ottenuto risultati comparabili ed in alcuni casi migliori dei metodi di stima dei fabbisogni basati su dati reali acquisiti in campo. Oltre a questi risultati, lo studio ha raggiunto ulteriori obiettivi: i) l'esperimento ha dimostrato la possibilità di adottare queste tecniche in maniera operativa su più fertilizzazioni di copertura e potenzialmente su tutta la superficie coltivata a cereali; ii) ha dimostrato come, laddove siano state utilizzate informazioni sullo stato nutrizionale derivate da remoto, è stato fornito meno azoto dove la coltura ha prodotto di più; iii) ha evidenziato come le diverse caratteristiche dei suoli hanno un effetto molto marcato sulla variabilità delle produzioni. Durante la stagione è stato correttamente stimata la reale necessità della coltura in ogni preciso momento e in alcuni casi (alcune parcelle in N3) non è stato somministrato fertilizzante perché

ritenuto non necessario senza aver intaccato la produzione finale. Questo fatto ha rappresentato un vantaggio per i metodi VR1 e VR2 e un limite per un approccio non spazializzato come UR, che benché sia già un metodo avanzato (ASB) che offre benefici in termini di efficienza e di quantificazione della dose di azoto, non sfruttando informazioni spazialmente distribuite ha avuto valori di ritorno economico RANC addirittura negativi in alcune aree del campo (-27 € t) che significa andare in perdita di guadagno già solo con il costo dei fertilizzanti.

In conclusione questo studio ha dimostrato l'applicabilità di informazioni a valore aggiunto derivate da immagini satellitari Sentinel-2 in un contesto operativo di coltivazione di cereali, ed ha permesso di evidenziare l'impatto di potenziali fattori limitanti come azoto e variabilità dei suoli per poterli gestire con informazioni quantitative e spazialmente distribuite costantemente aggiornate come supporto alle attività di agricoltura di precisione.

ii. Abstract

Research for new technologies applied to agriculture corresponds to the best way to know and interpret what happens in crops cycle to be able to manage them accordingly. This knowledge allows us to make decisions in the best possible way. Precision Agriculture (PA) is an approach that uses information and intelligent systems to manage crops wisely and rationally. Remote Sensing (RS) is a technology that allows us to have georeferenced and spatialized information in a recursive and constant manner for crop monitoring. Today we have the expertise to provide alternative activities and to offer a very wide range of complementary solutions for different agricultural applications especially for extensive crops such as cereals.

It is well known that if we want to reduce the environmental impact of crop production, we need to optimize our management strategies, maintaining or improving the production and reducing the inputs at the same time. Considering the amounts used and the significant environmental economical costs, the management of fertilization is a crucial factor in pursuing economic, environmental and use-efficiency objectives. The development of a fertilization strategy carried out according to the real needs of the crop, in a site-specific way and with variable rates depending on changing needs in space and time, is a key factor if we want to take careful decisions.

This research work stems from the need to outline operational methodologies for the management of nitrogen fertilization at variable rate, that can benefit from PA technologies and makes use of information derived from remote satellite imagery, to assess nutritional crop status. The selected crops for the case study are two of the most important cereals cultivated at national level, maize and durum wheat, both requiring essential fertilization to ensure adequate production. The activities were developed in an agricultural context with a strong technological vocation and applied research such as that of the company Bonifiche Ferraresi spa and the objectives were born from real needs to improve production techniques.

There are many different techniques and strategies available to adapt the nitrogen input (N) to the real crops needs, but many of the available methods are based on the use of proximal sensors that are costly and require field instrumentation that limits their extensive adoption in real farming conditions. For this reason, more and more attention has been paid to the development of advanced fertilisation management strategies that exploit satellite data, many of which are based on the assessment of the nitrogen nutritional status of crops. The research activity has been focused in this area with the scope to: i) test solutions for the estimation of crop nutritional status from satellite data and ii) implement a work-flow of fertilisation strategy in real farm conditions. The experimental design involved two main phases (I and II).

Phase I (F1) was devoted to study solutions to assess the nutritional status of maize and durum wheat by exploiting satellite Sentinel-2 (S2) observations. The characteristics of this optical multispectral satellite launched by the European Space Agency (ESA) are ideal for the development of PA applications based on crop status assessment. Moreover, the theoretical revisiting time of 5-days on any study area, makes this solution as a suitable tool for generating constantly updated information. In particular, estimation of nitrogen nutrition index (NNI) from Sentinel-2 (S2) satellite images was investigated to assess crop nutritional status by analyzing the role and the impact of soil properties variability in the proper evaluation of crop nitrogen deficiency. Vegetation indices (VIs) and biophysical variables (BVs), such as the green area index (GAI_S2), leaf chlorophyll content (Cab_S2) and canopy chlorophyll content (CCC_S2), derived from S2 imagery, were used to investigate plant N status and NNI retrieval, in the perspective of its use for guiding site-specific N fertilization. Two field trials (maize and durum wheat) were conducted by imposing to groups of plots in areas with different soil characteristics, different N treatments, in order to generate nutritional variability in the crop. Field data collection highlighted different responses of the crops to N rate and soil type in terms of NNI, biomass (W) and plant nitrogen concentration (Na%). For both crops, plots in one soil class (FOR1) evidenced considerably lower values of BVs and higher stress conditions with respect to other soil classes even for high N rates. Soil samples analyses showed for FOR1 soil class statistically significant differences for pH, compared to the other soil classes, indicating that this property could be a limiting factor for nutrient absorption, hence crop growth, regardless of the amount of N distributed to the crop. The correlation analysis performed between measured crop BVs and satellite based products (VIs and S2_BVs) showed that it is possible to: (i) directly derive NNI from CCC_S2 ($R^2=0.76$) and either Normalized difference red edge index (NDRE) for maize ($R^2=0.79$) or Transformed Chlorophyll Absorption Ratio Index (TCARI) for durum wheat ($R^2=0.61$); (ii) indirectly estimate NNI as the ratio of plant nitrogen uptake (PNUa) and critical plant nitrogen uptake (PNUc) derived using CCC_S2 ($R^2=0.77$) and GAI_S2 ($R^2=0.68$) respectively.

Following the results obtained in F1, Phase II (F2) of the study was devoted to develop methodologies and algorithms for the application of variable rate N fertilizations. Two different VRT approaches (VR1 and VR2) for spatially distributed nitrogen supply were tested and compared to a soil nutrient balance approach not spatially distributed (Uniform Rate - UR). Experimental fields for two new trials (maize and durum wheat) were divided into regular grid parcels, for which different N distribution strategies were randomly applied in near real time in the best moment during the crop season. Differentiated amounts of N were defined on the base of crop nutritional status assessment derived by satellite images acquired close to the fertilization period on the base of Phase I results.

The two VRT remote sensing based approaches, were focused on *i)* the assessment of difference between actually absorbed and critical N uptake (ΔPNU) (namely VR1) and *ii)* the calculation of Sufficiency Index (SI) as the ratio of a real-time sensed crop property to the same measurement from a reference known by statistically analyse VI variability in the field (namely VR2). Agronomic strategy performance for VR1 and VR2 were compared to the one obtained by an homogeneous fertilisation approach (UR) that defines nitrogen requirement on the base of field data for the computation of an agronomic simplified balance (ASB).

The evaluation of performance of the two remote sensing based methods for the definition of N needs (VR1 - VR2) in comparison to the standard method adopted by the farm (UR), was performed by analyzing yield maps. Three indicators were considered for each method (VR1, VR2 and UR): *i)* the average yield expressed in t ha^{-1} ; *ii)* the N use efficiency with Partial Factor Productivity of nitrogen (PFPN kg kg N ha^{-1}) and *iii)* the net return (RANC € t) based on the costs incurred.

In general, production for the VR method was less than the farm average by 24% for durum wheat and 18% for maize, despite the fact that the total amount of nitrogen used was lower than the farm average from 22% to 48% in durum wheat and around 40% in maize. The PFPN use efficiency indicator showed that for both wheat and maize the best method was the agronomic balance (UR) with 38.2 $\text{kg kg of N ha}^{-1}$ for wheat and 88.2 kg of N ha^{-1} for maize, followed by the VR1 method (ΔPNU) with 35.2 $\text{kg kg of N ha}^{-1}$ for wheat and 84.4 kg of N ha^{-1} for maize. From an economic point of view (RANC) the ΔPNU method generated a higher net profit in both crops of 647 € t (+20% than UR) for wheat and 2198 € t (+ 6% than a UR) for maize. The methods supported by satellite data have thus obtained comparable and in some cases better results than methods based on real soil properties data acquired in the field. However, it is important to underline how spatially distributed soil information is not commonly available or else very costly in normal farm condition. Hence, the obtained results can represent an alternative that can be easily scaled to large areas. In addition to these findings, the study has achieved further objectives: *i)* the experiment demonstrated the possibility of adopting these techniques for all the fertilizations in an operational manner and potentially over all fields cultivated with cereals; *ii)* it demonstrated that where remotely derived nutrition information was used, less nitrogen was supplied where the crop was in good nutritional condition without impacting on final yield ; *iii)* it showed that the different characteristics of the soils had a very marked effect on the variability of production and that it is therefore necessary to consider these information for a complete crop stress analysis in order to identify the real limiting factor in crop nutritional status and hence define the more appropriate management strategy. During the

season, the real need of the crop, at any precise moment, was correctly estimated and in some cases (some parcels) the fertilizers were not supplied, because it was considered unnecessary, without significantly affecting the final crop production. This fact represented an advantage for VR1 and VR2 methods and on the other hand highlighted the limit for a non-spatialized approach such as UR. The ASB method used can be considered already an advanced approach with respect to common fertilization based on fixed dose. ASB provides a quantification of the nitrogen dose on the basis of real soil properties. However, it does not consider seasonal variability due to the soil/crop/atmospheric interaction that produces within-field crop spatial variability, hence nutritional needs. In doing so, UR had a negative economic return value (RANC) for some areas of the field (-27 € t) which means going into economic loss already only with the cost of fertilizers.

In conclusion, this study demonstrated the added value of information derived from satellite images in an operational context of cereal cultivation. Results have highlighted the impact of the interaction of different limiting factors, such as nitrogen deficiency and soil properties variability, that need to be assessed, producing quantitative and spatially distributed information constantly updated for proper management in support to PA activities.

Keywords: precision agriculture, remote sensing, nitrogen, nutritional status, site-specific N fertilization, soil properties, biophysical variables, Sentinel-2.

iii. State-of-the-Art

Cereals cultivation requires sustainability in terms of production and environmental impacts. Nitrogen is one of the most important nutrients for plants and directly linked to crops yield. Soil N content varies spatially and temporally within a field and soil N availability depends on plant-soil-atmosphere interaction almost guided by the variations of soil properties and the presence of limiting factors. Crop N demand changes during the season depending on phenology and growing conditions and this requirement is met by soil N supply capacity and N fertilizations. But soil N availability and crop N uptake, and finally crop growth, are permanently interrelated during crop development, as explained by Gastal and Lemaire (2002), and the maximum potential growth is not singularly regulated only by crop N demand or soil N supply. N supply from soil and fertilizations and N accumulation in crops are very dynamic processes that should be considered through an integrated approach (Gastal and Lemaire 2002; Körschens et al. 2013) and a spatial relationships analysis (Casa and Castrignanò 2008) that takes in account spatial and temporal variability.

N mineral fertilizers are globally the most used substances for fertilization, with a world supply of ammonia (NH_3) of ~180 million tons (FAO 2015). Unfortunately, the large quantities used do not correspond to high levels of nitrogen use efficiency (NUE) (Caviglia et al. 2014; Cui et al. 2010; Dordas 2011; Lassaletta et al. 2014) because often N fertilizer are not correctly supplied. The largely used Yield goal (YG) method (Lory and Scharf 2003) only linked to target production or the economic optimum nitrogen rate (EONR) correlated to fertilizer costs often proved to be in contrast with NUE because it dramatically varies from location to another due to many factors (Franzen et al. 2016; Puntel et al. 2019; Ransom et al. 2020). Sustainable agriculture requires optimizing N management during the crop season, in order to achieve profitability and healthy environment (Bongiovanni and Lowenberg-Deboer 2004; Mariangela Diacono et al. 2013; Stamatiadis et al. 2017).

Diagnostic methods for determining crop N deficiency, result of primary importance for crop fertilization management. As mentioned before, plant N content follows a dynamic trend that depends on the specific needs at every stage of plant growth and on the nutrients availability. Starting from this assumption, Ulrich (1952) with a study on the nutritional requirements of plants, proposed the concept of the critical N concentration ($\text{Nc}\%$) defined as the minimum $\text{Nc}\%$ at a given time necessary to achieve potential non limiting production and maximum growth rate. These relationships allow the study of the dynamics of N uptake (Nu kg N ha^{-1}) in relation to plant growth (Novoa and Loomis 1981) considering the physiological aspects regulating N assimilation. Lemaire et al. (1984) showed that aerial dry matter accumulation (W t ha^{-1}) can be related to plant $\text{Nc}\%$ in grasses growing with non-limiting N supply and that from the constant relationship between $\text{Nc}\%$ and W was possible to

generate a function called the *critical N curve* independent of the experimental conditions. For %N values below this curve, N availability becomes a limiting factor for W development. Stable and similar relationships were found between the decline of N concentration (Nc%) in major C3 and C4 cultivated species in relation to their growth (Greenwood et al. 1990). It was clear that this relationship regulated many of the crop physiological processes, such as C assimilation and growth rate, and C and N allocation between organs (Gastal and Lemaire 2002) and consequently the yield. Based on these studies, Justes et al. (1994) proposed a diagnostic tool for the determination of the critical nitrogen dilution curve of an important crop such as winter wheat. Then many studies started to recommend the usage of crop specific N dilution curves for nutritional stress condition detection (Colnenne et al. 1998; G. Lemaire 1997; Plénet and Lemaire 2000). Nc % has not been used as a goal to reach during crop growth, but as a reference for optimal or non-optimal nutritional conditions in relation to real crop N concentration (Na%). An indicator of crop N status calculated from the ratio between Na% and Nc%, called *nitrogen nutrition index* (NNI), was proposed for the analysis and interpretation of crop growth variables by Lemaire et al. (1997). The same model was applicable also to Nu (kg ha⁻¹) using a specific crop coefficient and biomass accumulation data in order to obtain the critical N accumulation and even the NNI indicator (Lemaire and Gastal 1997; Gastal and Lemaire 2002; Plénet and Lemaire 2000).

Nevertheless, in order to provide operational nitrogen recommendation to farmers, the problem remained the collection of a very large amount of crop variables and the difficulty of doing many laboratory analyses. This approach initially changed because it was cost and time consuming but also because the use of geostatistics, agronomic models, remote sensing and precision agriculture techniques started to become a key component in agriculture support information generation (D J Mulla 1997). In the same way, the fast technological development of sensors, machineries and portable measuring instruments began to overcome the stumbling block of data acquisition. Soil or crop sampling strategies could be simplified by using geostatistics to derive and manage spatial patterns (Castrignanò et al. 1998; Goovaerts 1997; D J Mulla 1991) and by using modeling, for providing variables estimation (Brisson et al. 2003). Castrignanò et al. (2012) emphasized the importance of the spatial relationships among variables in multivariate geostatistics analysis for improving the estimation of single variables. For this reason, sensor-based approaches to collect crop and soil data and geostatistics analysis to gather more useful information to determine within-field variability has had a wide use (Casa and Castrignanò 2008). Soil electrical conductivity (Ec) representation through electromagnetic induction (EMI) sensor (Castrignanò et al. 2012; Corwin and Lesch 2003; Doolittle and Brevik 2014), or resistivity mapping (Banton et al. 1997; Samouëlian et

al. 2005) have been widely used as a proxy for the spatial and temporal variability of many soil physical and chemical properties (Y. Wang et al. 2015). Recently the full range of Veris iScan (Veris Technology Inc.) on-the-go multi-sensor offered both optical and electrical sensing components that allows high-resolution soil mapping of the main properties (pH, texture, temperature, EC, soil moisture, soil organic matter) greatly improved their prospects use. But even these very useful sensors are often used either for soil analysis or crop analysis by not considering the combined effects on production and failing to discriminate them.

As well as for soil survey, crops proximal sensors, such as SPAD meter (Minolta Camera Co., Osaka, Japan) or Dualex (Force-A, France) are recognized devices for the assessment of N deficiency (J. S. Schepers et al. 1992), by measuring the relative greenness of plants in a rapid and nondestructive manner also closely related with NNI (Debaeke et al. 2006; Yang et al. 2014). Many of these applications are based on the assumption of a strong relationship between leaf chlorophyll (LCC) and N content (Yoder and Pettigrew-Crosby 1995). But these hand-held chlorophyll meters (CM) are not suitable to be used for site-specific N management, because of the in large sampling effort that would be required to generate maps (Miao et al. 2009). The canopy chlorophyll content (CCC) is another important indicator of photosynthesis activity (Gitelson et al. 2005; Haboudane et al. 2002), which is related to the nitrogen concentration in green vegetation. It is determined multiplying the Leaf Area Index (LAI) (M. Weiss et al. 2004) by the leaf chlorophyll content (LCC). Being that CCC influences plant reflectance, crop multispectral sensors started to be developed not only for research purposes but also by agricultural industries and placed on board of a tractor or a spreader to vary management according to sensor readings. Handheld or on-board crop sensors, such as the GreenSeeker (NTech Industries Inc., Ukiah, California, USA), Crop Circle (Holland Scientific, Lincoln, USA) or Yara N-Sensor/Fieldscan (Yara International ASA, Germany) produce vegetation indices, such as the normalized difference vegetation index value (NDVI), correlated with leaf chlorophyll content, which are then used by algorithms to calculate optimal N application rates. Many studies tested this plant sensor-based diagnostic approach (Cao et al. 2015; Colaço and Bramley 2018; Franzen et al. 2016; Tremblay et al. 2009; Xia et al. 2016) and some operational commercial service (AgLeader OptRx system) are based on this kind of technology (Holland and Schepers 2010; Franzen et al. 2016). But the problem with these approaches remains that usually crop status references, i.e. N-rich and N-low strips, are needed and they are time consuming to implement in addition of having serious problems of representability (Ransom et al. 2020). Moreover, the empirical models used for the N prescription generation often need site-specific calibration or crop calibration often underestimated by the user. Chlorophyll fluorescence emission ratio has also been widely applied to crop N status monitoring

thanks to the development of specific instruments as in detail reported by Tremblay et al. (2012) in their review paper.

Recently, fast and low-cost acquisition systems, based on smartphone apps, demonstrated the added value of collecting many data of important crop variables, e.g. PocketN (Confalonieri et al. 2013; Confalonieri et al. 2015) with accuracy comparable to instruments already available on the market such as the well-known SUNSCAN (Delta-T, Cambridge UK) or the AccuPAR (Decagon, Pullman, WA, USA), LAI-2000 or LAI-2200 Plant Canopy Analyzers (Li-Cor, Lincoln, NE, USA). Smart applications are certainly seen with less mistrust by farmers than conventional tools and they can be widespread. They also have the advantage of being able to collect a lot of data many times during the season.

At the moment there are only limited means of easily obtaining accurate and constantly updated information on spatialized differences in crop N concentration or soil properties and almost always they need several tools to do so. This condition has been improved by the use of ancillary data provided by satellite, aerial or unmanned aerial vehicle (UAV) imagery and RS technologies offer today the major route to achieve fast and frequent data. Thanks to the progress of this technology, as highlighted by Reynolds et al. (2018), it became possible to use a combination of remotely-sensed and ground-based data to collect precise and near real-time data for satellite based product validation, for geostatistics analysis or as direct inputs to run models at any given location with the advantage, in this case, of producing spatially distributed outputs (Mirco Boschetti et al. 2015; Campos-Taberner et al. 2016; Gilardelli et al. 2019).

Basically, bridging the gap between the physical remote sensing measurements and the agronomic features, can be done using empirical or mechanistic approaches, or the combination of both, as described by Weiss et al. (2020) and Baret and Buis (2008). But the estimation of canopy variables from RS, is in some way problematic due to the ill-posed nature of the problem (Baret and Buis 2008). The retrieval of surface characteristics from reflectance measurements is in many cases an under-determined, problem due to the interaction of many not distinguishable factor. The most common approaches to retrieve agricultural variables are purely empirical approaches (needs of measured data) or mechanistic methods that consist of inverting models based on the radiative transfer models (RTM). Similarly, in hybrid methods (Berger et al. 2020), machine learning algorithms use simulated datasets, rather than measured data, to invert physically-based models. In their study, Weiss et al. (2020) called “primary” the variables that can be directly addressed from remote sensing, since they are involved in the process of radiative transfer (e.g. Green Area Index, surface temperature, soil moisture). These authors report an exhaustive list of primary variables that can be retrieved from

remote sensing data from solar, thermal and microwave domain, using active or passive sensors. Empirical approaches offer the advantage of directly inferring the secondary variables, such as crop nitrogen content (J. G.P.W. Clevers and Gitelson 2013; Delloye et al. 2018) and even if they are more applicable in many cases are too site-specific.

Satellite remote sensing has the potential to investigate vast land surfaces over space and time, thanks to the interaction of electromagnetic radiation in optical, thermal, microwave, and fluorescence domains. Optical data are provided from a wide range of satellite sensors providing images from a very high to low spatial, spectral, temporal and radiometric resolutions. While RS has been intensively and regularly used for global or regional scale crop monitoring with multi-spectral earth-observation system (Atzberger 2013; Defourny et al. 2019; Fritz et al. 2019), such as the Moderate Resolution Imaging Spectroradiometer (MODIS), the Medium Resolution Imaging Spectrometer (MERIS) or LANDSAT (MSS, TM, ETM+, OLI) there are still special needs for PA applications at the farm scale (Seelan et al. 2003). Many crop-related aspects of PA have been successfully investigated from RS, such as abiotic stress factors (F. Baret et al. 2007; Ghulam et al. 2008), weed or disease damages detection (López-Granados 2011; Mahlein et al. 2012), phenological metrics extraction (Bolton and Friedl 2013; M. Boschetti et al. 2009; Zeng et al. 2020), biomass accumulation or intra-field variability monitoring, yield (Basso et al. 2013; Bolton and Friedl 2013) and biophysical variables (Baret and Buis 2008; Weiss et al. 2000) estimation, rice flooding (Boschetti et al. 2014) dynamic investigation and many others (Weiss et al. 2020). Most of the studies have deeply investigated multispectral signal potential of currently available satellites. Also the category of hyperspectral sensors has shown excellent diagnostic capabilities thanks to the greater spectral resolution. Hyperspectral remote sensing is surely more reliable and sensitive than broad bands in estimating crop biophysical feature and in nutritional stress detection (Stroppiana et al. 2009), but this technology is not yet operationally available to be used in PA operational context (Fabio Castaldi et al. 2016; Inoue et al. 2012; Mariotto et al. 2013). In addition, the coarse spatial resolution remains a strong constraint for field scale output generation if hyperspectral sensing is not coupled to aerial or UAV vector or by exploiting constellations of private microsatellites able to acquire time series at <10 m resolution (Lu et al. 2020). UAVs have a relevant use mainly, due to their high spatial resolution. But the maximum available payload, the impact of calibration procedures (Weiss et al. 2017) and the lack of necessary autonomy of the batteries, limit the large scale monitoring capacity of this technology for extensive crops.

The availability of RS data has been greatly improved by recent launches of new private and public satellite systems (Gomasasca et al. 2019; M. Weiss et al. 2020). Among them, Sentinel-2 is a

European wide-swath, high-resolution, multi-spectral imaging mission built under Copernicus framework (Drusch et al. 2012) which consists in two twin-satellites flying in the same orbit, called Sentinel-2A and Sentinel-2B. They were launched by the European Space Agency (ESA) in 2015 to 2017 and they are equipped with a multiband sensor (Figure 1) with 13 spectral bands: four bands, B2 (490 nm), B3 (560 nm), B4 (665 nm) and B8 (842 nm) at 10 m, six bands, B5 (705 nm), B6 (740 nm), B7 (783 nm), B8a (865 nm), B11 (1610 nm) and B12 (2190 nm) at 20 m and three bands, B1 (443 nm), B9 (940 nm) and B10 (1375 nm) at 60 m spatial resolution.

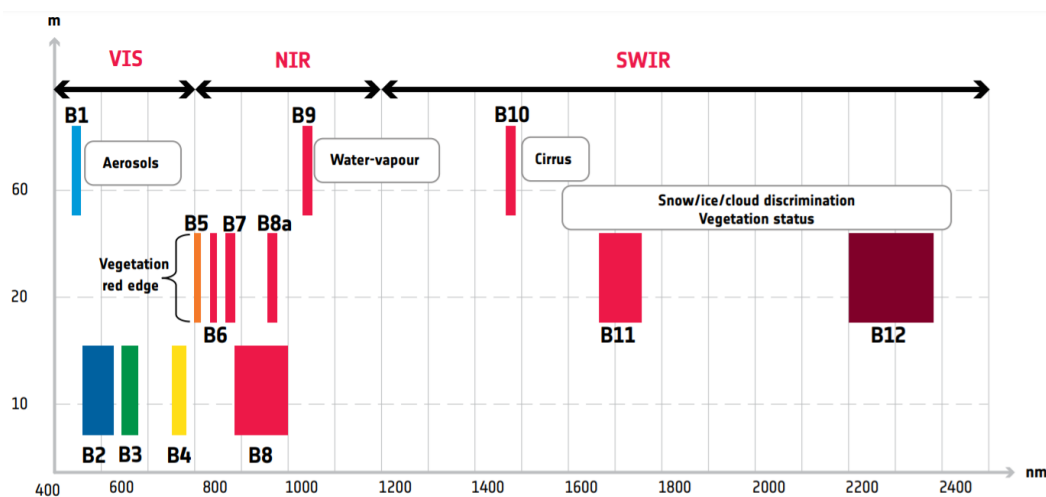


Figure 1: Spatial (meters) and spectral resolutions (wavelengths nm) of ESA Sentinel-2 satellite (source: <https://esamultimedia.esa.int/multimedia/publications/ESABulletin161/offline/download.pdf>)

The ESA strategy has been to provide free access to Sentinel imagery and digital image processing software “Sentinel toolboxes” such as SNAP (Sentinel Application Platform) for downstream services development. In fact, these satellites are proving to be particularly suitable for the development of PA applications, thanks to a 5-day revisiting time and to the decametric resolution compatible with crop variability detection at field level and crop management systems offered by variable-rate machinery.

Precision agriculture has the objective of adapting production strategies to crops needs and variability, increasing knowledge about the dynamics emerging during crop growth cycle. Since the 90s the development of PA (Bramley 2009; David J. Mulla 2013) has been sudden and constant and now it makes precision farming affordable and cost-effective and thus, almost operational thanks to all above mentioned developments (Weiss et al. 2020). Shared goals from global to local levels are the reduction of pollution, the decrease of waste and an improved production respecting environmental standards. But the stakeholders to be more involved are certainly the farmers, who

need support and detailed and precise information that can be combined to the use of a wide portfolio of machinery, tools and sensors already available on the market. The added value of information demand, in addition to data to be acquired, is a strong and actual need and it represents the great challenge of today for the consolidation of PA. This information reduces the burden of having to gather accurate crop management information from farmers, which is otherwise a major bottleneck for the widespread use of PA as highlighted by Reynolds et al. (2018).

Surely, fertilization management is something that can have a very significant impact on the improvement of agro-practices. In particular, the management of nitrogen is a particularly sensitive topic. Having detailed, consistent, and credible information at field-scale on the nitrogen nutritional status of crops is crucial in crop production processes interpretation. Having spatialized and possibly quantitative information, always timely, allows to apply precision farming techniques that start first from the knowledge of the phenomena.

The present research stems from this need and starting from what is already present in the literature at the operational level, aims at taking a step forward by conducting tests in support of site specific management of nitrogen in cereal crops through use of optical satellite images.

iv. Aim of the study

The aim of the present study was to investigate the possibility of using precision agriculture techniques supported by RS data on extensive cereal crops (maize and durum wheat) in an operational context. Field and satellite optical imagery data were analyzed, to retrieve agricultural variables by applying existing empirical methods and testing new ones. Field trials were carried out, to investigate the variability in crops nitrogen uptake and yield in relation to site-specific N applications. Sentinel-2 images were processed and tested to consider their operational use in order to provide in-season quantitative and spatialized information on plants nutritional status to support nitrogen management. Agronomic comparative tests on both crops were implemented to evaluate different methods derived from satellite based indicators and from agronomic balance in consideration of the high soil heterogeneity of the study area. The framework was the adoption of PA techniques to homogenize yield rationalizing production inputs and managing soil diversity, in a real farm context, that hosted the whole experimentation (Bonifiche Ferraresi SpA). Soil variability has an impact on farm crop production and plant N uptake sometimes shows effects independently from N rates supply. For this reason, the rationale of the study was to investigate the nutritional status during the season, as retrieved from satellite data, to bring out possible site specific limiting factors. An assessment of the applicability and the pros and cons of the developed and tested methods from a production, use

efficiency and economic point of view was made, comparing also the differences in relation to the two investigated crops.

v. Experiment design

The research work started with an initial phase of literature study (Phase 0) and was divided in two main phases, phase 1 (F1) and 2 (F2), distributed into three years (2018-2019-2020) of field trials with a final phase (phase 4) of results analysis (Figure 2). During this period all the satellite images necessary and functional to the goal of the study were acquired, together with the collection of field data, obtained during several instrumental field campaigns.

Phase I (2018-2019) was devoted to: *i*) the study of satellite detection capability of crop nutritional status for two cereals, maize and durum wheat, from Sentinel-2 images analysis, and their comparison with field data; *ii*) the implementation of open field trials in order to generate intra-field nutritional variability through VR fertilization interventions at different N doses during the season; *iii*) the analysis of the effects of different soil properties on the crop response to fertilization, by estimation of biophysical variables both from field sampling and from remote sensing; *iv*) the identification of algorithms for a spatial explicit quantification of the nitrogen dose, to be provided to the crop through VRT and site-specific prescription maps, based on the identification of actual nutritional crop needs. The results of Phase I studies were published in a peer-reviewed paper (Crema et al. 2020).

The aim of phase II (2019-2020) of the study was : *i*) test and develop algorithms for the creation of fertilization methods supported by satellite imagery (VR1 and VR2); *ii*) create and manage two experimental fields (maize and durum wheat) for the distribution, in full field and in near real time, of differentiated quantities of nitrogen, based on the crop spatial variability identified by satellite images; *iii*) perform site specific fertilizations through VRT application and prescription maps generation derived from 3 different methods (VR1-VR2-UR); *iv*) evaluate the performance of the different methods through the analysis of production data and different environmental and economic efficiency indicators.

In order to determine the effect of the treatments, a randomized block experimental design was tested in F1, evaluating the response of maize (2018) and durum wheat (2019) to different levels of nitrogen fertilization in different soil classes. Soil variability is a key productivity factor in the study area where the soil properties rapidly vary in space. The large size of the farm allowed the set-up of field experiments for situations with strong soil variability, as identified by geophysical maps and characterised by soil analysis. Five N classes, from very low to very high rates, laid down starting

from the farm protocol and the fertilization limits established by legislation, were chosen and replicated for all the soil classes identified. Each N rate defined was distributed for each 50 by 36 m plots whose dimension was based according to satellite data resolution and farm's spreader characteristics. A total of 20 parcels were collocated in each experimental field. During the season, field data of crop variables were collected, following a specific protocol established for each variable. Laboratory analysis was conducted to estimate crop traits and create a crop database. Satellite acquisitions were carried out during fertilization periods to investigate crop responses to fertilizer regimes and yield data were finally used to evaluate the effect of nitrogen in crop production.

In F2 activities conducted in 2020, different fields with intra-field soil variability, were selected for maize and durum wheat trials experimentation. N site-specific applications were carried out. This operational test followed a workflow starting from data acquisition, to information generation, to management application. Specifically, the three steps were: *i)* satellite data acquisition and processing, *ii)* prescription maps production using the developed algorithms and *iii)* fertilizer application using VRT machinery. Satellite data were obtained during the most important crop phenological stages in consideration of fertilization purpose. The production of the maps and the application of the fertilizer followed the usual timing and procedures of the farm. To guarantee such timeliness, maps were produced and used within 2 days from the satellite acquisition date. The experimental fields were divided into large parcels for the application of the N rates established in relation to the specific methods and no ground data were collected to force decision, in order to simulate a fully automated procedure. Final evaluation of crop production was conducted using yield maps generated by the harvester machines and fertilizer application maps gathered by the VRA spreader system.

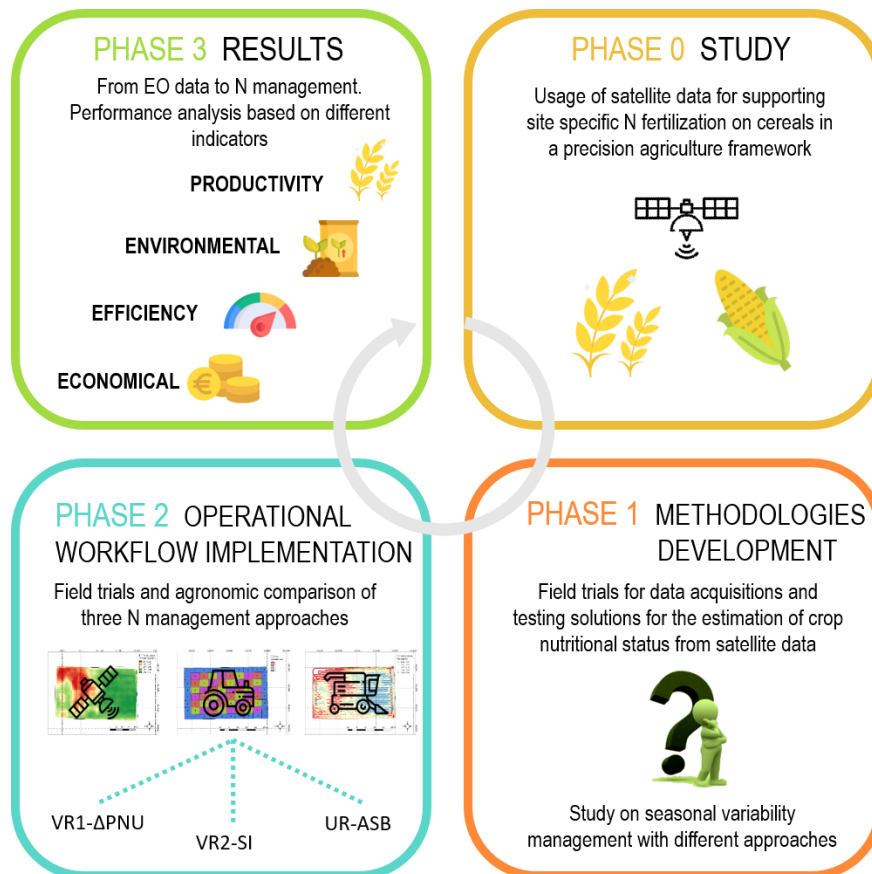


Figure 2: overall workflow of the research study divided in the main phases

1. Phase I - Nitrogen status monitoring from remote sensing

The results of this phase of the study have been published in a peer-reviewed paper (Crema A., Boschetti M., Nutini F., Cillis D., Casa R., 2020. Influence of Soil Properties on Maize and Wheat Nitrogen Status Assessment from Sentinel-2 Data. *Remote Sensing*, 12, 2175; doi:10.3390/rs12142175) but are reported here in greater detail.

1.1. Introduction

Soil properties variability greatly influences cereal crops production and interacts with a proper assessment of crops nutritional status, which is fundamental to support site specific management, able to guarantee a sustainable crop production. As reported by Cordero et al. (2019), several studies have demonstrated separately the potential advantages of crop spatial variability analysis with soil-based and plant-based methods (Diacono et al. 2013), in order to drive variable N fertilization. However, only few studies assessed the interaction between the two, hence the possibility to combine this information into a decision process (Cordero et al. 2019; Longchamps and Khosla 2015). The uncertainty in both plant N demand and soil N supply due to seasonality and growth potential often leads to non-optimal nitrogen management strategies (Lemaire et al. 2008).

Crop nutritional stress is usually assessed by considering N deficiency on the basis of plant analysis methods, with no direct measurements or consideration of soil properties information. If not considered, soil variability can prevent a correct diagnosis of the nutritional status of crops. The influence of soil properties variations in relation to crop nitrogen status assessment performed with proximal or RS technique is a topic that has not been much reported in literature.

One of the most widely used crop N status assessment methods relies on the estimation of the NNI, defined as the ratio of $N_a\%$ in the dry above-ground biomass to $N_c\%$, which is in relation to the specific above-ground biomass expressed in $t\ ha^{-1}$ (Lemaire et al. 2008). NNI can also be derived considering crop N content ($kg\ N\ ha^{-1}$), instead of concentration (%). In this case, NNI is computed using the ratio between actual plant nitrogen uptake (PNU_a) and critical plant nitrogen uptake (PNU_c), both expressed in $kg\ N\ ha^{-1}$ (Guerif et al. 2007; Plénet and Lemaire 2000). This is because the amount of N in the plant, expressed as a concentration or quantity, and biomass accumulation, have a relationship based on the theory of N dilution during plant growth (Greenwood et al. 1990). The critical nitrogen dilution curve derived from this theory (Justes et al. 1994) determines the minimum N concentration (N_c) or N content (PNU_c) (Plénet and Lemaire 2000) in the crop, for a specific moment, that allows for a normal growth with no biomass accumulation reduction. Besides the field estimation of NNI, this indicator can also be calculated by empirical relations with RS

vegetation indices or biophysical variables using proximal or satellite data. RS data can be used to derive both $\text{Na}\%$ and $\text{Nc}\%$ (via their relationships with W) or PNUa and PNUc . Many authors suggest that the latter option is preferable, because the relationship between RS data and total quantities of nitrogen present in the canopy is more robust (Houlès et al. 2007; Lemaire et al. 2008).

