Universitat de Lleida

Improvement of the nitrogen fertilization in irrigated Mediterranean environments

Ángel Maresma Galindo

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Universitat de Lleida

TESI DOCTORAL

Improvement of the nitrogen fertilization in irrigated Mediterranean environments

Ángel Maresma Galindo

Memòria presentada per optar al grau de Doctor per la Universitat de Lleida
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José Antonio Martinez Casasnovas

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A mi familia y pareja,
fuente de apoyo constante e incondicional

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Abstract / Resumen / Resum

Abstract

Application of mineral fertilizers, mainly nitrogen (N), is one of the most important methods that can be used to increase crop yields. Insufficient application of N can have serious economic consequences for the farmer, while excessive fertilization increases the risk of environmental pollution. For this reason, the quantification of the optimum in-season N requirement is an important step towards an economically and environmentally sustainable crop production system. High-yielding agricultural ecosystems in particular, such as those of the Ebro valley, suppose a challenge to N management. The present PhD thesis aims to contribute to the development of more sustainable agricultural systems by improving N efficiency. Soil sampling protocols and techniques, multispectral aerial images and double-annual cropping strategies and methods were analysed in this PhD thesis to improve N fertilization practices. Soil sampling protocols for N and organic matter (OM) were analysed to provide farmers with decision support tools for predicting crop N requirements. The results (study done in the USA) showed that sampling density should be adapted according to the object of study. Optimum soil sampling densities of 3.75 and 12.5 samples ha^{-1} were determined for OM and nitrates, respectively. Vegetation indices derived from multispectral aerial images differentiated among maize N status and determined in-season N requirements, contributing to better management of the timing and placement of N fertilizer. Green-based vegetation indices (VIs) were more accurate than red-based ones in predicting grain yield and in determining the optimum N rate for maize at V12 stage. In fact, the Green Chlorophyll Index (GCI) was the most notable of the VIs due to its ability to distinguish among maize N status up to 84% of maximum grain yield. In Mediterranean environments, the double-annual barley-maize system average annual grain and biomass yields of 20 and 35 Mg ha^{-1}, respectively, with N rates split between the two crops of 230-240 kg N ha^{-1} yr^{-1}. This fact shows the higher yield potential and stability of double-annual cropping strategies when compared with mono-cropping strategies. Moreover, double-annual cropping system contributed by adding complexity that allow to exploit the residual N content in the soil after harvest. Barley was especially able to use maize residual N and, when maize residual N was high, showed no yield response to N fertilization. In summary, the improvement in N use efficiency (NUE) in high-yielding environments will be more effective if several of the strategies studied in this research are used together.

Resumen

La aplicación de fertilizantes minerales, principalmente nitrógeno (N), es uno de los métodos más importantes que se pueden utilizar para aumentar los rendimientos de los cultivos. Aplicaciones insuficientes de N pueden tener graves consecuencias económicas para el agricultor, mientras que la fertilización excesiva aumenta el riesgo de contaminación ambiental. Por esta razón, la determinación de la dosis de N óptima para cada cultivo es un paso importante hacia un sistema de producción económicamente y ambientalmente sostenible. Los ecosistemas agrícolas de alto rendimiento en particular, como los del valle del Ebro, suponen un reto para la gestión de N. La presente tesis doctoral pretende contribuir al desarrollo de sistemas agrícolas más sostenibles mejorando la eficiencia del N. Con el objetivo de mejorar las prácticas de fertilización nitrogenada, se han analizado protocolos y técnicas de muestreo de suelos, imágenes aéreas multiespectrales y estrategias y métodos de doble cultivo anuales. Protocolos de muestreo del N y la materia orgánica (MO) del suelo fueron evaluados para proporcionar a los agricultores herramientas para la toma de decisiones y la predicción de los requerimientos de N del cultivo. Los resultados obtenidos (estudio llevado a en los EE.UU.) muestran que la densidad de muestreo debe adaptarse al objeto de estudio. Se determinaron que las densidades de muestreo óptimas eran de 3,75 y 12,5 muestras ha^{-1} para MO y nitratos, respectivamente. Los índices de vegetación derivados de imágenes aéreas multiespectrales diferenciaron entre los estados nutricionales (en cuanto a N) del maíz y determinaron los requerimientos de N, lo que contribuyó a una mejor gestión del momento y la localización del fertilizante nitrogenado. Los índices de vegetación basados en el verde (VIs) fueron más precisos que los basados en rojo para predecir el rendimiento de grano y determinar la dosis óptima de N para el maíz en estado de V12. De hecho, el Índice de Clorofila Verde (GCI) fue el más destacado de los VIs debido a su capacidad para distinguir entre estados nutricionales (en cuanto a N) del maíz hasta el 84% del rendimiento máximo de grano. En ambientes mediterráneos, los sistemas de producción en doble cultivo anual cebada-maíz obtuvieron rendimientos medios anuales de grano y biomasa de 20 y 35 Mg ha^{-1}, respectivamente, con aplicaciones de N divididas entre los dos cultivos de 230-240 kg N ha^{-1} año^{-1}. Este hecho demuestra el mayor potencial de rendimiento y estabilidad de las estrategias de doble cultivo anuales en comparación con las estrategias de monocultivo. Por otra parte, el sistema de doble cultivo anual contribuyó a añadir complejidad al sistema que permitió utilizar el N residual en el suelo después de la cosecha. La cebada fue especialmente capaz de utilizar el N residual de maíz y, cuando N residual del maíz fue alto, no mostró respuesta de rendimiento a la fertilización N. En resumen, la mejora de la eficiencia del uso del N (NUE) en sistemas de alto rendimiento será más eficaz si se utilizan conjuntamente varias de las estrategias estudiadas en esta investigación.

Resum

L'aplicació de fertilitzants minerals, principalment nitrogen (N), és un dels mètodes més importants que es poden utilitzar per augmentar els rendiments dels cultius. Aplicacions insuficients de N poden tenir greus conseqüències econòmiques per a l'agricultor, mentre que la fertilització excessiva augmenta el risc de contaminació ambiental. Per aquesta raó, la determinació de la dosi de N òptima per a cada cultiu és un pas important cap a sistemes de producció econòmicament i ambientalment sostenibles. Els ecosistemes agrícoles d'alt rendiment, en particular, com els de la vall de l'Ebre, suposen un repte per a la gestió de N. La present tesi doctoral pretén contribuir al desenvolupament de sistemes agrícoles més sostenibles millorant l'eficiència del N. Amb l'objectiu de millorar les pràctiques de fertilització nitrogenada, s'han analitzat protocols i tècniques de mostreig de sòls, imatges aèries multiespectrals i estratègies i mètodes de producció en doble cultiu anuals. Protocols de mostreig del N i la matèria orgànica (MO) del sòl van ser avaluats per proporcionar als agricultors eines per a la presa de decisions i per a la predicció de les necessitats de N del cultiu. Els resultats obtinguts (estudi fet als EUA) mostren que la densitat de mostreig se ha d'adaptar a l'objecte d'estudi. Es van determinar que les densitats de mostreig òptimes eren de 3,75 i 12,5 mostres ha^{-1} per a MO i nitrats, respectivament. Els índexs de vegetació derivats d'imatges aèries multiespectrals van diferenciar entre els estats nutricionals (pel que fa a N) del blat de moro i van determinar les necessitats de N, la qual cosa va contribuir a una millor gestió del estadi fenològic i de la localització del fertilitzant nitrogenat. Els índexs de vegetació basats en el verd (VIs) van ser més precisos que els basats en el vermell per predir el rendiment de gra i determinar la dosi òptima de N per al blat de moro en estat de V12. De fet, l'Índex de Clorofil·la Verd (GCI) es l'índex que va ser el més destacat dels VIs causa de la seva capacitat per distingir entre estats nutricionals (pel que fa a N) del blat de moro fins al 84% del rendiment màxim de gra. En ambients mediterranis, els sistemes de doble cultiu anual ordi-blat de moro van obtenir rendiments mitjans anuals de gra i biomassa de 20 i 35 Mg ha^{-1}, respectivament, amb aplicacions de N dividides entre els dos cultius de 230-240 kg N ha^{-1} any^{-1}. Aquest fet demostra el gran potencial de rendiment i estabilitat de les estratègies de doble cultiu anuals en comparació amb les estratègies de producció en monocultiu. D'altra banda, el sistema de doble cultiu anual va contribuir a afegir complexitat al sistema que va permetre utilitzar el N residual en el sòl després de la collita. L'ordi va ser especialment capaç d'utilitzar el N residual de blat de moro i, quan N residual del blat de moro va ser alt, no va mostrar resposta de rendiment a la fertilització N. En resum, la millora de l'eficiència de l'ús del N (NUE) en sistemes de alt rendiment serà més eficaç si s'utilitzen conjuntament diverses de les estratègies estudiades en aquesta investigació.