From the literature, two approaches have been used to estimate NNI from satellite data: 1) direct methods (DM) and 2) indirect methods (IM). DM rely on the possibility to estimate NNI by “direct” relationships with VIs. According to Chen (2015), we can use: i) a “mechanistic approach” (DM_1) that require first to estimate $\text{Na}\%$ and W to calculate NNI, or ii) a “semi-empirical approach” (DM_2), based on the direct definition of a parametric regression between VIs and NNI values that are usually estimated with in situ data. Many authors have successfully used DM of NNI from RS using multispectral (Ata-Ul-Karim et al. 2014; Huang et al. 2015; Xia et al. 2016; Zhao, Ata-Ul-Karim, et al. 2018; Zhao, Duan, et al. 2018) or hyperspectral data (Cilia et al. 2014; R. Wang et al. 2014) for optimizing the timing and the rate of N fertilizer applications. The study of Nutini et al. (2018) tested a DM_1 approach in an operational workflow, for producing NNI maps based on the combined use of high-resolution satellite images and ground-based estimates of crop parameters using smart apps.

IM are based on a different paradigm: RS data are firstly used to retrieve BVs from RS, such as LAI and CCC. Then, from these estimates, a relationship with PNUa and W is adopted to derive PNUc according to a crop-specific dilution curve. Once PNUa and PNUc are estimated, NNI is calculated. IM approaches have the advantage to exploit RS data to estimate optically related BVs, hence to be a physically based solution. BVs estimation can be performed using: i) parametric regression methods using VIs (Blondlot et al. 2005; Jan G.P.W. Clevers et al. 2017; Feng et al. 2015; Guerif et al. 2007), ii) non-parametric machine learning algorithm (e.g. Neural Network, SVR, PLSR etc.). The latter can be implemented or by inverting radiative transfer models (RTM), e.g. adopting Look Up Table approaches (Berger et al. 2018), or by hybrid methods (Berger et al. 2020; Castaldi et al. 2015; Delloye et al. 2018; Upreti et al. 2019). For a detailed review of the different available approaches see Verrelst et al. (2019). Recently ESA Sentinel Application Platform (SNAP) toolbox, reached an operational stage in providing BVs (LAI, $f\text{APAR}$, $f\text{cover}$, CCC) through a biophysical processor tool based on a hybrid approach employing neural networks (Weiss and Baret 2016). This greatly facilitates the operational adoption of RS products in agricultural management decision support systems.

Under this framework, the objectives of the F1 study were to investigate the robustness and representativeness of nutritional status assessment through the computation of NNI from Sentinel-2

for maize and durum wheat, in relation to different nitrogen fertilization levels and soil properties. A field experiment for two consecutive crop seasons was set up to interpret the potentiality and limits of NNI in identifying nitrogen deficiency.

More specifically, Sentinel-2 acquisitions were exploited to analyze spatial and temporal variability of crop status from RS with the following objectives: *i)* compare the performances of direct and indirect NNI estimation approaches; *ii)* assess the added value of the operational S2-BV product with respect to ad hoc calculated VIs.

1.2. Materials and Methods

1.2.1. Study area and experimental design

The study area is located in Northern Italy, in the Eastern part of the Ferrara province, in the Po valley. This flat area, between the city of Ferrara and the Adriatic Sea, derives from extensive land reclamation works concluded at the beginning of the 20th Century. This operation generated new arable lands from zones that were previously permanently flooded marshland. This explains a great deal of heterogeneity in soil properties, affecting yields and crop growth in agriculture activities. The whole experimentation was located in Jolanda di Savoia (FE) (lat. 44°52'26" N, long. 11°56'24" E, elev. -3 m) and has been hosted by Bonifiche Ferraresi Spa farm, that represents the largest Italian farm in terms of utilized agricultural area. This company is currently active in the experimentation and application of PA technologies on extensive crops. The many crops cultivated and the great variability of the soil of the farm, require careful management of agricultural practices to standardize the production. For this reason, the experimentation was carried out in a context where it was possible to have a large data source and an availability of machineries and tools suitable for PA. The agronomical practices and all the operations regarding crops needs were executed by machineries and technicians of the farm in collaboration with subcontractors for specific tasks.



Figure 3: location of the experimental field n°45 within the Bonifiche Ferraresi Spa farm at Jolanda di Savoia (Fe)

A field experiment was carried out, in the 2018 and 2019 seasons, respectively on maize and durum wheat. A plot of land of around 16 ha (Figure 3), identified in the farm with the number 45, was chosen and sown with maize hybrid SY HYDRO (Syngenta FAO 700) in 2018 and with durum wheat ODISSEO (Syngenta) in 2019. In order to select the field for the field trial, we took advantage of some data held by the farm. In fact, previously, in 2015, the farm conducted a survey for the determination of soil properties using an Automatic Resistivity Profiler (ARP) geophysical sensor (Piori et al. 2013) as well as soil sampling. From these data, a soil map of the whole farm (Figure 4b) and a resistivity map (Figure 4a) were derived by the surveying company SOING (Livorno, Italy). This information was used to identify areas with contrasting soil properties potentially related to different productivity.

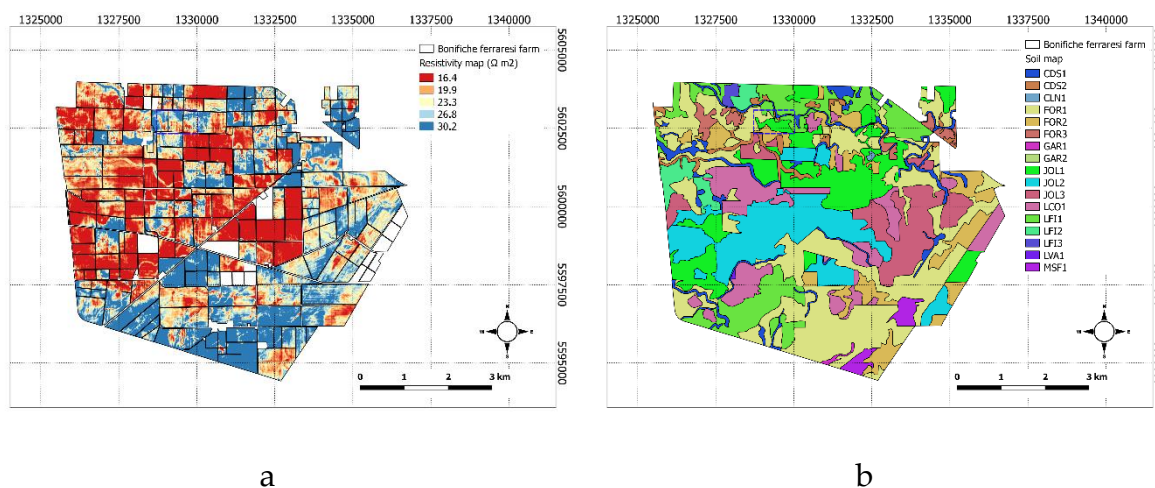


Figure 4: Bonifichè Ferraresi farm: a) electrical soil resistivity map (Ω m) 0-50 cm depth profile (2015); b) soil map (2015) with soil classes

The selection criteria of the experimental field were *i*) the presence of a certain degree of spatial variability in the resistivity map and *ii*) the presence of different soil classes within the field. Four different soil classes, coded as CDS2, JOL3, JOL1, FOR1 were found within the field n° 45 (Figure 5b) and a portion of land crossing the field from side to side with different resistivity properties was also present in the field (Figure 5a).

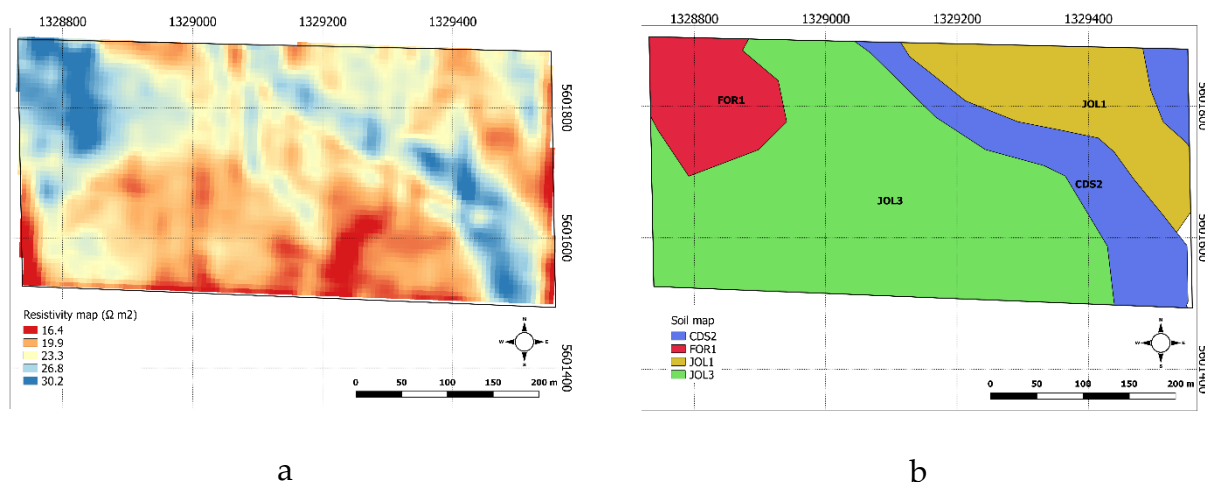


Figure 5: Experimental field: a) electrical soil resistivity map (Ω m²) 0-50 cm depth profile and; b) soil map with soil classes

The field spatial variability was therefore exploited for the localization of the plots in which to carry out the experimentation. A total of 20 plots were established, in which 5 N fertilizer rates were applied with 4 replicates (Figure 6b). Each replicate was located inside one of the identified soil classes (Figure 6a) with the goal of maximizing the intra-field nutritional variability. Plot size, 36 m width by 50 m length, was established based on the width of a variable rate (VR) fertilizer spreader,

i.e. Sulky model x36 (Sulky-Burel, Chateaubourg, France) available at the farm, and for being large enough to contain at least 10 pixels of a 10 m resolution S2 image.

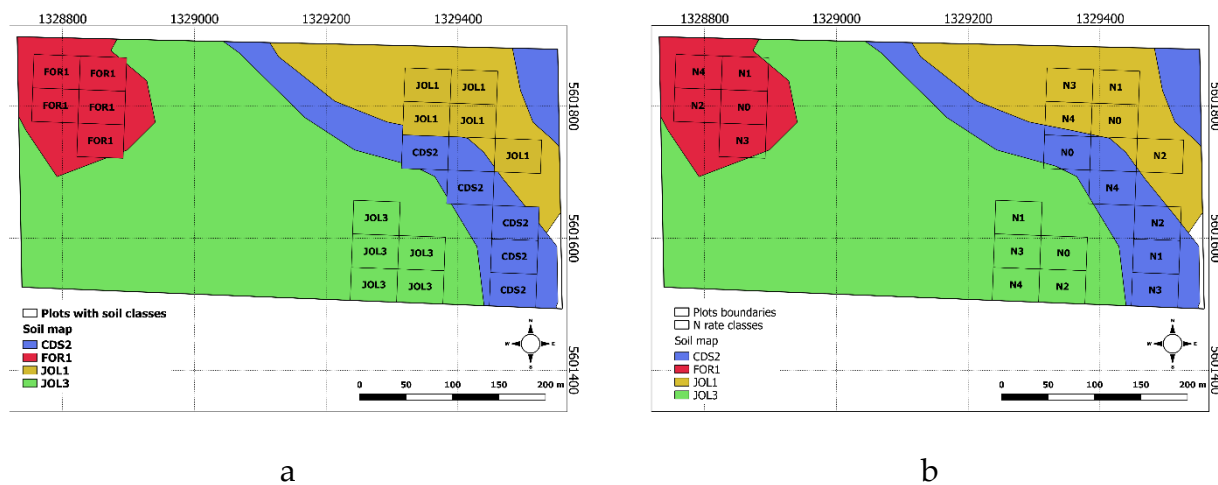


Figure 6: Experimental field: a) localization of the 20 plots based on the soil map (5 plots for each soil class); b) N replicates (5 N rates)

The 5 N treatments applied to maize and wheat, respectively in the 2018 and 2019 season, are reported in Table 1. For both crops, two N treatments (N1 and N2) were below the standard N prescription rate of the farm, in order to create N limiting conditions, whereas two treatments (N3 and N4) were above, to create N non-limiting conditions. Plots with the N0 treatment were considered as non-fertilized controls, but actually received 10 kg N ha^{-1} because a uniform basal dressing was applied for both crops at sowing, with 125 kg ha^{-1} of 8-18 fertilizer (i.e. 10 kg N ha^{-1}), as a standard practice at the farm. A uniform basal dressing with 200 kg ha^{-1} Pheoscor (NPK 0-16-0, CaO-MgO-SO₃ 8-2-22) was also applied before sowing for both crops, whereas no K fertilizer was applied because of the good soil content of this nutrient.

Table 1: Experimental design details.

Year	Crop	N treat. code	N rate (kg N ha^{-1})	BBCH ^a	N application date	BBCH ^b	Field campaign date	BBCH ^c	S2 acquisition date
2018	Maize	N0	10						
		N1	90	00 ^d	17-Apr	12	09-May	13	19-May
		N2	180	13	21-May	15	29-May	16	03-Jun
		N3	300	16	01-Jun	30	18-Jun	50	23-Jun
		N4	350	60	26-Jun	65	03-Jul	65	08-Jul
		Standard	240						
2019	Durum Wheat	N0	10						
		N1	60	00 ^d	20-Oct				
		N2	120	22	04-Mar	25	19-Mar	25	20-Mar
		N3	180	237	06-Apr	31	19 Apr		

N4	240	51	02-May
Standard	160		

^a Crop growth stage BBCH (Lancashire et al. 1991) at the date of the N fertilization

^b Crop growth stage BBCH at the date of the field campaign

^c Crop growth stage BBCH at the date of the S2 acquisition

^d 00 refers to the N application at the sowing date (basal dressing).

During the season, top dressing N fertilizations using prescription maps were carried out according to key phenological stages (Table 1), with the exception of N0 plots. Application maps (as-applied maps) recorded by the VR spreader system, allowed to check the amount of N provided and the accuracy of the spreading in terms of geolocation. In the 2018 season, the last N application at flowering was carried out with a uniform distribution of N, due to the absence of VR machinery for liquid fertilizer distribution. The field was irrigated by means of a drainage sub-irrigation system during the 2018 maize season (Table 2). Other practices for pest, diseases and weeds control were carried out according to usual farm management practice for both crops. An effort was made to obtain non-limiting conditions for all factors, except for nitrogen fertilization.

Table 2: Agronomic details of field experiments for maize in 2018 and wheat in 2019.

	Variety	Density	Plowing date	Planting date	Harvest date	Irrigation
Maize	SY	8.8 seeds m ²	2-Apr 2018	17-Apr 2018	25-Aug 2018	Sub-irrigation
Wheat	ODISSEO	200 kg ha ⁻¹	20-Oct 2018	12-Nov 2018	15-Jun 2019	No irrigation

1.2.2. Field data collection

1.2.2.1. Soil characterization

In April 2018, an intensive soil sampling campaign was carried out, stratified in the more contrasting areas identified by the resistivity map (Figure 7a) of the experimental field, collecting a total of 36 soil samples, from a 0–30 cm depth. The number of samples was chosen in order to have 9 points for each of the soil classes (Figure 7b) as a compromise between accuracy and resources availability.

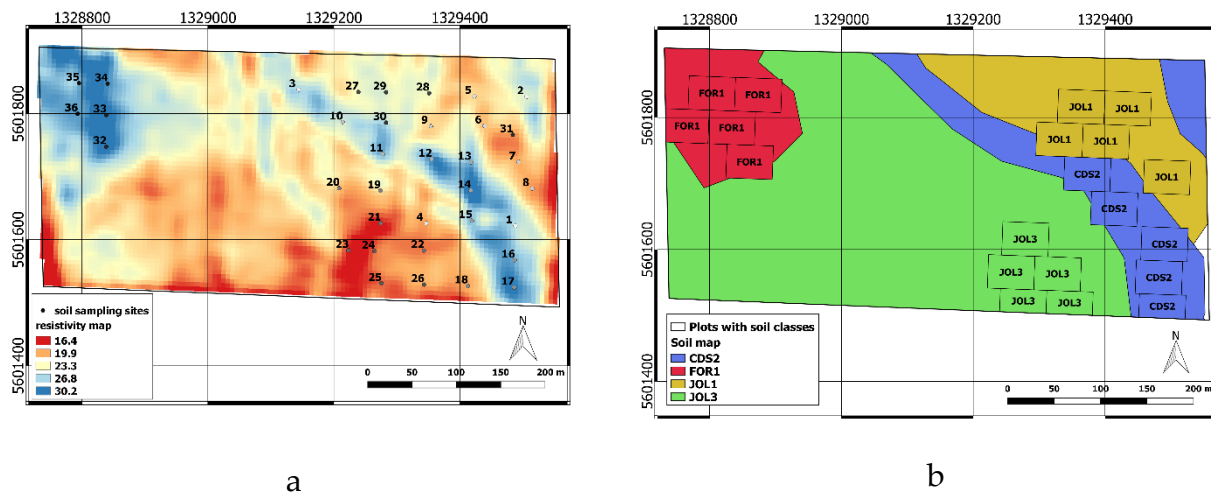


Figure 7: localization of the 36 soil samples collected in the field

The localization of the samples was established inside the experimental plots and outside following a regular scheme in order to have a good representativeness of the data. The soil classes description based on FAO World Reference Base (WRB) for soil resources (Micheli 2008) is reported in Table 3.

Table 3: Soil type of experimental field (n° 45) description.

Soil class	wrb_class*
CDS2	Calcaric Gleyic Fluvisols (Siltic, Geoabruptic)
JOL1	Fluvic Thaptohistic Gleysols (Clayic, Hurnic, Sulfudic)
JOL3	Calcaric Fluvic Gleysols (Protosalic, Protovertic, Sulfudic, Clayic)
FOR1	Calcaric Fluvic Gleyic Cambisols (Protovertic, Humic, Clayic)

Several soil cores of 2 cm diameter were collected at each sampling point (Figure 8). For each site the samples taken were merged and then a part was kept for subsequent laboratory analysis. Each sample was geo-referenced, labelled with the number of the corresponding parcel and stored in a refrigerator awaiting the analysis.



Figure 8: a) sampling operations in the field; b) detail of soil sample (profile 0-30 cm)

Soil texture, soil organic matter (SOM), total nitrogen, total organic carbon (TOC), pH, C/N were measured in the laboratory using official standard analytical protocols (see §1.3.1).

1.2.2.2. Crop characterization

Along the season, field measurements were carried out to acquire ground data. These surveys had the purpose of collecting crop biophysical variables during the season and related to the plants response to the different fertilization regimes. The objective was to report the effects of the nitrogen supply combined to different soil properties with regard to crop development. The list of collected BVs is shown in Table 4:

Table 4: List of ground collected crop biophysical variables (BVs)

Acronyms	Biophysical Variable	Unit	Method/instruments
Cab	Leaf chlorophyll content	mg cm ²	DUALEX
LAI	Leaf area Index	-	Digital Hemispherical Photographs (DHP)
W	Dry Above Ground Biomass	t ha ⁻¹	Weight
Na	Plant Total Nitrogen concentration	%	Kjendalh
BBCH	Phenology development	-	BBCH

Early in the season, before the starting of the field campaigns, a protocol to guide the surveys was drawn up, for standardizing the measurements. The protocol has been partly derived from the methodology for the validation of medium spatial resolution land satellite products described in the VALERI project (Leroy et al. 2000). The spatial sampling strategy has been defined in order to correctly use local ground measurements. The VALERI methodology and the protocol used in similar work (Camacho et al. 2014; Delloye et al. 2018) were considered to set the minimum number of ESU (Elementary Sample Unit) at the optimal location to get a good description of the geostatistics of the BVs considered and to generate robust relationships between the measured BVs and the corresponding spatial resolution of the satellite images (Leroy et al. 2000). The ground data collection date was as close as possible to satellite acquisition in order to obtain as much correspondence as possible between the *in situ* and remote data. During the maize season, 4 sampling campaigns were carried out, whereas only 2 campaigns took place during the durum wheat season because of the early crop lodging. Unfortunately, not all the collected data were finally used, due to cloud contamination problems in satellite images, mainly occurring during the durum wheat season (see § 1.2.3).

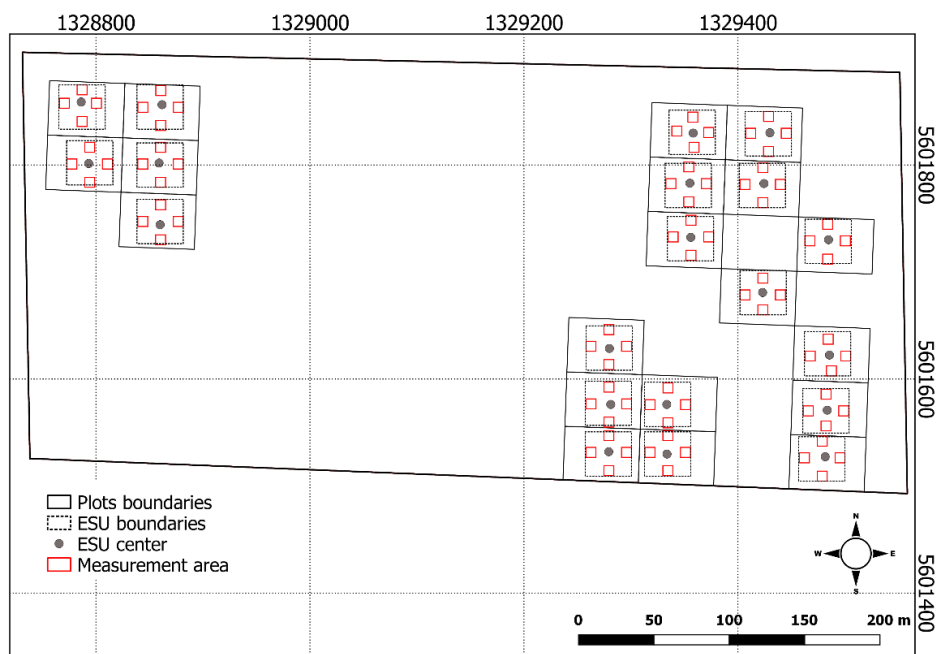


Figure 9: Plots boundaries with Elementary sampling unit (ESU) and measurement area of field 45

An ESU of 20 by 20 m size, was defined inside of each plot and a Trimble GNSS receiver was used to record the center of each ESU (grey marker in Figure 9). Starting from this point, four sampling areas (red square of Figure 9) of 3 by 3 m were created based on the 4 cardinal point and shifted by around 10 m (Figure 9). Inside any of this measurement area, a sampling scheme was prepared to guide LAI (Figure 10) and Dualex (Figure 11) measurements thus avoiding the personal selection of the best plant or location. The problem of the unaware choice of plants is something that happens especially for crops such as maize where plants per square meter are not as numerous as for other cereals and so the experimenter is led to select the best looking plants (Leroy et al. 2000).

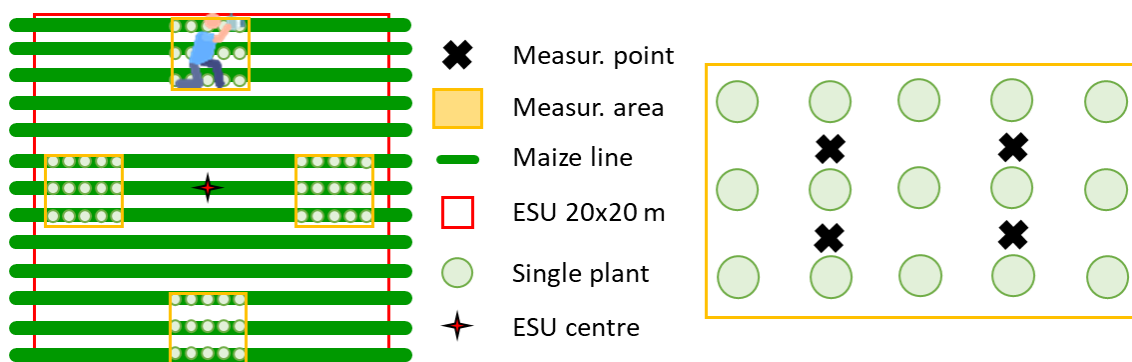


Figure 10: sampling scheme of LAI data acquired by Digital Hemispherical Photographs on maize culture. On left side the red boundaries of the ESU with the orange boundaries of the four sampling areas. On the right side the zoom of a sampling area with the localization of the plants (green circle) and the measurement points (black marker)

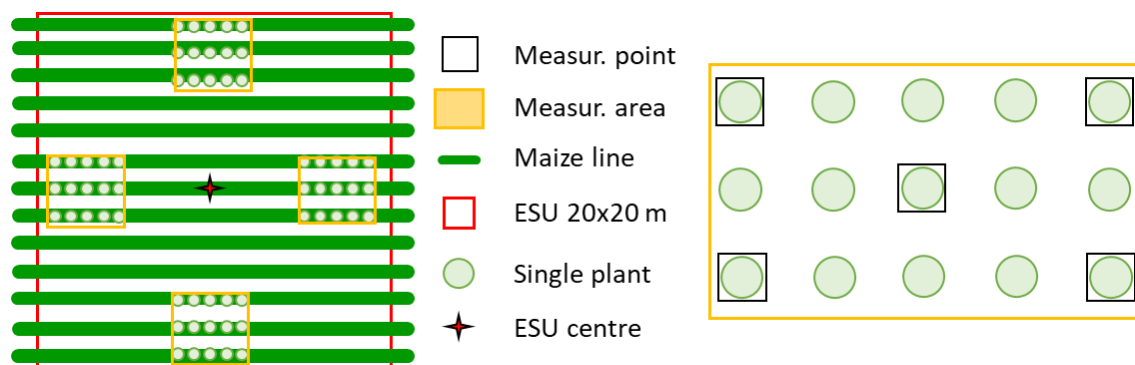


Figure 11: sampling scheme of Leaf Chlorophyll Content acquired by Dualex on maize culture. On left side the red boundaries of the ESU with the orange boundaries of the four sampling areas. On the right side the zoom of a sampling area with the localization of the plants (green circle) and the measurement points (black square)

A Canon digital camera with a fish-eye lens was used to acquire 16 Digital Hemispherical Photographs (DHP) in each ESU (4 in each measurement area of Figure 10) at each date and processed with the Can_Eye V6.494 software (Demarez et al. 2008) to estimate crop green area index (GAI). GAI includes all the green parts of the crop and not just the leaves, as for the leaf area index (LAI). The effective_GAI (GAI_eff), computed by the Can_Eye software, was used and not the true_GAI, considering the fact that the former is more theoretically related to the LAI estimated from satellite images (Delloye et al. 2018; Duveiller et al. 2011; Marie Weiss and Baret 2016) (Duveiller et al. 2011; Weiss and Baret 2016; Delloye et al. 2018).

Leaf chlorophyll content ($Cab - \mu \text{ cm}^{-2} \text{ leaf}$) readings from 60 plants in each ESU (15 in each measurement area of Figure 11) were acquired with the Dualex (Force-A, Orsay, France) at each field campaign. The Dualex records also the flavonoid content (Flav), the anthocyanins content (Anth) and the nitrogen balance index (NBI), calculated as the ratio between Cab and Flav content. This index has been used to assess N nutritional status in wheat and maize (Cartelat et al. 2005; Tremblay et al. 2012).

GAI_eff and Cab were then used to compute the canopy chlorophyll content (CCC) by multiplying these two variables. Above ground biomass (W) was measured by harvesting 8 plants for each ESU for maize (approximately the number of plants by square meter) and 1 m² of wheat crop, which were then dried in the oven at 80° C until constant weight, then converted in tons per hectare ($W \text{ t ha}^{-1}$). The whole plant samples were then finely ground and used for N concentration (Na%) determination using the Kjeldahl method (Nelson and Sommers 1973). The plant phenological stages were identified and registered using the BBCH scale (Biologische Bundesanstalt, Bundessortenamt and CHemical industry) (Lancashire et al. 1991). A list of data collected during measurement campaigns is given in Table 5.

Table 5: List of ground collected crop biophysical variables both for maize and durum wheat

Biophysical Variable	N° ESU	N° sampling area per ESU	N° measures per area	N° measures per ESU	Total measures per campaign
Cab	20	4	15	60	1200
LAI	20	4	4	15	300
W	20	1	1	1m ²	20
Na	20	1	1	1	1
BBCH	20	4	2	8	160

1.2.3. Satellite data

Sentinel-2 SAFE (Standard Archive Format for Europe) level-2A (Bottom-of-Atmosphere BOA) cloud free images, both from S2-A and S2-B, were downloaded from Copernicus Open Access Hub (<https://scihub.copernicus.eu/dhus/#/home>) and processed using the sen2r R toolbox (Ranghetti et al. 2020) to produce L2A products and obtain the VIs listed in Table 7. The images are organized in the Sentinel-2 tiling grid divided into square tiles (granules) of approximately 100 km on each side. The Relative Orbit number including the study area was R022 and the granule code was 32TQQ. The ESA SNAP biophysical processor toolbox (Weiss and Baret 2016) was used to retrieve the satellite-derived biophysical variables (S2_Bvs) LAI_S2 and CCC_S2. LAI_S2 estimated from RS includes all the green parts of the plant and therefore can be called GAI (Duveiller et al. 2011). This variable is similar to the GAI_eff output of CAN_EYE (Weiss and Baret 2016; Delloye et al. 2018; Duveiller et al. 2011). CCC_S2 is a quantitative variable (g of chlorophylls a+b per m² of soil) calculated at canopy level, strongly related to leaf nitrogen content (Houlès et al. 2007; Delloye et al. 2018) and it is directly estimated from the SNAP toolbox (LAIxCab). S2Toolbox level2 biophysical variables algorithms implemented in SNAP (Weiss and Baret 2016) allow to estimate GAI and CCC and not Cab. Since Cab estimation is fairly complicated due to the gradient of Cab content in the plant and the complexity of canopy structure that influence the radiometric response, the CCC variable is provided, to avoid ambiguities between the GAI and Cab estimation during the inversion process (Weiss and Baret 2016). The spectral domain where Cab can be estimated with high accuracy is in the visible and near infrared and also GAI is influencing the reflectance in this domain, especially for dense canopies (Xia et al. 2016). For this reason, CCC is used as indicator of N stress (Baret et al. 2007; Delloye et al. 2018; Weiss et al. 2000), because it includes information on the leaf nitrogen content and the photosynthetic capability (Green et al. 2003). All the products were provided with 10 m of spatial resolution.

Table 6: List of Sentinel-2 images used in F1 with corresponding phenological stage (BBCH)

Crop	Date	BBCH
Maize	19-May	13
Maize	03-Jun	16
Maize	23-Jun	50
Maize	08-Jul	65
Wheat	20-Mar	25

In the 2018 season, four S2 images acquired after N applications and close to field measurement dates were processed, whereas only one useful S2 image close to the field campaign dates was available in 2019 (Table 6), due to cloud occurrence and lodging problems that affected wheat crop before flowering. A vector layer with plot boundaries was used to extract Vis/BVs values from images considering an internal buffer area. Pixels values were averaged for each plot and compared with ground-measured variables.

Table 7: List of vegetation indices used in the present study

Index	Name	Reference
NDVI	Normalized Difference Vegetation Index	(Rouse, J. W., R. H. Haas, J. A. Schell 1973)
NDRE	Normalized difference red edge index	(Barnes et al. 2000)
MSAVI	Modified Soil-Adjusted Vegetation Index	(Qi et al. 1994)
GNDVI	Green Normalized Difference Vegetation Index	(Gitelson et al. 1996)
MCARI	Modified Chlorophyll Absorption in Reflectance Index	(Daughtry et al. 2000)
MCARI2	Modified Chlorophyll Absorption in Reflectance Index 2	(Haboudane et al. 2004)
MTVI2	Modified Triangular Vegetation Index 2	(Haboudane et al. 2004)
OSAVI	Optimized Soil Adjusted Vegetation Index	(Rondeaux et al. 1996)
TCARI	Transformed Chlorophyll Absorption Ratio Index	(Haboudane et al. 2002)
TCARI/OSAVI	Transformed Chlorophyll Absorption Ratio Index/ Optimized Soil Adjusted Vegetation Index	(Haboudane et al. 2002)
TCI	Triangular chlorophyll index	(Hunt et al. 2012)

1.2.4. Dilution curve theory and NNI determination

The critical N dilution curves of both maize and durum wheat crops were calculated using Equation 1, adopting the crops specific coefficients (a , b) proposed for maize and wheat by (Plénet and Lemaire 2000) ($a = 3.4$ $b = 0.37$) and (Justes et al. 1994) ($a = 5.35$, $b = 0.442$) respectively.

$$Nc \% = aW^{-b} \quad (1)$$

W is above-ground biomass (t ha^{-1}), a represents the plant N concentration when W is 1 t ha^{-1} and b is a dimensionless coefficient. N_c % is constantly considered 3.4% for $W < 1 \text{ t ha}^{-1}$ for maize and 4.4% for $W < 1.55 \text{ t ha}^{-1}$ for wheat according to the literature (Plénet and Lemaire 2000; Justes et al. 1994).

The NNI was calculated using Equation 2 as the ratio between the actual plant N concentration (N_a %) and the critical plant N concentration (N_c %):

$$NNI = \frac{N_a}{N_c} \quad (2)$$

and was used to define conditions of N deficiency ($NNI < 0.9$), N surplus ($NNI > 1.1$) and optimal status ($0.9 < NNI < 1.1$). NNI can also be determined as the ratio between the actual plant N uptake (PNU_a , kg ha^{-1}) and the critical plant N uptake (PNU_c , kg ha^{-1}). PNU_a and PNU_c were calculated by multiplying W by N_a % and N_c % respectively.

$$\Delta PNU = PNU_a - PNU_c \quad (3)$$

Excess or deficit of nitrogen uptake can be determined by the relationship between the nitrogen absorbed by the crop (PNU_a kg ha^{-1}) and its biomass (Guerif et al. 2007). Then a quantitative indicator ΔPNU (kg ha^{-1}) (Houlès et al. 2007) can be determined using Equation 3 and directly used to calculate the N rates (increase or reduction of planned dose) to be considered for site specific fertilization purposes.

1.2.5. NNI estimation from remote sensing

In this study two approaches have been tested for NNI calculation from RS data. For method 1 (DM) a linear regression between selected Vis and field measured N_a % and NNI were derived, while for method 2 (IM) $S2_Bvs$ were first calculated from SNAP biophysical toolbox and then linear regressions to measured PNU_a and W were exploited in order to determine NNI. The studies of Houlès et al. (2007) and Guerif et al. (2007) proposed three indirect methods to estimate PNU_a and PNU_c from RS data, and then for calculating ΔPNU or NNI, evaluating the third scheme as the best in terms of prediction. This scheme consists of calculating PNU_a directly from CCC using experimental relationships and then obtaining PNU_c by estimating W from GAI. The NNI values obtained from the two methods were then compared with field measured NNI. In order to further test the information content of NNI as a proxy for nitrogen deficiency diagnosis, an NNI map for all the

2018 maize cultivated fields was produced with S2 data and compared to field level reported yield. The regression model between VIs and BVs calculated from S2 images and crop variables collected during field experiments were analyzed using statistical parameters using R 3.5 version software (R Core Team 2018). The performance of the different regression models was estimated by analyzing the coefficient of determination (R^2), mean absolute error (MAE), root mean square error (RMSE) (Table 8).

Table 8. Statistics used to compare model's performance in regression analysis; p is value derived from RS (Vis and S2_BVs) and o is the observed value (BVs). $\bar{O}$ is the mean value of o based on N observations.

Statistic	Equation
Coefficient of determination	$R^2 = 1 - \frac{\sum (p_i - o_i)^2}{\sum (p_i - \bar{o}_i)^2}$
Mean Absolute Error	$MAE = \frac{1}{N} \sum_{i=1}^N p_i - o_i $
Root Mean Square error	$RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^N (p_i - o_i)^2}$

The results of soil laboratory analyses were assessed using one way-analysis of variance (ANOVA) and Tukey HSD post hoc test ($p < 0.05$) in statistical software R 3.5 version (R Core Team 2018) to evaluate differences in soil properties among soil classes.

1.3. Results

1.3.1. Soil variability in the experimental field

In general, laboratory analyses revealed that the field had a clay soil (63%) with generally good fertility, as indicated by high total organic carbon (TOC) and soil organic matter (SOM) (6%), moderate total N content (0.34%) and good C/N (10.25%). The soils of the farm are all fairly young, very deep and with a lot of organic matter typical of peaty soils. They are alluvial soils with very high intra-field variability. Fertility, understood as the content of nutrients such as SOM, N and TOC, is not generally a problem, but their availability is linked to the high variability of other elements and frequent problems of water stagnation. The complete results of the laboratory analyses carried out on each sample are given in the following Table 9:

Table 9: Results of single soil sample analysis

ID	Soil class	N total (%)	TOC (%)	C / N	SOM (%)	Clay (%)	Silt (%)	Sand (%)	pH (-)	texture
1	CDS2	0.324	3.48	10.76	6.00	56.25	34.72	9.03	7.53	Clay
2	CDS2	0.345	3.72	10.78	6.41	65.63	22.66	11.71	7.53	Clay
3	CDS2	0.339	3.37	9.96	5.82	60.36	25.38	14.26	7.59	Clay
4	JOL3	0.297	2.72	9.15	4.69	70.23	21.57	8.20	7.59	Clay
5	JOL1	0.310	3.00	9.67	5.18	65.87	27.26	6.87	7.55	Clay
6	JOL1	0.267	2.63	9.87	4.54	69.80	22.83	7.37	7.64	Clay
7	JOL1	0.325	3.41	10.49	5.88	61.11	28.47	10.42	7.55	Clay
8	JOL1	0.349	3.50	10.02	6.03	67.53	22.26	10.20	7.50	Clay
9	JOL1	0.299	3.01	10.07	5.19	61.94	27.34	10.72	7.58	Clay
10	CDS2	0.298	3.09	10.37	5.32	51.42	23.84	24.74	7.59	Clay
11	CDS2	0.307	3.23	10.52	5.57	62.42	30.77	6.81	7.61	Clay
12	CDS2	0.316	3.02	9.56	5.21	68.07	22.56	9.37	7.55	Clay
13	CDS2	0.345	3.53	10.24	6.09	63.48	26.19	10.33	7.56	Clay
14	CDS2	0.341	3.40	9.96	5.86	67.98	20.13	11.90	7.57	Clay
15	CDS2	0.316	3.24	10.26	5.59	65.28	21.53	13.20	7.55	Clay
16	CDS2	0.346	3.75	10.84	6.46	68.93	18.79	12.29	7.68	Clay
17	CDS2	0.365	4.02	11.02	6.92	71.05	17.53	11.42	7.45	Clay
18	JOL3	0.326	3.44	10.55	5.93	65.64	23.53	10.83	7.60	Clay
19	JOL3	0.301	2.63	8.73	4.53	64.52	23.08	12.41	7.68	Clay
20	JOL3	0.319	3.40	10.65	5.86	57.67	23.84	18.49	7.63	Clay
21	JOL3	0.356	3.58	10.05	6.17	65.08	24.10	10.83	7.46	Clay
22	JOL3	0.286	2.82	9.87	4.87	64.13	20.76	15.12	7.68	Clay
23	JOL3	0.323	3.30	10.22	5.69	64.54	19.04	16.42	7.28	Clay
24	JOL3	0.318	3.28	10.33	5.66	61.49	24.91	13.60	7.19	Clay
25	JOL3	0.448	4.44	9.91	7.66	58.53	23.36	18.12	4.81	Clay
26	JOL3	0.306	3.43	11.19	5.91	66.57	23.60	9.83	7.50	Clay
27	JOL1	0.352	4.05	11.51	6.98	59.88	25.74	14.38	7.66	Clay
28	JOL1	0.327	3.54	10.82	6.10	57.73	27.34	14.93	7.73	Clay
29	JOL1	0.322	3.77	11.73	6.50	58.53	27.15	14.32	7.75	Clay
30	JOL1	0.332	3.53	10.61	6.08	58.92	25.88	15.20	7.69	Clay
31	JOL1	0.299	3.60	12.02	6.21	61.52	22.30	16.18	7.60	Clay
32	FOR1	0.445	4.81	10.81	8.30	63.36	17.39	19.25	5.01	Clay
33	FOR1	0.432	3.78	8.75	6.52	57.83	33.41	8.76	4.99	Clay
34	FOR1	0.408	4.54	11.12	7.82	61.02	28.67	10.31	5.20	Clay
35	FOR1	0.292	2.48	8.52	4.28	66.66	31.02	2.32	7.30	Clay
36	FOR1	0.433	4.37	10.11	7.54	62.34	23.75	13.91	4.89	Clay

The comparison among soil classes showed a statistically significant difference in pH and in total N (%) for FOR1 class with respect to the others (Table 10). The differences in pH, sub-acidic for FOR1 (pH=5.45) compared to the sub-alkaline of the other classes (pH~7.5), respect to N total content, are considered quite important because of: *i)* the general high amount of N total in the soil; *ii)* the potential impact of pH on elements availability in the soil for plant nutrition.

Table 10: Results of the soil sampling carried out in the experimental field.

Soil Class	N total (%)	TOC (%)	C / N (%)	SOM (%)	Clay (%)	Silt (%)	Sand (%)	pH (-)
CDS2	0.33 a	3.44	10.39	5.93	63.72	24.01	12.28	7.56 a
JOL3	0.33 a	3.30	10.07	5.7	63.84	22.78	13.39	7.24 a
JOL1	0.32 a	3.40	10.68	5.87	62.28	25.66	12.06	7.63 a
FOR1	0.40 b	4.00	9.86	6.89	62.24	26.85	10.91	5.48 b
Mean	0.34*	3.54	10.25	6.1	63.02	24.82	12.16	6.98*

* Significant differences among means ($p < 0.05$). The letters a,b,c represent statistically significant difference among groups ($p < 0.05$) in post-hoc test; * significant differences among means ($p < 0.05$).

1.3.2. Crop response to fertilization and soil influence

1.3.2.1. Plant growth and yield

During the 2018 season, maize was harvested at the phenological phase of milky stage to produce silage of maize mash (grains and maize cobs). The harvest was weighed at the farm weighbridge showing a yield of 14.2 t ha^{-1} . In 2018, the farm harvested 278 ha of silage maize in total, with an overall average yield of 18.22 t ha^{-1} , therefore the experimental field produced less than the farm average (considering FAO 600-700 classes) and less than the variety yield potential (HYDRO FAO 700). The 1st of June 2018 during phase BBCH 15 the whole farm and the experimental field were affected by a hailstorm that caused serious damage to plant foliage with different intensity for each field.

For the maize experiment, crop response to variable N fertilization showed different patterns depending on N rate and soil classes. In general, it is expected that maize biomass would increase with increasing N rates (Cordero et al. 2019; Xia et al. 2016; Justes et al. 1994). However, in our case, biomass response was strongly influenced by soil properties as shown in Figure 12 and the biomass trends are not always correlated with increasing N rates indicating that other limiting factors might have affected crop N uptake.

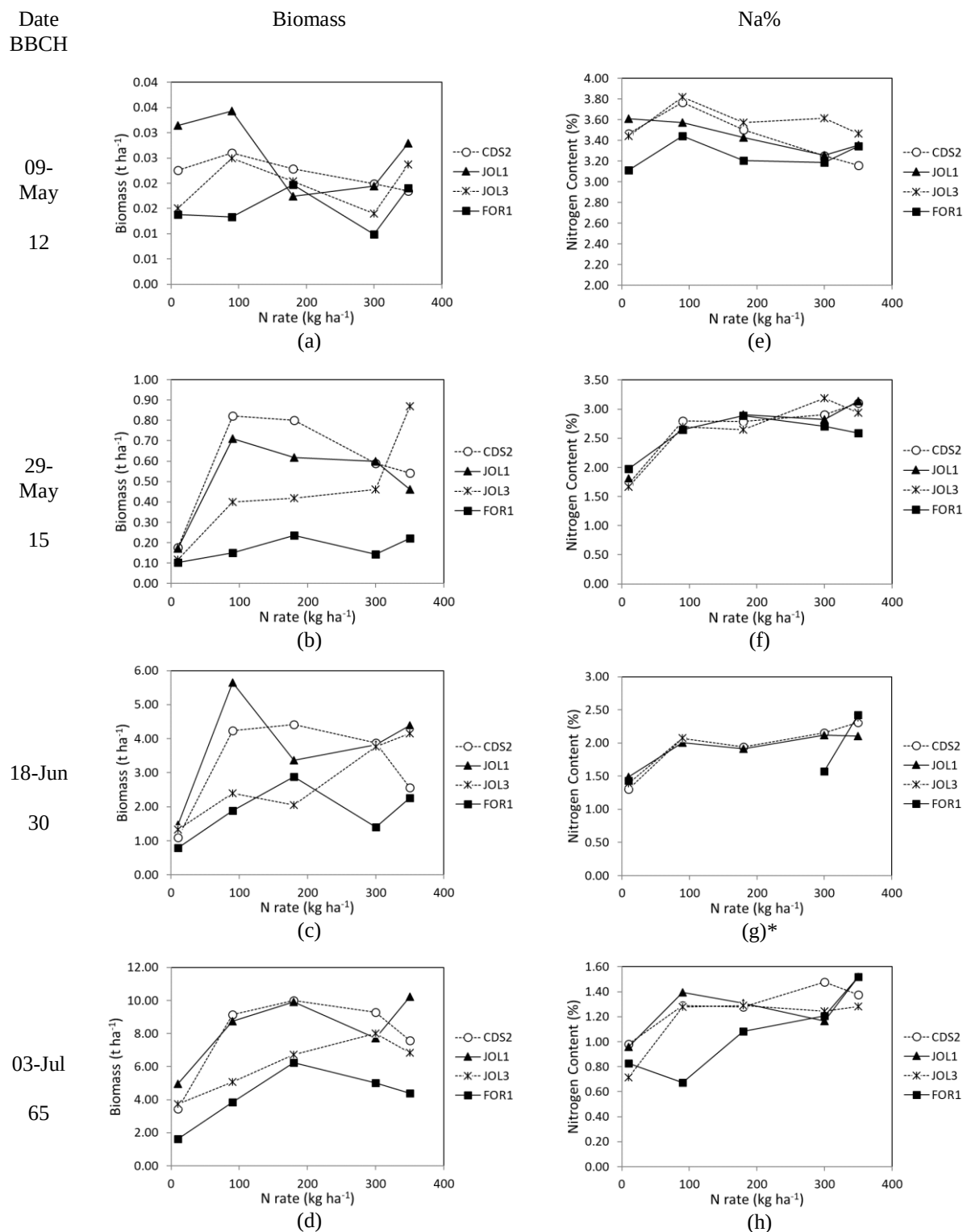


Figure 12: Response of maize above ground biomass and Na% to N fertilizer rates, measured during the 2018 field campaigns in the different soil classes (see Table 10): a) 09-May BBCH 12 b) 29-May BBCH 15; c) 18-Jun BBCH 30; d) 03-Jul BBCH 65. Plant nitrogen concentration e) 09-May BBCH 12, f) 29-May BBCH 15; g) 18-Jun BBCH 30; h) 03-Jul BBCH 65. * Na% missing values for FOR1 class due to samples contamination

On the first measurement date (09th of May, BBCH 12) the effect of N fertilization on maize biomass was still not visible (Figure 12a). On the other dates, whereas for most soil classes a rate of

90 or 180 kg N ha⁻¹ allowed to reach the maximum biomass, the plots with soil type FOR1 showed a remarkably lower response to N (Figure 12b, c and d). This effect was probably due to other nutritional limitations, caused by the low pH of this soil class (Table 10). For example, it can be seen from Figure 11, that the JOL1 soil class is characterized by increasing biomass values according to N application on both phases, BBCH 15 (Figure 12b) and BBCH 65 (Figure 12d), whereas the FOR1 soil class remained stable with a lower biomass accumulation. In general, a limited or negative effect on biomass production was obtained for the highest N rates in this experiment. In particular, the range of measured biomass values at flowering (Table 11), which are expected to be related to the final yield, highlights how the biomass values reached in JOL1 almost doubled the biomass level achieved in the FOR1 soil class and how the biomass in the different soil classes are statistically different.

Table 11: Maize above ground biomass (t ha⁻¹) measured during the last field campaign of 2018 (BBCH 65) and analyzed by the ANOVA method (different letters are significantly different at $p < 0.05$).

Soil Class	Max	Min	Mean	Tukey's post-hoc test
JOL1	10.2	5.0	8.3	a
CDS2	10.0	3.4	7.9	ab
JOL3	8.0	3.7	6.1	ab
FOR1	6.3	1.6	4.2	b

Different N treatments showed a substantial effect on Na% changes across different growth stages except for the first measurement date (Figure 12). During subsequent growth phases, similar patterns of response of Na% to N rates were observed among the soil classes. Unlike biomass, Na% showed increasing concentration values for increasing N rates in all soil classes. The FOR1 class achieved high concentrations of nitrogen in highly fertilized plots as opposed to what was seen for biomass (Figure 12f, g, h).

Concerning the durum wheat 2019 experiment, marked effects of different soil classes of the field, on crop response to N rates, were also observed. In Figure 13, the results of only 2 field measurement dates are presented, due to lodging problems caused by strong winds occurring later in the season. At the BBCH 31 stage, plots in JOL1 registered the highest biomass values (Figure 13b) in all N treatments (reaching 3.24 t ha⁻¹ at the highest N4 rate), while plots in FOR1 yielded the lowest biomass, less than half of the total biomass of JOL1 at the highest N rate (1.1 t ha⁻¹). The interpretation of Na% trend in wheat is more complex with respect to maize, since no clear response to N rate is visible (Figure 13), especially for the FOR1 soil class, which showed low Na% values for high levels of N fertilization. These results confirm differences of crop response to different soil classes to the N

fertilization in both years. In some zones of the field (JOL1, CDS2), the crop managed to reach substantial W and Na% values, even if it was less fertilized, whereas in others it was not able to produce high biomass even if it was highly fertilized (FOR1). An apparent non responsiveness of crop biomass and N content to N fertilization rates in a specific zone of the field, was observed for both seasons and crops, and can be associated to static variables of soil properties, such as in this case the pH.

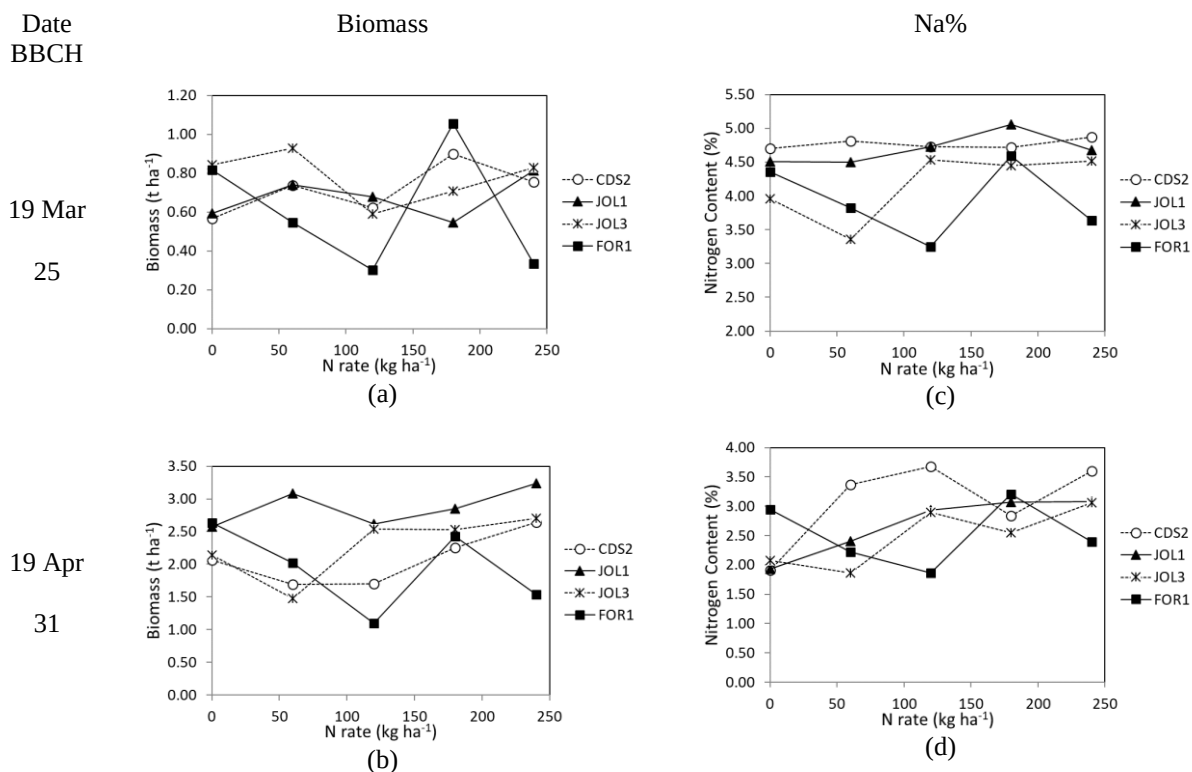


Figure 13: Response of wheat above ground biomass (W) and Na% to N fertilizer rates, measured during the 2019 field campaigns in the different soil classes (see Table 10): a) W at 19 Mar BBCH 25; b) W at 19 Apr BBCH 31 c) Na% at 19 Mar BBCH 25; d) Na% at 19 Apr BBCH 31.

1.3.2.2. Nutritional status – NNI values

Figure 14 provides a representation of crop nutritional status for each measured plot in relation to N supply, data are plotted in the W (x-axis) and Na space (y-axis) and compared to the critical N from the dilution curves for maize (Plénet and Lemaire 2000) and durum wheat (Justes et al. 1994).

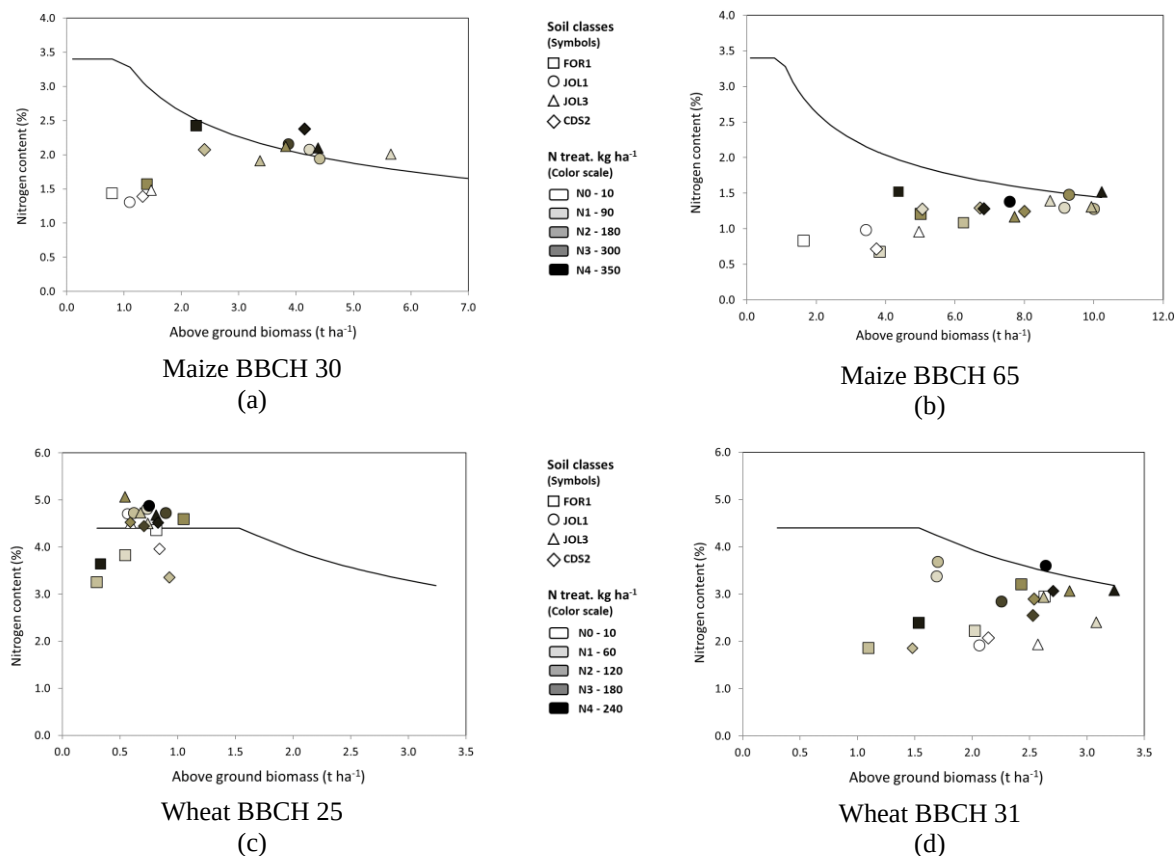


Figure 14: Relationships between biomass and N content for different soil classes at different N rates compared to crop specific critical N dilution curve for maize and wheat.

The calculated NNI values ranged between 0.42 and 1.12 and 0.43 and 1.15 for maize and wheat respectively revealing the condition of plot with N deficiency ($NNI < 0.9$). NNI values under varied N applications did not show a clear gradient as expected and observed in other studies (Zhao, Duan, et al. 2018).

In Figure 14, the grayscale of the symbols, from light grey to black, is proportional to the N application. It would be expected that brighter grey points (N0 and N1 treatments) would have been more distant from the critical curve with respect to the darker ones (N3 and N4 treatments). This is visible only for the points corresponding to the low doses (N0-N1-N2), but it is not equally respected for the high doses (N3 and N4), evidencing how other factors might have limited nitrogen uptake and plant growth even in non-limiting N conditions. The NNI values for N0 and N1 generally decreased during all BBCH phases, whereas for N3 and N4 treatment NNI remained substantially stable (data not shown). NNI values for N3 and N4 treatment in FOR1 soil class did not reach the value of 1 for both the considered seasons and crops. This finding indicates a sub optimal N condition (Figure 14) whereas CDS2 and JOL2 were very often higher than 0.9. Substantial differences were observed under different soil classes, where classes FOR1 and JOL1 markedly differed in NNI values

especially in N4 treatment. FOR1 had a constant limiting factor that influenced N uptake regardless of N applications.

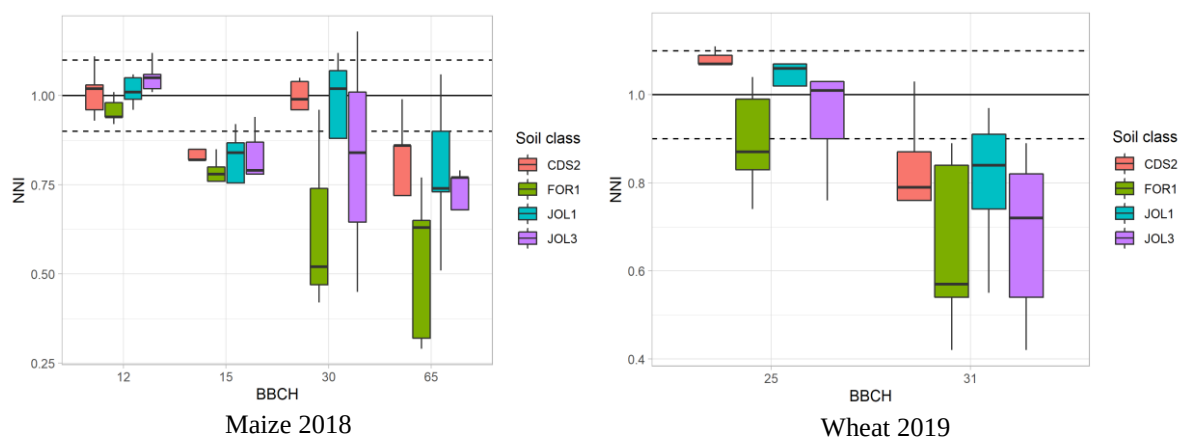


Figure 15: Boxplot of NNI values during different phenological phases according to soil classes identified in Table 10. All 5 N treatments are included in each boxplot. The horizontal continuous line indicates the N sufficiency, horizontal dot lines at NNI of 0.9 and 1.1 represent good nutritional conditions of the crop.

The boxplot of Figure 15 shows how NNI varied during the growth seasons for maize (2018) and wheat (2019). The general non-optimal nutritional condition of maize and wheat crop in the experiment, especially late in the growth season, is highlighted by many $N_a\%$ values lower than the critical concentration represented by the dilution curve. Only a few plots reached optimal nutritional condition with NNI values greater than 0.9. The effects of soil class properties on NNI were statistically non-significant ($p < 0.05$), but FOR1 class constantly had values unequivocally lower than 0.9 during the season independently of the fertilization rate. Instead, all the plots in classes CDS2 and JOL1 had higher values and no stress for maize (wheat) at BBCH 12 and 30 (25) was detected. The different behavior emerged in the different areas of the field regardless of the nitrogen fertilization class, suggesting that the capability of the plants to absorb nutrients and therefore nitrogen depended on different properties of the soil and not only on the quantity of nitrogen that was given with fertilizers. In this case the sub-acid pH of FOR1 class limited more the other classes the absorption of nitrogen available in soil and provided by fertilization.

1.3.3. Remote Sensing estimation of NNI

1.3.3.1. S2 BVs accuracy

Before considering NNI estimation with indirect methods, S2_BVs retrieval performance, as obtained from the SNAP biophysical processor, was analyzed (Figure 15). From our experimental data GAI_S2 for maize are well correlated with field estimates ($R^2=0.87$ and $RMSE=0.58$). Lower

performances are obtained for wheat ($R^2=0.41$ and $RMSE=0.81$) probably due to smaller dataset, however Sentinel-2 retrievals generally produced higher values than the field measurements, i.e. over-estimation (Figure 16). The obtained accuracy metrics are comparable to those reported by (Delloye et al. 2018) and also in their study the trend of GAI_S2 values showed some bias (over-estimation).

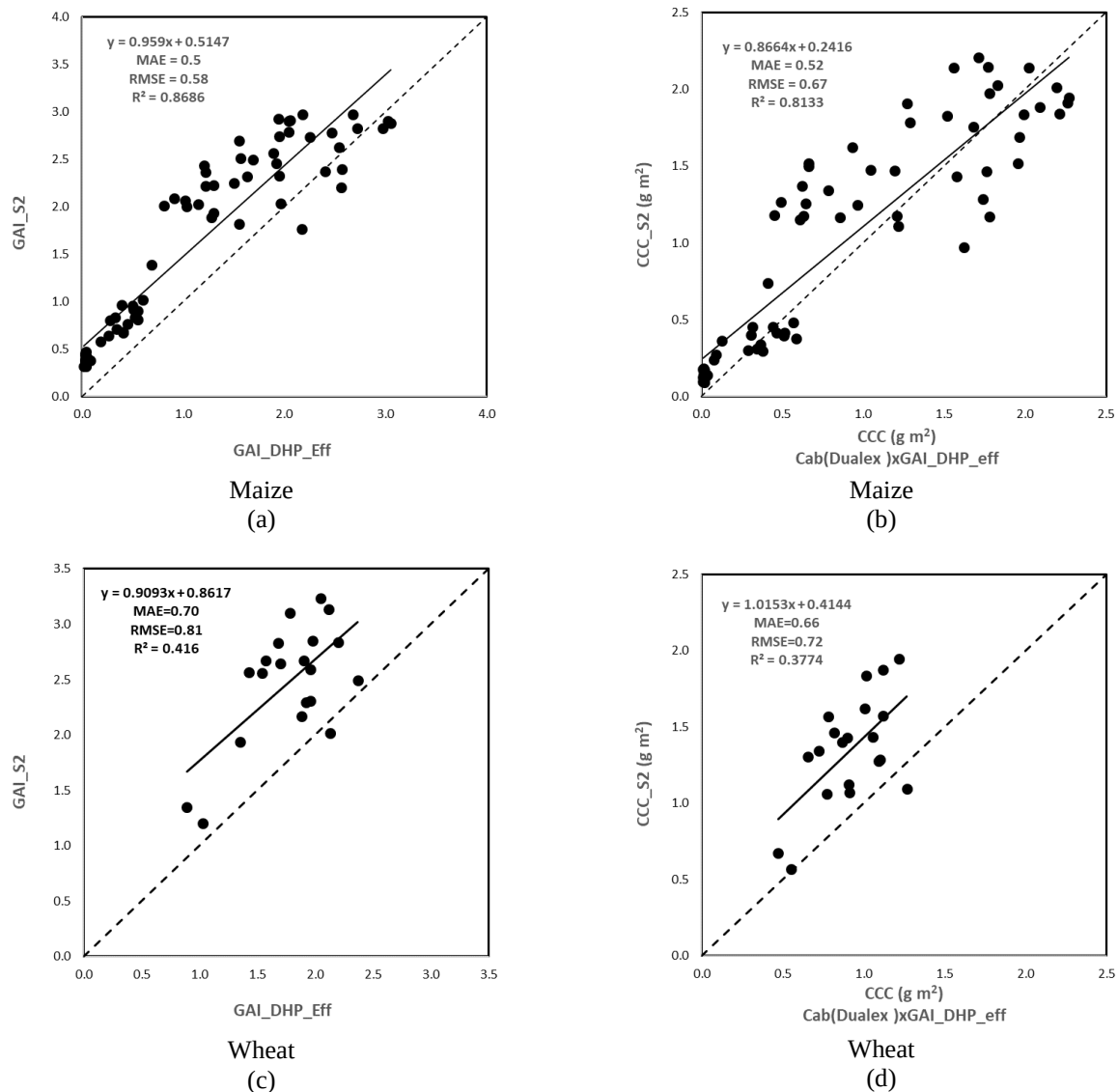


Figure 16: Scatterplot and statistics computed for GAI and CCC retrieved from Sentinel-2 images. Relationships in maize crop from 2nd leaf stage to silking stage (4 S2 images) between: a) GAI estimated from S2 (GAI_S2) and measured GAI (GAI_DHP_effective); b) CCC from S2 (CCC_S2) and measured CCC (Cab Dualex x GAI_DHP_eff). Relationships in wheat crop for tillering between: c) GAI from S2 (GAI_S2) and measured GAI (GAI_DHP_effective); d) CCC estimated from S2 (CCC_S2) and measured CCC (Cab Dualex x GAI_DHP_eff).

For what concerns CCC_S2 for maize, the estimations are well correlated with field measurements ($R^2=0.81$ and $RMSE=0.67$) even though there is an overestimation at the beginning of the season and an underestimation towards the end. Worse performances were again obtained for wheat, with $R^2=0.38$ and $RMSE=0.72$. It is important to note that in 2019 for wheat a shorter

phenological period was monitored due to cloud contamination in EO data that did not allow to analyze images when field data were acquired. These results are in general in agreement with those reported in the literature (Delloye et al. 2018; Sehgal et al. 2016).

1.3.3.2. Crop nutritional status estimation

1.3.3.2.1. Direct method

The analyses for maize crop 2018 data (Table 12) show that there were highly significant relationships between NNI and NDRE ($R^2=0.79$; RMSE= 0.26; MAE= 0.2) and GNVI ($R^2=0.77$; RMSE= 0.27; MAE=0.22) (Figure 17).

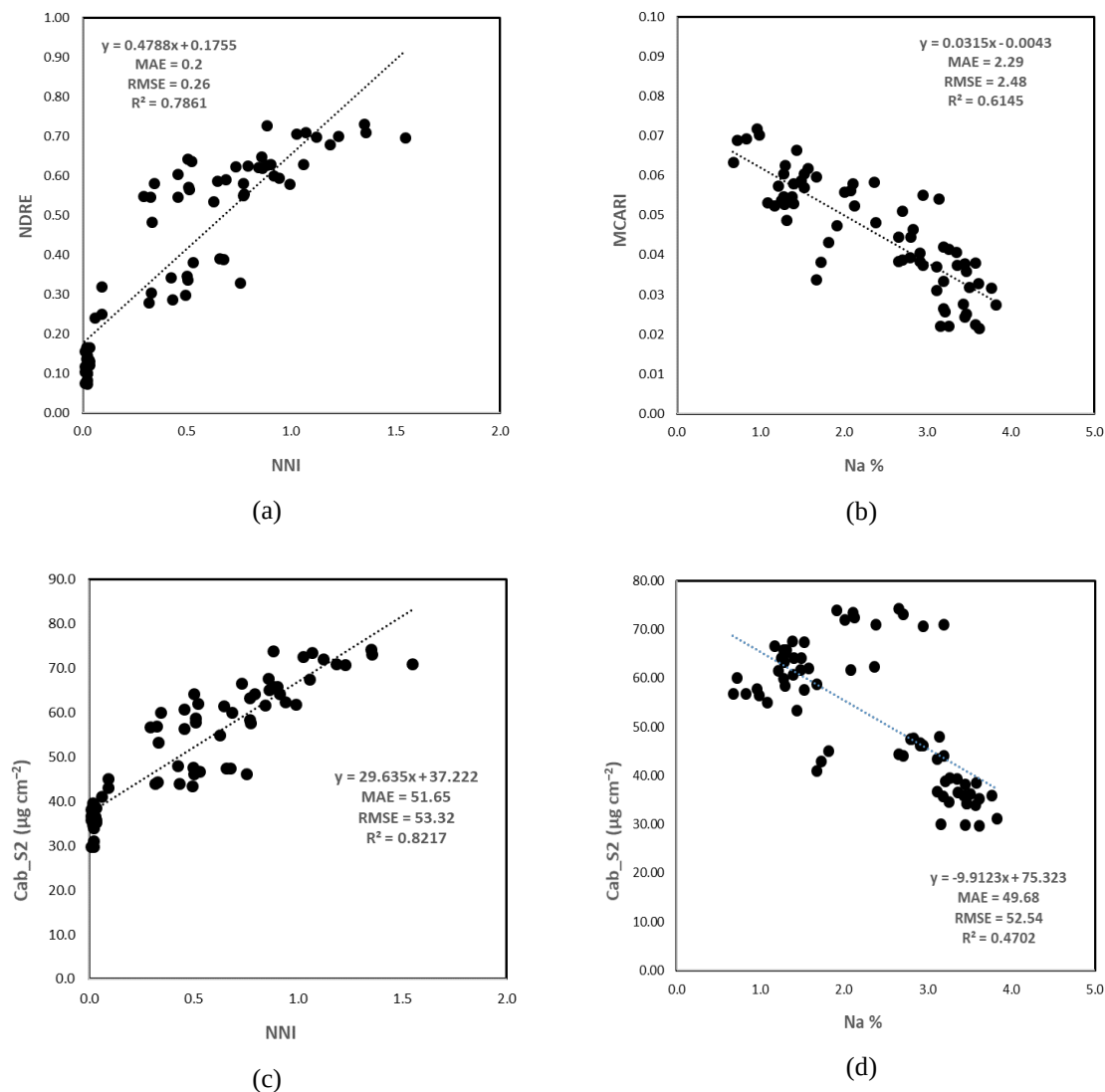


Figure 17: Scatterplots and statistics for S2_BVs and VIs derived from Sentinel-2 data (4 S2 images) and field data from 2nd leaf stage to silking stage in maize crop 2018. Relationships between: a) Normalized difference red edge index (NDRE) and Nitrogen Nutrition Index (NNI) from ground data; b) Modified Chlorophyll Absorption in Reflectance Index (MCARI) and plant nitrogen content (Na%) from ground data; c) Chlorophyll content (Cab_S2 from S2 (GAI_S2/CCC_S2) and Nitrogen

Nutrition Index (NNI) from ground data; d) Chlorophyll content (Cab_S2 from S2 (GAI_S2/CCC_S2) and plant nitrogen content (Na%).

The highest R^2 and the smallest RMSE confirm the usefulness of VIs that exploit the Red-edge spectral region (e.g. NDRE) to provide prediction of leaf N content as mentioned in many studies (Cammarano et al. 2011; Feng et al. 2015; Li et al. 2014; Miao et al. 2009). We also found a good relationship between Na% and MCARI ($R^2=0.71$; RMSE= 2.48; MAE= 2.29), as previously shown for the same crop by Cilia et al. (2014), but in general VIs achieved worse results as compared to S2_BV for retrieving BV as W, CCC and PNUa. For this reason, these results suggest using VIs to directly derive NNI instead of calculating W and Na% and then indirectly deriving NNI values.

Table 12: Coefficients of determination for the linear regressions between field measured or calculated BVs and satellite (S2) derived Vis and S2_BVs for maize crop 2018. Only statistically significant values are reported.

Variable	W	GAI DHP	GAI DHP	Cab	CCC1 ^a	CCC2 ^b	Na	NNI	PNUa
Unit	t ha ⁻¹	Effective	True	µg cm ²	g m ²	g m ²	%		kg ha ⁻¹
GAI_S2	0.68	0.87	0.91	0.25	0.77	0.81	0.56	0.74	0.75
CCC_S2 (g m ²) ^c	0.65	0.84	0.88	0.28	0.77	0.81	0.46	0.76	0.77
Cab_S2 (g m ²) ^d	0.54	0.77	0.83	0.30	0.69	0.76	0.47	0.82	0.70
GNDVI	0.56	0.78	0.86	0.26	0.67	0.74	0.58	0.77	0.66
MCARI	0.34	0.48	0.56		0.32	0.37	0.71	0.33	0.29
MCARI2	0.53	0.76	0.84	0.23	0.64	0.71	0.59	0.74	0.63
MSAVI	0.55	0.77	0.85	0.23	0.65	0.72	0.63	0.73	0.63
MTVI2	0.53	0.76	0.84	0.23	0.64	0.71	0.59	0.74	0.63
NDRE	0.51	0.75	0.83	0.26	0.64	0.71	0.56	0.79	0.64
NDVI	0.48	0.72	0.80	0.21	0.59	0.66	0.56	0.76	0.60
OSAVI	0.53	0.75	0.84	0.24	0.63	0.70	0.61	0.74	0.62
SAVI	0.56	0.78	0.86	0.24	0.66	0.73	0.61	0.73	0.65
TCARI	0.43	0.64	0.72	0.28	0.54	0.62	0.54	0.74	0.54
TCARI/OSAVI							0.47		

^a CCC1 expressed as GAI_DHP_effective multiplied by Cab Dualex;

^b CCC2 expressed as GAI_DHP_true multiplied by Cab Dualex;

^c CCC_S2 expressed as LAIxCab (SNAP BV product);

^d Cab_S2 expressed as GAI_S2 divided by CCC_S2

An analysis of the relationships between S2 BV and nutritional status indicators was also performed (Table 12). No index reached the performance of the GAI_S2 for biomass estimation ($R^2=0.68$) confirming the correctness in the use of this variable to derive W. Chlorophyll related variables (CCC_S2 and Cab_S2) resulted well correlated with NNI, comparably to VIs analysis, but not so well with Na%. A better relationship was found between NNI and leaf chlorophyll Cab_S2 ($R^2=0.82$) where Cab is estimated as the ratio of the two variables output (S2_BVs) from the SNAP Sentinel-2 biophysical processor, i.e. CCC_S2 and GAI_S2. Being Cab_S2 a derived secondary

variable, the use of CCC_S2 ($R^2=0.77$) in NNI estimation could be preferred to that of the leaf chlorophyll content. In general, good results were found for BV retrieval (W, CCC, PNUa) that suggest the possibility to use both direct or indirect methods to derive NNI.

Table 13: Coefficients of determination for the relationship between field measured or calculated BVs and satellite (S2) derived Vis and S2_BVs for wheat crop 2019. Only statistically significant values are reported.