General Introduction

General Introduction

Agriculture is currently facing unprecedented challenges globally. There is a need to increase world food production to meet global demand (Bodirsky et al., 2014), while reducing productions costs and sustaining the environment. Nowadays, the main method to increase yields and to maintain or restore soil nutrients is the application of mineral fertilizers, mainly nitrogen (N) (Hirel et al., 2011). Indeed, N is often considered the most limiting nutrient for crop production (Fageria and Baligar, 2005). Although the benefits of adding N fertilizer to agricultural systems are straightforward, they are accompanied by substantial economic and environmental costs (Robertson and Vitousek, 2009). Insufficient application of N can have serious economic consequences for the farmer, whereas excessive fertilization increases the risk of environmental pollution (Khan et al., 2001). In most intensive agricultural production systems, over 50% and up to 75% of the N applied to the field is not used by the plant (Raun and Johnson, 1999). This means that more than half N used for crop fertilization is currently lost into the environment (Lassaletta et al., 2014). Hence, improving nitrogen-use efficiency (NUE) in worldwide cropping systems is utterly necessary, as it is one of the most effective means of increasing crop productivity while decreasing environmental degradation (Cassman et al., 2002; Davidson et al., 2015).

Traditionally, N fertilization of field crops, such as maize, has been adjusted by yield-based N recommendations methods (Mulvaney et al., 2006). However, without a widespread reliable soil test to predict soil N mineralization, variation in soil N supply (mineralizable soil N) provides a significant challenge to the yield-based approach, as does uncertainty about how yield goals should be determined (Sawyer et al., 2006).

Crop N requirements change from year to year, and quantifying the optimum in-season N requirement is an important step towards an economically and environmentally viable crop production system (Sripada et al., 2005). The accurate quantification of N requirements in maize becomes especially interesting in high-yielding environments, such as those of the Ebro Valley (NE Spain), where high N rates are applied in order to cover crop N requirements.

1. The Ebro Valley (semiarid irrigated area in NE Spain)

The Ebro Valley is an extensive area located in the northeast of Spain (Fig. 1), characterized by a semiarid climate, with average annual rainfall ranging from 200 to

400 mm. Despite its semi-aridity, this valley is one of the most important areas for agriculture and livestock farming in Spain, due to the presence of considerable irrigation infrastructure. In the Ebro Valley there are 906,000 ha irrigated with an average water consume of 7.370 hm^3 yr^{-1} (CHE, 2017). The irrigation surface is divided into flood-irrigation (55%), sprinkle irrigation (25%), and drip irrigation (20%), this latter mainly for fruit production (CHE, 2017). Alfalfa (*Medicago sativa* L.), wheat (*Triticum aestivum* L.) and especially maize (*Zea mays* L.), are the most important field crops in the Ebro valley and have higher yields in comparison with other rainfed production areas of the world (Cela, 2011).

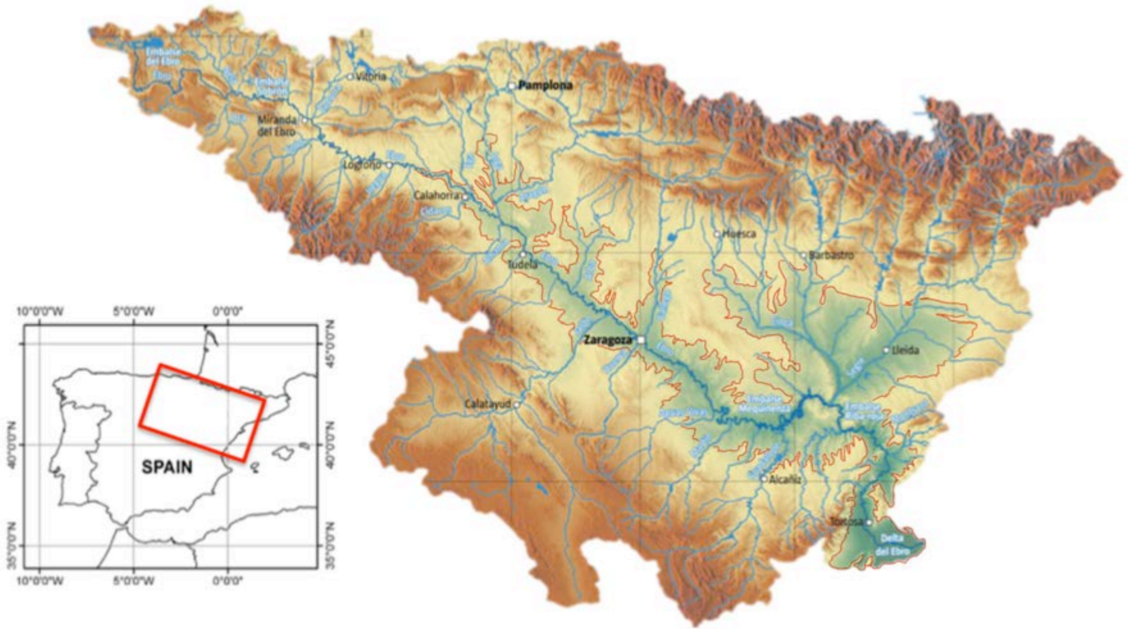

Figure 1. Ebro river basis (MAPA). Adapted from: Confederación Hidrográfica del Ebro (2017). The blue shady area around rivers represents the main irrigated zone.

Average maize yields in the area range from 10 to 15 Mg ha^{-1} (14% moisture) under sprinkler irrigation (Berenguer et al., 2008; Cela et al., 2011; Yagüe and Quílez, 2010), although under good agronomical conditions, the most efficient farms can produce more than 18-19 Mg ha^{-1} (Biau et al., 2011). High yielding maize grown in Spanish agro-systems require water but also a satisfactory input of available nitrogen (N) and a long growing season.

Data from surveys in the Ebro Valley about N fertilization in maize indicate that farmers apply rates of 318–453 kg N ha^{-1} yr^{-1} (Cavero et al., 2003; Isidoro et al., 2006; Sisquella et al., 2004). As total plant N uptake is normally between 250 to 300 kg ha^{-1} (Berenguer et al., 2008; Cela et al., 2011; Yagüe and Quílez, 2010), there is a high risk of not using N efficiently.

2. How could be the NUE improved in high-yielding environments?

Many approaches for improving the NUE of high-productivity annual cropping systems have been identified (Cherry et al., 2008). Some of the more outstanding are:

I) Provide farmers with decision support tools that allow them to better predict crop N requirements and avoid overfertilization.

II) Better manage the timing, placement, and formulation of fertilizer N in cropping systems to ensure N is available where and when plant demand for N is greatest.

III) Adjust crop rotation to add complexity that improves to take up more available N.

2.1. Provide farmers with decision support tools

The determination of the soil organic matter (OM) and N, as major determinants and indicators of soil fertility and quality, is important to have real information of the agricultural productivity (Al-Kaisi et al., 2005; Fageria and Baligar, 2005; Reeves et al., 1997). However, within field variability is affected by both temporal and spatial processes (Sogbedji et al., 2001; Wall et al., 2010). Thus, the determination of available N can vary spatially and temporally among fields influencing the N optimal rates to achieve maximum yields. Soil sampling protocols for OM and N determination could be used to determine patterns in soil N supply potential that will contribute for site-specific applications of N fertilizer.

2.2. Timing and placement of N fertilizer

The temporal and spatial change in crop N requirements within the same field entails a challenge to N fertilization. Image-based remote sensing can be used to monitor seasonal variability of soil and crop characteristics. Multispectral aerial images could be used to detect N deficiencies, predict grain yield and determine N fertilizer requirements of maize for site-specific application (Blackmer et al., 1996; Scharf and Lory, 2002; Sripada et al., 2005).

2.3. Crop rotations to add complexity

One of the simplest means for capturing more of the N added to annual cropping systems is to include cover crops in a rotation (Robertson and Vitousek, 2009). In particular environments the wheatear conditions allow cover crops to achieve maturity and being harvest (secondary crop), considering the system as double-annual cropping.

In double-annual cropping systems, soil is covered during larger period of the year than with monocropped systems. This entails several benefits, as prevention of soil erosion by wind and water (Hirel et al., 2011), an increase of total dry matter production (Lloveras, 1987a, 1987b; Yagüe and Quílez, 2013), increase of field gross margin (Gil, 2013;) per land unit, and a reduction of the NO_3^--N run-off (Gabriel and Quemada, 2011; Heggenstaller et al., 2008; Krueger et al., 2012) (Figure 2), among others.

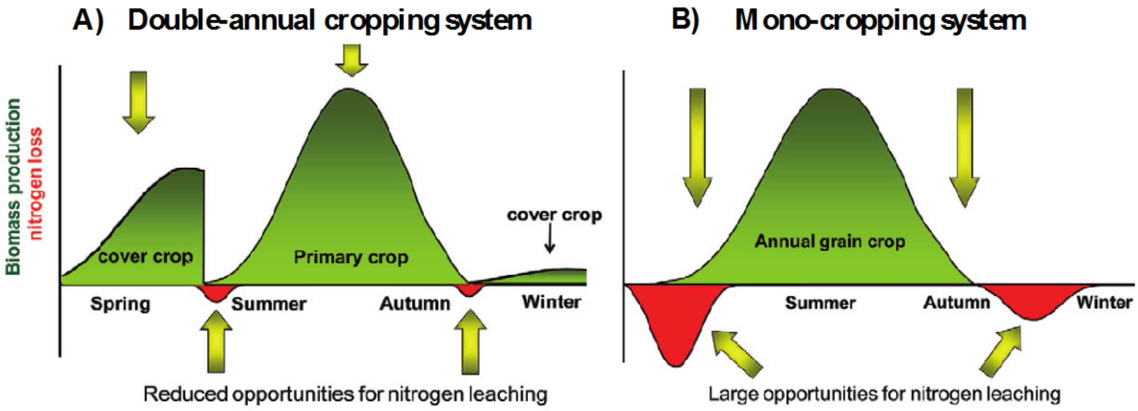

Figure 2. Hypothesized representation of the seasonal dynamics of dry matter production and NO_3^--N leaching in (A) double-annual cropping system and (B) mono-cropping system. Adapted from: (Heggenstaller et al., 2008).

2.4. Personal view

In summary, the improvement of the NUE in high-yielding environment will be more effective if several approaches are used together. Farming N fertilization practices could start by soil sampling to estimate the soil N supply for the growing season and to determine the N fertilization. Subsequently, in-season N determination of maize N status by image-based remote sensing could help to fine-tune the N fertilization rates estimated for each part of the field. These practices will reduce the N application to farming systems while maintaining productivity, as it will be more adjusted to crop N requirements. The NUE of monocropping systems could also be improved by the implementation of another crop in the inter-crop period that will use the residual N of the main crop.

3. The challenge

The present Ph.D. thesis aims to contribute to the development of more sustainable agricultural systems by improving the nitrogen (N) efficiency. Soil sampling protocols, multispectral aerial images and double-annual cropping strategies were the analysed in this research to improve the N fertilizer practices that will impacted yields and crop's profitability. Farmers have been encouraged to:

I) Adjust soil sampling densities according to the soil parameter that want to be determined.

II) Adjust maize N fertilization at V12 stage with the aid of multispectral aerial images.

III) Introduce double-cropping (barley-maize) strategies, which are more N efficient systems than traditional monocropping maize in the Ebro Valley.

Finally, it is need to remember and maintain the social mandate to develop a safe agricultural system that uses efficiently the nutrients to provide the maximum yields while reduce the waste of fertilizers while maintaining the sustainability of the cropping systems.

4. General Objectives

The main objective of this thesis was to explore nitrogen (N) management strategies to improve the efficiency of N fertilization, particularly in high-yielding irrigated Mediterranean agro-ecosystems. A better N management will contribute to increase yields and profitability while decreasing environmental pollution risk.

To achieve this general objective, different experiments were conducted in field trials located in Gimenells, Almacelles and Algerri (Ebro Valley, NE, Spain). Moreover, results from a field trial in Cayuga County (USA) were evaluated with the aim of extrapolating new perspectives for continuing with the improvement of N management in Mediterranean conditions in the future.

The main objective was divided into several specific objectives as follows:

1. To study the effects of soil sample density on field N determination and its spatial and temporal variability.
2. To determine in-season maize N requirements by the use of vegetation indices derived from multispectral aerial images acquired by a) Unmanned aerial vehicles and b) Aircraft.
3. To evaluate the profitability and sustainability of the double-annual barley-maize cropping system, determining the optimum annual N rate and its temporal distribution for agronomic, economic and environmental goals.

5. Outline of the present Thesis

This Thesis is divided into seven sections: the general introduction, four experimental chapters, general discussion and general conclusions. The four experimental chapters are independent and presented in the format of journal articles. For this reason, some parts, such as the material and methods sections, may contain some repetitions. One of the chapters has been already published in a scientific SCI journal, while the rest are currently under revision

In particular, Chapter I was carried out in USA as part of an international research experience in Cornell University during the duration of the Ph.D.

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Chapter I

In-Field Variability of Illinois Soil Nitrogen Test and Loss-on-Ignition for Nitrogen Management

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Abstract

The Illinois soil nitrogen test (ISNT) with loss-on-ignition at 500°C (LOI_{500}) adjustment was effective in identifying fields or areas within fields with high soil N supply potential in New York. However, spatial and seasonal variability in ISNT-N and LOI_{500} can impact interpretations. Our objectives were to determine (1) impact of soil sample density on ISNT-N, (2) implications of a change in spatial and temporal variability of ISNT-N results, (3) probability of obtaining an accurate interpretation as impacted by sampling intensity, and (4) impact of using LOI_{500} equivalents (derived from LOI_{360}) on ISNT-N interpretation. Two 4-ha corn (*Zea mays* L.) fields were sampled (150 samples/field, 0-20 cm depth; 64 in regular grid, the remainder in a pattern that optimized lag distance distribution; fall-applied manure in one field) in July and after corn harvest. Semi-variograms were constructed to investigate spatial dependence. Nitrate showed the weakest spatial dependence. Increasing the sampling intensity to 15 cores per 4-ha (3.75 samples ha^{-1}) reduced the CI for ISNT-N interpretation to < ±6% resulting in consistent classification. The LOI_{500} and LOI_{360} results were well correlated ($r^2 = 0.78$). Use of LOI_{500} equivalents derived from LOI_{360} resulted in identical soil N supply classifications for 92% of the samples. We conclude that practical and effective sampling protocols for soil N supply potential in the northeast cannot be based on soil nitrate and should be 3.75 samples ha^{-1} or more where greater accuracy is needed for ISNT-N interpretations, while LOI_{500} equivalents can be used to derive ISNT-N critical values.

Abbreviations: Soil Organic matter, SOM; nitrogen, N; pre-plant nitrate test, PPNT; pre-sidedress nitrate test, PSNT; Loss-on-ignition, LOI; Soil organic carbon, SOC; Illinois soil nitrogen test, ISNT;

1. Introduction

Soil organic matter (SOM) and soil nitrogen (N) are major determinants and indicators of soil fertility and quality, being closely related to soil productivity in agricultural ecosystems (Reeves, 1997; Al-Kaisi et al., 2005; Fageria and Baligar, 2005). Nitrogen is often the most limiting nutrient for crop production; insufficient application of N can have serious economic consequences for the farmer, whereas excessive fertilization increases the risk of environmental pollution (Khan et al., 2001). As a result, efficient use of N is important for the economic sustainability of food, feed, and fiber production. In addition, the dynamic nature of N and its propensity for loss from soil-plant systems into the environment create unique challenges for agriculture and environmental management (Fageria and Baligar, 2005).

Yield-based N recommendation methods have predominated field-based for several decades, despite the fact that their original intent was to provide long-term generalized fertilizer N recommendations on a more regional scale (Mulvaney et al., 2006). Soil is the principal source of N for most field crops which obtain 50-80% of its N requirement from the soil even where N fertilizer is applied at higher rates (Kundu and Ladha, 1995). Knowing the total amount of N available for a crop at times when the crop can utilize it will greatly enhance field-based N management. However, a better understanding of within field variability is needed too as soil N dynamics, SOM content, and nitrate availability are affected by both temporal and spatial processes (Wall et al., 2010; Sogbedji et al., 2001), with the greatest influences in the top 10 cm of the soil (Wall et al., 2010). Studies by Robertson et al. (1988) and Cambardella et al. (1994) have shown that soil pH, SOM, and assorted mineral element concentrations can vary by an order of magnitude at spatial scales of 5 m or less. This with-in field variability in soil N supply can greatly impact the assessment of N availability and hence the N use efficiency of any applied N.