Variable	W	GAI DHP	GAI DHP	Cab	CCC1 ^a	CCC2 ^b	Na	NNI	PNUa
Unit	t ha ⁻¹	Effective	True	µg cm ²	g m ²	g m ²	%		kg ha ⁻¹
GAI_S2	0.36	0.42	0.39		0.37	0.35			0.33
CCC_S2 (g m ²) ^c	0.35	0.37	0.37		0.37	0.38			0.27
Cab_S2 (g m ²) ^d	0.28	0.21	0.27		0.30	0.38			
GNDVI	0.45	0.45	0.44		0.46	0.46			0.39
MCARI		0.32	0.20		0.18	0.10	0.48	0.47	0.31
MCARI2	0.36	0.46	0.36		0.36	0.29	0.35	0.35	0.42
MSAVI	0.36	0.46	0.36		0.36	0.29	0.35	0.35	0.42
MTVI2	0.47	0.46	0.45		0.48	0.48			0.39
NDRE	0.42	0.50	0.43		0.41	0.36	0.32	0.31	0.45
NDVI	0.39	0.48	0.39		0.38	0.31	0.36	0.36	0.44
OSAVI	0.36	0.46	0.36		0.35	0.28	0.38	0.38	0.43
SAVI				0.34			0.61	0.61	
TCARI				0.42			0.40	0.40	
TCARI/OSAVI		0.29		0.23			0.54	0.53	0.29

^a CCC1 expressed as GAI_DHP_effective multiplied by Cab Dualex;

^b CCC2 expressed as GAI_DHP_true multiplied by Cab Dualex;

^c CCC_S2 expressed as LAIxCab (SNAP BV product);

^d Cab_S2 expressed as GAI_S2 divided by CCC_S2

For wheat crop 2019 data (Table 13) only one S2 image was available during the key phenological phases and close to the field measurements, due to cloud occurrence and the lodging problems that affected the crop before flowering. The partial results for wheat were affected by dependence of the relationship with crop phenology and environmental conditions (Delloye et al. 2018; Hamblin et al. 2014) and cannot be a valid exportable predictive model. Despite this, TCARI obtained the best performance in Na% ($R^2=0.61$, RMSE=4.28) and NNI ($R^2=0.61$ RMSE=0.87) retrieval for direct estimation.

1.3.3.2.2. Indirect method

Indirect methods require the capacity to directly estimate different biophysical variables related to crop status. The advantage of using PNUa instead of NNI indicator is because it is possible to quantitatively estimate crop N deficit/surplus (Guerif et al. 2007; Houlès et al. 2007) and then use this amount to make N prescription maps. This analysis was performed only on maize data because S2 data were available across the whole 2018 crop season. Figure 18 shows the relationship between

GAI_S2 and W for maize, described by a logarithmic function, as expected, with good accuracy $R^2=0.84$ and $RMSE=2.60$. The variable GAI_S2 can be used to estimate through empirical relationships W. From these estimates PNUc can be derived using the crop specific critical canopy nitrogen content curve. To further calculate ΔPNU , it is crucial to accurately estimate PNUa. The correlation between CCC_S2 and PNUa shows a R^2 of 0.77 that is in line with what achieved by Delloye et al. (2018) and Xia et al. (2016), but with a higher RMSE ($RMSE=61.12$; $MAE=41.26$).

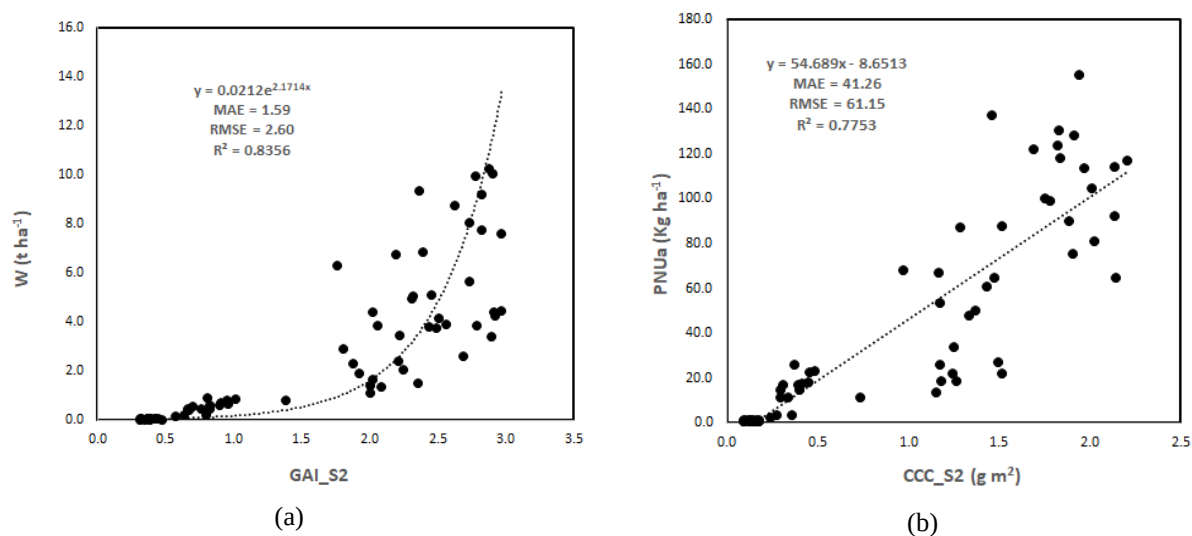


Figure 18: Relationships between biophysical variables retrieved from Sentinel-2 (4 S2 images) and ground measured in maize crop 2018; (a) above ground biomass (W) and Green Area Index from S2 (GAI_S2); (b) Nitrogen uptake (PNUa) and Canopy Chlorophyll Content from S2 (CCC_S2).

NNI estimates derived from the S2 image of 8th of July with the two methods (direct and indirect) showed similar results if compared with NNI field data. For a single image, with the direct method using the NDRE index we achieved a R^2 of 0.62 ($RMSE=0.21$), whereas with the indirect method using NNI obtained by the ratio between PNUa and PNUc, we obtained a R^2 of 0.63 ($RMSE=0.27$).

1.3.4. NNI map and yield relation

A NNI map for all the 2018 maize fields of the farm was produced and compared to reported yield at field level. The S2 image of the 8th of July, corresponding to the end of maize vegetative phase, was used to generate a NNI map using the NDRE relationship (Figure 18). The NNI map represents, at the beginning of flowering, when the fertilization period is concluded, a proxy of the potential end-of-season yield, if no other limiting factors are occurring (e.g. water stress, pest, disease or lodging).

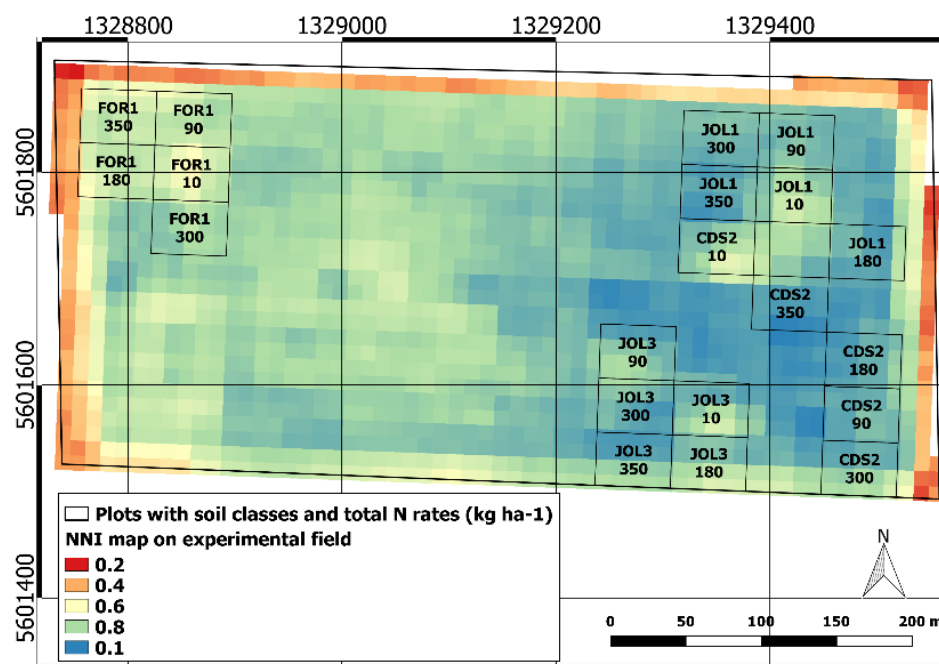


Figure 19: NNI map derived on the experimental field with the direct method from the NDRE of the S2 image of 08-July. Rectangles show the locations of the plots, the corresponding soil classes (see Figure 1) and total N rates provided during the season (kg N ha^{-1})

The NNI map derived using NDRE showed the variability and the low NNI values of the crop in the experimental field (Figure 19) especially for FOR1 plots (top left of Figure 19). Anova test confirmed that field level NNI values were well correlated with the final yield and maize group (FAO class 600-700) according to nutritional conditions at flowering. The most productive fields had the higher NNI values and vice versa and the NNI map generally predicted well the final yield (Table 14). Correlation analysis between NNI and yield revealed a significant positive correlation (Pearson's $r = 0.6$).

Table 14: Yield and NNI mean values (directly derived using S2 image of 08 July) of each maize field of the farm analyzed using ANOVA method (different letters are significantly different at $p < 0.05$).

Maize field ID	NNI mean value	Tukey's post-hoc test	Yield t ha ⁻¹
9	0.90	a	22.1
175	0.90	a	18.5
10	0.89	a	24.2
11	0.88	ab	24.1
258	0.86	bc	19.5
8	0.83	cd	17.8
308	0.83	d	18.0
312	0.82	d	13.0
368	0.78	e	19.5
Exp. Field	0.77	e	14.2
331	0.69	f	19.5
467	0.68	f	16.0
92	0.58	g	10.5
91	0.5	h	16.0

1.4. Discussion

It is recognized that seasonal N status assessment can be performed using the NNI indicator (Lemaire, Gilles; Meynard, J., M. 1997), however, not much investigation is present in literature about the interaction between detected crop N deficiency and actual source of crop growth limiting factors. NNI can be used to highlight which zones of a field present crop anomalous conditions. $NNI > 1$ represents “luxury consumption” and $NNI < 1$ “nitrogen deficiency”. Usually this information is used to tactically modulate nitrogen fertilization with VRT machinery, providing the appropriate amount of fertilizer. However, in some cases, plant N deficiency is not related to nitrogen availability, but to other factors that must be identified and taken into account, to support the most appropriate fertilization strategy. The study completed in F1, highlighted that NNI is an appropriate tool for the diagnosis of the nutritional status of the crops, however soil properties can have an impact on crop N uptake, showing an effect independent from N rates supply. The capacity of NNI to assess crop nutritional status in relation to soil properties was confirmed for two consecutive crop seasons. In our case, measured NNI showed that the experimental plots located in an area of the field with acidic soil (FOR1) had lower NNI values than all the other plots in the other soils' types both for maize and wheat. High N rates did not increase Na% and consequently crops biomass in the problematic soil area of the field (FOR1), as expected and as demonstrated by other studies (Justes et al. 1994; Plénet and Lemaire 2000; Zhao, Ata-Ul-Karim, et al. 2018). In addition to this, the high rate plots in FOR1 class reached only half of the biomass accumulation (4 t ha⁻¹) as compared to the best performance of JOL1 and CDS2 soil classes (8 t ha⁻¹) and the two classes are statistically different considering this

variable. It is well known that most plant nutrients are optimally available to plants within soil pH range between 6.5 and 7.5 and soil classes of the field experiment belonged to this range except FOR1. Nutrients absorption depends on elements availability in the root zone and the growth rate potential, even in N excessive condition, is regulated by the soil N supply (Zhao et al. 2018). N fertilization can temporarily reduce soil pH in the surface soil layer after the supply, especially at high amounts as demonstrated by Schroder et al. (2011). In presence of acidic soils, this effect may be even higher causing greater problems in nutrient absorption, with a combination of yield limiting factors. The study of Gastal and Lemaire (2002) highlighted that nitrogen assimilation needs to be considered in the context of inter-regulation of multiple crop physiological processes (i.e. C N assimilation and allocation) and soil N availability.

Besides this consideration, overall the estimated NNI values during the season have revealed areas with a general sub-optimal crop growth in the experimental field which resulted in an average yield (14.18 t ha^{-1}) much lower than the average of all the maize fields of the farm (18.22 t ha^{-1}) confirming the goodness of NNI to indicate crop yield potential (Gilles Lemaire et al. 2008). NNI calculated from RS during the season resulted in a dynamic diagnostic tool to assess spatial explicit crop N status and limitation in N uptake. Chlorophyll content estimation for crop stress status monitoring has been considered in many studies and for site-specific N management (Han et al. 2002; Miao et al. 2009) but the relationship between chlorophyll content and Na% remains highly empirical (Houlès et al. 2007; Lemaire et al. 2007) because it is usually influenced by variety, phenology and study area, limiting the generalization capability of the method using only chlorophyll content information (F. Baret et al. 2007; Delloye et al. 2018). RS DM are proposed as a rapid and robust way for nutritional status assessment to be used as an alternative to direct field measurements (Xia et al. 2016; Zhao, Ata-Ul-Karim, et al. 2018). Information on actual nitrogen content or concentration, or direct indication of nitrogen deficiency (NNI) from remote data are of great help in crop monitoring.

From the 2018 maize experiment we found that the direct estimation of NNI using VIs or S2_BVs derived by Sentinel-2 is feasible, as demonstrated by general good correlation metrics comparable to other studies. The best performance was obtained using Cab_S2 secondary variable ($R^2=0.82$) slightly better than using CCC_S2 ($R^2=0.77$) or NDRE ($R^2=0.79$). This performance is similar to what reported by Li et al. (2014). Our results indicated also that Na% can be directly derived using MCARI with an agreement of $R^2=0.71$ confirming the conclusions of Cilia et al. (2014). However, biomass estimation directly from VIs resulted not reliable. So, the direct “mechanistic approach” (DM_2) was not performing as expected. These results confirmed the possibility to directly derive NNI (DM_1)

with VIs and/or S2_BVs as a suitable solution for operational crop monitoring to support site specific nitrogen fertilization. The NNI derived maps showed comparable results in terms of correlation with DM ($R^2=0.62$, RMSE=0.21) and IM ($R^2=0.63$, RMSE=0.27) approaches.

Indirect method (IM) derives NNI from the separate estimations of W and PNUa. The advantage of using such approach is that estimated PNUa can be used to compute Δ PNU, this is a promising quantitative approach to define nitrogen absorption deficit of a crop (Martine Guerif et al. 2007; Houlès et al. 2007; Gilles Lemaire et al. 2008). PNUA (kg ha^{-1}) is a quantitative variable preferable than a variable expressed in percentage as Na, because it avoids an intermediate step to convert N content from a mass basis to an area basis (Roderick et al. 1999). Experimental results demonstrated that the biophysical variable CCC estimated from S2 is well related with PNUa ($R^2 = 0.78$) confirming the results of previous studies (Delloye et al. 2018; Xia et al. 2016). S2 estimated GAI can be used to estimate W ($R^2=0.84$), since this variable is necessary to calculate PNuc according to crop specific dilution curves. The quantitative deficit or surplus values given by the indicator Δ PNU is thus suitable for adjusting N supply in a site-specific fertilization management.

1.5. Conclusions

The first part (F1) of the study highlighted that soil types have an effect on the diagnosis of the nutritional status of the crops in response to N rates supply. Sentinel-2 data can be exploited to estimate nutritional deficit or surplus computing NNI with direct and indirect methods. In addition to this indicator using indirect methods, it is possible to quantitatively define the required N amount to supply with VRT machinery by calculating Δ PNU. However, this information is not sufficient, and it is necessary to take in account the potential causes of crop nutritional status, such as soil properties variability. In the experiment presented here, a limiting factor for an optimal crop growth was identified in the soil properties (pH) that reduce the capacity of the crop to uptake N, also in conditions of high N availability in the soil and supplied by fertilization. This influences the interpretation of N status, because even though a nitrogen deficit is detected (i.e. $\text{NNI} < 0.9$) a supplementary provision of fertilizer (Δ PNU) would not be efficient. The experiment confirmed that different soil types within a cultivated field can have an effect on growth, independently of N rates provided and consequently impact on dry matter production and final yield. Indeed, in the location of a field where yield potential is low, added N fertilizer profitability can be reduced as demonstrated for example by Ma and Biswas (2015). These results confirm that, since crop N absorption is a very dynamic and complex process, site-specific management in a PA paradigm requires a combination of spatial explicit information: static maps describing variability of soil properties and seasonal information related to actual crop nutritional status achievable from RS data. The information from soil characterization is potentially

complementary to crop N status monitoring, and could improve NUE and site specific N fertilization strategies of cereals cropping systems.

2. Phase II - Full-scale comparison of variable rate nitrogen fertilization methods

2.1. Introduction

In precision agriculture, diagnostic tools for spatial and temporal analysis of crop N content (% N on dry biomass or N uptake by the plant) variability are used and, thanks to VRT site specific practices are implemented to increase final NUE. As such, other than assessing the reliability of crop information generation, in order to be able to effectively implement a site-specific fertilization, it is necessary to address the problems related to an effective application of precision fertilization. In particular, it is important to understand which are the best methods and what is the best overall N distribution strategy. These aspects are equally important as the accurate in-field crop variability detection, i.e. the retrieval of biophysical variables and the final estimation of nutritional status. Conceptual models are not enough, because there is a great need to address the concrete difficulties in managing the agronomic practices such as nitrogen fertilization. These aspects are of paramount importance, especially for farmers who are the key stakeholders in this process and the end-users of the implemented solutions. The active participation of farmers into PA chain is required to take the opportunity for the development of the whole sector.

In literature is possible to find two main approaches utilized for spatial variability management and consequent precision N modulation (Gaikwad and Sawant 2016; Said Nawar et al. 2017): i) delineation of uniform management zones (MZ) on the basis of many different ancillary geo-spatial information layers (e.g. soil or crop sampling, soil maps, historical yield maps records, archive of Vis images); ii) adoption of seasonal zoning system (SZS) based on remote and/or proximal sensing data, able to spatially explicitly assess crop nutritional status. The main difference between these two approaches is the idea of constant or not constant spatial and temporal consistency of plant response to soil condition hence to nutritional stress. Soil-based and plant-based methods have been deeply investigated, but often independently, whereas the study of both soil and crop properties correlated with crop productivity should be considered (Cordero et al. 2019).

The approach of “static” MZ delineation, aims to emphasize within field/farm differences of soil properties that change greatly in space but slowly over time (Delloye et al. 2018). The delineation and characterization of homogeneous areas within a field helps the farmer to manage few zones with theoretically equivalent yield potential and similar characteristics (A. R. Schepers et al. 2004). The studies of Khosla et al. (2002) and Inman et al. (2005) obtained an increased NUE, by adjusting N rates according to the soil characteristics, whereas Basso et al. (2013) used yield maps in addition to soil properties for constant MZ delineation purposes because yield includes static and seasonal effects in its variability (Buttafuoco et al. 2017). More recent studies tested the performance of different

cluster analysis methods for zones delineation, based also on historical archives of remotely sensed layers (Gavioli et al. 2019; Pascucci et al. 2018). This methodology has the advantage of identifying within-field yield potential variability in the absence of yield map records. Different clustering methods are available even already implemented in free software such as Management Zone Analyst (MZA) developed by Fridgen et al. (2004) that uses a fuzzy k-means clustering algorithm to delineate MZ from geo-referenced field information.

“Seasonal” identification of spatial and temporal variability of crop nutritional status has been largely investigated using remote sensing (RS) techniques for different crops (Basso et al. 2016; Cammarano et al. 2014; David J. Mulla 2013). This approach aims to derive crop related biophysical information that can change during the season and that are connected to potential crop stresses, in particular N deficiency. Temporal variability rapidly changes (Casa and Castrignanò 2008) and satellite images are now the main supplier of recursive and cost-effective information. Changes related to the appearance of pathologies or possible stresses even associated to weather conditions can have a very fast development and spatially affect crops in a non-uniform way. For this reason, the temporal consistency of the information provided by satellites such as the ones from S2 platform is of primary importance.

F2 research was devoted to test and compare in real farm condition three different AP methods for N dose definition to support fertilisation: a Uniform Rate (UR) approach based on soil information was compared to two variable rates based on remote sensing data, VR1 and VR2. In particular, VR1 was based on F1 results, BVs and N content retrieval from optical multispectral data and semi-empirical approach, while VR2 was based on a sufficiency index approach proposed by Holland and Schepers (2013). The objectives of F2 activities were: *i*) to define quantitative methodologies for supporting N prescription for maize and winter wheat; *ii*) to address a large-scale operative test facing all top-dressing fertilizations steps (Nt1-Nt2-Nt3); *iii*) to assess the performance of different N management strategies from an economic, environmental and agronomic point of view; *iv*) to assess the influence of soil characteristics on the different fertilization management strategies.

2.2. Materials and Methods

2.2.1. Study area and experimental design

A field experiment was carried out, in the 2019 and 2020 seasons, respectively on maize and durum wheat, in order to apply methods and results achieved in F1. Two different fields were selected based on the cultivation plan of the private farm Bonifiche Ferraresi SpA (BF). The farm hosted the F2 trials and carried out the agronomic operations, providing machineries and technicians. The fields

used were different from those used for F1 experiments. The selected varieties (Table 15) were Dekalb DKC6728 for maize crop (FAO 600) and Odisseo (SIS) for durum wheat, corresponding to the same variety used in F1 and a widely grown variety in the BF farm and also at a national level.

Table 15: Agronomic details of field experiments for maize in 2018 and wheat in 2019.

	Variety	Density	Plowing date	Planting date	Harvest date	Irrigation
Maize	DKC6728	7.8 seeds m ²	20-Feb 2020	4-Apr 2020	28-Aug 2020	Sub-irrigation
Wheat	ODISSEO	200 kg ha ⁻¹	25-Oct 2019	4-Nov 2019	24-Jun 2020	No irrigation

During the cultivation cycle of both cereals, 3 N fertilization (Nt1-Nt2-Nt3) had been planned to be operationally carried out. The experimental methodology was aimed at providing a comparison between 3 different distribution approaches regarding the definition of the N dose needed for the crop (Table 16). Two of these methods, Variable Rate methods (VR1-VR2), exploited crops N needs estimation through the use of indicators (Δ PNU, SI) derived from Sentinel-2 satellite data, whereas the third, Uniform Rate (UR) method, defined crop N needs as output of an agronomic simplified balance (ASB) that used soil properties data of the experimental fields. The ASB method is the standard method commonly used in the BF farm for N prescription definition and it represented the “standard” for comparison. The method generates a unique output suitable to providing a N dose, partitioned in three moments, but spatially uniformly spread in the field. Theoretically, the ASB method can also be used for VR applications by calculating the balance and applying the dose on different Management zones (MZ) defined a priori. However, spatially distributed ASB does not allow to deal with seasonal spatial variability. Instead, the S2 derived methods produce maps with variable N dose to be applied with a variable rate spreader following a site-specific prescription addressed by seasonal within-field variability.

Table 16: Description of the 3 methods used for nitrogen prescription creation

Name	Description	Acronym	Methodology	Code
ASB	Agronomic simplified balance	UR	Standard	1
Δ PNU	Delta Plant Nitrogen Uptake	VR1	Remote	2
SI	Sufficiency Index	VR2	Remote	3

Both VR1 and VR2 methods included the analysis of Sentinel-2 images acquired close to the fertilization date, to define the crop nutritional status during the season, whereas the UR method defined in a static way the crop needs at the beginning of the season. The algorithms developed in F1 were applied to S2 images and Bvs_S2 maps produced were exploited to first assess intra-field variability and then to define spatial explicit dosage of the fertilizer to be used. The final prescription maps for each method were generated for the entire experimental fields. The different prescriptions

derived from the three methods were subsequently applied dividing the fields in big parcels on a regular grid and attributing alternatively a different method for each cell. Three maps were generated, one for each method, and merged into a single final map with the assigned prescription based on the parcels scheme. The details of the schemes are provided in section 2.2.5 for durum wheat and 2.2.6 for maize.

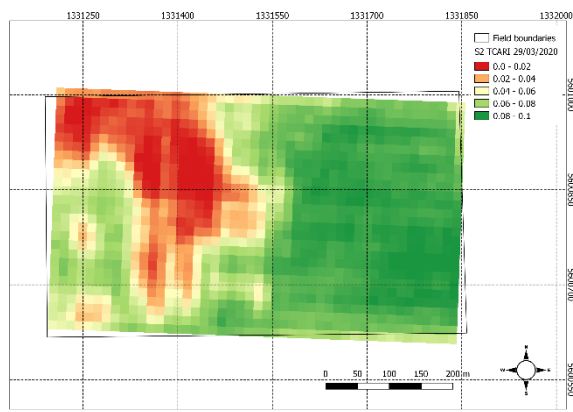
2.2.2. Satellite data

Five Sentinel-2 images were used in the 2020 experiment for both crops (Table 17), respectively three for durum wheat and two for maize. Unfortunately, due to clouds contamination problems of the images in early maize season it was not possible to use S2 data for Nt1 planned at 2nd leaves stage. But this was not a serious problem, because as seen in F1, the crop's cover fraction in this period is very low and the ground response in the spatial reflectance signal is very high.

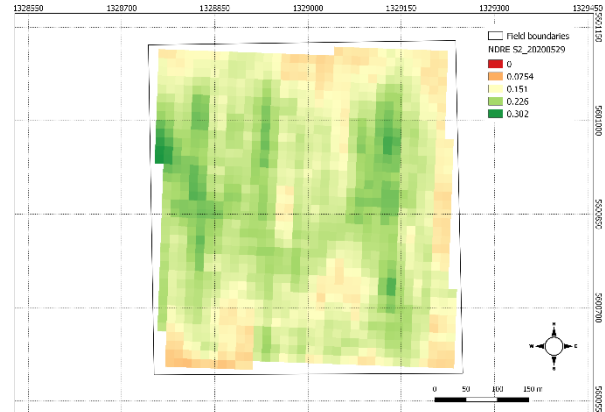
Table 17: acquisition dates of Sentinel-2 images used for fertilizations and corresponding crop phenology

Acquisition date	Crop	Fertilization	Phenology
08/02/2020	Durum wheat	Nt1	Tillering
29/03/2020	Durum wheat	Nt2	Stem elongation
08/05/2020	Durum wheat	Nt3	Inflorescence emergence
-	Maize	Nt1	2° leaves
28/05/2020	Maize	Nt2	6° leaves
27/06/2020	Maize	Nt3	Flowering

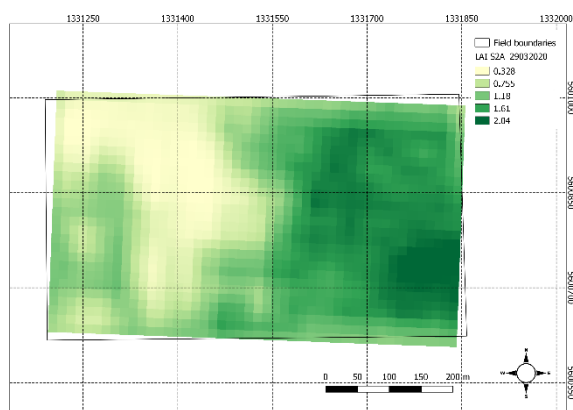
The images were downloaded in L2A format and processed using sen2r package (Ranghetti et al. 2020) and ad hoc R scripts to derive vegetational indices (NDRE - MSAVI - TCARI). From those images the operative products provided by the SNAP software toolbox (s2tbx) were also obtained corresponding to biophysical variables such as the Leaf Area Index (GAI_S2) and the Canopy Chlorophyll content (CCC_S2). In Figure 20 VIS and BVs obtained from two different S2 images are presented, one per crop, showing the high within-field variability in both trials with particular effect in durum wheat field experiment (Figure 20 left column panels).



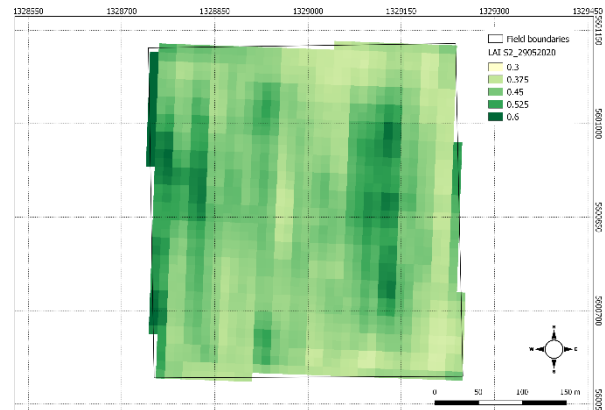
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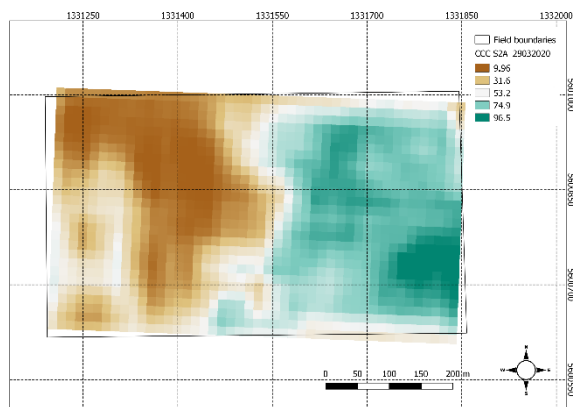
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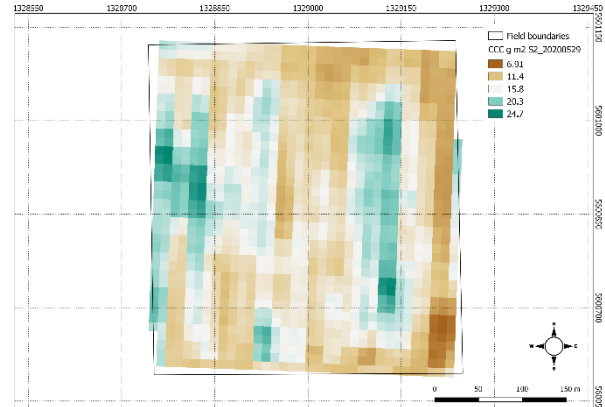
c



d



e



f

Figure 20: S2 images of 29-March for durum wheat a) TCARI; c) GAI_S2; e) CCC_S2; and 29-May for maize b) NDRE; d) GAI_S2; f) CCC_S2

2.2.3. N fertilization prescription methods

2.2.3.1. VR1 – ΔPNU

The first (VR1) method of variable dose definition based on seasonal crop nutrient status assessment, is conceptually derived from Nitrogen Nutritional Index (NNI) retrieval from remote sensing data (direct and indirect estimation form S2) as demonstrated in the studies carried out in F1. NNI can be calculated using the ratio between the actual concentration of N (Na%) and the critical concentration of N (Nc%) of the plant but can be also determined as the ratio between the effective (PNUa kg ha⁻¹) and the critical uptake of N in the plant (PNUc kg ha⁻¹) on the base of the theory of the critical dilution/uptake curve of nitrogen in crop (M Guerif et al. 2007; Plénet and Lemaire 2000). As it emerged in the first part of the study, it is possible to directly estimate the NNI from remote sensing data via vegetation indices such as NDRE and MSAVI or indirectly through the estimation of biophysical variables such as dry biomass (W), used to calculate PNUc, by GAI_S2 and PNUa by CCC_S2 and then calculate NNI and/or ΔPNU that is a quantification of deficiency/excess of N in crop as given by the formula:

$$\Delta PNU = PNUa - PNUc \quad (4)$$

The quantitative indicator ΔPNU (kg ha⁻¹) (M Guerif et al. 2007) can be determined using equation (4) and used directly to calculate nitrogen rates (increasing or reducing the planned reference dose) for site-specific fertilization. The reference N dose (DR) for each fertilization period (Nt1-Nt2-Nt3) is established at the beginning of the season on the basis of the total N dose (DT) to be supplied, taking into account the varietal requirements and the fractionation established by the farmer (e.g. 50-20-30 %). The final output of the prescription map, in a vector format, corresponds to the application (Napp) expressed in kg ha⁻¹ of nitrogen (or fertilizer to be used) and resulting from DR minus ΔPNU (eq. 5). Obviously ΔPNU can be negative, if the crop is in nitrogen deficit, or positive if the crop is in luxury consumption.

$$Napp = DR - \Delta PNU \quad (5)$$

The ΔPNU can hypothetically be considered as it is, to directly calculate Napp rate and the consequent map, without considering DR, but instead considering this N amount to fill only the gap of the lack of nitrogen for an optimal state of the crop. However, this entails the risk of a lack of the

minimum fertilizer intake established by the variety protocol, especially as regards maize, which is a very demanding crop from the point of view of nitrogen. This latter approach, however, could potentially be applied to some of the planned fertilizations (Nt1-Nt2-Nt3) of wheat or other cereals which are much more susceptible to nitrogen excess (e.g. rice). The use of the indirect method offers the possibility to derive quantitative information through the estimation of the real and critical uptake of the crop and can generate directly usable information.

Despite the good results achieved with both the indirect (IM) and the direct methods (DM), the latter was discarded for the following reasons: *i)* the empirical models found with the DM only allowed to calculate NNI, or Na% and PNUa; *ii)* the use of same VIs to estimate different BVs (W, Na% and PNUa) could generate redundant and auto-correlated information; *iii)* NNI estimated with the DM does not provide quantitative information on N rates to be supplied; *iv)* the spectral response synthesized in VIs is influenced more by biomass than nutrients content.

Instead, we chose to use the indirect method because: *i)* Bvs_S2 operative products showed good correlations with PNUa; *ii)* it allowed to use the relationships between GAI_S2 and W to accurately derive crop biomass accumulation and consequently the PNUc; *iii)* it is possible to calculate PNUa from the CCC_S2 and not from VIs which are more sensitive to biomass variability than chlorophyll content, especially in dense canopy. *iv)* it allows to obtain an indicator expressed in kg ha⁻¹ of N to be used to quantify the fertilizer at any time. The list of inputs used for Δ PNU indirect estimation are listed in Table 18.

Table 18: Bvs derived from S2 and necessary to indirectly calculate Δ PNU

Bvs	Unit	Name	Method
GAI_S2	-	Leaf Area Index	Operational product
CCC_S2	g cm ²	Canopy Chlorophyll Content	Operational product
W	t ha ⁻¹	Dry biomass	Estimation
Nc	%	Critical N	Estimation
PNUa	kg ha ⁻¹	Plant Nitrogen Uptake actual	Estimation
PNUc	kg ha ⁻¹	Plant Nitrogen Uptake critical	Estimation

The methodological steps involved: *i)* to obtain GAI_S2 and CCC_S2 from the best identified Sentinel-2 image, in relation to fertilisation planned period, and *ii)* to apply the relationships obtained in F1 to estimate the necessary inputs for the calculation of Δ PNU. With regards to wheat, the regressive models defined in F1 study were not used for all the F2 N applications. The crop early lodging and the cloudiness of the available data in F1 affected the results and the remaining data were not sufficient to have solid relationships between variables. In any case, both F1 experiments (Crema et al. 2020) and the literature (Delloye et al. 2018; M Guerif et al. 2007; Marie Weiss and Baret 2016)

highlighted that the best variable for estimating PNUa is CCC_S2. Regarding maize crop the empirical model used (eq.6) was the one obtained in F1 (see § 1.3.3). For wheat two different approaches were selected: in Nt1 and Nt2 the regressive model employed was the one proposed by Delloye et al. (2018) and shown in eq.7, whereas in Nt3 the model was the one found in F1 and shown in eq. 8.

$$PNUa (kg ha^{-1}) = (0.5469 * CCC_S2) - 8.6513 \quad (6)$$

$$PNUa (kg ha^{-1}) = (39.968 * CCC_S2) + 11.809 \quad (7)$$

$$PNUa (kg ha^{-1}) = (89.317 * CCC_S2) + 0.3759 \quad (8)$$

The W was derived using the eq. 9 and eq. 10 for wheat and maize respectively. For maize we used the regressive model obtained from field W data and GAI_S2 values derived from Sentinel-2 (See § 1.33 for details).

$$W (t ha^{-1}) = (0.4054 * GAI_S2) - 0.0231 \quad (9)$$

$$W (t ha^{-1}) = 0.0212e^{2.1714*GAI_S2} \quad (10)$$

Then, knowing W, it was possible to calculate firstly the Nc % and the PNUc of both crops for any specific moment through eq. 11 and 12. The Nc% of maize and durum wheat crops was calculated using eq. 11 and the crop-specific coefficients (a, b) proposed for maize and wheat by (Plénet and Lemaire 2000) (a = 3.4 b = 0.37) and (Justes et al. 1994) (a = 5.35, b = 0.442),

$$Nc \% = aW^{-b} \quad (11)$$

where W is the above ground biomass in t ha⁻¹, a represents plants N% when W is 1 t ha⁻¹ e b is a coefficient. Nc% is considered constant at 3.4% for W<1 t ha⁻¹ in maize and 4.4% for W<1.55 t ha⁻¹ in durum wheat (Plénet and Lemaire 2000; Justes et al. 1994).

To obtain $PNUc$ in $kg\ ha^{-1}$ from W expressed in $t\ ha^{-1}$ it was necessary to divide the whole by conversion factor 10 (eq. 12):

$$PNUc\ (kg\ ha^{-1}) = (W * Nc\%)/10 \quad (12)$$

From $PNUc$ it was then possible to calculate ΔPNU by subtracting $PNUa$ as reported in eq.13:

$$\Delta PNU\ (kg\ ha^{-1}) = PNUc - PNUa \quad (13)$$

In this way, and differently with respect to eq. 1, N deficiency is stated with positive values expressed in $kg\ ha^{-1}$ of N that are lacking in the crop at that time to achieve an optimal nutritional status. This condition highlights the risk of nitrogen stress resulting in a slowdown in biomass accumulation. For this reason, for the final calculation of N_{APP} (eq. 14) the DR has been increased or decreased according to the indicator ΔPNU in order to allow greater supply if there were situations of stress and reductions if there were conditions of luxury consumption with a $PNUa$ greater than $PNUc$.

$$N_{APP}\ (kg\ ha^{-1}) = DR + \Delta PNU \quad (14)$$

N_{APP} was then converted into a prescription map with the $kg\ ha^{-1}$ of the fertilizer chosen for each application.

1.1.1.1. **VR2 – Sufficiency index approach**

The second (VR2) method of N rate prescription is an approach based on the general shape of an N fertilizer response function (sensor index vs. N rate) and the relationship between N rate and in-season crop proximal sensing (K. H. Holland and Schepers 2010). It relies on the use of an indicator called Sufficiency index (SI) proposed by (Peterson et al. 1993) and then used by K. H. Holland and Schepers 2010 to develop an N application model compatible with proximal sensing for implementation in variable-rate applicator (VRA) systems. To our knowledge, it has not been previously reported using remote sensing instead of proximal sensing data, therefore an adaptation for such case has been developed.