The exact number of samples needed to represent soil variability in a field has been a matter of discussion since the 1920s (Linsley and Bauer, 1929). Geostatistical methods provide tools to describe spatial variation quantitatively and can be used predictively (Webster and Oliver, 1992). A variogram describes the spatial correlation structure in variables examined and will allow for identification of spatial patterns (Baxter et al., 2003) that can be used to design optimal sampling strategies for individual fields. Without prior background information on the field, geostatistical modeling for soil sampling strategies typically results in more accurate estimates of field averages than random sampling (Franzen and Peck, 1995). However, determination of spatial patterns of a field requires collection and analysis of a large number of soil samples, which is costly and time-consuming. This is especially the case with soil parameters that have low or moderate spatial structures such as soil nitrate (Cambardella et al., 1994; Cambardella and Karlen, 1999; López-Granados et al., 2002; Mallarino and Wittry, 2004).

Two soil tests that have been used to guide N management of corn are the pre-plant nitrate test (PPNT) and pre-sidedress nitrate test (PSNT). In the humid northeastern region of the USA, the spatial and temporal variability in soil nitrate tend to be large and neither test considers the fraction of the organic N that can be mineralized during the crop growing season. Thus, the PPNT is not a common test in the Northeast, while the PSNT has only been adopted by a small number of farmers and farm advisors in the region.

Soil organic matter typically shows much less spatial and temporal variability in agricultural fields but laboratory measurements of various forms of SOM can be time-consuming and costly. Wet chemical oxidation methods require the use of hazardous materials and automated dry combustion equipment is expensive with time-consuming maintenance. The loss-on-ignition (LOI) method, where soil is exposed to a high temperature for a set amount of time, is more rapid and inexpensive. The results of LOI analyses (combusted 360°C for 2h in a muffle furnace) and analyses of soil organic carbon (SOC) were highly correlated (r^2 ranging from 0.94 to 0.98) for soils of the north central USA (Konen et al., 2002). As a result, the LOI methodology was integrated into many basic soil fertility assessment packages by both Land Grant University and commercial analytical facilities. However, LOI results are seldom used to develop N recommendations as the methodology itself does not distinguish among various forms of organic matter, and unique relationships exist for different soil geographic areas (Konen et al., 2002). This limited usefulness of an LOI assessment was shown in the eastern region of the USA by Klapwyk and Ketterings (2006) who concluded that LOI results alone could not predict N responsiveness of corn in NY soils.

The Illinois Soil Nitrogen Test (ISNT) was introduced 15 years ago as a means to estimate soil N supply potential for field-specific adjustment to N recommendations. The ISNT estimates ammonium-N plus a labile pool of soil N that can mineralize during the growing season and supply mineral N to the growing crop (Khan et al., 2001; Mulvaney et al., 2001). The test does not measure soil nitrate but rather gives an estimate of soil N supply potential from organic N sources.

Research in NY showed ISNT-N results, adjusted for SOM determined by LOI, to be usefulness in adjusting soil N supply potential estimated, specifically in manured field and fields where corn is rotated with hay (Klapwyk and Ketterings, 2006). For example, Ketterings et al. (2013) showed that for fields with optimal ISNT-N, manure could replace starter N without a decline in corn silage yield or quality but starter N fertilizer application was needed for optimal yield in fields deficient or marginal in ISNT-N and without a manure history. Lawrence et al. (2009) presented an overall accuracy of 83% of the ISNT-N with LOI adjustment of critical values in determining responsiveness to N fertilizer in 2^{nd} or higher year corn after hay, while PSNT only predict correctly the 47% of the times.

In the work by Klapwyk and Ketterings (2006), Ketterings et al. (2003) and Lawrence et al. (2009), LOI was estimated by heating the soil samples at 500°C for two hours (Storer, 1984), hereafter referred to as LOI_{500}. Many routine soil testing facilities includes determination of SOM by LOI but use 360°C (Schulte and Hopkins, 1996), hereafter referred to as LOI_{360}. Results can differ depending on the composition of the soil (Matthiessen et al., 2005). Where LOI_{500} and LOI_{360} are similar, or linearly correlated, it is hypothesized that LOI_{360} data could be used (possibly with an adjustment factor) to interpret ISNT-N results in NY. Further research is needed to evaluate this hypothesis across a larger number of soil samples and soils.

Although the studies in NY show promise for use of ISNT-N with LOI adjustment to fine-tune N guidelines for corn, the ISNT and LOI research conducted in NY did not take into account within-field spatial variability. As pointed out by Ruffo et al. (2005), there is limited research on the spatial and temporal variability of ISNT-N while such variability can have implications for sampling density for accurate assessment of this pool of soil organic N. A better understanding of the spatial dependency of ISNT-N, LOI, soil nitrate, and the relationships between LOI_{500} and LOI_{360} is needed to develop more accurate and precise recommendations for soil sampling for N management.

The objectives of the current study were to (1) evaluate the impact of soil sample density (number of samples per field) on field-level ISNT-N during the growing season and after harvest, with and without manure application, (2) quantify implications of a change in spatial and temporal variability of ISNT-N results, (3) determine the maximum coefficient of variation (CV) accepted and probability of obtaining a mean within the accepted CV as impacted by sampling intensity, and (4) determine the relationship between LOI_{500} and LOI_{360} and implications of use of an LOI_{500} equivalent (based on LOI_{360} data) on ISNT-N interpretation.

2. Materials and methods

2.1. Sites

Two 4-ha corn (*Zea mays* L.) fields in Cayuga County, NY, in a corn and after alfalfa (*Medicago sativa* L.) were sampled and analyzed. Field 1 was 200 by 200 m while field 2 was 300 by 130 m (Figure 1).

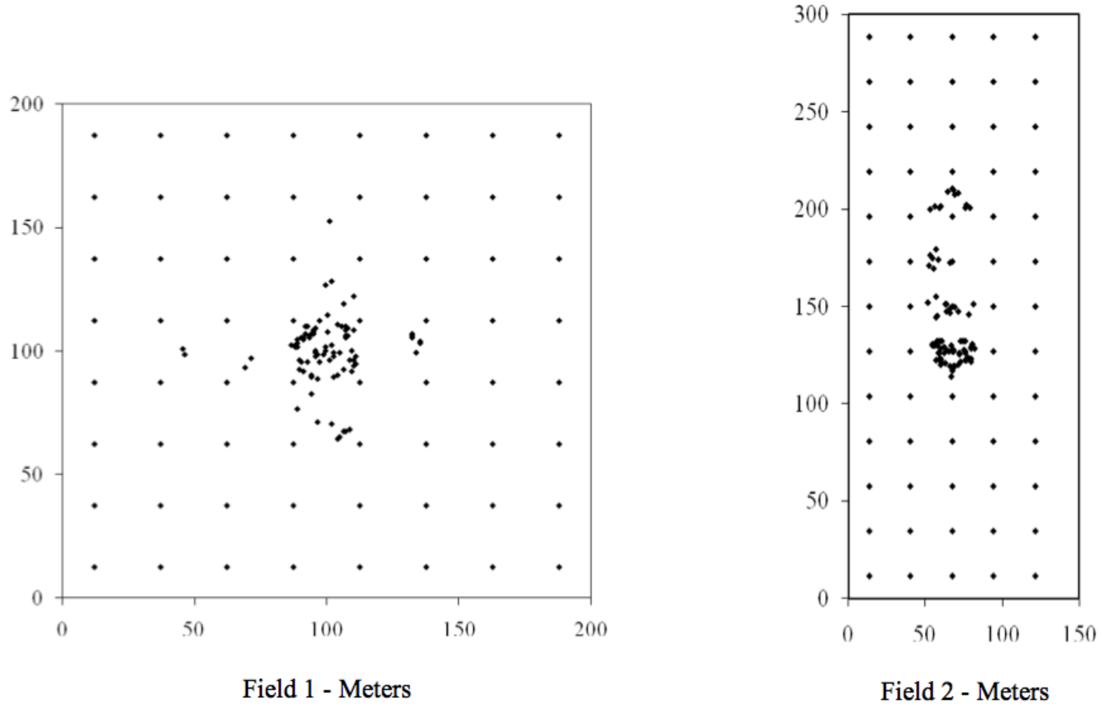

Figure 1. Soil sampling schemes for spatial and seasonal variability assessment of corn fields in Aurora, NY. The sampling protocol consisted of a regular grid sampling to ensure coverage of the fields and more intense sampling within a smaller area of the field to give the most uniform distribution of lag distances possible using lag distance classes of 9 m. The regular grid included 64 points spaced 25 m by 25 m (Field 1) or 23 m by 27 m (Field 2).

The soils were Lima silt loams (fine-loamy, mixed, active, mesic Oxyaquic Hapludalf) with within-field slopes between 0 and 3%. At planting in early May, 170 kg

ha^{-1} of 18-13-0 (N-P_2O_5-K_2O) was banded 5 cm below and to the side of the corn seed, and in mid-June, 45 kg ha^{-1} of N were sidedressed as a 30% urea ammonium nitrate solution. For further details on the two fields and assessment of spatial and temporal variability in soil test phosphorus, see Grandt et al. (2010).

2.2. Soil Sampling and Analysis

Fields 1 and 2 were sampled twice, once in the first week of July and again two wk after harvest at the end of Nov. Field markers were used to identify sampling locations. For each sampling season, 150 samples were taken per field to enable geostatistical analysis using semi-variograms (Webster and Oliver, 1992). The protocol of Warrick and Myers (1987) consisting of a regular grid sampling to ensure coverage of the fields with additional points was followed. Lag distance classes of 9 m were used to give the most uniform distribution. No two samples were closer than 1.2 m to each other. The regular grid included 64 points spaced 25 m apart in Field 1 while in Field 2 samples were 23 m by 27 m apart (Figure 1). These grid distances were chosen to balance funding limitation and the risk of sampling at too large a grid spacing to assess spatial variation (Grandt et al., 2010; Lauzon et al., 2005).

A composite of six individual cores was taken in each sample point. Samples were taken randomly from within a 0.6 m diameter of the actual point while avoiding sampling of the fertilizer band (76 cm row spacing for corn in both fields). Each core was taken to a depth of 0.2 m, the recommended depth for soil sampling in NY (Ketterings et al., 2003). Soils were analyzed for Morgan extractable nitrate (Morgan, 1941), organic matter by LOI for 2 h at 500°C (Storer, 1984) and 360°C (Schulte and Hopkins, 1996), and ISNT-N according to Khan et al. (2001) with the enclosed-griddle modification (Klapwyk and Ketterings, 2005). The LOI_{500} and ISNT-N results were classified using the ISNT-N/critical ISNT-N ratio: (i) “deficient in soil N supply potential” (ratio <0.93), (ii) “marginal in soil N supply potential” (ratio 0.93-1.07) and (iii) “optimal in soil N supply potential” (ratio >1.07) according to Ketterings et al. (2013).

Field 2 received a manure application of 110 Mg ha^{-1} after corn harvest using a drag hose and Aerway aeration system (Holland Group of Companies, Norwich, Ontario) set at 15° (sharpest angle). Manure contained 0.98 g kg^{-1} organic N, 1.58 g kg^{-1} ammonium-N, 0.36 g kg^{-1} P, and 1.71 g kg^{-1} K. The field was sampled two weeks after manure application. Field 1 did not receive manure. It was seeded to a cereal rye (*Secale cereale* L.) cover crop within days after corn harvest.

2.3. Statistical analysis

Conventional statistics to indicate the degree of overall variation, and geostatistics to examine whether or not that variability is spatially structured, were used to analyze the spatial features of the measured variables. Of the 600 soil samples that were analyzed for this study, the results of twelve samples were deleted. For these twelve samples, the standard deviation of LOI_{500} and estimated LOI_{500} from LOI_{360} exceeded ±3, more likely reflecting a sample labeling or laboratory analytical error.

For each field and sampling season, descriptive statistics (mean, standard deviation, minimum, maximum) were derived from samples taken in the regular grid (64 samples) using SAS$^{®}$ 9.4 software (SAS Institute Inc., Cary, NC, USA). Changes in soil test means between sampling seasons (within the same fields) were tested using a two-tailed, paired t-test and, additionally, the 95% CIs of the mean soil test levels for both fields and both seasons were determined. A simulation of sampling intensities of 3, 5, 10, 15, 20, 25, 30, 35, and 50 samples per 4-ha field for each sampling season was carried out using simple random sampling without replacement of grid samples. For each variable, 1000 simulations were done to reduce the range in results to less than 5% for the most unfavorable sampling intensity tested (3 samples per 4-ha field) using the Microsoft$^{®}$ Excel 2007 software (Microsoft Corp., Redmond, WA). The probability that the estimate produces a value within the mean and acceptable CV of 6.4% of nitrate, LOI_{500}, LOI_{360}, ISNT-N and ratio ISNT-N/Critical ISNT-N for a group of samples of each size was calculated. A maximum CV of ± 6.4% was accepted to reduce the risk of incorrect ISNT-N/critical ISNT-N ratio classification between the deficient and optimal N supply potential; the ratio is classified as “deficient in soil N supply potential” when <0.93 (extra N needed for corn production) and “optimal in soil N supply potential” when >1.07 (no extra N needed for corn production) (Ketterings et al., 2013).

Non-directional semivariograms were derived with GS+$^{®}$ 9.0 software (Gamma design, Plainwell, MI, USA) using all samples for each field and sampling time. Skewness of data distribution suggested normal distribution of soil parameters (skewness of between −0.5 and 0.5) and hence no transformation was used for geostatistical analysis. Lag distance classes of 9 m were used and models allowed for the presence of a non-zero nugget value. The best-fit model (linear, spherical, exponential, or gaussian) was determined by GS+® based on the residual sum of squares (RSS). In addition to the semivariograms themselves, parameters derived from the analysis including nugget (C_o), range, sill variance ($C + C_o$), and the proportion of the estimated total sample variation (the "sill"; $C + C_o$) explained by structural variance (C) was calculated. The proportion structural variation index [$C/(C+C_o)$] ranges from 0 to 1; a higher index value indicates that variability in the dataset is strongly structured (Gross et al., 1995; Wang et al., 2009). Here we classified spatial correlation based on the proportion structural variation index: (i) index < 0.25 = weak spatial structure, (ii) 0.25 < index > 0.75 = moderate spatial structure, and (iii) index > 0.75 = strong spatial structure.

A generalized linear model (GLM) was used to compare LOI_{500} and LOI_{360} values using JMP® pro 12 software (SAS Institute Inc., Cary, NC, USA). A multiple regression model was used to determine if the time of sampling (season) or manure addition (field) impacted the relationship between LOI_{500} and LOI_{360}:

$$\begin{aligned} LOI_{500} = {} & B_o + B_1\,(LOI_{360}) + B_2\,(\text{field}) + B_3\,(\text{season}) + B_4\,(LOI_{360})\,(\text{field}) \\ & + B_5\,(LOI_{360})\,(\text{season}) + B_6\,(\text{field})\,(\text{season}) + B_7\,(LOI_{360})\,(\text{field})\,(\text{season}) \end{aligned}$$

Moreover, the converted values from LOI_{360} to LOI_{500} were used in the formula together with the ISNT-N results to predict the N supply potential of the field, after which the predictions (deficient, marginal and optimal) were compared with the ones obtained using the original LOI_{500} and ISNT-N data. The comparison was done three ways: (1) using one conversion equation per field (sampling times combined), (2) using an equation that combined fields but excluded the fall sampling where manure had been applied, and (3) using one equation that combined both fields and sampling seasons.

3. Results and discussion

3.1. Descriptive Statistics

Nitrate was not impacted by time of sampling for Field 1 (Table 1), presenting average values of 5.7 and 5.9 mg kg^{-1} in summer and fall, respectively. In contrast, the mean nitrate value of Field 2 increased (P <0.05) by two fold (from 7.0 to 13.5 mg kg^{-1})

from summer to the fall consistent with the impact of manure addition on available N (Ketterings et al., 2003). In both fields, high variability was observed among samples and between sampling seasons agreeing with N heterogeneity of soils (Cambardella et al., 1994).

Table 1. Descriptive statistics of soil test values for two corn fields. Field 2 received manure in the fall 2 weeks before soil sampling.