The SI is defined as the ratio of a real-time sensed crop property, to the same variable measured in a reference optimal area of the field and can be applied to any VIs or in-season crop biophysical variable (eq. 15)

$$SI = \frac{VI_{pixel}}{VI_{reference}} \quad (15)$$

SI varies from 0 to 1, VI pixel is the sensor's read for a specific zone of the field (i.e. average on homogeneous zone or value at pixel level from a raster image) and VI reference is the VI value of the non-N limited crop for the same field. Knowing SI from any sensor is possible to apply the model using eq. 16:

$$N_{APP} (kg\ ha^{-1}) = (MZ_i * N_{opt} - N_{PreFert} - N_{OM}) \sqrt{\frac{(1-SI)}{\Delta SI * (1 + 0.1 * e^{m * (SI_{threshold} - SI)}}} \quad (16)$$

where MZ_i is a coefficient (between 0 and 2) that varies taking into account the different yield potential of the soil in different areas, N_{OPT} is the EONR (Expected Optimal Nitrogen Rate) or the maximum N rate established by the farmer, $N_{PreFert}$ is the sum of fertilizer N applied prior to crop sensing and/or in-season N application, N_{OM} is the N credit for the average organic matter content within the field. SI is the sufficiency index, ΔSI is the sufficiency index difference parameter, m is the back-off rate variable ($0 < m < 100$) and $SI_{Threshold}$ is the back-off cut-on point. The ΔSI parameter is the difference between the SI of an area of the field with the best N availability ($SI = 1.0$) and the area in the worst nutritional condition at that moment.

Originally, in this method, a N-rich strip was established in the field, by providing non-limiting N, with the aim of having a real reference on the condition of nutritional stress absence. Many algorithms (Franzen et al. 2016; David J. Mulla 2013; Ransom et al. 2020) developed for proximal sensors for fertilization management take advantages of such N-rich crop reference strips in the field. But creating a N-rich strip as a reference for the purpose of calibrating active sensors can be unpractical for the farmers, therefore Kyle H. Holland and Schepers 2013 proposed a new model using a virtual reference concept. The virtual-reference concept uses the histogram of VI values (Figure 21), collected driving through the field, to examine the frequency distribution function and then takes the 95-percentile cumulative value from the histogram as the VI reference for good N

nutritional status. Then N recommendation established during fertilization is based on the crop reference value that is continuously updated while driving again through the field.

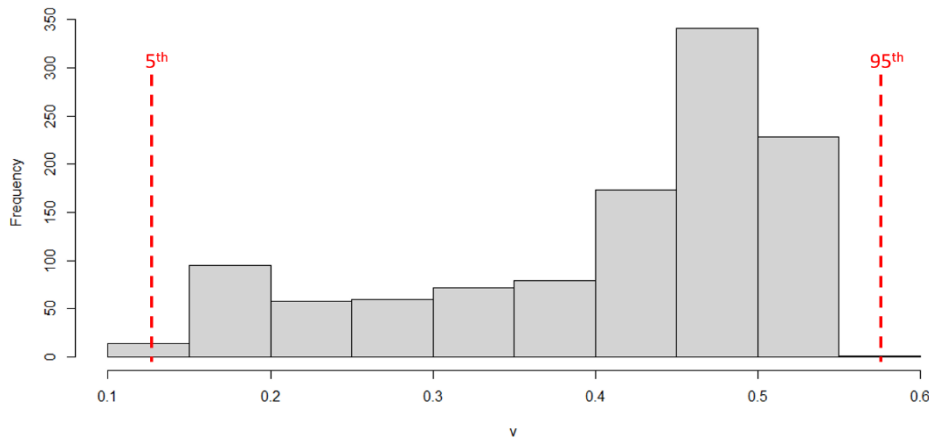


Figure 21: frequency distribution of VI (NDRE) values of durum wheat derived from S2 image of 08th May 2020

For VR2 treatment in our experiment, a modified version of this method, conceived to be used with remote sensing data, was applied. SI was derived from the satellite S2 image, determining SI(0) from the 5th percentile of the cumulative frequency distribution of all VI pixel values of the field (Figure 21), whereas the 95th percentile of VI pixel values was used as a reference of a well fertilized area. This approach is based on the assumption that in every field there is an area, for that context and at that moment, representing a crop that grows without stress and that can be a virtual relative reference. As such, in this way this method could be applied to any field and any crop using satellite data.

$$SIO = \frac{VI \text{ percent.5}^\circ}{VI \text{ percent.95}^\circ} \quad (17)$$

$$\Delta SI = 1 - SIO \quad (18)$$

First of all, for each S2 image, the frequency histogram of the best VI correlated with PNUa emerged from the data analysis of F1, i.e. NDRE for maize and TCARI for wheat, was derived, after which the percentiles (5th and 95th) were obtained for the calculation of SIO using eq. 17 and ΔSI with eq. 18. For Napp calculation, using eq. 16, the coefficient MZi has not been used, in order to not obscure the response effect of different soils and to not define potential yield classes a priori. The

N_{prefert} and N_{OPT} values used in each fertilisation (Nt1-Nt2-Nt3) were respectively the DT and DR values according to the fertilisation plan.

1.1.1.2. **UR – Agronomic balance**

The third method (UR) is not spatially distributed, and it is fundamentally based on a simplified agronomic balance (ASB) of soil nutrients, a commonly used method to achieve adequate restoration of soil fertility and to provide sustainability of agricultural systems (Cobo et al. 2010; Roy et al. 2003). Nitrogen balance is quantified by considering, as field average, the main N outputs and changes in soil total N storage from N inputs. The N inputs considered are: N fertilization from inorganic N fertilizers, from manures and amendments; atmospheric N depositions (rain, snow, and dry deposition); biological N fixation; crop residues. The N outputs considered are: crop N removal (grain and biomass); N losses (through N leaching, NH_4 volatilization, denitrification, gas emissions, surface runoff). From the many equations available for full or simplified methods available (Roy et al. 2003) we used the model shown in eq 19 for the calculation of seasonal N requirement (N_{app} kg N ha^{-1}):

$$N_{\text{app}} = a - b + c \quad (19)$$

Where a is the crop N requirements determined by the target yield; b is the N contribution supplied by soil fertility (soil properties), c is the amount of N losses. The detailed methodologies of any single member calculation is not reported here, because it is a non-disclosed intellectual property of the IBFS spa company, but this model allows to derive crop N requirements of the entire growth cycle starting from the YG and the chemical-physical properties of the soil at field scale. This method, which is commonly used by the BF farm, is already in itself an advanced non-standard method, because benefits from soil properties maps and could also consider crop rotation, N application maps and yield maps as data. UR has been taken as a reference for the performance comparison with the other 2 methods (VR1-VR2). Differently from the variable rate methods, UR is not generally a spatially distributed method, and the output (N_{app}) is a single prescription. It could be partially distributed aggregating areas defined homogenous due to soil properties similarities (MUZ) or using spazialized data as yield maps, N application maps and soil properties maps.

2.2.4. **Data analysis**

Data analysis for the evaluation of the performance of the three N application models (VR1-VR2-UR) was carried out by using yield data of experimental trials derived from yields maps

generated by the harvester. In addition, the values of the amount of nitrogen (total N applied kg ha⁻¹) distributed and recorded in the application maps during the site-specific distribution, were taken into account. Two indicators were selected to determine which of the methods offered the best performance considering different aspects. Partial Factor Productivity of Nitrogen (PFPN kg kg N ha⁻¹) (Casa et al. 2011; Cordero et al. 2019; Cui et al. 2010; Novoa and Loomis 1981) has been used for NUE evaluation through eq 20:

$$PFPN = Y/Nt \quad (20)$$

where Y is the yield and Nt is the total N supplied with fertilization, both expressed in kg ha⁻¹. For the economic aspect consideration, the net return above N fertilizer cost incurred was estimated (RANC € t) (Bachmaier and Gandorfer 2009; Casa et al. 2011; Cordero et al. 2019) achieved with eq 21:

$$RANC(\text{€ ha}^{-1}) = Y * Pg - Nt * PN \quad (21)$$

where Y is the yield (t ha⁻¹), Pg is the price of wheat/maize (€ t⁻¹), Nt is the N fertiliser rate (t ha⁻¹) and PN is the price of a unit N (€ t⁻¹). PN was obtained from average fertilizers prices in the years 2019 and 2020 (<https://teseo.clal.it/?section=concimi>) and Pg was obtained from average prices in 2020 derived from CLAL (<https://www.clal.it>). In this way, the overall assessment was carried out not only in terms of yield but also in terms of efficiency of the method and from the point of view of economic convenience. This approach provides different elements that can be considered for the choice of the more suitable method for a decision maker.

The single yield point values recorder by the combine harvesters mapping systems have been interpolated using ordinary kriging technique for yield maps generation and with geostatistical multivariate analysis using cokriging (D J Mulla 1997). This allowed to compare N treatments carried out in the field. The pseudo cross-variogram generation allows estimation of the variable at an unsampled location, by computing a related empirical spatial function (Myers 1991; Webster and Oliver 2008). Using the yield values taken from the plots of any N treatment, it has been possible to generate a coregionalization model (Casa and Castrignanò 2008) between pairs of treatments (e.g VR1 vs UR) in order to estimate the potential spatialized differences that would be expected if both

treatments had been applied to the whole field. Statistical analysis was performed using R software version 4.0.3 (R Core Team, 2020) and R Studio version 1.4.1 (RStudio Team, 2020).

2.2.5. Test Case I – Durum Wheat 2020

The field selected for the trial was about 12 ha and is identified in the farm with the code 187 (Figure 22). It contains a single class of soil called JOL1 that refers to a clay or clay-loamy soil, very deep and greyish brown.

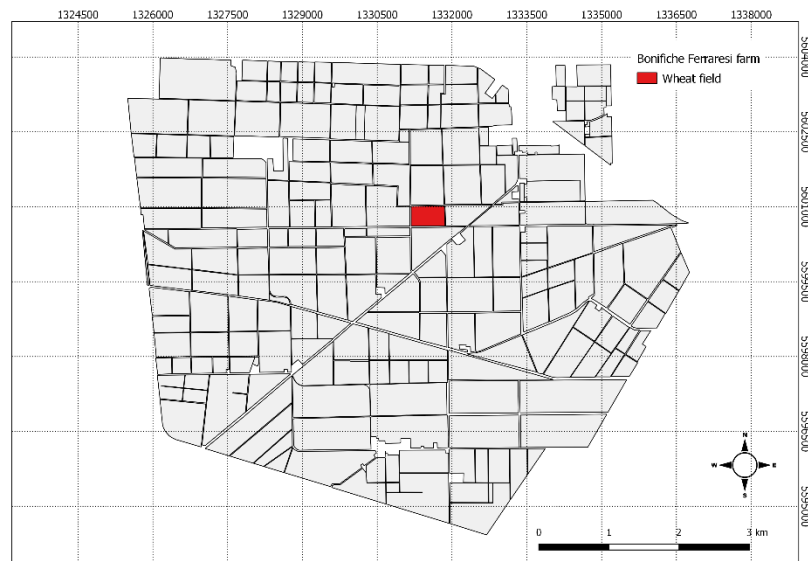


Figure 22: localization of wheat experimental field (n° 187) inside of Bonifiche Ferraresi Spa sited in Jolanda di Savoia (Fe)

Despite the presence of one soil class, the resistivity map of the experimental field indicates a certain degree of variability of the soil properties as reported in Figure 23. The variable range values is similar to experimental field (n°45) used F1 suggesting an experimental condition similar to the previous but not the same.

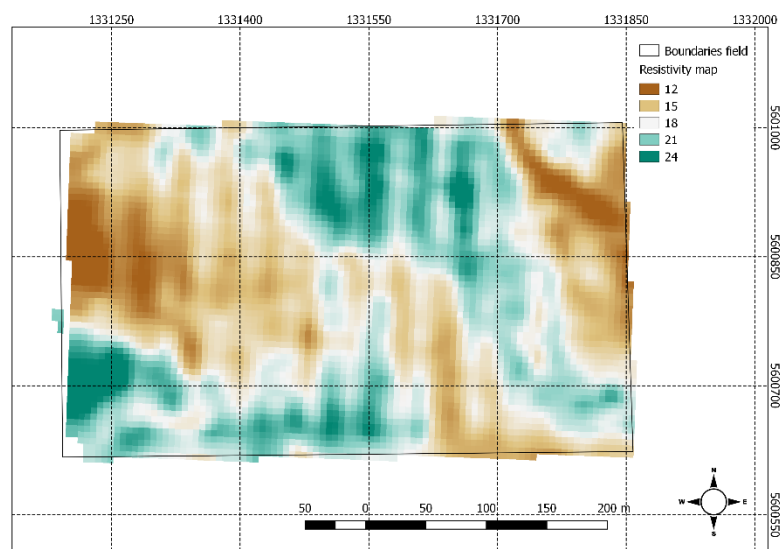
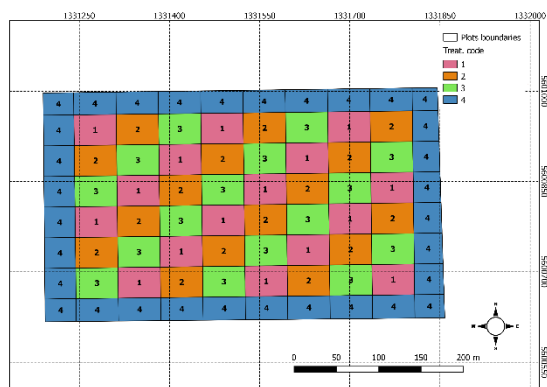
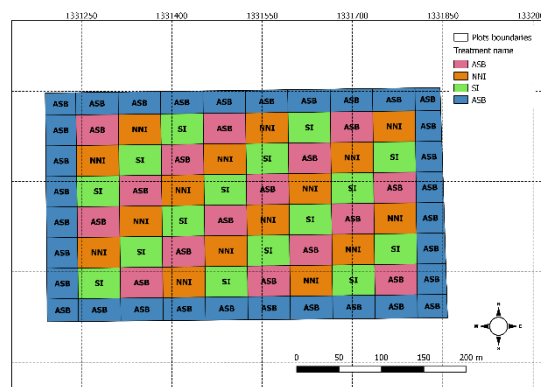


Figure 23: resistivity map of durum wheat experimental field n° 187

The field experimental layout was set up by dividing it into zones with a regular grid of 50 by 36 meters plots, as shown in Figure 24a. To each zone of the three methods (ASB, SI, Δ PNU) was assigned one, for a total of 48 plots (Figure 24b). In the perimeter of the field a buffer zone was created, indicated with code 4 in Figure 24a, fertilized with method 1 (UR) but which was not considered for the analysis of the results.



a



b

Figure 24: a) virtual grid pattern of field n°187 labeled with the respective code; b) virtual grid pattern labeled with N treatment method

Crop sowing was carried out on the 04/11/2019 with durum wheat Odisseo variety, i.e. the same variety of the trial conducted in F1. At sowing, a starter fertilizer equal to 10 kg ha⁻¹ was supplied in combination with the seeds. The seeds rate followed the recommended number of viable seeds, i.e. 350-400 seeds m⁻², corresponding to approximately 180-200 kg ha⁻¹. The fertilizations plan of durum

wheat was established in order to carry out three top dressing interventions (Nt1-Nt2-Nt3) during the season at the most important phenological stages regarding crop nutrients needs: tillering, stem elongation, booting. The fertilization protocol, as required by the company strategy and experimentation planning, followed the scheme reported in Table 19.

Table 19: wheat trial N fertilization seasonal plan (2020)

Fertilization	Crop	Date	Phenology (BBCH)	Product	Application
Nt1	Wheat	24/2/2020	tillering	Ammonium Nitrate 33%	Variable rate
Nt2	Wheat	11/04/2020	stem elongation	Urea 46%	Variable rate
Nt3	Wheat	09/05/2020	booting	Ammonium Nitrate 33%	Variable rate

The amounts of nitrogen used for the definition of fertilizer prescription maps at any interventions is reported in Table 20, Table 21 and Table 22. The reference varietal protocol for establishing the fractionation quota used for the VR1 and VR2 methods was derived from the suggestions of the seed company (30-50-20 %) while the maximum permissible doses for VR1 and VR2 were derived from the guiding lines of integrated production of the Emilia-Romagna Region 2020.

Table 20: Total N rate (DT) and reference N rate (DR) kg ha⁻¹ for any fertilization

	SI	ΔPNU	ASB	Variety protocol
DT	160	160	88	160
DR tillering	48	48	26	48
DR stem elongation	80	80	40	80
DR booting	32	32	13	32

Table 21: Reference rate (DR) kg ha⁻¹ of fertilizers for any fertilization

	SI	ΔPNU	ASB	Product
DR tillering	143	143	79	Nitrate 33%
DR heading	174	174	86	Urea 46%
DR booting	96	96	39	Nitrate 33%

Table 22: N fractionation (%) of total rate (DT) and reference rate (DR) for any fertilization

	SI	ΔPNU	ASB	Variety protocol
DR tillering	30	30	30	30
DR heading	50	50	45	50
DR booting	20	20	15	20

The final amount of fertilizer expressed in kg ha⁻¹ was derived differently, depending on the method used: for VR1 (ΔPNU) and VR2 (SI) it was calculated starting from the closest Sentinel-2 images to the fertilization date, whereas for the UR treatment (ASB) a spreadsheet with the Simplified

Agricultural Balance (IBFS) was used to obtain the amount of product according to the soil characteristics of the field. Since a single class of soil, in terms of pedological type, was present in the field, despite the apparent resistivity variability (Figure 22), the UR rate was the same from time to time in all the parcels.

The geo-referred prescription maps in a vector shapefile format (Figure 25a) where uploaded on board the machineries system and the N target rate received (Figure 25b) where verified before the operations. Thanks to the fertilizer variable rate system, the operations were then recorded in as-applied map, that were used to check if the correct N amount had been actually supplied in the right area (Figure 25c). The N rates applied, derived from the as-applied maps, were cleaned from outliers data and then averaged over each plot (Figure 25d).

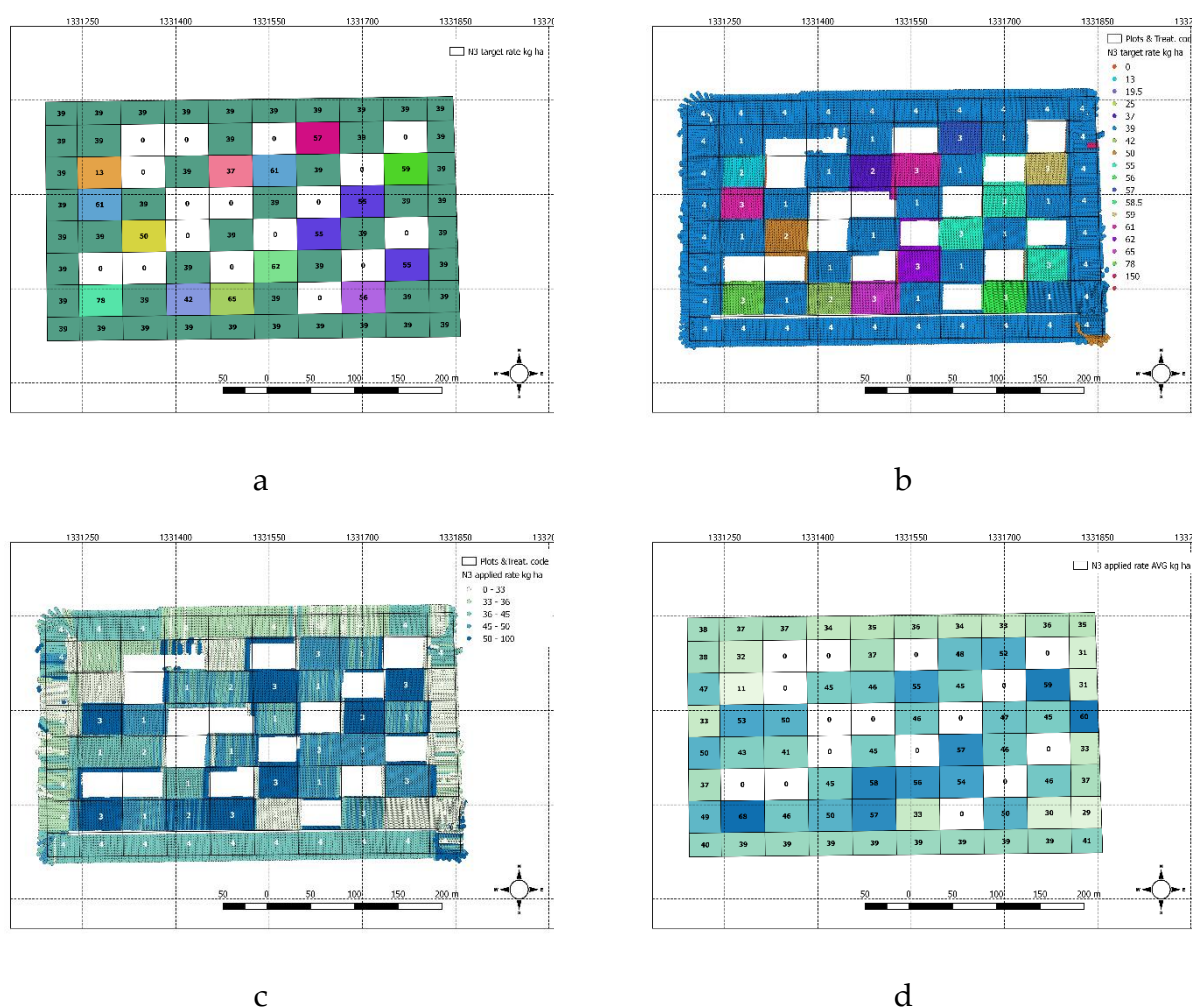


Figure 25: a) N3 prescription map (kg ha⁻¹) of ammonium nitrate; b) N target rate (kg ha⁻¹) of the application map; c) the applied rate (kg ha⁻¹) of the application map; d) average amount (kg ha⁻¹) per plot actually applied;

For the operations in the field an ISOBUS Sulky x36 spreader with satellite guide and John Deere Greenstar monitor was employed, operated by BF staff. For fertilizations details and maps see § Annex.

The durum wheat was harvested on the 6th July 2020 using a John Deere combine harvester S770 equipped with a John Deere yield mapping system. A yield map (Figure 26a) was produced using the mapping systems of the machinery and this data was explored for anomalies analysis. For instance, the map (Figure 26a) reveals a big red patch in the left side of the field, whereas the right side is the most productive. The red area corresponds to the zone where during the season the crop had intense damages (flooding) and where it produced very low values of grains (almost zero t ha⁻¹ in some areas). The final yield was 2.5 t ha⁻¹, which is very low for this variety.

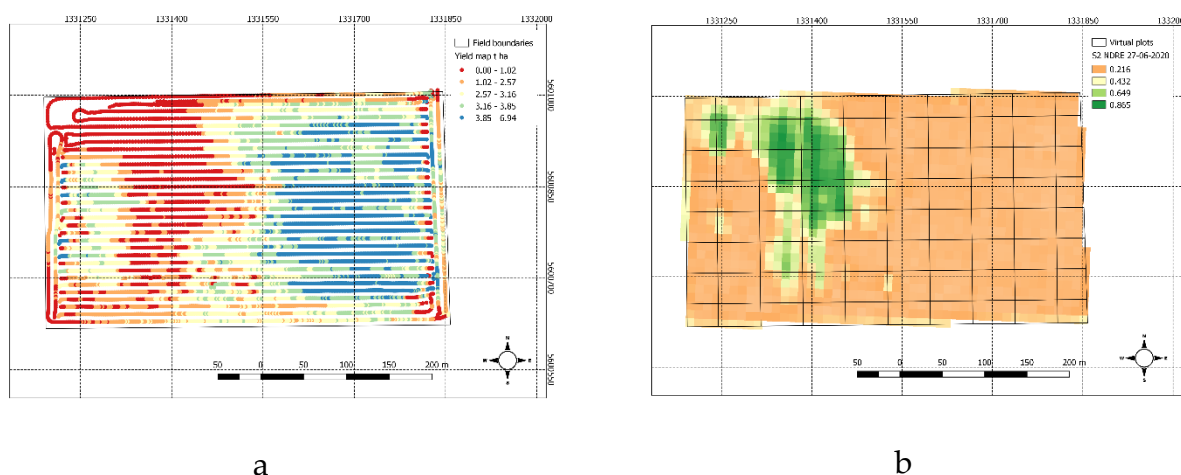


Figure 26: a) yield map (t ha⁻¹) of durum wheat ; b) NDRE derived from Sentinel-2 image acquired on 27th June 2020

To better highlight how low production was related to the lack of crop in Figure 26b, a map of the NDRE vegetational index, derived from the S2 image acquired 10 days before harvest (27th June 2020) was calculated. As shown in Figure 26b, the low values of the index (orange color) is well representing the homogeneity of the phenological phase of maturation and senescence of the wheat. On the other hand, large areas with very high index values can be found due to the presence of numerous green weeds grown in the areas left bare due to the absence of crop during the season (see § Annex).

2.2.6. Test Case II – Maize 2020

The field chosen for maize trial (12 ha) is identified in the farm with the ID code 55 (Figure 27).

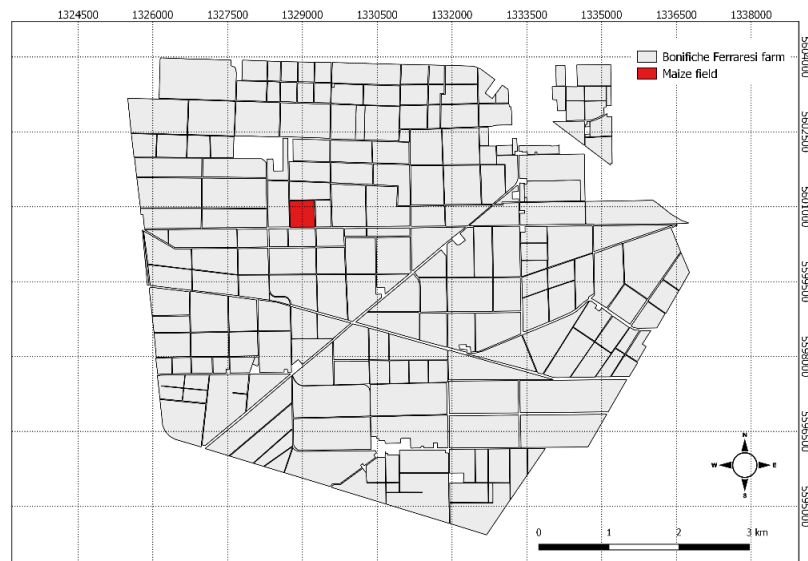


Figure 27: Location of experimental field ID 55 within the territory of Jolanda di Savoia (Fe) owned by Bonifiche Ferraresi Spa

The resistivity map shows a variability of soil characteristics related to soil texture, as shown in Figure 28, with values ranging between 12 and 26 Ω m. No soil sampled data were acquired during the experiment and the complete soil properties description were taken by the farm archive generated in 2015.

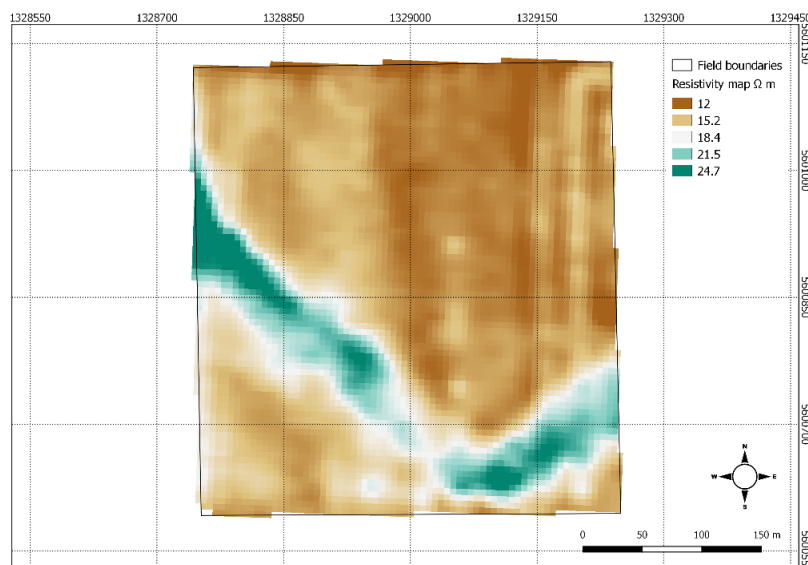


Figure 28: ID 55 resistivity map, profile 0-50 cm.

The soil map identifies 3 different soil types (CDS1, JOL1, LCO1) within the field, as shown in Figure 29 and as described in Table 23. In particular, the CDS1 soil class has historically been regarded as including the areas with the lowest yield performance as compared to the others soil classes (farm statistics). This soil class refers to those areas of inactive old riverbed with shallow soils and abrupt passages in both topsoil and subsoil. The experimental scheme was conceived as to consider and analyze the effects of these soil characteristics on the fertilization strategies.

Table 23: Description of soil types in field 55

Soil class	Weaving	Wbr class	Description
JOL1	clayey and silty clay phase	Fluvic Thaptohistic Gleysols (Clayic, Hurnic, Sulfidic)	Very dark greyish brown soils, very deep, clayey or clayey loam and rich in organic carbon already in topsoil. Organic with moderately decomposed peat usually below 100 cm.
CDS1	silty clayey and loamy loamy silty phase	Calcaric Gleyic Fluvisols (Humic, Arenic, Geoabruptic)	Soils Light olive brown or greyish brown, shallow, with abrupt texture passage from clay loam to clay loam in topsoil, from sandy francs to sandy francs in subsoil, very calcareous
LCO1	silty clayey phase	Calcaric Fluvic Gleysols (Clayic, Humic)	Soils Very dark greyish brown, dark bluish grey in the rice fields, very deep, weakly calcareous with a weakly alkaline reaction in topsoil and neutral in subsoil

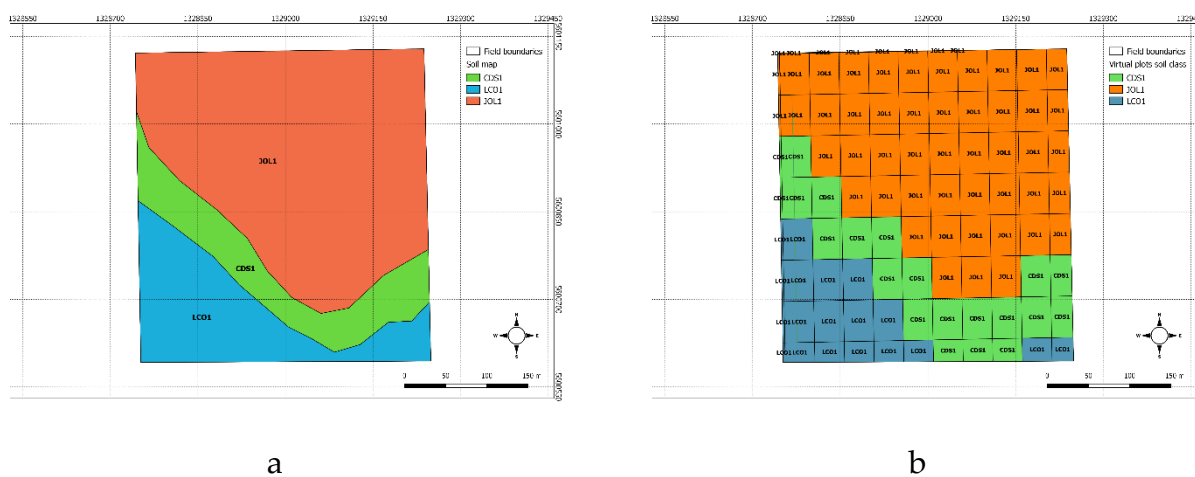


Figure 29: soil classes of field ID 55 derived from the soil map (2015); b) experimental plots layout with the corresponding soil class

The field was divided into plot areas, according to a regular grid of 50 by 36 meters. A soil class (Figure 29b) and a fertilization method (Figure 30a) between ASB-UR, VR1-ΔPNU and VR2-SI was then assigned to each plot, for a total of 48 plots created in the field. In the perimeter of the field a

buffer zone was created with parcels equally fertilized with UR methods code 4 () but not considered for the analysis of the results.

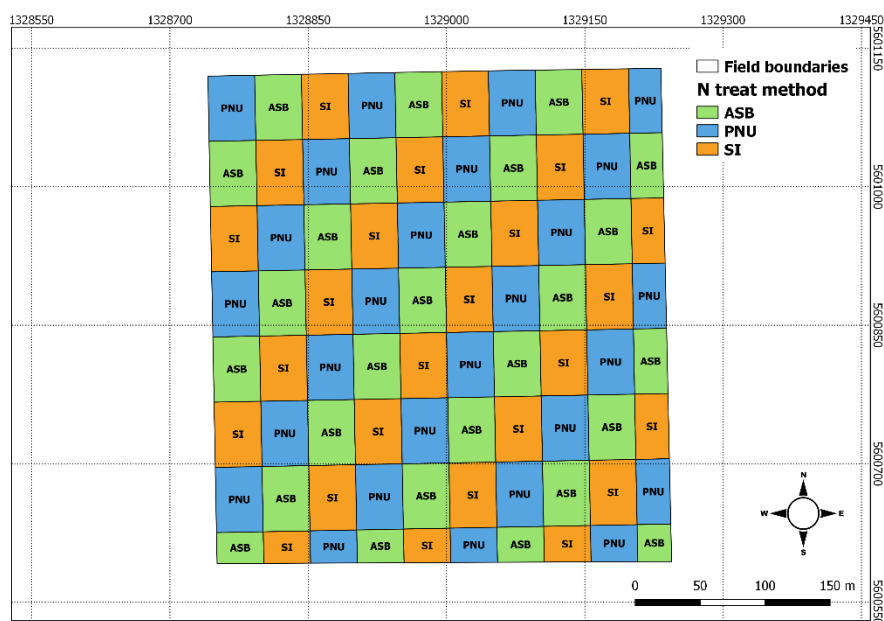


Figure 30: a) virtual plots labeled with fertilization method; b) virtual plots labeled with the corresponding treatment code

The variety selected for the maize trial was DKC6728 (FAO class 600). Sowing was carried out on the 4th april 2020 with a constant dose of seed corresponding to 78000 seeds ha⁻¹ (7.8 plants m⁻²) with 75 cm interrow spacing, combined with a fertilisation for starter effect of about 10 kg ha⁻¹ of nitrogen (recorded as N0). The fertilisation protocol, as required by the farm strategy and the experimentation planning, followed the scheme described in Table 24 and Table 25. Maize fertilization plan included three top dressing fertilizer applications (Nt1-Nt2-Nt3) during the season in the key phenological stages.

Table 24: maize trial N fertilization seasonal plan (2020)

Fertilization	Crop	Date	Phenology	Fertilizer	Distribution
Nt0	Maize	04/04/2020	Pre-sowing	N-K	Uniform
Nt1	Maize	06/05/2020	3° leaves	Urea 46%	Variable
Nt2	Maize	01/06/2020	6° leaves	Urea 46%	Variable
Nt3	Maize	06/07/2020	flowering	N30 Liquid	Variable

Table 25: N supply amount of maize trial with overall N rate (Total N - Nt0-Nt1-Nt2-Nt3), the total N rate (DT_N - Nt1-Nt2-Nt3) used for the trial, the reference N rate (DR_N) used at any application $kg\ ha^{-1}$, and the amount in % and $kg\ ha^{-1}$ provided at any fertilization Nt0-Nt1-Nt2-Nt3 for any soil class

Soil class	Total N $kg\ ha^{-1}$	DT_N $kg\ ha^{-1}$	Nt0 %	Nt1 %	Nt2 %	Nt3 %	Nt0 $kg\ ha^{-1}$	Nt1 $kg\ ha^{-1}$	Nt2 $kg\ ha^{-1}$	Nt3 $kg\ ha^{-1}$	DR_{N1} Urea 46%	DR_{N2} Urea 46%	DR_{N3} Liquid 30%
CDS1	159	147	7	40	43	17	12	59	63	39	128	138	100
JOL1	215	204	5	40	48	12	12	81	97	39	177	211	100
LCO1	214	203	5	40	48	12	12	81	97	39	176	210	100

The three N applications were planned to be in VR mode through the use of prescription maps and the reference rates (DT_N and DR_N) described in Table 25. The total reference (DT_N) was derived from the simplified nutrient balance spreadsheet used at the farm (IBFS) to obtain a different amount of product depending on soil properties of the 3 soil classes present in the field. For a soil class (CDS1) a lower DT_N was provided than for the other two classes. The fractions (%) to be allocated of the planned three fertilizations (Nt1-Nt2-Nt3) defined the single DR_N for each intervention. Whereas for ASB the rates were defined a priori and remained unchanged, the fertiliser amount for VR1- Δ PNU and VR2-SI was instead based on Sentinel-2 images and the computations defined by the algorithms previously reported. The VR mode was applied to Nt2 and Nt3 fertilizations while for the first fertilization date (Nt1) the dose was the same for all three N management treatments. For the within-field operations we used a Sulky x36 fertilizer spreader with satellite guide and John Deere monitor (Greenstar), operated by BF company workers. Regarding the N3, the application of liquid fertilizer in variable mode, by using a crop sprayer (Mazzotti) equipped with satellite guide and monitor Trimble (see § Annex) was tested, for the first time in the farm.

The maize crop was harvested on the 28th of August 2020 with CLAAS 980 harvester equipped with CLAAS original yield mapping system and a data collection and management system called Climate Fieldview. The average yield was $12.8\ t\ ha^{-1}$ and the raw yield map data reported in Figure 31 shows how a certain degree of variability was present in the field at the end of the season.