Statistic	Field 1 (no manure)				Field 2 (with manure)			
	Summer	Fall	Change	†	Summer	Fall	Change	†
NITRATE-N	--- mg kg^{-1} ---		--%--		--- mg kg^{-1} ---		--%--	
Mean	5.7	5.9	2.9		7.0	13.5	93.4	‡
SD	4.4	5.6	26.9		3.9	9.4	141.0	
Minimum	0.05	0.05	0.0		0.05	0.05	0.0	
Maximum	17.5	23.2	32.7		23.7	44.6	88.4	
LOI_{360}	--- g kg^{-1} ---		--%--		--- g kg^{-1} ---		--%--	
Mean	41.9	41.1	-1.9	‡	41.3	41.8	1.4	
SD	4.7	4.4	-6.8		4.1	4.0	-2.2	
Minimum	30.3	31.2	3.0		28.2	28.6	1.4	
Maximum	56.7	54.4	-4.1		51.3	52.5	2.3	
LOI_{500}	--- g kg^{-1} ---		--%--		--- g kg^{-1} ---		--%--	
Mean	48.1	45.9	-4.7	‡	47.0	49.5	5.4	‡
SD	5.6	5.0	-11.8		4.9	4.6	-5.2	
Minimum	34.9	34.8	-0.3		33.0	34.9	5.8	
Maximum	63.4	62.1	-2.1		62.4	61.8	-1.0	
ISNT-N	--- mg kg^{-1} ---		--%--		--- mg kg^{-1} ---		--%--	
Mean	300.3	288.5	-3.9	‡	291.7	295.8	1.4	
SD	35.4	37.1	4.6		30.7	35.6	15.9	
Minimum	207.5	197.7	-4.7		190.1	192.4	1.2	
Maximum	392.8	412.2	4.9		363.4	411.5	13.2	
ISNT-N / Critical ISNT-N	--- ratio ---		--%--		--- ratio ---		--%--	
Mean	1.08	1.06	-2.2	‡	1.06	1.06	-0.6	
SD	0.09	0.10	18.0		0.08	0.10	19.7	
Minimum	0.85	0.79	-7.1		0.75	0.75	-0.5	
Maximum	1.32	1.36	3.1		1.27	1.36	7.2	

† The significance of the difference between the summer and fall seasons was calculated using a two-tailed, paired t-test.‡ Fall mean significantly different from summer mean at $P < 0.05$.

The mean LOI_{500} value of Field 1 decreased from 48.1 g kg^{-1} to 45.9 g kg^{-1} from summer to fall, consistent with Wall et al. (2010) who reported higher LOI_{360} values during summer in four of the five sites in their work. For field 2, the mean LOI_{500} was 47.0 g kg^{-1} in the summer and 49.5 g kg^{-1} in the fall, possibly reflecting the manure application that took place after harvest of the corn. The mean LOI_{360} values followed the same trends but where manure had been applied (field 2) differences in LOI_{360} between sampling seasons were smaller for LOI_{500}, consistent with the higher temperatures used to determine LOI_{500} (Schulte et al., 1991; Matthiessen et al., 2005).

The mean ISNT-N value of Field 1 was 3.9% higher in the summer sampling round than in the fall sampling. A lower value in the fall is consistent with Wall et al. (2010) who showed a decline in available N later in the growing season as crops take up N (Wall et al., 2010). The average ISNT-N level of Field 2 was not impacted by sampling time, suggesting the manure addition compensated for a decline in ISNT-N possibly due to N mineralization (Jokela, 1992) and reflecting a temporary increase in ammonium-N when sampling takes place within 4-5 weeks after manure addition (Klapwyk et al., 2006).

The ratio ISNT-N/Critical ISNT-N, in Field 1, was 2.2% lower in the fall than in the summer, primarily affected by the reduced ISNT-N in fall. This difference could be meaningful for individual fields, as it changed the N supply potential classification according to Ketterings et al. (2013) from optimal “No extra N is needed” in summer to marginal “May extra N is needed” in fall. No significant differences were detected in the ISNT-N/Critical ISNT-N ratio between seasons in Field 2, reflecting increases in both LOI and ISNT-N following application of manure in the fall.

3.2. Spatial Statistics

Nitrate presented moderate spatial structure (Table 2), with the exception of fall sampling in Field 1 where a weak spatial correlation was found ($C/(C+C_o) = 0.16$), probably affected by the linear model of the semivariogram that was fitted to the data. However, in both sampling seasons and fields, all semivariograms had relatively low r^2 values ($r^2 \approx 0.5$), which impact the reliability of the interpretations of spatial structure.

Spatial dependence can be soil depth specific. For example, Robertson et al. (1988) showed that 20% of the total variance was represented by the nugget in a 0.5-ha study where samples were taken over 0-15 cm depth. However, López-Granados et al. (2002) found no spatial correlation for nitrate distribution in the top soil (0-10 cm) but strong spatial correlation in the subsoil (between 25 and 35 cm depth). Several studies presented moderate structure in nitrate distribution with ranges < 20 m (Jackson and Caldwell, 1993; Gross et al., 1995). Cambardella et al. (1994) showed moderate nitrate spatial dependency in the non-till fields while weak dependency in the plowed fields, suggesting extrinsic management such as tillage and residue removal affected the spatial dependency. In a follow-up study, Cambardella and Karlen (1999) showed moderate spatial dependence for nitrate in fields treated with inorganic fertilizer but random distribution in organically fertilized (manure/municipal sludge-kilns dust mixture) fields. Ranges varied from 0 to 182 m depending of soil depth, consistent with the results of the current study where the ranges in both seasons were about 80 and 30 m for Field 1 and Field 2, respectively.

Table 2. Best fit models† for semi-variograms of nitrate-N, loss-on-ignition at 360°C (LOI_{360}) or 500°C (LOI_{500}), Illinois soil nitrogen test-N (ISNT-N), and the ratio of ISNT-N and critical ISNT-N (based on Ketterings et al., 2013) for two corn fields sampled in summer and following corn harvest in the fall. Field 2 received manure in the fall two weeks prior to soil sampling.

Field	Season	Skew	Model	Nugget (C_o)	Sill ($C + C_o$)	Range	($C/C + C_o$)	r^2	RSS
Nitrate-N				--- (mg kg^{-1})^2 ---		(m)			
1	Summer	0.21	Spherical	8	27	75	0.71	0.42	485
	Fall	0.40	Linear	27	32	87	0.16	0.57	18
2	Summer	0.75	Spherical	4	10	25	0.63	0.36	27
	Fall	0.59	Exponential	42	97	34	0.57	0.48	1108
LOI_{360}				--- (g kg^{-1})^2 ---		(m)			
1	Summer	0.49	Spherical	0.011	0.311	79	0.97	0.91	0.008
	Fall	0.49	Spherical	0.220	0.212	50	0.90	0.90	0.003
2	Summer	0.09	Spherical	0.007	0.167	57	0.96	0.83	0.005
	Fall	-0.47	Spherical	0.028	0.162	38	0.83	0.66	0.006
LOI_{500}				--- (g kg^{-1})^2 ---		(m)			
1	Summer	0.47	Spherical	0.035	0.421	79	0.92	0.93	0.011
	Fall	0.58	Spherical	0.073	0.289	75	0.75	0.91	0.005
2	Summer	-0.11	Spherical	0.059	0.240	52	0.76	0.61	0.018
	Fall	-0.13	Spherical	0.074	0.214	49	0.66	0.80	0.004
ISNT-N				--- (mg kg^{-1})^2 ---		(m)			
1	Summer	0.36	Spherical	76	1661	74	0.95	0.90	260117
	Fall	0.43	Spherical	125	1512	41	0.92	0.93	103863
2	Summer	0.15	Spherical	42	927	53	0.96	0.77	210819
	Fall	-0.32	Exponential	537	1189	43	0.55	0.51	175705
ISNT-N / Critical ISNT-N				--- (ratio)^2 ---		(m)			
1	Summer	0.16	Spherical	9.20E-04	0.010	68	0.91	0.89	1.07E-05
	Fall	0.18	Spherical	3.51E-03	0.118	37	0.70	0.90	4.57E-06
2	Summer	0.12	Spherical	8.70E-04	0.007	53	0.87	0.84	5.38E-06
	Fall	-0.39	Exponential	1.50E-04	0.009	13	0.98	0.30	9.90E-06

† The best fit model was based on 150 samples taken per sampling round per field.

In contrast to nitrate, LOI_{360} and LOI_{500} showed a high spatial correlation (Figure 2) with higher $C/(C+C_o)$ for the summer sampling than for the fall sampling for both fields. Field 2 showed the biggest differences between the two sampling seasons, most likely reflecting increased variability resulting from the manure application after corn silage harvest. The LOI_{500} results were impacted more by the manure application than the LOI_{360} results. The lowest spatial correlation in LOI, independent of temperature of combustion, was found for the fall sampling in Field 2 ($C/(C+C_o)$ = 0.66). The LOI range was between 40 and 80 m for both fields and seasons, with smaller ranges when manure was applied.

Baxter et al. (2003) reported comparable results, with an LOI range distance of 49 m. These ranges are smaller than ranges reported in Cambardella et al. (1994), who showed range values for SOC that exceeded 100 m and Cambardella and Karlen (1999) who determined ranges from 75 to 182 m by the automated dry combustion method. Similarly, Geypens et al. (1999) reported bigger ranges for SOC (160 m) determined by the Walkley-Black method. Our results are different from those obtained by Jackson and Caldwell (1993), who found a lack of autocorrelation of SOM at scales greater than 1 m using the colorimetrically with potassium dichromate method suggesting site to site differences, possibly impacted by field management and manure management histories.

Field cultivation history can also impact spatial dependence as shown by Robertson et al. (1993) who examined SOC spatial dependence for both a 4-ha cultivated and a 1-ha uncultivated field. Their results showed a moderate correlation in the cultivated field, in contrasting with the strong correlation of the LOI in our study, but also suggested a range of 50 m.

Both ISNT-N and ISNT-N/Critical ISNT-N reflected a high spatial structure (Table 2). Fall sampling in Field 2 showed the lowest correlation coefficient, potentially reflecting the impact of manure application on spatial dependency and supporting the recommendation to not sample fields for ISNT-N within 4-5 weeks after manure addition (Klapwyk et al., 2006). The ranges for ISNT-N varied from 41 to 74 m, smaller than range values presented by Gardner et al. (2008) who found spatial correlation over distances of 100 m. Range values of ISNT-N/Critical ISNT-N were consistent with the values presented for LOI and ISNT-N for each field and season.

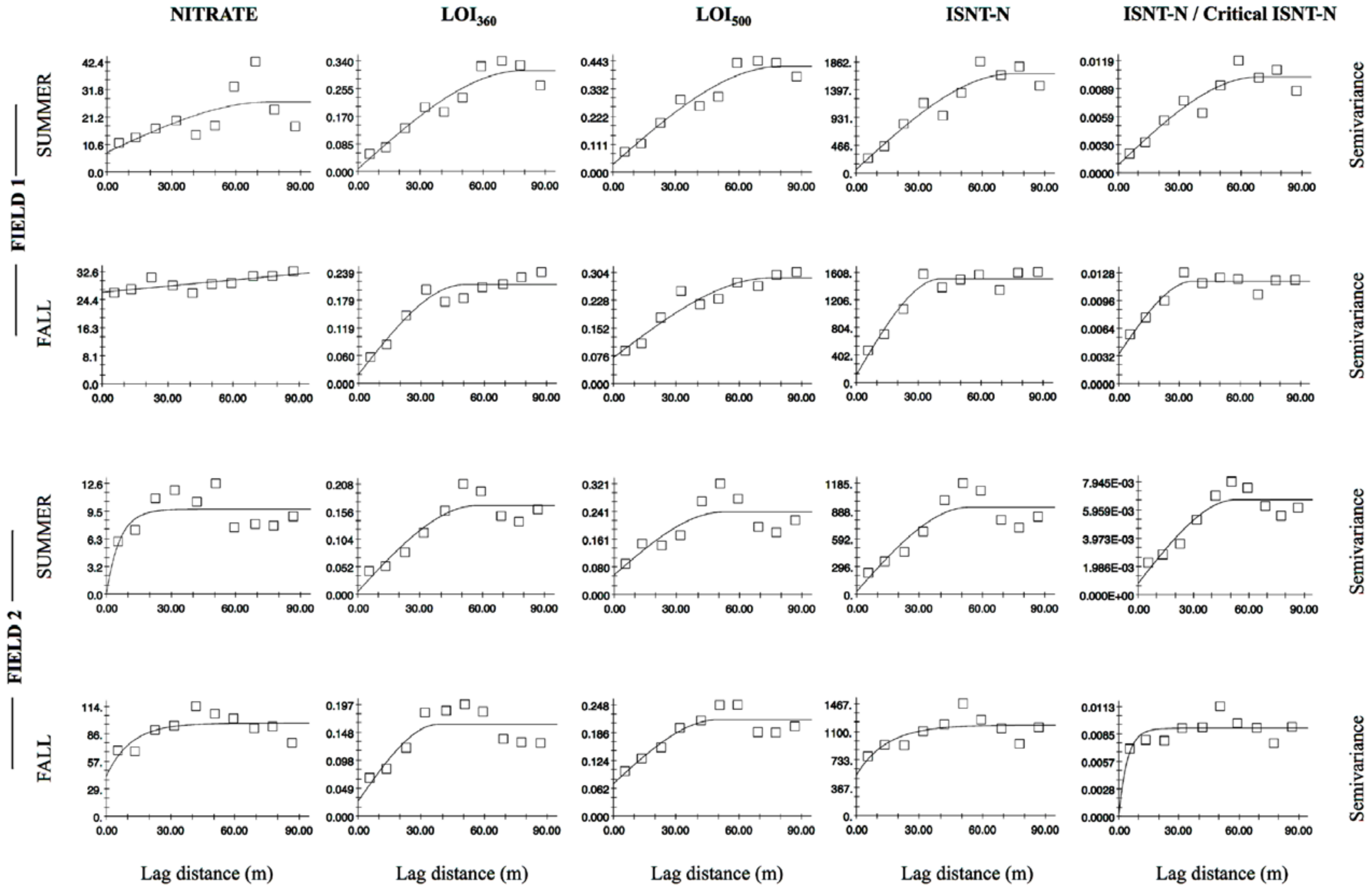

Figure 2. Graphs of best fitted semi-variograms for nitrate-N, loss-on-ignition at 360°C (LOI_{360}), at 500°C (LOI_{500}), Illinois Soil Nitrogen Test-N (ISNT-N) and ISNT-N/Critical ISNT-N in two corn fields during summer and fall. Field 2 received manure in the fall two wk prior to soil sampling.

A greater spatial dependency was found for summer sampling as compared to fall sampling. Ranges around 75 m and 55 m were determined for $LOI_{360,}$ LOI_{500}, ISNT-N, and ISNT-N/Critical ISNT-N for Field 1 and 2, respectively. Fall sampling reflected shorter ranges and greater variation among sampling points. In this study, the two fields showed similar ranges, possibly reflecting similar parent material and management history (both fields were managed by the same farmer). Robertson et al. (1993) suggest that the spatial pattern and scale of soil variability can differ markedly among edaphically identical sites and that these differences can be related to disturbance history. Thus, Kerry and Oliver (2004) concluded that average variograms that are not parent material specific would not provide a suitable guide to sampling on all parent materials.

Assessment of spatial dependence of additional fields varying in soil type and management history is recommended if field-specific guidance is to be developed. Our results, however, show the limited spatial dependence of nitrate compared to LOI and ISNT-N and hence greater potential for use on farms of LOI and ISNT-N as indicators of soil N supply.

<u>*3.3. Sampling Simulation*</u>

The smallest 95% CIs obtained in the simulation were 1.2 mg kg^{-1}, 1.2 g kg^{-1}, 1.3 g kg^{-1}, 10 mg kg^{-1} and 3%, for nitrate, LOI_{360}, LOI_{500}, ISNT-N, and ISNT-N/Critical ISNT-N, respectively (Table 3). Increasing the number of samples from 1 to 15 per 4-ha field reduced the 95% CI and also increased the probability of obtaining value within the mean ± 6.4% CV (to >95%) for all parameters except nitrate (Table 3). This is consistent with findings by Wollenhaupt and Crawford (1997) who showed that soil properties with larger CV values (such as nitrate) require more intensive sampling than properties with smaller CV values.

The simulations showed large variability of soil nitrate and low accuracy in determining an average field value. Even with a sampling density of 50 cores in a 4-ha field (12.5 samples per ha), there was only an 85% of probability of obtaining an estimate within ± 6.4% of the true average of the field. These findings are consistent with work by Cameron et al. (1971) who showed nitrate-N levels were more closely related to weather conditions than soil zones.

Table 3. Average confidence interval (and the probability of obtaining an estimate within the mean ± CV of 6.4%†) of nitrate-N, loss-on-ignition at 360^{o}C (LOI$_{360}$) or 500^{o}C (LOI$_{500}$), Illinois soil nitrogen test-N (ISNT-N), and the ratio of ISNT-N and critical ISNT-N (based on Ketterings et al., 2013) as a function of the number of subsamples taken in two corn fields during summer and fall. Field 2 received manure in the fall 2-wk prior to soil sampling.