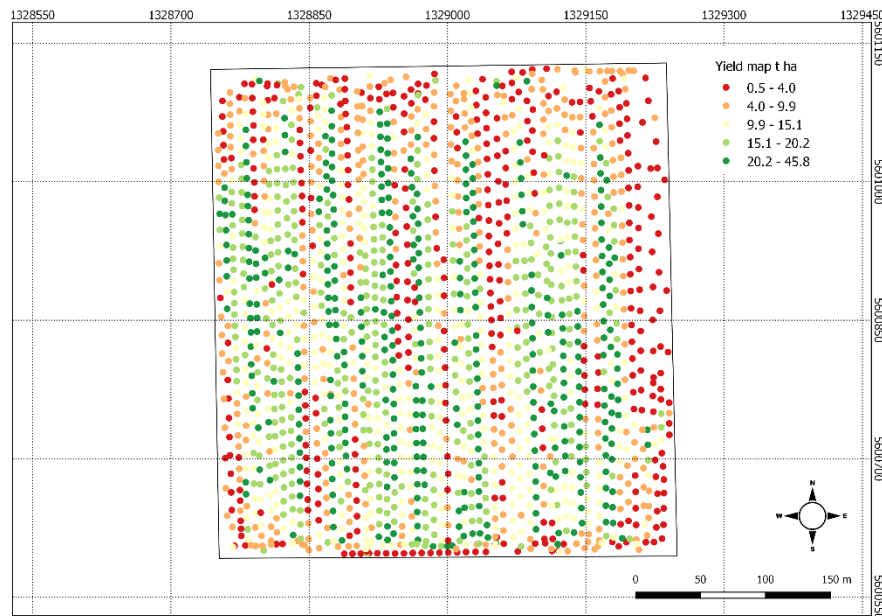


Figure 31: maize yield map generated by CLAAS harvester mapping system

The raw point map data downloaded from the harvester mapping system was then elaborated to generate a new interpolated map with continuous values over the entire field surface. As seen from the map, there are many low values positioned continuously and placed vertically. These spots represent areas where there were crop failures problems caused by animals (rodents) that damage maize plants. This has led to large areas near the internal channels used for the drainage of water with very few plants per square meter (see § Annex). These yield values therefore do not correspond to wrong values but still represent a problem for statistical analysis of data, as described in chapter 2.3.2.

2.3. Results

2.3.1. Test Case I – Durum Wheat 2020

Regarding the trial on durum wheat on field ID 187, first it has to be noted that the experiment has been successfully and entirely conducted in an operational manner on a field scale. A primary result was the operational use of S2 images for supporting the fertilizations throughout the season and the supply of the prescription map the day after the acquisition. Considering the crop response and the overall season trend, the average final yield was 2.5 t ha^{-1} , which is less than what would be expected from this variety. The yield map (Figure 26a) revealed that a large portion of the field, located in the North West side, had serious growth problems during the season. About one third of the experimental field was compromised by such adverse events and 10 out of 48 experimental plots (20% of the total trial) failed to yield, while others 7 plots resulted very compromised (Figure 32).

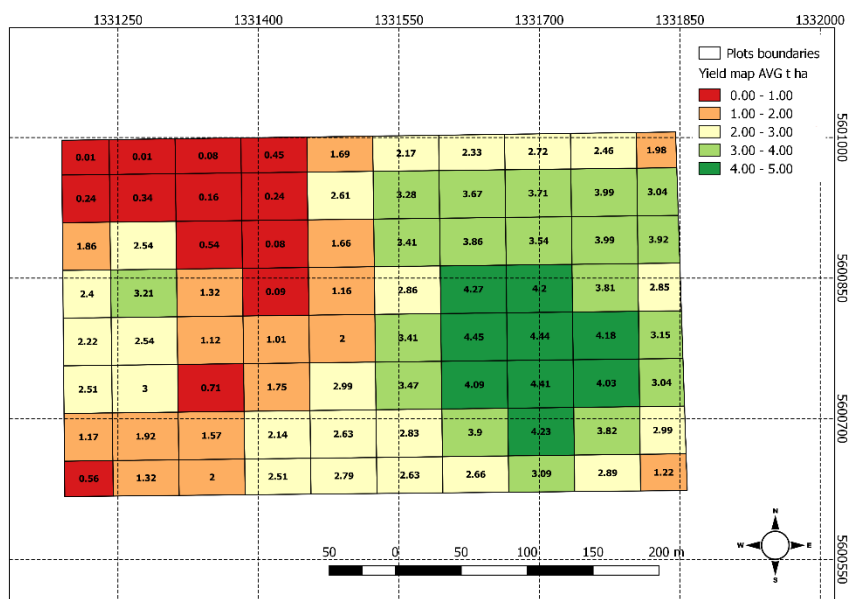
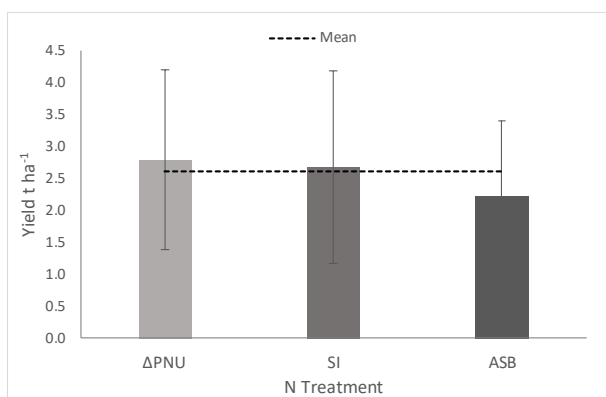
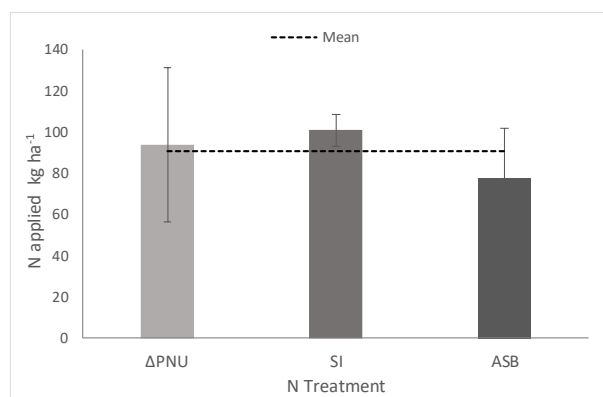


Figure 32: yield averaged plot by plot (t ha⁻¹) of durum wheat field ID 187

Excluding the zones where the crop was not present, the average yield was 3.5 t ha⁻¹, with peaks of 6 t ha⁻¹. The farm field average for durum wheat during the 2020 season was 4.6 t ha⁻¹ with the highest values around 6 t ha⁻¹. As there are no different soil classes within the field, it was not possible to verify the presence of different production performance due to the soil effect.



a



b

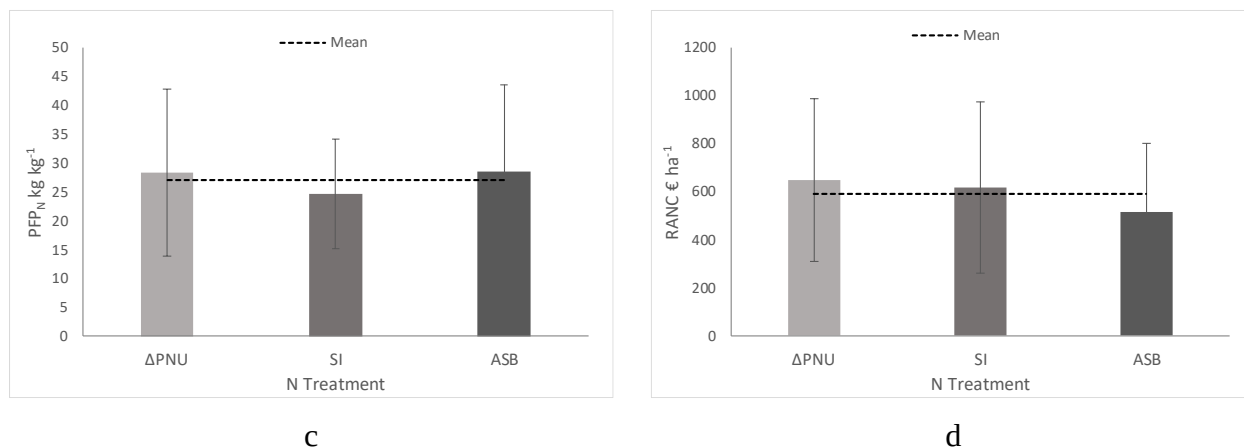


Figure 33: durum wheat average value on N treatment a) yield; b) yield and total N supplied; c) partial factor productivity (PFPN kg of grains by kg of N ha⁻¹ supplied); d) economic return (RANC € t⁻¹)

Regarding N treatments, some differences on final yield were observed. Figure 33a shows that considering the average yield, the plots that produced the most were those with the treatment VR1-ΔPNU (2.8 t ha⁻¹), followed by those with treatment VR2-SI (2.7 t ha⁻¹) and then by those with a uniform dose ASB-UR (2.2 t ha⁻¹). Comparing the average yield per type of N management method with the average dose of nitrogen supplied during the season (Figure 33b), we found that the ΔPNU method was the most productive (+4% yield than SI) without being the one that used the most nitrogen rate (-9% N than SI). The SI method was the one that used the highest N total rate (+29% N than ASB) while the ASB method produced the least (-20% yield than ΔPNU), but was also the most conservative (-20% N than ΔPNU) in the amount of nitrogen used (80 kg ha⁻¹). In any case, all the methods used a considerably lower amount of nitrogen than what is normally adopted in standard practice or permitted by legislation (160 kg ha⁻¹ of N). Table 26 summarises the results of the N needs definition methods used in the experiment.

Table 26: N treatments performance on durum wheat considering: yield (t ha⁻¹), total N applied during the season (N supplied kg ha⁻¹), nitrogen use efficiency (PFPN kg kg ha⁻¹), net return above N cost (RANC € t)

N treatment	Yield t ha ⁻¹				RANC € t				PFPN kg kg ha ⁻¹				N supplied kg ha ⁻¹			
	Min	Mean	Max	Δ%	Min	Mean	Max	Δ%	Min	Mean	Max	Δ%	Min	Mean	Max	Δ%
VR1-ΔPNU	0.1	2.8	4.4	0	8.6	647.3	1042.6	0	2.7	28.4	46.5	-0.5	32	94	127	-20
VR2-SI	0.2	2.7	4.5	-4	40.7	616.5	1043.3	-5	5.1	24.6	36.8	-13	44	101	138	-29
UR-ASB	0.0	2.2	4.4	-20	-26.8	513.4	1052.1	-21	0.1	28.5	54.9	0	61	78	94	0

Besides the nitrogen consumption, if we consider the partial factor productivity of nitrogen (PFPN), i.e. the kg of grain produced for every kg ha⁻¹ of N used, as an indicator of the nitrogen use efficiency (NUE), it appears that the methods UR and ΔPNU are equivalent (28 kg ha⁻¹), while SI

obtains performances clearly worse up to 14% less than the other two methods (i.e. 25 kg ha⁻¹) (Figure 33c). Using an economic indicator such as the net return on nitrogen costs (RANC € t⁻¹), it appears that the VR1-ΔPNU method obtains a better result than the other methods (Figure 33d) with an average value of about 647 € t⁻¹ (Table 26) and maximum values of RANC above 1000 € t⁻¹. In addition, if for the maximum values of RANC all methods obtained similar results, as for the minimum values we noted that the UR method achieved even negative values (-26 € t⁻¹). This means that the ASB-UR method, without seasonal information provided from remote sensing, was not able to manage areas with bad crop conditions or even with complete crop failure, losing money already taking into account just fertilizer costs. Instead, the other methods have decreased or even not applied at all fertilizer in the compromised areas, thus allowing to save costs thanks to the information provided by satellite images.

Analyzing the N needs estimations and the N recommendations for N seasonal plan generation, different patterns can be observed. The points in the scatterplots of Figure 34 represent the average of the yield values and the N rate values for each plot of the field. From the Figure 34a and b, it emerged that, despite the linear correlation between yield and N supplied, different clusters of points are present. Looking at the distribution of the points in the graphs and at the trendline, it is clear that they may not represent well the effects of fertilization. The cluster with the lowest values of N rate and yield corresponds to those of non-fertilized plots at the N2-N3 applications dates and with the heavy weeds contamination. By removing these data from the analysis, we obtained a trendline with an inverse relation that, however, is more easily readable. Figure 34c and d describe how during the season the more productive plots were the less fertilized. This seemingly contradictory result is not surprising, since the plots received an N rate based on N status derived from remote sensing and thus less fertiliser was provided where the crop showed a good nutritional status. As we can see from Figure 34c and d this lower rate, provided to plots in good condition, did not reduce the final production, but on the contrary permitted to reduce, in both VR methods, the total N amount supplied varying spatially the distribution of the fertilizer.

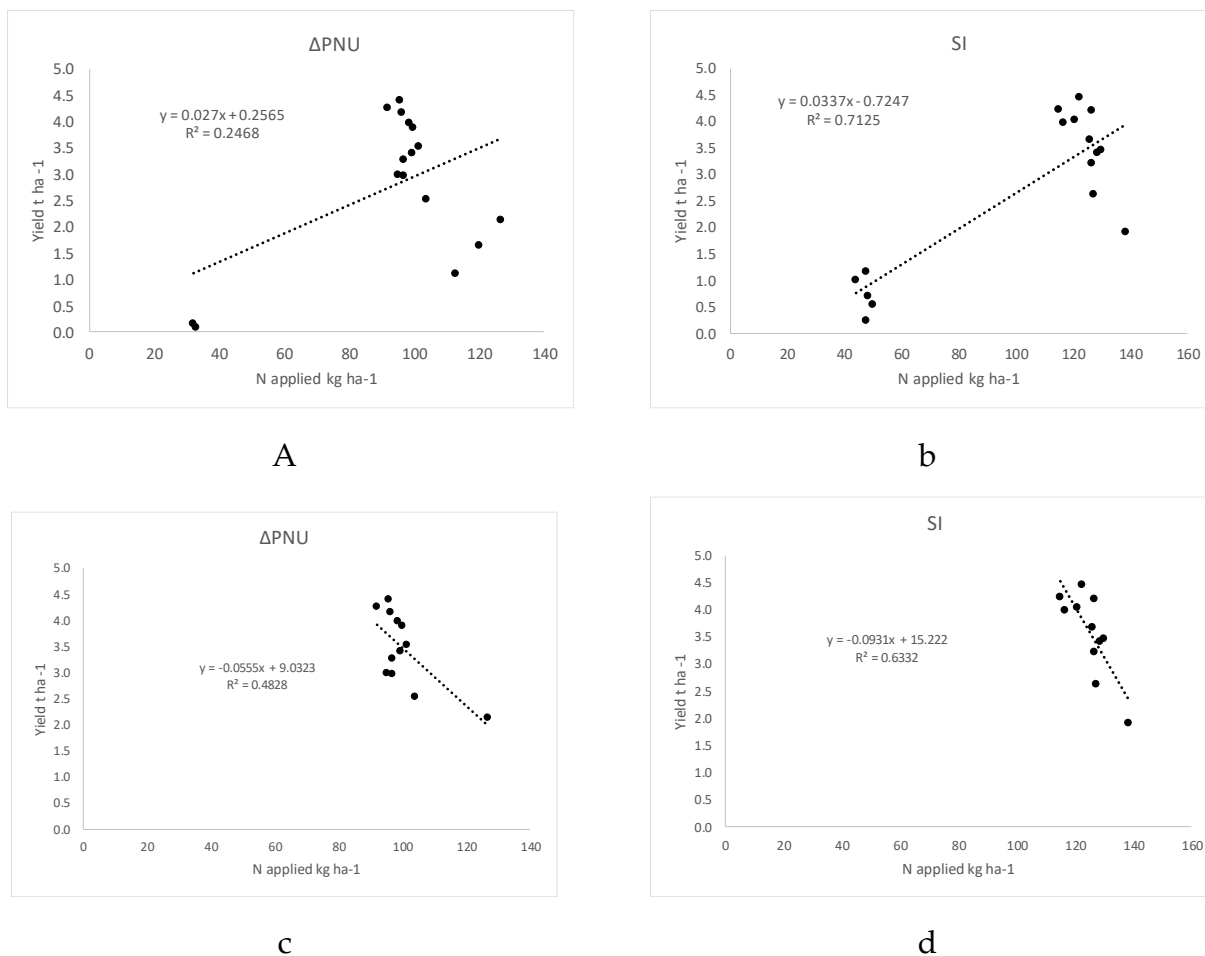


Figure 34: scatterplot between yield and total N applied in each plot for methods Δ PNU (a) and SI (b); panel c and d represent relation for data with yield > 2 t ha⁻¹ for methods Δ PNU and SI respectively.

Considering instead the relationship between the satellite-derived PNUa estimation and the final yield, there is a strong correlation between the data, with values of $R^2=0.95$ and $R^2=0.96$ for VR1- Δ PNU and VR2-SI plots respectively (Figure 35). This means that the crop nutritional status was correctly estimated during the season. The points of Figure 35 represent the mean value of the sum of the PNUa estimations from S2 images before Nt1-Nt2-Nt3 in each plot. The plots that produced the most were also those that absorbed the most nitrogen during the season, confirming the precise estimation of N status.

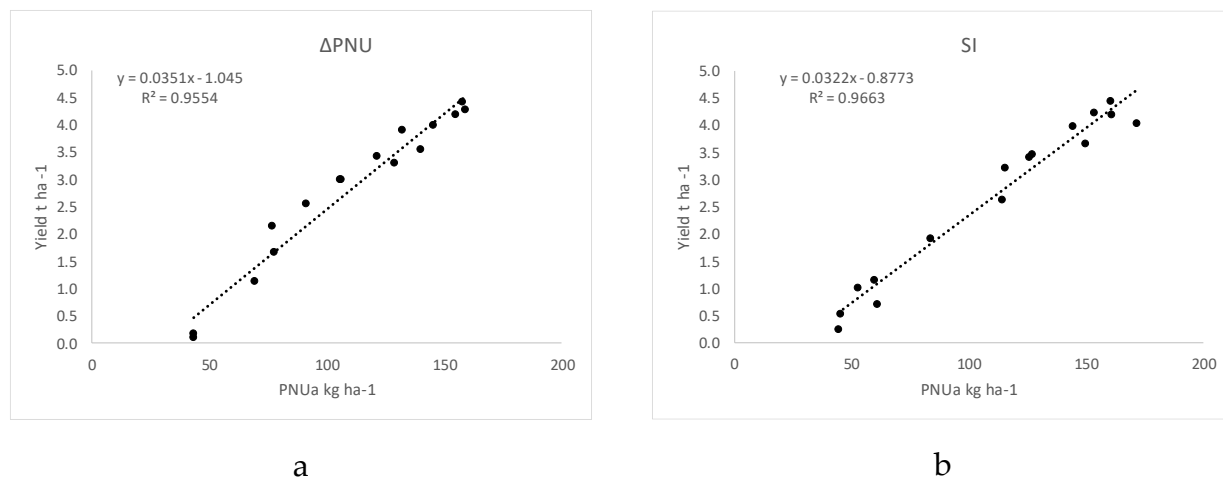


Figure 35: scatterplot between yield and cumulative PNUa estimated from image x, y and z (Nt1+Nt2+Nt3) in each plot for methods a) ΔPNU ; b) SI

In addition to the information about the plant uptake, Figure 36 shows the results of the adopted strategy, suggested by the algorithms. Any point of Figure 36a and b are the plot mean value of the sum of PNUa and total N supplied related to the final yield. This figure shows not only that the nutritional status was correctly estimated during the season, but also that the N rates were correctly adjusted during the season and that the crops with the larger availability of nitrogen (supplied and absorbed) reached higher yields and vice versa, as it usually happens. The clusters observable in both graphs of Figure 36 are the plots where the plants were not present which were (correctly) not fertilized. Otherwise, this would have represented a waste of fertilizer with potential economic and environmental impact. The N recommendation tools based on satellite data did not affect the yield with a lower than standard investment in nitrogen and a better economic return (RANC) and efficiency (PFPPN) then UR-ASB.

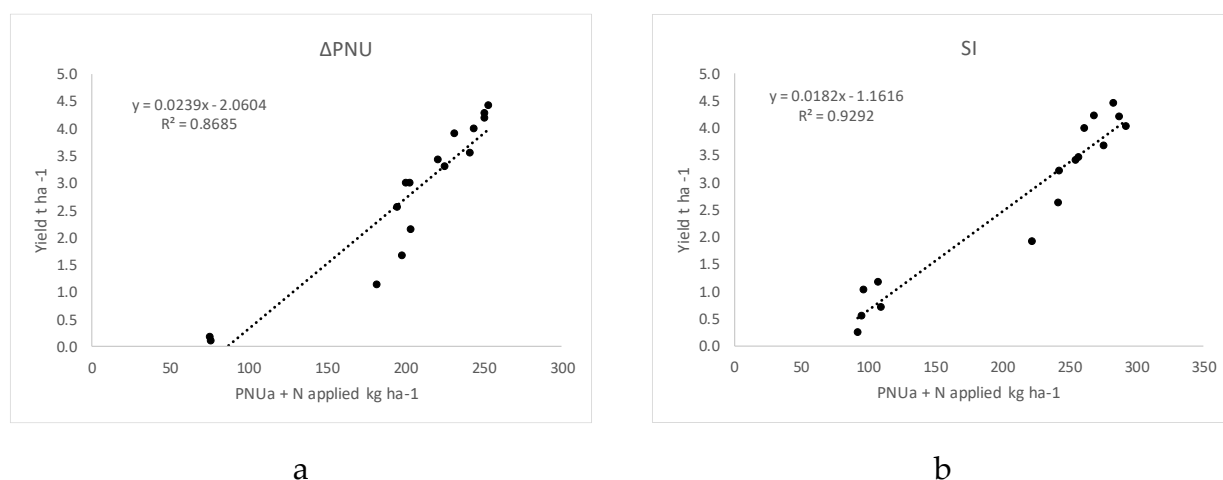


Figure 36: scatterplot between plot mean value of the PNUa added to the total N supplied and the plot average yield for methods a) Δ PNU; b) SI

The lack of information derived from satellite when using an approach as ASB-UR does not permit to perform this kind of analysis on the strategy adopted and on crop response to fertilization, other than in terms of final yield. Despite this, some considerations can also be made on this method as reported by the graphs in Figure 37. Conversely to the other methods, the inverse correlation in Figure 34cd is not present in Figure 37a where there is no correlation ($R^2=0.08$) between the final yield and the total N rates supplied. The plots with uniform treatment defined by the nutrient balance, which received the same dose of nitrogen, had different yield results, presumably due to the nutritional status of the crop and the characteristics of the soil. We are therefore seeing a yield variability due to factors not attributable to nitrogen fertilization. Furthermore, the dispersion of points in Figure 37a along the horizontal axis (N supplied) is generated by the difference between the quantity of nitrogen actually distributed (derived from the application map of the spreader) in each plot and the quantity of nitrogen prescribed by the ASB-UR method that had to be a uniform rate (Figure 37b). The scatter plot in Figure 37c highlights the relation between N prescription (target) and application ($R^2=0.9$) with an overestimation for low values and an underestimation for increasing target N values. The non-perfect set-up of the spreader varied by a $\pm 15\%$ the applied dose compared to the target one. Nitrogen recommendations were not linearly related with yield.

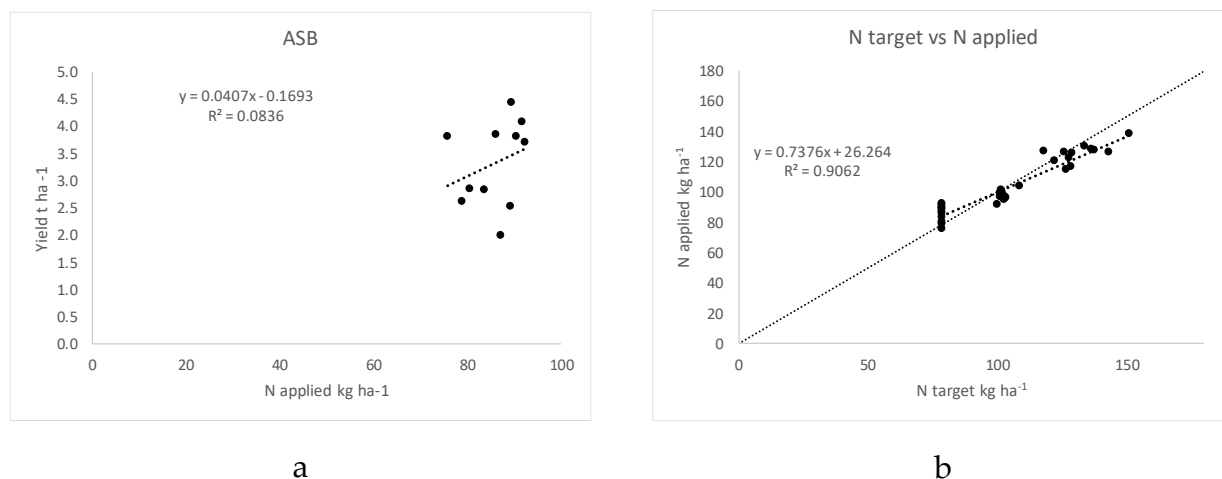


Figure 37: a) scatterplot between yield and total N applied for each plot of ASB method; b) scatterplot between N target and total N applied for each plot of ASB method.

The analysis conducted with geo-statistical techniques using pseudo co-kriging did not produce reliable results, probably as a consequence of the large areas with low or zero yield values. A further test was conducted using a subset of data corresponding to the eastern part of the field, but again no

reliable results were obtained. Therefore, it was not possible to determine which of the three N recommendation tools tested during the trial would generate more yield if carried out over the whole field. Figure 38ab shows the yield maps of the VR1- Δ PNU and ASB-UR methods spatialized over the whole field by pseudo co-kriging derived from the application of the coregionalization model obtained using the punctual yield values of their respective plots. As we can see the two maps are very similar and the differences (Figure 38c) are very few and concentrated in the non-productive part of the field. Furthermore, Figure 38d shows that the statistical reliability of these differences is not significant.

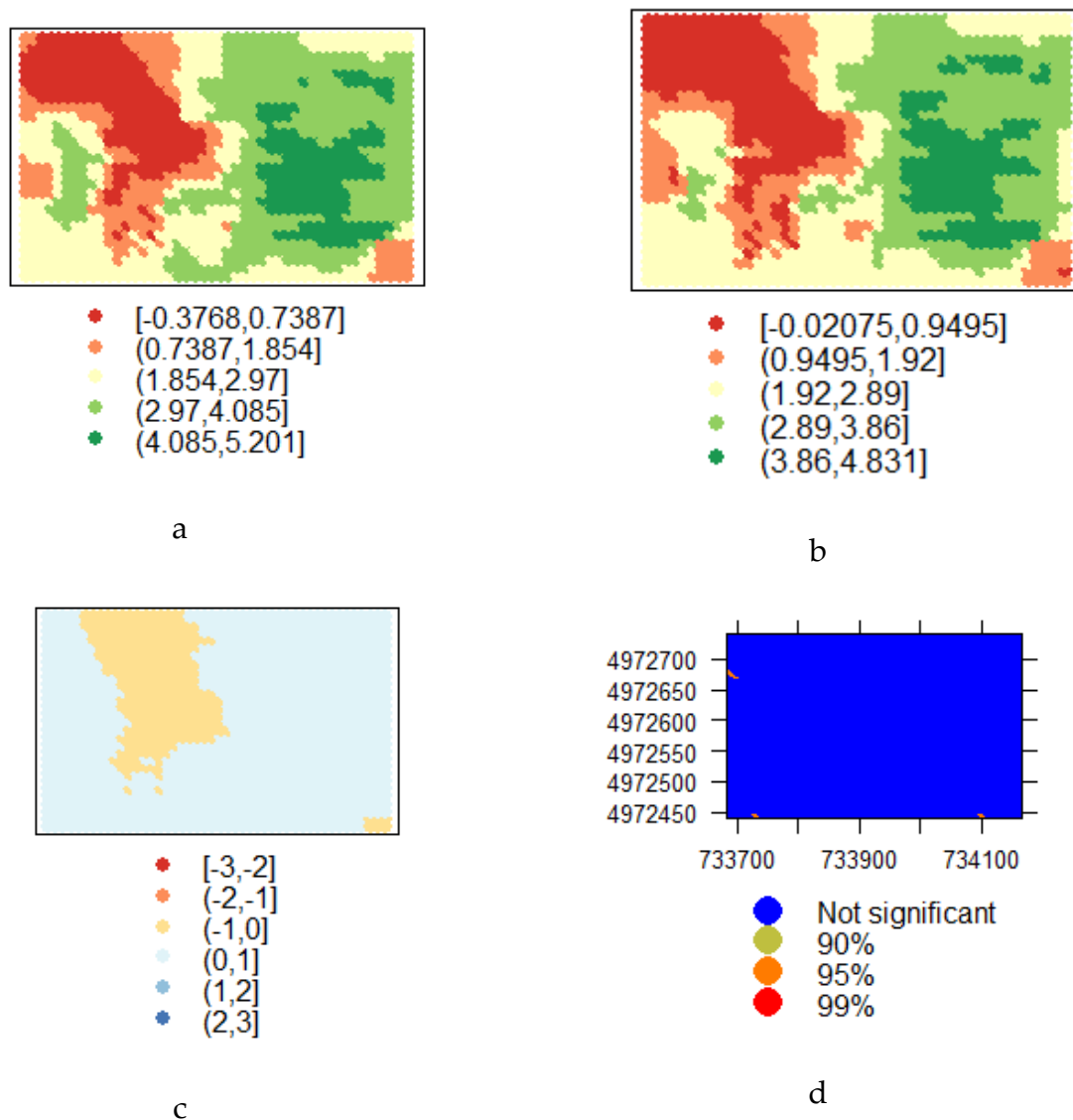


Figure 38: a) Δ PNU Yield map estimation (t ha⁻¹); b) ASB Yield map estimation (t ha⁻¹); c) yield map differences between Δ PNU and ASB (t ha⁻¹); d) map of significance differences

2.3.2. Test Case II – Maize 2020

Also the maize experiment for the comparison of variable-rate N fertilization methods, was carried out in an operational manner covering the whole 2020 season. The crop was harvested to produce whole meal maize mash (grains and maize cob harvested at about 30% moisture) for silage and on average the yield was 12.8 t ha^{-1} with peaks of over 30 t ha^{-1} and a minimum of almost 0 t ha^{-1} due to areas where the crop did not grow. If we consider only the 48 parcels useful for the analysis of the results, excluding the areas affected by crop failure and the anomalous data, the average yield was 13.6 t ha^{-1} (Figure 39).

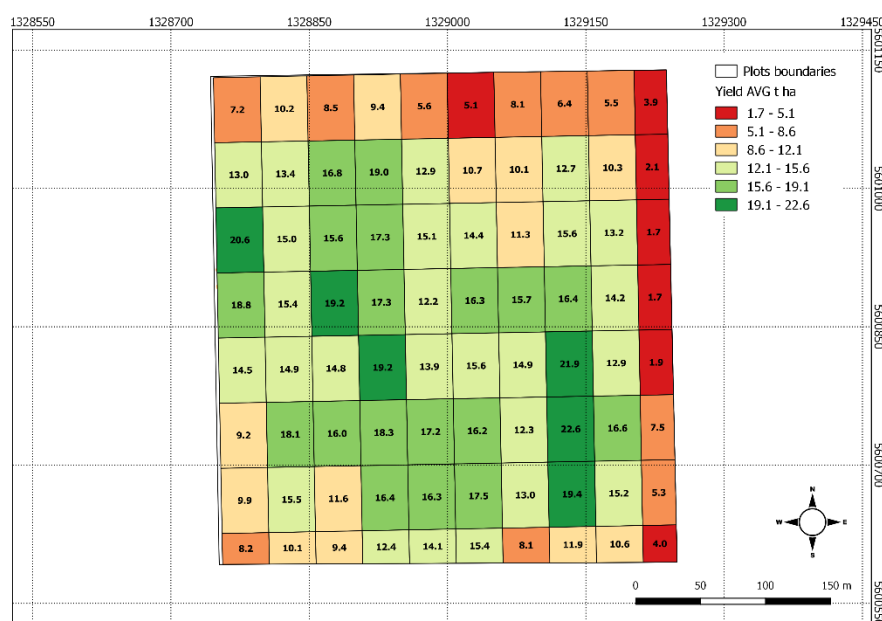


Figure 39: yield averaged plot by plot (t ha^{-1}) of maize field 55

The comparison with the maize overall farm average yield shows how in general field 55 produced lower than the average farm (16.7 t ha^{-1}) and much lower than the best field (20 t ha^{-1}). Extensive damage was caused by rodents, especially in the proximity of drains, which greatly reduced the actual presence of plants stand with respect to the planned one. The yield performance was however obtained with a much lower N fertiliser use (160 kg ha^{-1} mean value for field 55) than the standard reference of 260 kg N ha^{-1} and the farm 2020 average of 175 kg N ha^{-1} used for maize fields.

Table 27: N treatments performance on maize considering: yield (t ha^{-1}), total N applied during the season (N supplied kg ha^{-1}), nitrogen use efficiency (PFPN kg kg ha^{-1}), net return above N cost (RANC € t)

N treatment	Yield t ha^{-1}				RANC € t				PFPN kg kg ha^{-1}				N supplied kg ha^{-1}			
	Min	Mean	Max	$\Delta\%$	Min	Mean	Max	$\Delta\%$	Min	Mean	Max	$\Delta\%$	Min	Mean	Max	$\Delta\%$
VR1- Δ PNU	10.3	14.1	18.3	0	1580	2198	2861	0	57	85	109	4	143	168	181	0
VR2-SI	9.6	13.3	16.0	6	1471	2067	2507	6	54	84	117	5	131	161	180	4
UR-ASB	10.1	13.3	16.3	6	1546	2072	2556	6	62	88	125	0	127	154	165	8

In addition to the average yield, the analysis conducted (Table 27) shows how the plots managed with method VR1- Δ PNU produced more (14.1 t ha^{-1}) than the others which adopted different N management methods (13.3 t ha^{-1}) (Figure 40c). However, for this treatment there was a slight increase in amount of fertilizer (Figure 40d), that lowered the N use efficiency (PFPN) compared to the average (86 kg ha^{-1}) and to the balance method (ASB-UR) which obtained slightly better results (88 kg ha^{-1}) (Figure 40a).

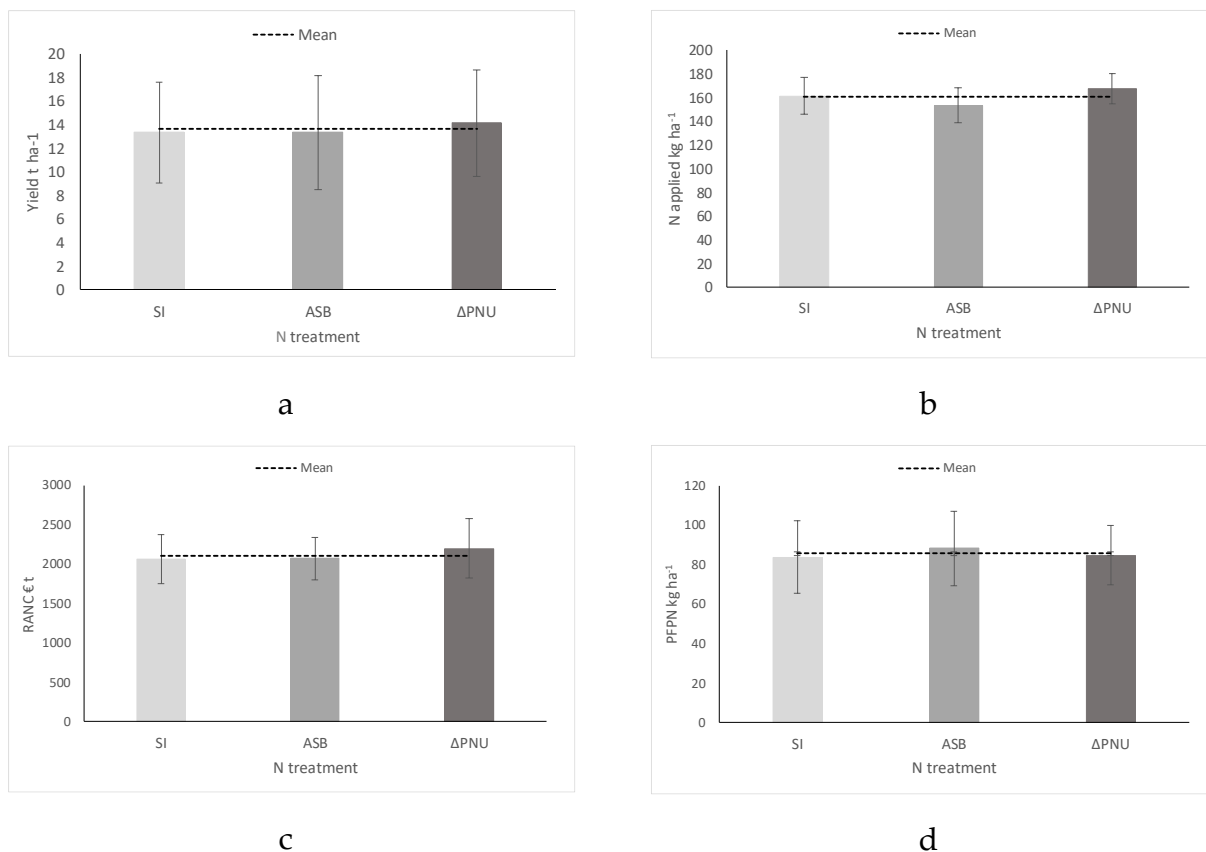


Figure 40: average value on N treatment of: a) maize yield t ha^{-1} ; b) maize total N supplied kg N ha^{-1} ; c) maize economic return (RANC € t^{-1}); d) maize partial factor productivity (PFPN $\text{kg of grains by kg of N ha}^{-1}$ supplied)

From an economic return point of view (RANC), however, despite the lower PFPN values obtained (109 kg ha^{-1}), the VR1- Δ PNU method was confirmed to be the one that guaranteed the best

average economic return (2198 € ha⁻¹), as well as maximum return (2861 € ha⁻¹), i.e. more than 100 € ha⁻¹ higher than the other N management tools (Figure 40b).

As described in chapter 2.2.6 there were areas with different soil types inside field 55 that allowed to perform an evaluation of method efficiency in the three soil classes. As shown in Figure 41, the three soil classes had a very different response depending on the N management tool considered. Not always the increased N investment generated a higher yield as happened for example for the VR2-SI method in LCO1 and VR1-ΔPNU in CDS1 which did not obtain a better response (Figure 41 and Table 28).

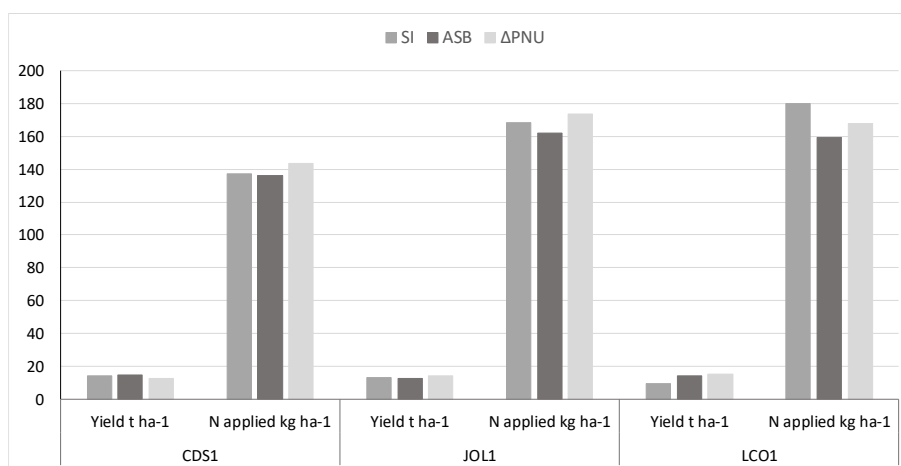


Figure 41: maize average yield t ha⁻¹ and total N kg ha⁻¹ applied in the three N treatments and for the three soil classes

To confirm that soils with different characteristics generate different crop responses, Figure 42a shows how the N usage in soil class CDS1 was better than the overall average of three N treatments compared, highlighting that the crop in this area of the field could use better each unit of nitrogen supplied. As for the economic return (Figure 42b) we notice that in general the differences in performance are not so easily interpreted because of the different values that do not bring out a better situation than another but in general we can say that the soil class CDS1 has obtained the best RANC independently by the N management tool used.

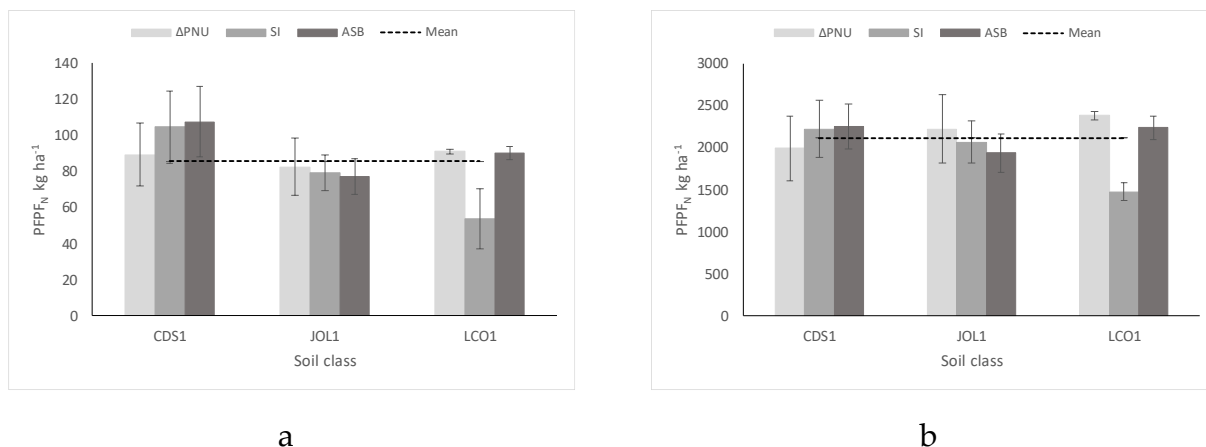


Figure 42: maize average PFPN (a) and RANC (b) in the three N treatments and for the three soil classes

The Table 28 shows how on average the yield in the CDS1 class was quite high (14 t ha⁻¹) compared to the low total fertilizer rate supplied (138.4 kg ha⁻¹).

Table 28: average yield and total N supplied broken down by soil classes and N treatments

	CDS1			JOL1			LCO1			Class Mean		
	Yield t ha ⁻¹	N tot kg ha ⁻¹	RANC € t	Yield t ha ⁻¹	N tot kg ha ⁻¹	RANC € t	Yield t ha ⁻¹	N tot kg ha ⁻¹	RANC € t	Yield t ha ⁻¹	N tot kg ha ⁻¹	RANC € t
SI	14.2	137.3	2222	13.3	168.5	2065	9.6	180.0	1471	13.3	161.4	2067
ASB	14.4	136.2	2254	12.5	162.0	1934	14.4	159.5	2235	13.3	153.6	2072
ΔPNU	12.8	143.7	1993	14.3	173.9	2220	15.3	168.0	2381	14.1	167.5	2198
Treat. Mean	14.0	138.4	2178	13.4	168.5	2082	13.8	167.0	2141	13.6	160.9	2112

Analyzing, in addition to the indicators performance, the effectiveness of the variable distribution in comparison to the homogeneous, we obtain further results. For what regards the use of N according to the management tools and the distribution mode, Figure 43 shows how, also for maize, as was previously observed for wheat, the areas that received less N amounts did not suffer a drop in yield. This finding shows that the rationalization prescribed did not affect the crop in terms of yield.

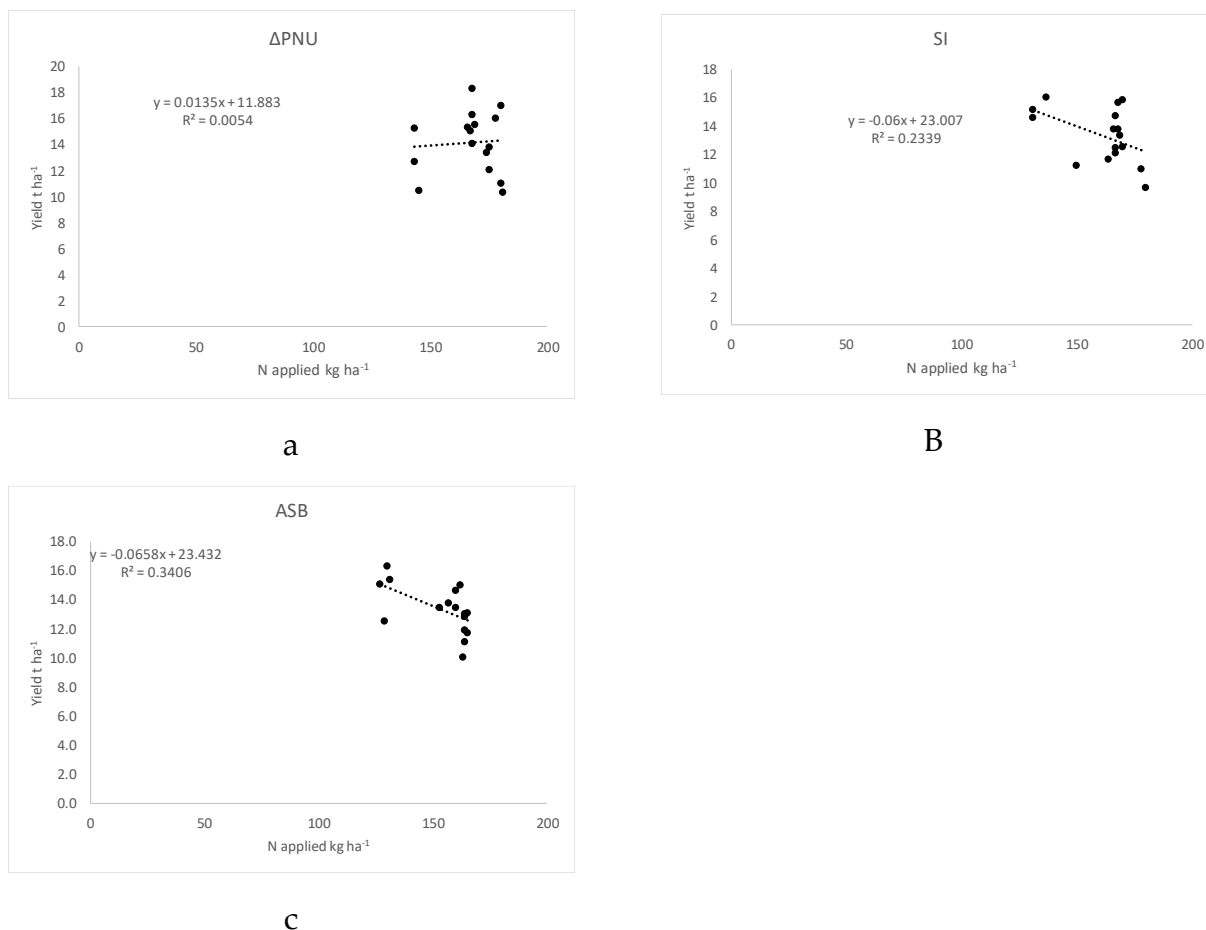


Figure 43: relationships between yield and total N applied for each plot for the experimental treatments a) ΔPNU; b) SI; c) UNI

This result is visible for the VR2-SI method (Figure 43b) where the higher yield values correspond to the lower values of N applied, but in general the effect is masked because of two main reasons. The clusters with the lower total N applied values correspond to the plots in CDS1 soil class that influenced the representation of data, given the low total N supplied rates and the good yields for plots in this soil class, regardless of the method applied. The ASB used for DT determination, prescribed a different total crop N need for CDS1 class (less N requirement) as compared to the other soil classes. The CDS1 soil class is typically considered less productive than other classes, and it refers to those areas of old riverbeds historically more problematic (farm interview), but with an excellent soil potential productivity, i.e. high soil organic carbon (SOC), good C/N and high total soil N. The second issue was the very low yield values recorded in the field by the machinery that have vertical regular pattern, potentially an artefact, and that affected the real average yield value for many plots. The red stripes visible on maize yield map (Figure 44b) correspond to real crop failure caused by animal damage and recorded as low yield points in the yield map. Even performing an outliers removal from the dataset before interpolation, these points were not removed because considered not

outliers and because this could cause the removal of low but reliable yield values in other areas. These bad data flattened the variability by altering the representation of the N fertilizations effects on final yield generating then bad data visualization and statistics.

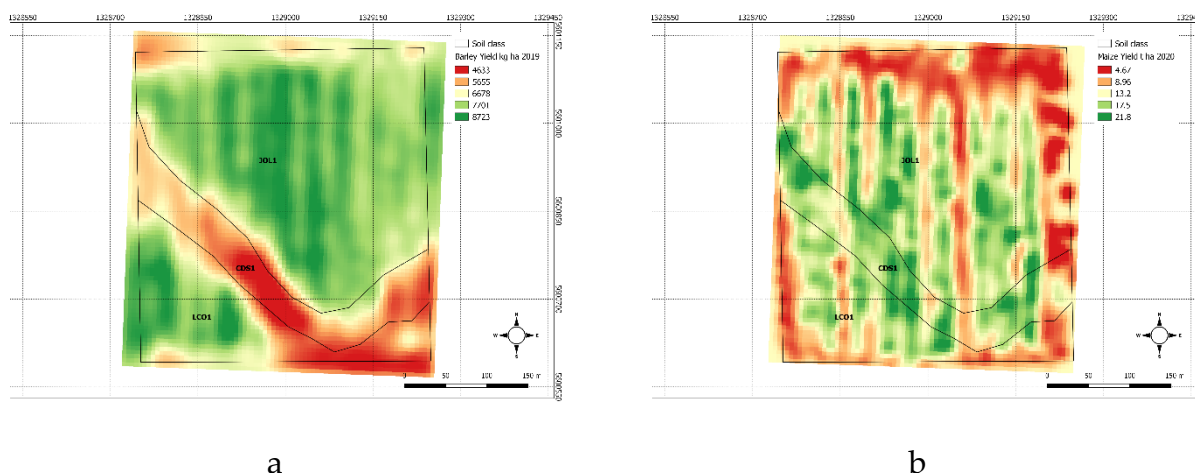


Figure 44: yield data interpolation of: a) barley 2019; b) maize 2020

Independently from the erroneous data, when comparing yield map of different years of field 55, even if with different crops, interesting patterns can be noticed. Figure 44a shows barley yield map of 2019 and Figure 44b maize yield map of 2020 with overlapped the boundaries of the soil classes present in the field 55. Despite the annual variability, the overall productivity trend of the CDS1 area is completely different. During the 2019 season, as usually happens, this area of barley field reached lower yield performance compared to the rest of the field because generally affected by early stress for a non-irrigated crop like barley, since it has a coarser soil texture. Instead, in the 2020 season, this portion of the field not only did not show stress, but on the contrary it produced slightly more than the remaining part of the field. The 2020 yield spatial consistency is potentially attributable to the fertilization management and to the general good season for maize crop with regards to the study area that benefited from regular rains in June and July without generating water stress. Probably, with a condition of water abundance, the plants suffered more on peaty, deep and heavy soils rather than on shallower soils such as the CDS1 which drained the water more and allowed better nutrients absorption without showing stress.

The fertilizer distribution accuracy performed by the spreader showed even in maize trials an inaccurate supply compared with the established target rates. The scatterplot of Figure 45 shows how despite a good correlation ($R^2=0.92$), there was an underestimation of the effectively applied rates compared to that target rates. This inaccuracy, which in some cases considering the sum of the 3 fertilizations (Nt1 Nt2 Nt3) reached the 60 kg of N ha⁻¹, can potentially invalidate the benefits of a

site-specific application of the fertilizer. Moreover, the lack of the N amount established by the algorithms has probably contributed to generate growth deficits of maize with consequently yield reduction..

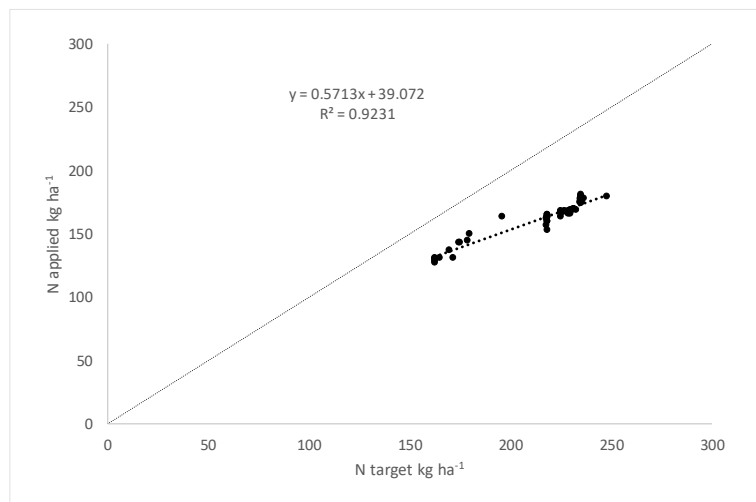


Figure 45: scatterplot between N target present in the prescription maps (Nt1-Nt2-Nt3) and the N effectively supplied and recorded in the application maps (N applied) in maize

The analysis using pseudo-co-kriging geostatistical techniques provided an estimation of how the yields would have been if only one of the N management methods tested had been applied across the field. The test compared one by one all the yield maps obtained from the three different N treatments. Only in one test the analysis generated yield maps with significance differences (Figure 46d) and was the comparison of the VR1- Δ PNU and ASB-UR methods with significant differences ($z > 90\%$) for 24% of the total surface area. The spatialization of the VR1- Δ PNU and ASB-UR methods and their comparison (map differences, Figure 46c) showed how ASB-UR and Δ PNU provided a relatively higher yield for the 64 and 36% of the area respectively. Considering only the areas with significant differences (24% of the total) emerged that 58% (42%) of this area would have produced more if the ASB-UR (VR1- Δ PNU) method was adopted. In addition, the average yield for the VR1- Δ PNU method (Figure 46a) would have been 15 t ha⁻¹ while the average yield for the ASB-UR method would have been 15.4 t ha⁻¹ (Figure 46b).

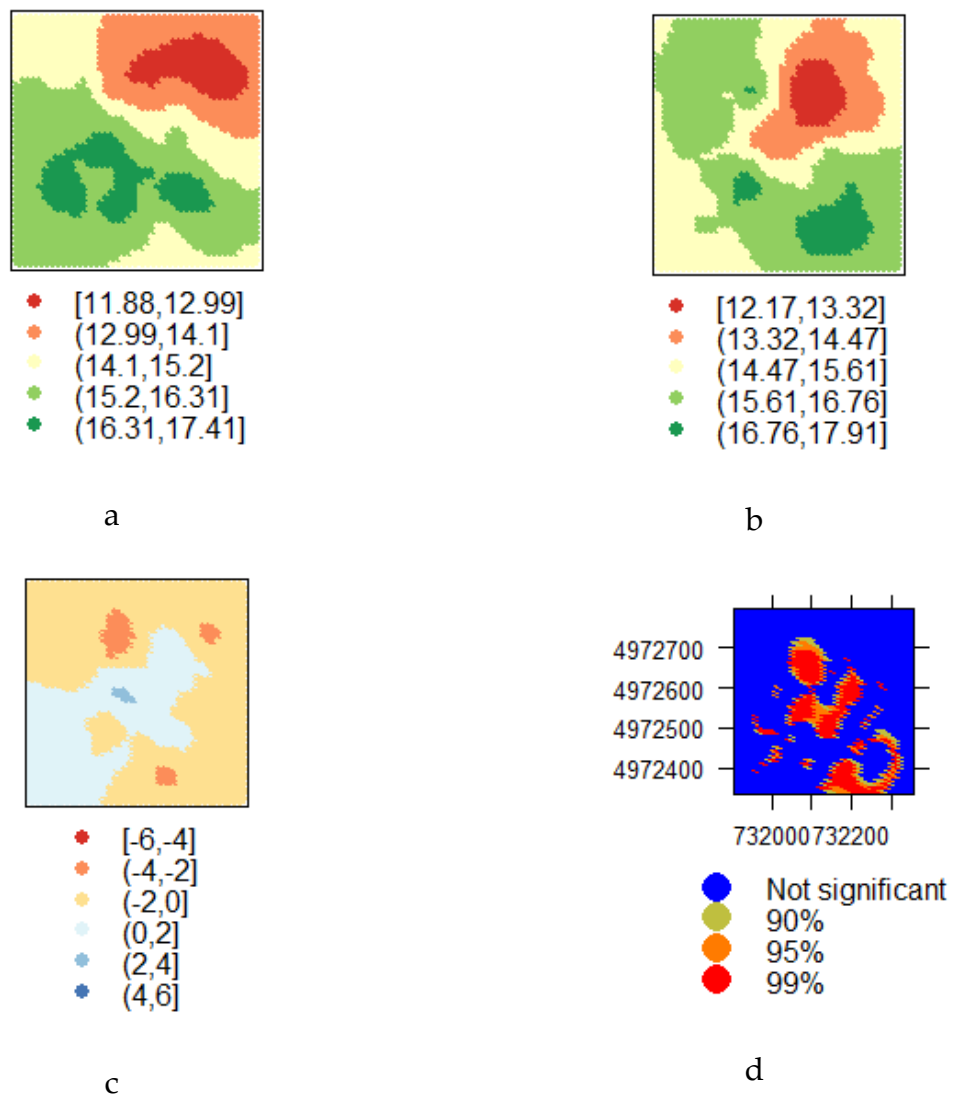


Figure 46: a) Δ PNU Yield map estimation (t ha^{-1}); b) ASB Yield map estimation (t ha^{-1}); c) yield map differences between Δ PNU and ASB (t ha^{-1}); d) map of significance differences

2.4. Discussion

Important indications regarding the performance of the tested methods can be drawn from the results achieved.

2.4.1. Crop management

The field experiments carried out in this study demonstrated the feasibility of applying in real farming conditions advanced tools for rational N fertilizations, supported by remote sensing data, throughout the season by modulating different fertilisers in space and time. All methods highlighted how seasonal crop-based and soil-based information are very helpful in a perspective of sustainable N management. In a context of soil types with high levels of organic matter and nitrogen content, such as those of the study area, over-fertilisation can be above all an environmental issue, with no

greater return on yield and costs reduction. As found from F1 experimental results, high N rates did not correspond to greater biomass accumulation, as a consequence of strong difference of soil characteristics that must be taken into account for precision farming applications. The findings of the F2 experiments, devoted to real N management during the season, revealed that, on both crops tested, that on average the Δ PNU method was the one that produced the most without suggesting an higher N supply. In addition, if we exclude from the analysis those plots from field areas in which serious crop damage had occurred, in both crops all tested methods reached average and maximum yield values equal to farm overall production, but with significantly lower N supply and indicating an overall better economic efficiency. This result, obtained over two years and over two crops, clearly showed that N was not the only limiting factor and that the soil N uptake by the crop is a consequence of not only by N supply with fertilizations but also by soil conditions that influence the efficiency of nutrients absorption (Fageria and Baligar 2004).

Soil conditions vary depending firstly on the physico-chemical and biological properties of the soil and then on weather conditions and management that interact with them, defining the real nutrient-supplying capacity of the soil (Havlin and Heiniger 2020). For example, the JOL1 soil type is a fertile soil typically considered highly productive (farm statistics) and not generally managed with high level of fertilization, because it is considered in any case able to attain good yields. This aspect was confirmed from results of F1 experimentation, where for this class the maximum biomass accumulation was achieved at intermediate N rates of about 200 kg ha⁻¹ (maize) and 120 kg ha⁻¹ (durum wheat). For what regards findings from F2 activities we can highlight the following consideration for the two crops.

For wheat the production in some areas of the field reached maximum yield values around 6 t ha⁻¹ with about 100 kg ha⁻¹. The general moderate or weak crop response to high N rates seems to show that N supplied by fertilizations is not completely absorbed by the crop, and consequently in these conditions, nitrogen is probably not the only limiting factor. In the VR2-SI managed plots, the yield did not increase despite a higher total amount of N than for all other methods. In ASB-UR managed plots, the N rate was about half of the standard dose (85 vs 160 kg N ha⁻¹) and less than 20% if compared to VR methods tested. It is important to remind that, if we do not consider the plots with crop damage on average the yield was 3.3 t ha⁻¹ compared to 3.5 t ha⁻¹ of the other VR methods. Probably with very fertile soils the very hot spring (2020) encouraged SOM mineralization and favorable soil conditions for nutrients absorption.

Instead in the areas with intense crop damage the resistivity map highlighted possible differences in soil characteristics just in the area where the greatest stagnation occurred. A cluster analysis (k-

means) was performed for finding subgroups within the resistivity data set and to identifying the right number of clusters to group data (Silhouette method) using R software version 4.0.3 (R Core Team, 2020). With a cluster analysis performed both with the first layer of depth (0-50 cm) and the three layers available (50-100 cm and 100-150 m), we obtained the potential presence of two classes with different characteristics (Figure 47). Probably these are local differences not highlighted by the soil map that represents the field as homogeneous (JOL1) for characteristics but that in reality locally had areas with different crop response.

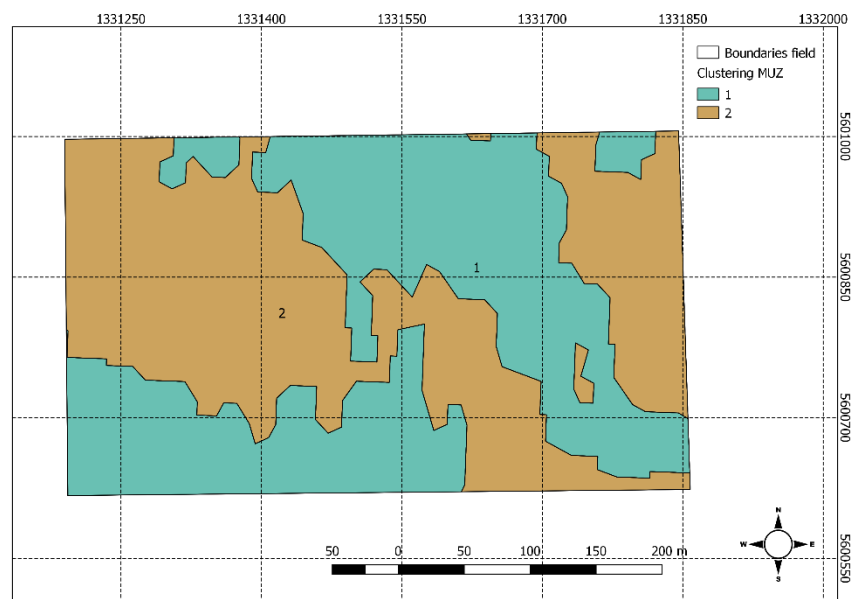


Figure 47: experimental field n° 187 (wheat): b) clustering of resistivity profile 0-50 cm depth

For maize the soil class JOL1 was the class, regardless of the N management method used, with the worst performance in terms of yield (12.5 t ha^{-1} against 13.6 t ha^{-1} field averages). This class along with LCO1 was also the one that received the highest nitrogen dose regardless of the N management method. Instead, the CDS1 soil class was the one that produced the most (+4% compared to JOL1) and received less nitrogen than all other classes (-20%). CDS1 class represents a shallow soil with abrupt passages between the phases that often generate crop stress, caused by primarily by water deficit and secondarily by nutrient deficits. The 2020 season, which had rains distributed over the entire growing season of maize, probably favored growth in this zone due to the sandy subsoil, as opposed to other classes that have deep soil, clayey and peaty subsoils and that may have had problems of water stagnation and soil saturation in the subsoil, as happened in the experiment of wheat with soil JOL1. Even in this case nitrogen does not seem to have been a limiting factor, but on the contrary, it seems that other factors have favored the overall good nutritional status in an area

usually characterized by stress and which received on average only 138 kg ha⁻¹ of nitrogen. The good nutritional status was also confirmed by the fact that parcels with VR methods within the CDS1 class received from 16% (VR1-ΔPNU) to 21% (VR2-SI) less nitrogen than the VR parcels within other soil classes (JOL1-LCO1). Satellite images allowed to detect favorable N status conditions and consequently the algorithms indicate a reduced N rates without impacting on final yield.

2.4.2. Importance of seasonal monitoring

The within field crop spatial variability occurred during the trials demonstrated the importance of seasonal monitoring to efficiently identify actual crop status in a spatial explicit way and how such information can be derived from satellite images. The problems due to biotic and abiotic stresses are constantly and seasonally present, but with the characteristic of being variable in time and space (Basso et al. 2011; Cammarano et al. 2020; Maestrini and Basso 2018). The possibility of having recursive and spatialized information through the operational data provision of S2 platform, allowed to implement VR strategy for fertilizations management (VR1-2) during the season based on crop monitoring at the field scale (Defourny et al. 2019). This is an advantage compared to the standard ASB-UR method that, despite being an advanced approach with respect to fix dose varietal dependent fertilisation scheme, because it uses measured soil properties to modulate the crop needs, is static in time and it is based on an a priori decision that is not adapted to manage seasonal events. This was demonstrated in both field trials. The test on wheat, performed on a field considered homogeneous, having been classified into one soil class (JOL1) according to farm information, revealed very different responses caused by soil-crop-weather interaction. The test on maize instead, conducted in a field with different soil classes, showed conflicting results compared to what was expected on the base of the historical response of crop to the different soil types. This confirms that optimal nitrogen management would require both soil-based and crop-based information (Cordero et al. 2019; S Nawar et al. 2017). This would be required in order to: i) consider the properties of the soil as an important productive factor for defining fertilisation strategy and understanding “temporally constant” limiting factors; ii) exploit in-season information on crop growth spatial variability and nutritional status to adapt the strategy according to seasonal trends.

By analyzing overall yield farm data on wheat and maize 2020, it emerged that nitrogen has been accurately dosed with an average of 175 kg ha⁻¹ for maize and 105 kg ha⁻¹ for durum wheat, always below the crop standard rates. The N supplied has been differentiated between fields and with choices made a priori by the farm according to the soil classes with good results in terms of yield. But the dispersion of the data and the absence of correlation in the Figure 48ab, shows that increasing N rates

do not correspond to increasing yields in both crops, suggesting a margin for optimising N provision, by filling the gap of highlighted lack of efficiency in fertilisation. This does not mean that the soil nutrient balance approach (i.e. ASB) adopted at farm level is not a proper solution, but only that the effect of seasonal variability emerged had not been completely taken into account. This impacted on the final yield, RANC and on the overall efficiency of fertilizations (low yields and high doses of nitrogen), which indeed did not happen in the trials with the VR methods (high yields low doses of nitrogen). The review work of (Said Nawar et al. 2017) clearly highlighted that many studies from the last decade showed that when zoning techniques are used for variable-rate nutrient application, farm efficiency increased when this is compared to traditional uniform-rate application methods. The possibility of estimating the N uptake or the overall crop status allows not only to rationalize the nitrogen provision, but also to optimize the response in terms of nitrogen absorbed and final yield with potential financial and environmental benefits.

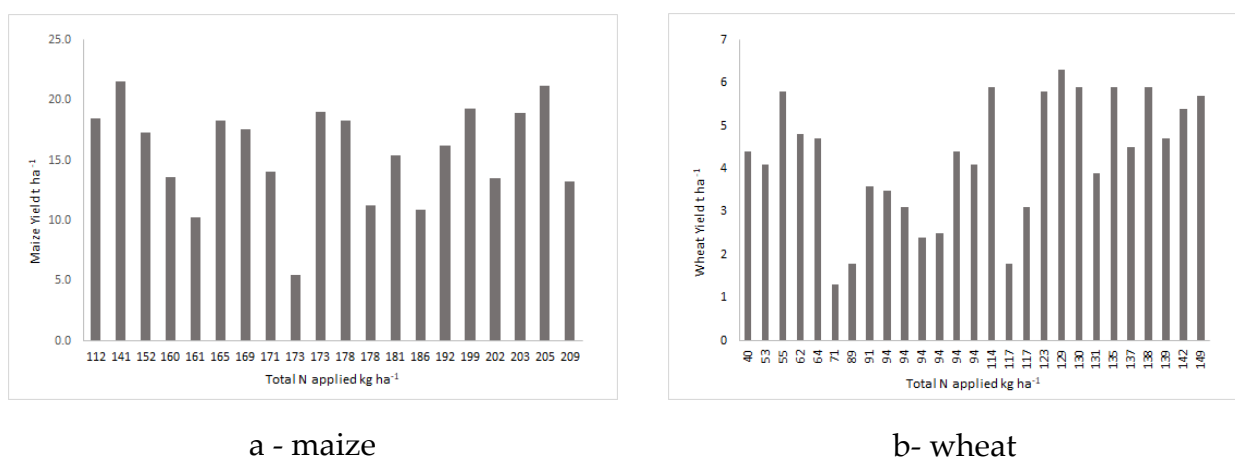


Figure 48: barplot of yield mean values in 2020 and total N supplied, calculated field by field at farm level, (a) maize (b) durum wheat

2.4.3. N fertilizer recommendation tools

The N management tools tested in this study can be considered purely empirical approaches for VR1- Δ PNU and VR2-SI and physically based approach for UR-ASB. The first method (VR1- Δ PNU) is a spatial plant-based quantification of crop N demand based on the actual crop nutritional status, the second method (VR2-SI) is a spatial plant-based rationalization of the amount of fertilizer a-priori established on the base of overall field statistics, the third method (UR-ASB) is a (spatial/non spatial) soil nutrient balance approach for the quantification of N to be supplied with fertilizations to compensate crop needs. In general, most of the operational field data collection systems rely on reported or previously defined empirical statistical relationships between remotely derived products

and variables of interest, that in our case are those related with nitrogen uptake (Mariangela Diacono et al. 2013; M. Weiss et al. 2020). In particular, as also demonstrated in this experiment, these approaches can provide, after model calibration, information in a fast way (near real time) without particular computational effort. For this reason, such methods are the most adopted in operational applications. It is important to remind that often the used relationships are affected by the representativeness of the calibration dataset that reduce statistical robustness and exportability in other contexts (space and time).

Using the methods mentioned above, the experimental results demonstrate that the total N amount can be reduced (on average -40% for wheat -30% for maize) if compared to the standard dose (160 kg N ha⁻¹ for wheat and 240 kg N ha⁻¹ for maize) normally suggested by consultants and adopted by the farmer in this area. As we have seen, yields were lower than the standard, probably due to crop damage, but certainly no negative environmental externalities were generated. On the contrary, the overall economic benefit was comparable to literature (Gandorfer and Rajsic 2008) or better, especially for maize (Casa et al. 2011). Among the tested approaches, variable rate N management does not always increase grain yield with respect to uniform N application and this finding agrees with previous results (Cordero et al. 2019; B. L. Ma et al. 2014; Vizzari et al. 2019). In both crops, in some parts of the field, the results were equal to the fields that produced more at the farm level and the strategies used showed good overall results in environmental, economic and use efficiency terms. Indeed, not only crop yields, but also N efficiency and environmental and economic returns should be considered, for agroecosystem improvement (Jin et al. 2012; Khosla et al. 2002).

The VR1- Δ PNU method, thanks to the crop N dilution theory, allowed using a quantitative indicator that gave information about the N nutritional status. Then the information about N deficiency or surplus were used to quantify the right N amount without generating environmental risks due to lack of absorption. The recursive crop analysis before fertilizations, through satellite acquisitions, allowed to monitor the growth and consequently to identify some problematic areas in the field. The apparently external factor (abiotic or biotic stress) that influence crop production should be always taken into account and the importance of having a monitoring instrument, without being in the field, demonstrated here its importance. The VR1- Δ PNU method correctly suggested not fertilizing some areas, as it would have been a waste of product because it would not be absorbed by plants. Varying spatially the N rates VR1- Δ PNU reached the same N use efficiency of ASB-UR for wheat crop (PFPN 28 kg ha⁻¹) and only the 4% less for maize crop but always getting a better economic return (RANC +20% for wheat and +6% for maize). Of course, determining the optimum amount of fertilizer to apply is a critical issue and in some cases an overestimation of PNUa, derived

from CCC_S2, emerged during the calculation of ΔPNU . The correct estimation of variables such as W and PNUa is essential to avoid errors and a wider calibration dataset would allow to improve statistical relationships. The possible estimation of the critical and real uptake also allowed us to calculate the NNI giving indications on the overall nutritional status. As shown in Figure 49, the NNI calculated field by field on the entire farm from S2 images acquired at flowering, explains in advance 50% of the final yield variability. This information shows the possibility to make a coherent representation of variability with productivity and therefore to have a tool to manage it in advance. Contrary to what is shown in Figure 48, where the applied rates were not consistent with final yields, the VR1 diagnostic tool could allow to extend the economic and efficiency benefits, obtained from the experimental fields, at the entire farm or for a district including different farms.

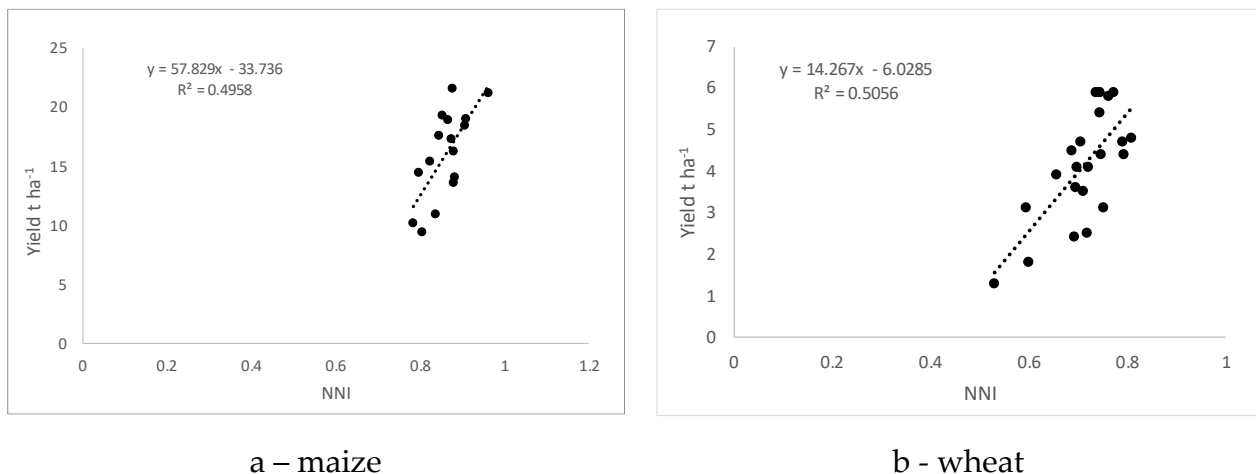


Figure 49: scatterplot between mean yield values and mean NNI values, derive from S2 images 11th of august 2020 (maize) and 23th of may 2020 (wheat), calculated field by field at farm level (a) maize (b) durum wheat

As for the first VR method, also for the VR2-SI, the satellite images allowed to modulate the uniform dose on the basis of the variability found. The virtual reference calculated within the field generally assigned a higher average dose than the other two methods especially on durum wheat. This was because the N rate increased for low reference index values and decreased for high values (good nutritional conditions). The presence of many areas of bad crop conditions, due to failure, in both trials affected the algorithm results, the method tried to overall increase the dose to compensate for the growth deficit. This approach is certainly valid to flatten variability, but shows its limits in those highly compromised areas, because it tends to allocate higher rates than economically optimal as seen in other studies (Franzen et al. 2016; Ransom et al. 2020). In fact, the PFPN of this method was the lowest with -5% for maize and -14% for wheat when compared to ASB-UR whereas the RANC was equal to ASR-UR in maize. The virtual reference approach to algorithm calibration is far less likely

to generate erroneous N recommendations than the N-rich approach because minimizes the potential for nutrient imbalances caused by excess N availability in the N-rich strip (Franzen et al. 2016; Kyle H. Holland and Schepers 2013). In addition, satellite images that include large portions of territory allow us to have statistical metrics on many fields at once. With a field-by-field survey, it is potentially possible to make comparisons by grouping fields by sowing date or by variety, thus having a more robust proxy for plant N status. The virtual reference in fact can be even more useful and representative if used within groups of fields assumed similar thus avoiding possible anomalous references.

The spatially uniform N recommendation method ASB-UR, was very reliable in terms of correct definition of nitrogen rate. The advantage of using measured data on soil properties allows to quantify N needs on the real characteristics avoiding losses and environmental risks. The difficulty of constantly having up-to-date information on soil properties and/or on fertilisation inputs and crop removal is the major limitation for nutrient balance approaches. Potentially, however, adopting data fusion techniques and multi-source information, such as yield and as-applied maps produced by variable rate machinery, soil properties maps and crop N status maps remotely derived during the growing season, this method can be very promising because it adopt a physically based approach in a spatially distributed way (Fabio Castaldi et al. 2016; Ge et al. 2011). The integration of soil-based and plant-based methods (Miao et al. 2018) to drive fertiliser applications can be considered a future promising tool for N use efficiency improvement (Cordero et al. 2019) and overall farm system sustainability (M. Weiss et al. 2020). On maize trials, soil classes with ASB-UR plots had different reference rate (DR) on the basis of soil properties, so a sort of simulation of the spatially distributed dose was tested, but only with a priori criteria and not with in-season information. This has led to several results showing that without seasonal information and without site specific management systems the UR method highlights its limitations to the advantage of VR methods (S Nawar et al. 2017). In addition to the above-mentioned positive and negative aspects of each method, there are further considerations to be made. Important aspects to consider for an operational use of any of these N management tools are the timing of fertilizations and the use of machineries suitable for variable rate distribution with satellite guidance and mapping capability. With regards to the first aspect, the Nt1 fertilizations are the most problematic and those with the least potential added value. This is because the satellite images when the crop is in a phenological phase of second leaves (maize) have an unfavorable signal to noise ratio (crop cover fraction very low) and because during early tillering phase (wheat) the N uptake is usually satisfied by the soil N supply. The most important intervention, both from an agronomic point of view and with regard to satellite derived information accuracy, are

the fertilization Nt2 at stem elongation for durum wheat, and for maize at the sixth leaves and at flowering. The latter was also successfully tested on maize in VR mode during the 2020 field trial, with liquid fertilisation exploiting a sprayer (Mazzotti) with on board monitor (Spektra Agri) for map prescription reading. Plant N uptake is rapid for maize between the sixth leaves and silking, and for wheat from late tillering until heading. N stress during these phases can strongly affect final yields. Specific site fertilizations carried out in these stages are the most effective because they can intervene on nutritional deficits allowing the crop to recover whereas, regarding Nt3, they contribute to nutrient accumulation in grains and consequently to better final yield and quality.

vi. Final conclusions and future perspectives

The future model of agriculture has to adequately respond to the challenge of competitiveness balancing growth in production with sustainable management practices that reduce, or possibly eliminate negative externalities. The farm production efficiency improvement should be accompanied by a reduction in environmental impacts and by an improved inputs management as suggested, among others, by the implementing rules of precision agriculture. The objectives of this research were to evaluate the performance of different N fertilizer recommendation tools, on different crops and across diverse soils, exploiting information derived from satellite images and spatially differentiated fertilizations in a perspective of precision agriculture practices adoption. An agronomic comparison between advanced N management systems pre-operationally available was operated analyzing the results with different indicators for highlights pros and cons of each method. Remote sensing provides a nondestructive way of spatially and temporally monitoring vegetation deriving indicators and crop variables useful for reaching many objectives of agricultural policies. Satellite data available from Sentinel mission provides a resource for a real-time assessment of crop status during the season able to guarantee new adoptable methodologies to guide agro-practices. The results of the study showed how many limiting factors for an optimal crop growth was identified during the trials (soil pH, biotic or abiotic stress, wheather conditions, soil properties variability) that reduce the capacity of the crop to uptake N, also in conditions of theoretical high N availability, i.e. soil N content and N supplied by fertilization. The data obtained from the experiments in F1 generally confirmed that plant N uptake and consequently biomass accumulation not only depends on the total amount of N supplied by fertilization and that the selected NNI indicator was coherently linked to trials outcomes being able to identify low and high production conditions. This revealed the importance of monitoring seasonal plants N status, but also highlighted the needs of linking this information to soil properties, for a complete support of N site-specific fertilization in a PA framework. These findings contributed to the

set up of the F2 experiments where 2 variable rate tools for N managing based on two different satellite image based approaches (VR1- Δ PNU and VR2-SI) were compared to N rate quantification generated by an agronomic simplified balance (ASB-UR) based on soil sampled data. The SI and Δ PNU tools generally performed slightly better than ASB. Δ PNU showed the best results in terms of yield and economical return (RANC). The ASB generally performed better in terms of use efficiency (PFPN), because of the high total nitrogen and organic matter content of the soil defined as low N needs to be supplied by fertilization. In any case with the remote N status estimation methods (both SI and Δ PNU) the plots that produced the most were also those that received the least total N, indicating coherent N rates quantification. The VR algorithms tested, allow a good seasonal adaptability for different conditions without field data collection. A proper N rationalization and distribution was therefore carried out without affecting the yield (especially in the undamaged areas). The indicators used showed that although there were crop damages, due to abiotic and biotic stressors that lowered the average yield, the results obtained were equal to those at farm level and comparable with those available in the literature for similar studies.

Data availability and processing time are of crucial importance for applications at field scale because the management practices have to be in agreement with the environmental context. Although empirical relationships are typically calibrated over experimental observations and for this reason they are sensitive to the conditions of acquisition they are still the most used because they well adapt to the local scale. Other non-regressive techniques are usually demanding in computer resources and time, and are thus hardly used in an operational context. In addition, empirical approaches offer the advantage of directly inferring the secondary variables not directly achievable by the inversion of radiative transfer models. In this particular study area, with high soil variability and high overall fertility, but with a high seasonal imbalance, these techniques have been operationally applied with good results.

Some aspects to be taken into account for a potential operational integration of these nitrogen variable rate management tools, are the fertilisation timing overall strategies and the use of machineries suitable for variable rate distribution with satellite guide assistance and mapping capability. Investment in agro-mechanical technology is considered to be a necessary condition for the adoption of precision agriculture techniques. Today, many services are being developed to support farmers, by providing data and information derived from satellite images. Through agricultural contractors they provide specialized, seasonal or temporary services to farmers. This is considered a good option to the burden of data collection and for the management of spatialized information on soil, plant and that, as we have seen in this study, are essential for a rational use of input but also for

a better overall farm efficiency and economic return. Further experiments and tests are needed in different study areas and exploiting data acquired on different seasons to deeply investigate the effect of different soil properties and N supply for crop N deficiency diagnosis and to explore the possibility of using more remotely derived data to gather information both on soils and crops. The use in combination of sensors with data fusion techniques should allow the use of soil and crop ancillary information in addition to historical variability derived by satellite archives and yield data. The perspective goes towards an increasing adoption of technology in agriculture with a use of different tools in a complementary way to have an overall view of the production processes in order to achieve sustainable farming objectives.

vii. Annex

In this chapter details of the trials carried out in F2 are given with relative annotations and comments on crop growth and on the development of operations flows conducted during the season.

a. Test case I (F2)– Durum wheat

i. Seeding

The seeds rate followed the recommended number of viable seeds 350-400 seeds m^{-2} corresponding to approximately 180-200 kg ha^{-1} . From the analysis of the application map, it can be noted () that the Southern side of the field had a higher dose of around 220 kg ha^{-1} due to inaccuracy of the machinery.

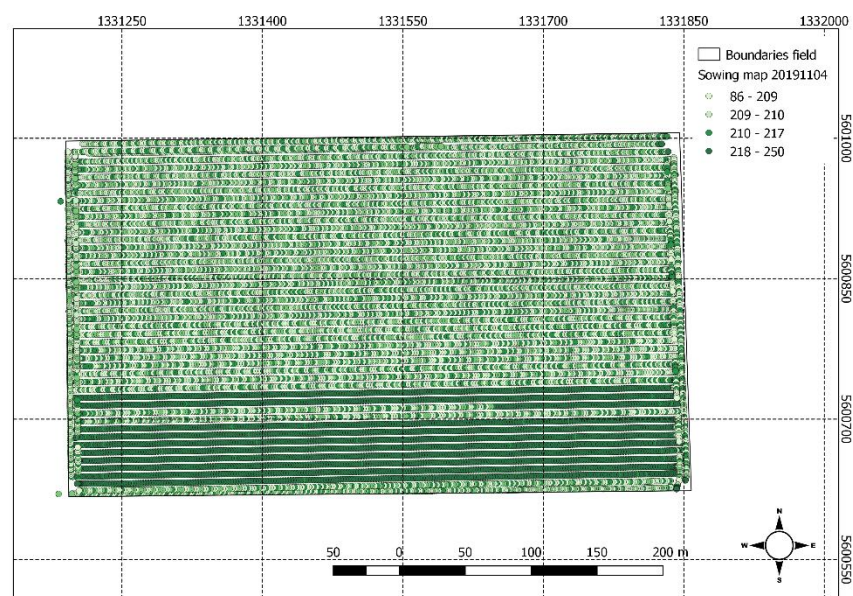


Figure 50: wheat sowing application map as recorded by machinery (kg ha^{-1})

ii. Fertilization

1. Nt1

The Nt1 fertilization was planned for the phenological phase of tillering and a differentiated rate of fertilizer (ammonium nitrate prilled 33.5%) was given to each plot of VR1 and VR2 as shown in the prescription map of Figure 51. The corresponding S2 used as input of both VR1 and VR2 algorithms was acquired on 08th February 2020 and defined a variable dose between 33 to 53 kg N ha⁻¹ considering the DR of tillering of 48 kg N ha⁻¹ (Table 20) as reference to modulate the rates, while the UR a priori established a constant rate of 26 kg N ha⁻¹ (Table 20) Finally a prescription was assigned at each plot the fertilizer amount according N management method.

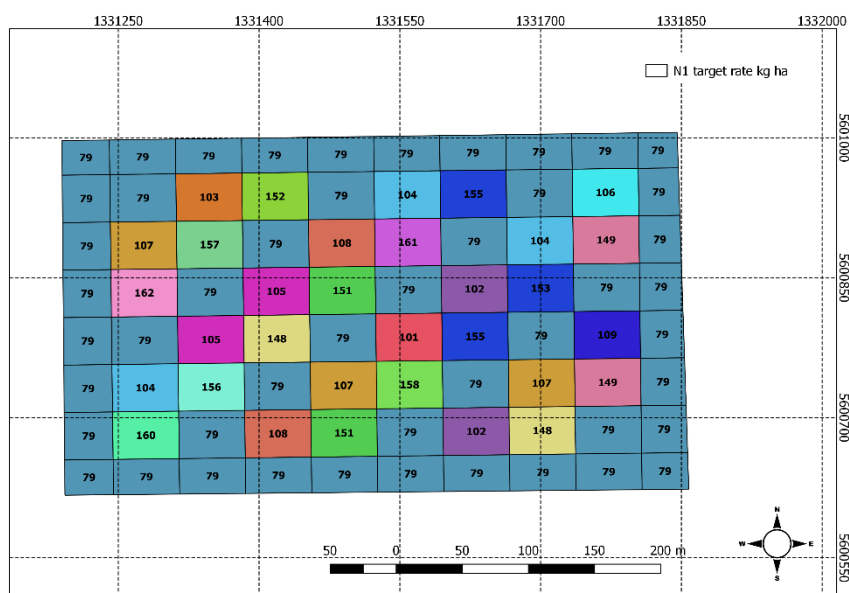


Figure 51: wheat Nt1 prescription map expressed in kg ha⁻¹ of ammonium nitrate (33% N)

On the 24th of February 2020, the first variable-rate Nt1 fertilization was applied and the corresponding application maps with the actual supply of product was derived (Figure 52a). This layer is particularly useful to understand if the established quantity was actually distributed in any single location. In Figure 52b, instead, a statistic per parcel (average of points) is represented to obtain the value of the quantity actually applied in each zone and the deviation from the target quota.

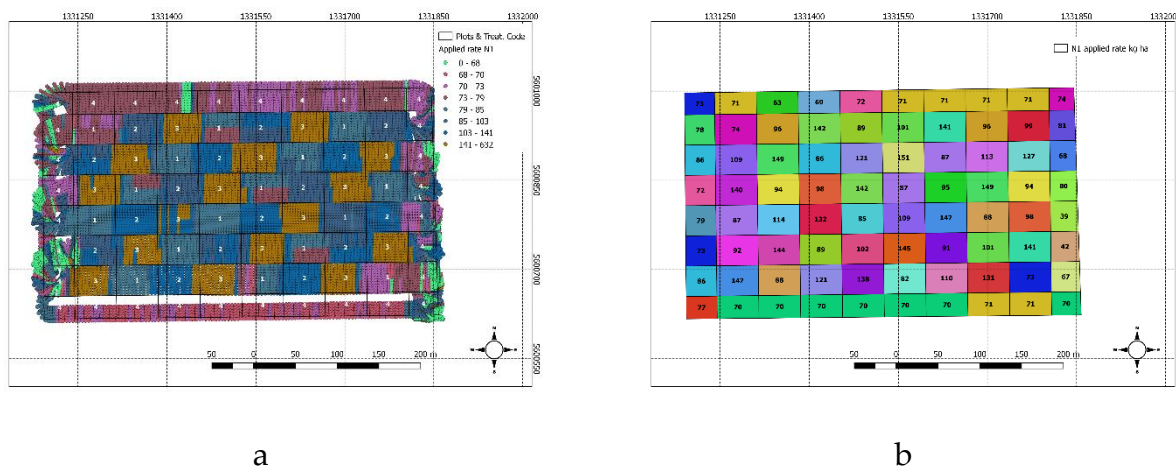


Figure 52: a) map of application (points) with visualized the amount of fertilizer (Ammonium nitrate) actually distributed kg ha⁻¹; b) the averaged quantity of fertiliser actually distributed per parcel kg ha⁻¹

2. Nt2

The Nt2 top dressing was planned for the stem elongation phenological phase and the S2 image used was acquired on 29th March 2020. This phase is phenologically very important for the crop because it is the period in which 50% of the DT is generally supplied. In Figure 53 the final Nt2 prescription map is presented, which defined a variable dose between 54 and 72 kg ha⁻¹ of N for VR1 and VR2 treatments depending on crop variability. The UR dose derived from the ASB was equal to 40 kg ha⁻¹ of N. As for Nt1 also for Nt2 a different prescription of UREA 46% was assigned to each plot according to the scheme of Figure 24. It should be noted that in some plots (green plots of Figure 53) the assigned rate was equal to 0 because the crop in these areas has suffered damages that generated big areas with failed plants within the field. For those plots where remote information was available and useful to identify this problem, fertilisation was not provided to avoid economic losses and potential environmental damage (leaching). For UR, plots falling in the areas but with no external information (variability from satellite images) the uniform rate with the prescribed amount of fertilizer was nevertheless supplied.

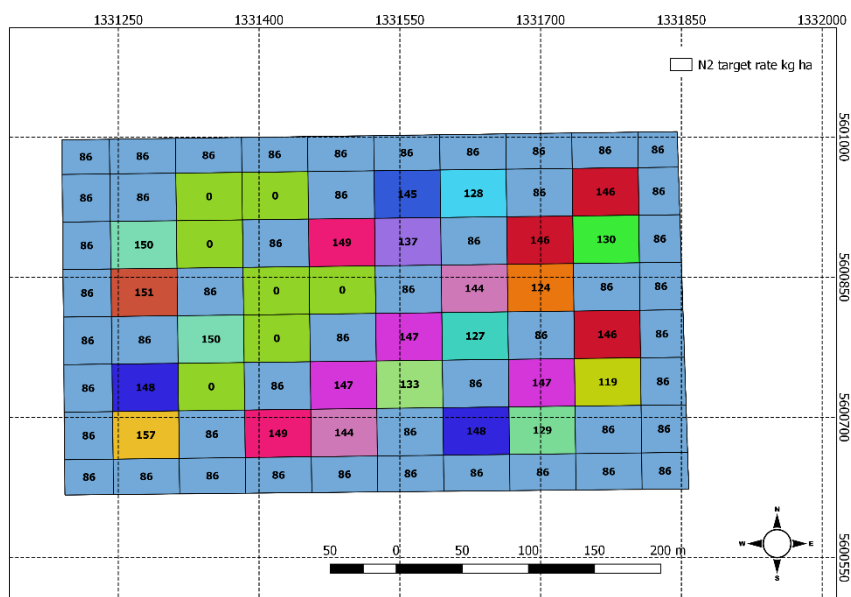
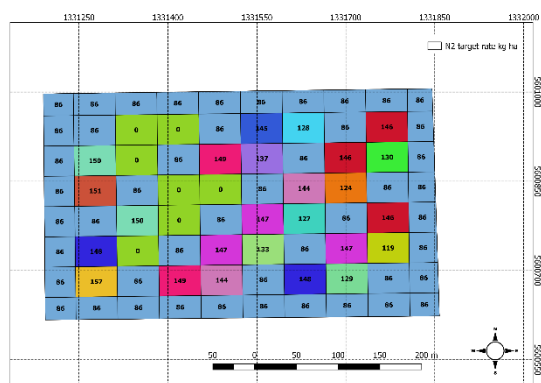
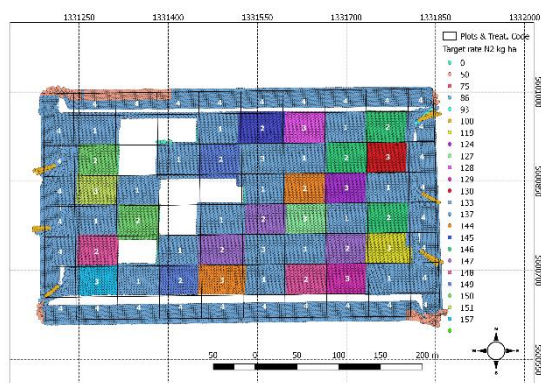


Figure 53: wheat Nt2 prescription map expressed in kg ha^{-1} of product (UREA 46% N)

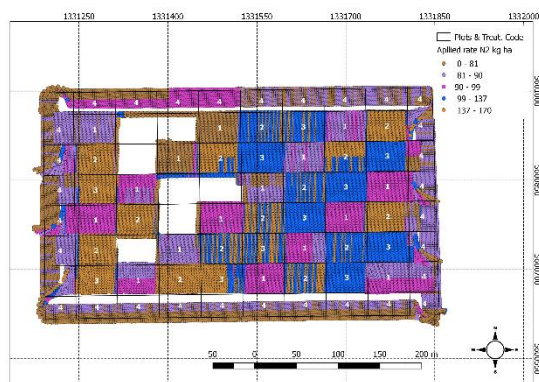
Figure 54c showed the application map of fertilisation employed on the 11th April 2020. We note that some plots have been correctly not fertilized (white plots in Figure 54a) as from the target rate visible in Figure 54a. Others received the correct amount, but some other plots had some variations of the applied quota with respect to the target as visible in Figure 54d and in Figure 54b with the representation of the average of fertilizer applied in any plot.



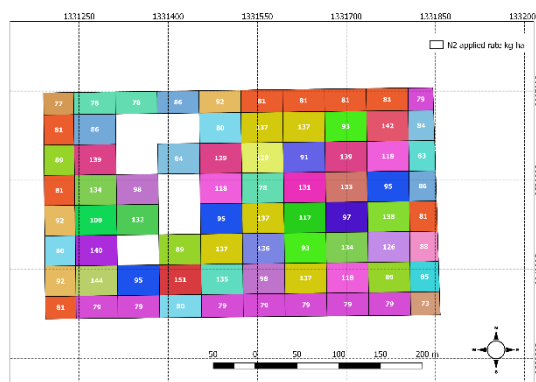
a



b



c

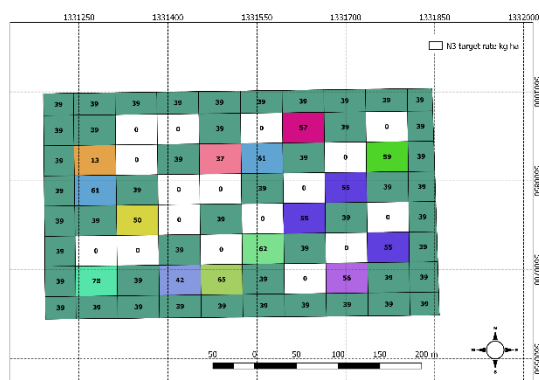


d

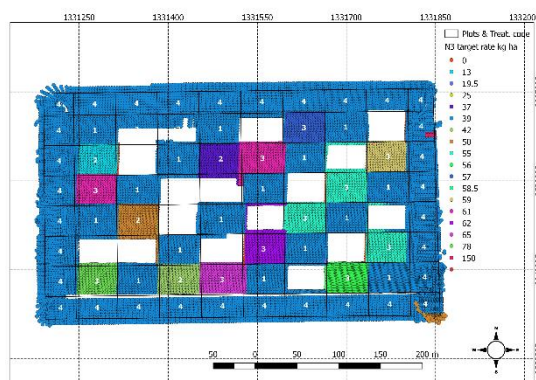
Figure 54: a) Nt2 prescription map (kg ha^{-1}) of UREA; b) N target rate (kg ha^{-1}) of the application map; c) the applied rate (kg ha^{-1}) of the application map; d) averaged amount (kg ha^{-1}) actually applied;

3. Nt3

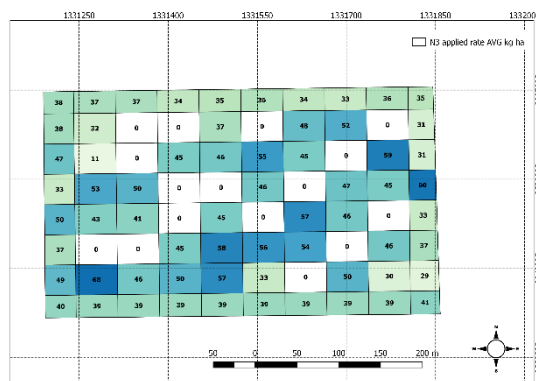
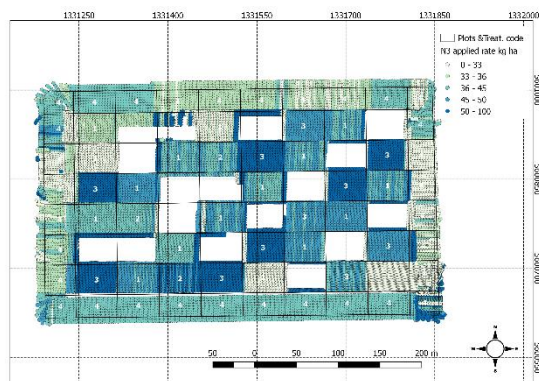
The last top fertilization Nt3, scheduled for the pre-flowering booting phase, was carried out on 08th May 2020 using the prescription map visible in Figure 55a and the ammonium nitrate as the selected fertilizer.



a



b



c

d

Figure 55: a) Nt3 prescription map (kg ha^{-1}) of ammonium nitrate; b) N target rate (kg ha^{-1}) of the application map; c) the applied rate (kg ha^{-1}) of the application map; d) averaged amount (kg ha^{-1}) actually applied;

As in the case of Nt2 fertilisation, again for some plots a fertiliser rate of 0 kg ha^{-1} was prescribed (white areas of Figure 55). These plots did not receive any N rate (Figure 55cd) for the same reasons of Nt2 and because the plants had not recovered strength, but indeed many had died. On the contrary, some others plots did not receive a N quota because the crop status analysis provided by the VR1 algorithm showed a nutritional status of the crop that did not require intervention, as the crop was not in N deficit. The UR N rate derived from ASB was equal to 13 kg ha^{-1} of N while VR2 N rate varied from 15 to 23 kg ha^{-1} of N.

iii. Harvest

The pictures in Figure 56. corresponds to the zone where during the season the crop had intense damages (flooding and then crop failure) and where it produced very low values of grains (almost zero in some areas). In the post-sowing period, during the month of November, heavy rainfalls caused flooding conditions for a long period of time. The presence of surface water and then saturated soil for many days hampered the crop development hoped for the germination and the growth in the early stages. This caused widespread plant death and large areas with lack of crop as seen in the images shown in Figure 56.





Figure 56: crop damages in the early stages of the durum wheat (April 2020)

The pictures taken in the field highlighted the typical wet spot or “drown out” patches (often circular spot) that represents increased salinity due to water logging (Seelan et al. 2003). This increasing on the topsoil often causes irremediable damages to the crop for all the season. In addition, during March and April the lack of rainfall and a higher temperature than average, together with the presence of hard and saline soil crust generated from the prolonged flooding of November, caused further severe crop stress and a high plant mortality rate. Then during the season these areas were completely occupied by weeds that remaining green until the harvest as shown in the photos of Figure 57.



a



b



c



d

Figure 57: Pictures of the harvest of wheat: a-b) crop with no weeds infestation; c-d) area with strong weeds infestation

b. Test case II (F2) – Maize

Regarding experimental design, in order to set up plots that could be completely covered by the working width of the Sulky spreader (36m), the guide lines collected during the seeding were used as reference to orient the plots grid (Figure 58). This was done is because the field is internally divided into areas separated by channels for water drain that forced the correct alignment of the plots to the passes of the operating machines, in order not to compromise the correct fertilizer distribution. This was also a test for using complementary ancillary information generated by machines and often not reused for other purposes despite their potential value.

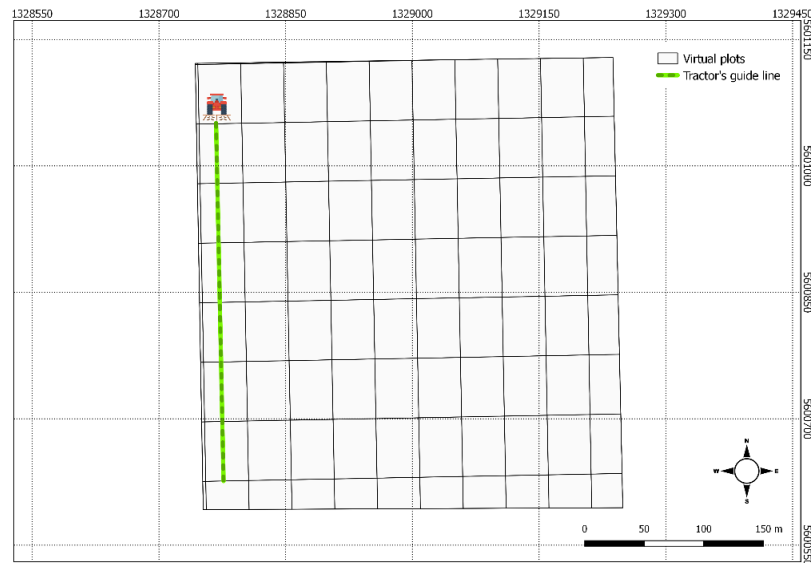


Figure 58: guideline used for the orientation of experimental plots

i. Fertilization

1. Nt1

The Nt1 fertilization was planned for the phenological phase of 2nd-3rd leaves (BBCH 12-13). It was applied a differentiated dose in the different soil classes (CDS1 and LCO1-JOL1), but not differentiated for each plot, as shown in the prescription map of Figure 60a.



a



b

Figure 59: photos of maize field 55 taken on 29/04/2020 (BBCH 12 – 2nd leaves)

At this crop development stage, the use of satellite images is demanding because the reflectance recorded by the satellite sensor is largely influenced by the soil and just marginally by plants (Figure 59a). This is because the crop's cover fraction is still low due to the limited crop growth (Figure 59b). For this reason, it was however initially decided to apply a differentiated N rate in the three soil classes, but not differentiated for N treatments, as shown in Figure 60a. The fertiliser used was urea (46% N).

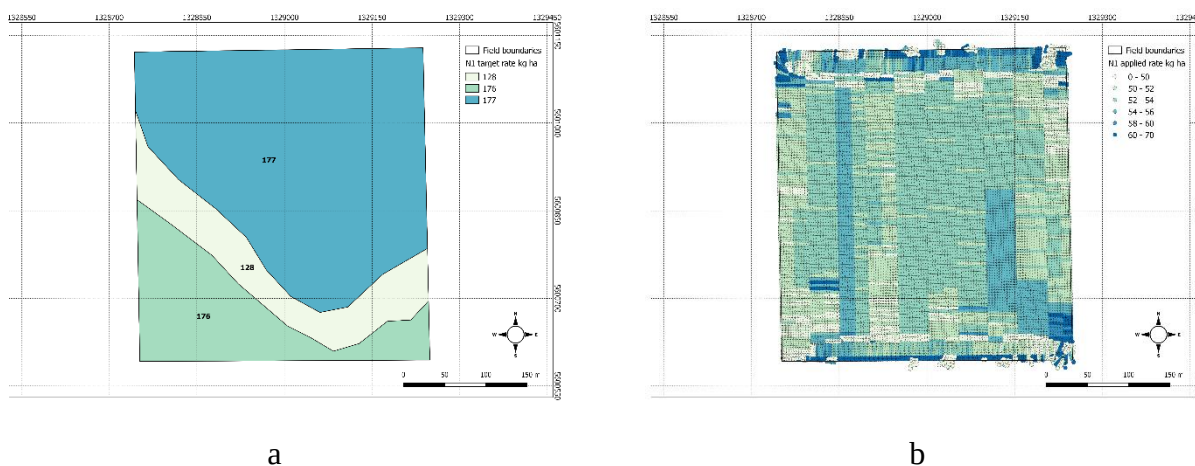


Figure 60: a) maize Nt1 prescription map (kg ha⁻¹ of UREA) ; b) maize Nt1 application map (kg ha⁻¹ of UREA)

Unfortunately, for unclear reasons, the fertilization which occurred on the 6th of April 2020, recorded on Nt1 application map a fertilizer supplies in a constant mode of about 50 kg ha⁻¹ of urea, equal to 23 kg ha⁻¹ of N. Probably the spreader provided an equal dose for a malfunction not detected during the operation. As shown in Figure 60b, the N rate provided varied not according to the prescription, but according to the normal flow variation attributable to the spreader.

2. Nt2

The Nt2 fertilization was carried out, as planned, at 6th leaves phenological stage. The VR algorithms utilized the Sentinel-2 image acquired on 28th of May 2020 to define the crop N requirement. This phase represents an optimum time to acquire information on crop nutritional status and growth deficit, both from the point of view of the reliability of the spectral signal and from the agronomic point of view. In fact, the cover fraction is no longer affected by the presence of soil and maize at this stage has a rapid growth and it is possible to perform fertilizations without damaging the crop with the passage of the machinery. At this stage it is still possible for the crop to recover growth deficits, because the plants have not yet expressed their full potential, whereas later interventions are usually not performed because of the high risk of foliar damage. The standard

fertilization procedure considers 6th leaves stage fertilization as the last N supplied, but the high N amount usually represents in many cases more an environmental issue than an agronomic advantage. The crop N uptake in this phase is not at the maximum level and the most used N fertilizer without urease inhibitors after some days are no more available and subject to ammonia losses.

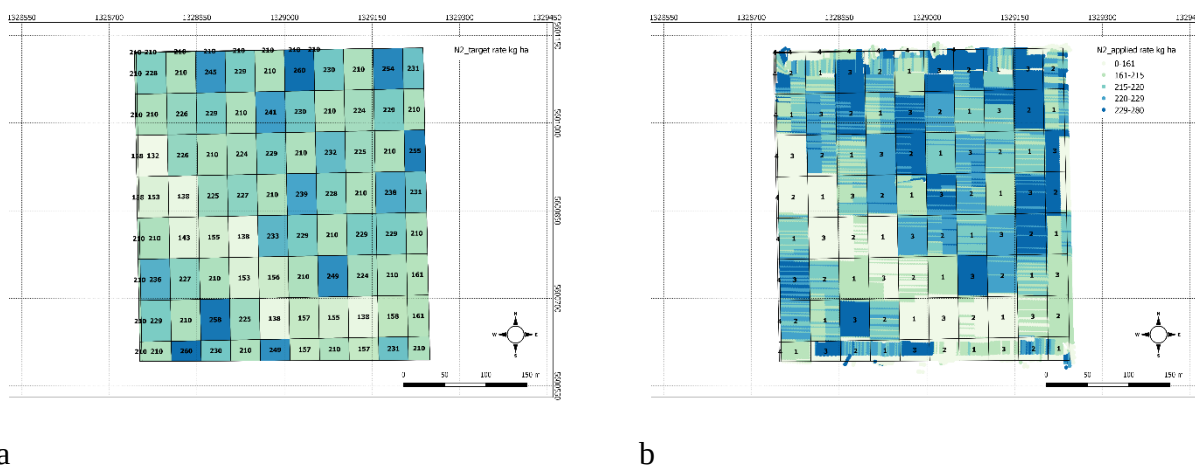


Figure 61: a) maize N2 prescription map (kg ha⁻¹ of UREA) ; b) maize N2 application map (kg ha⁻¹ of UREA)

Figure 61a reports the Nt2 prescription map with a fertilizer rate ranging from a minimum of 130 to a maximum of 260 kg ha⁻¹ of urea corresponding to 59 to 116 kg N ha⁻¹. The DR_{N2} used as reference were diversified for the soil classes and amounted to 97 kg N ha⁻¹ for JOL1 and LCO1 and 63 kg N ha⁻¹ of for CDS2. The application map of Figure 61b shows how for the Nt2 intervention the spreader worked in a VR mode correctly providing the right amount in the right place.

3. Nt3

The fertilization during the phenological phase of flowering is commonly performed with a uniform N rate with foliar application or liquid application at soil level (water + fertilizer), but in general it is rarely carried out by farmers. At this stage, the plant N uptake reached the seasonal maximum and intervention at this time is considered useful for grain accumulation and not for green biomass accumulation. In this way, however, a N dose not previously used can be supplied to the crop without the risk of environmental losses and lack of absorption. It is an alternative strategy for minimizing the risk of Nt2 over-fertilization. In addition, fertilization using a crop sprayer with hose drops system allow the application of water soluble fertilizers through fertigation to ensure that the nutrients are carried into the soil in solution. The foliar application is effective for the application of minor nutrients, whereas it is not possible to use fertilizer with higher nitrogen percentage without risk of damage due to scorching of the leaves. For this test we used a crop sprayer with hose drops

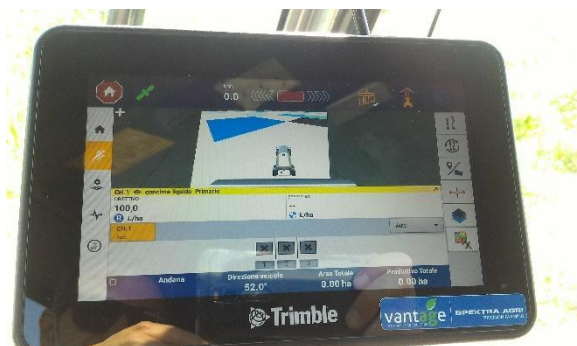
system (Mazzotti) (Figure 62ab) with satellite guide and on board monitor Trimble (Spektra Agri) (Figure 62c) able to read prescription maps and a N30 fertilizer.



a



b



c

Figure 62: a e b) crop sprayer with hose drops system (Mazzotti); c) monitor Trimble with prescription map on background

This operation represented an unprecedented test for the BF farm, to understand if it was possible to supply a liquid fertilizer in VR mode at an advanced phenological stage such as flowering. For the definition of Nt3 fertilizer rate, the Sentinel-2 image of the 27th of June 2020 was used and a DR_{N3} of 40 kg N ha^{-1} of that generated a range of doses (continuous values) from 100 to 140 l ha^{-1} . Subsequently, these continuous values have been translated to discrete classes on the prescription map (Figure 63) because the water management hydraulic system of the sprayer can operate only variations of about $\pm 10\%$. The minimum fertilizer amount was provided in the UR plots with 100 l ha^{-1} of product (about 40 kg ha^{-1} of N). The VR1- Δ PNU method varied the dose from 44 to 48 kg N ha^{-1} , whereas the VR2-SI method established a rate of 40 kg N ha^{-1} almost everywhere except in 2 plots where the rate was 48 kg N ha^{-1} .

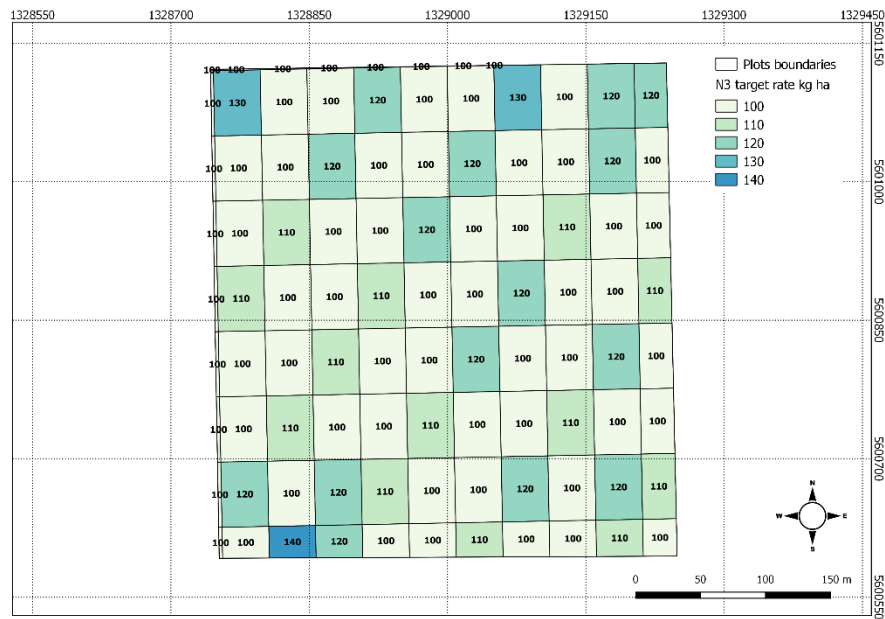


Figure 63: a) maize N3 prescription map l ha^{-1} of N30



Figure 64: maize crop failures due mainly to animals (rodents) occurring mostly along the water ditches.

Figure 42

As shown from the yield map (Figure 44b) there were many low yield areas positioned continuously and aligned vertically. These areas were near the water ditches used for the drainage of

water and the crop failures problems were caused by animals that damaged maize plants (Figure 64). This generated large spots with very few plants per square meter that affected the overall yield but also the yield map.

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List of publications

Crema, A., G. Vandini, M. Boschetti, F. Nutini, D. Cillis, and R. Casa. 2019. “Interaction between Soil Variability and Maize Nitrogen Status Assessment from Sentinel-2.” In *Precision Agriculture '19*, 453–459. The Netherlands: Wageningen Academic Publishers. doi:10.3920/978-90-8686-888-9_56.

Crema, Alberto, Mirco Boschetti, Francesco Nutini, Donato Cillis, and Raffaele Casa. 2020. “Influence of Soil Properties on Maize and Wheat Nitrogen Status Assessment from Sentinel-2 Data.” *Remote Sensing* 12 (14). MDPI AG. doi:10.3390/rs12142175.

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