		Number of samples in a 4-ha field									
Field	Season	3	5	10	15	20	25	30	35	50	All
Nitrate-N						± mg kg^{-1} (%)					
1	summer	4.4 (15)	3.6 (18)	3.0 (23)	2.4 (24)	2.2 (29)	2.0 (34)	1.8 (38)	1.7 (47)	1.5 (77)	1.4 (100)
	fall	6.0 (7)	5.0 (12)	3.6 (16)	3.0 (24)	2.6 (26)	2.3 (30)	2.1 (37)	2.0 (42)	1.7 (68)	1.5 (100)
2	summer	3.9 (19)	3.4 (25)	2.6 (27)	2.2 (32)	1.9 (39)	1.7 (46)	1.6 (48)	1.5 (54)	1.3 (85)	1.2 (100)
	fall	9.1 (14)	7.5 (18)	5.4 (26)	4.5 (32)	3.9 (37)	3.5 (46)	3.2 (51)	2.9 (59)	2.5 (83)	2.2 (100)
LOI$_{360}$						± g kg^{-1} (%)					
1	summer	5.8 (59)	4.8 (73)	3.5 (92)	2.9 (97)	2.5 (99)	2.3 (100)	2.1 (100)	1.9 (100)	1.6 (100)	1.4 (100)
	fall	5.2 (64)	4.3 (77)	3.1 (92)	2.6 (98)	2.2 (100)	2.0 (100)	1.8 (100)	1.7 (100)	1.4 (100)	1.3 (100)
2	summer	5.5 (61)	4.5 (72)	3.3 (90)	2.7 (97)	2.4 (99)	2.1 (100)	1.9 (100)	1.8 (100)	1.5 (100)	1.3 (100)
	fall	5.3 (63)	4.2 (77)	3.1 (93)	2.5 (99)	2.2 (100)	2.0 (100)	1.8 (100)	1.7 (100)	1.4 (100)	1.2 (100)
LOI$_{500}$						± g kg^{-1} (%)					
1	summer	7.0 (60)	5.8 (70)	4.2 (89)	3.5 (96)	3.0 (98)	2.7 (100)	2.5 (100)	2.3 (100)	1.9 (100)	1.7 (100)
	fall	5.9 (63)	4.9 (77)	3.6 (92)	2.9 (99)	2.6 (100)	2.3 (100)	2.1 (100)	1.9 (100)	1.6 (100)	1.5 (100)
2	summer	5.8 (62)	4.9 (74)	3.6 (92)	2.9 (98)	2.6 (99)	2.3 (100)	2.1 (100)	1.9 (100)	1.6 (100)	1.4 (100)
	fall	5.4 (65)	4.7 (80)	3.3 (95)	2.8 (99)	2.4 (100)	2.1 (100)	2.0 (100)	1.8 (100)	1.5 (100)	1.3 (100)
ISNT-N						± mg kg^{-1} (%)					
1	summer	46 (54)	39 (71)	28 (86)	23 (95)	20 (98)	18 (99)	16 (100)	15 (100)	13 (100)	11 (100)
	fall	46 (54)	37 (65)	27 (85)	22 (94)	20 (98)	18 (100)	16 (100)	15 (100)	12 (100)	11 (100)
2	summer	40 (59)	33 (74)	24 (88)	20 (96)	17 (99)	16 (100)	14 (100)	13 (100)	11 (100)	10 (100)
	fall	41 (62)	33 (74)	25 (88)	20 (96)	17 (99)	16 (100)	14 (100)	13 (100)	11 (100)	10 (100)
ISNT-N / Critical ISNT-N x 100						± % (%)					
1	summer	12 (71)	10 (86)	7 (98)	6 (100)	5 (100)	4 (100)	4 (100)	4 (100)	3 (100)	3 (100)
	fall	12 (66)	10 (80)	7 (94)	6 (99)	5 (100)	5 (100)	4 (100)	4 (100)	3 (100)	3 (100)
2	summer	11 (75)	9 (85)	7 (97)	5 (100)	5 (100)	4 (100)	4 (100)	4 (100)	3 (100)	3 (100)
	fall	11 (71)	9 (83)	7 (96)	6 (99)	5 (100)	4 (100)	4 (100)	4 (100)	3 (100)	3 (100)

† The 6.4% CV was calculated using 100 randomly selected sets of samples (25 m grid) to determine the average confidence interval for each sample size.

Ferguson et al. (1996) concluded that grid soil sampling is an accurate means of developing variable rate N application maps in some fields. However, they also recognized that a recommendation system based on grid-sampling to determine annual residual soil nitrate-N is not likely to be widely adopted because of the time and expense involved.

Increasing sampling intensity from 3 to 15 samples per 4-ha reduced the 95% CI for LOI_{360} from ±5.5 to ±2.8 g kg^{-1} and for LOI_{500} from ±6.2 to ±3 g kg^{-1}. For ISNT-N the CI reduced from ±42 (3 samples) to ±22 mg kg^{-1} (15 samples) while the CI for the ISNT-N/Critical ISNT-N ratio was reduced from ±12 to ±6%. Our results demonstrated as sampling density increases, the variation between samples decreases, consistent with the results of Franzen and Peck (1995) for P, K and pH, and by Grandt et al. (2010) for P.

Intense sampling is important primarily when field means are close to critical classification values such as the agronomic critical soil test value or an environmental threshold level (Grandt et al., 2010). In our study, at a sampling density of 3 samples per 4-ha (0.75 samples ha^{-1}), in the Field 1 the mean ISNT-N/Critical ISNT-N value for the fall sampling round was 1.06 with a CI of ±12%. Given a critical value of 1.07 to separate optimal from marginal in NY (Ketterings et al., 2013), a low sampling intensity could impact interpretations. Increasing the sampling intensity to 15 samples per 4-ha (3.75 samples ha^{-1}) reduced the CI to < ±6% which classified the field as marginal in most cases. These findings stress the importance of use of ranges for soil test interpretations, as done in NY for interpretation of ISNT-N data (Ketterings et al., 2013), as well as the importance of increasing sampling intensity to 3.75 samples ha^{-1} or more when field means are close to fertilizer or manure application agronomic or environmental cutoffs.

Despite the reduction in spatial correlation with manure addition in fields (Table 2), the simulation of sampling at different intensities did not suggest that a recently manured field needed a higher sampling density to obtain the same accuracy as a non-manured field. Although fall sampling, or sampling at least 2 mo after the most recent fertilizer application, did result in more stable results for P and K in studies by Lockman and Molloy (1984) and for N in studies by Wollenhaupt and Crawford (1997), our results agree with Grandt et al. (2010) who concluded that the manure application in this study did suggest greater intensity of soil sampling for P within fields was needed.

Consistent with these findings, current sampling guidelines suggest sampling to be delayed until 4-5 weeks after manure application to avoid elevated ISNT-N values (Klapwyk et al., 2006), but do not suggest a need for additional samples after manure or other fertilizer application (Cornell Cooperative Extension, 2014).

Sampling intensity determined in this study for the various soil parameters might be extrapolated to bigger fields as Cameron et al. (1971) reported that the number of samples needed to estimate the field average did not increase drastically with an increase in field size. It must, however, be recognized that in the sampling protocol used in this study, six subsamples were taken within very short distance and composited to obtain enough samples mass to conduct the various laboratory analyses. Thus, micro variability across short distances (< 0.6 m) was averaged across the six subsamples and could not be evaluated in this study.

3.4. Relationship between LOI_{500} and LOI_{360}

Results of LOI_{500} and LOI_{360} were closely related (Table 1) although manure application in Field 2 impacted the intercept but not the slope of the relationship between LOI_{500} and LOI_{360} (Table 4; Figure 3). The lack of a change in slope suggests that manure application increased LOI_{500} and LOI_{360} equally.

Table 4. The results of a multiple regression between organic matter as determined by loss-on-ignition at 500°C (LOI_{500}), time of sampling (season), field (independent variables)† and organic matter as determined by loss-on-ignition at 360°C (LOI_{360}, dependent variable).

Parameter	Fit regression			
	Summary			
r^2	0.8165			
r^2 adjusted	0.8143			
	Parameter Estimates			
Term	Estimate	Standard Error	*t* ratio	P > |t|
Intercept	4.2732	0.9059	4.72	<0.0001
LOI_{360}	1.0434	0.0217	48.07	<.0001
Season	0.1262	0.0927	1.36	0.1738
Field	-5.9880	0.0927	-6.46	<0.0001
Season*LOI_{360}	-0.0226	0.0217	-1.04	0.297
Field*LOI_{360}	0.0232	0.0217	1.07	0.2842
Season*Field	-0.8453	0.0927	-9.12	<.0001
Season*Field*LOI_{360}	-0.0180	0.0217	-0.83	0.4067

† Season is a binary variable to distinguish between two sampling rounds (summer and fall). Field is a binary variable to distinguish between two fields, one of which received manure two weeks prior to fall sampling.

The r^2 values of the correlations between the two LOI methodologies were 0.82 (summer) and 0.85 (fall) for Field 1 versus 0.78 (summer) and 0.75 (fall) in Field 2. The differences between fields could reflect soil composition differences (Veres, 2002) as heating above 450°C can destroy inorganic carbonates (Davies, 1974; Schumacher, 2002). Similarly, Howard and Howard (1990) concluded that due to differences in the nature of the organic matter among soils and horizons within a soil type, knowing more about the soils is essential before converting LOI values, consistent with the conclusion reached by Konen et al. (2002) that unique relationships exist for different soil geographic areas.

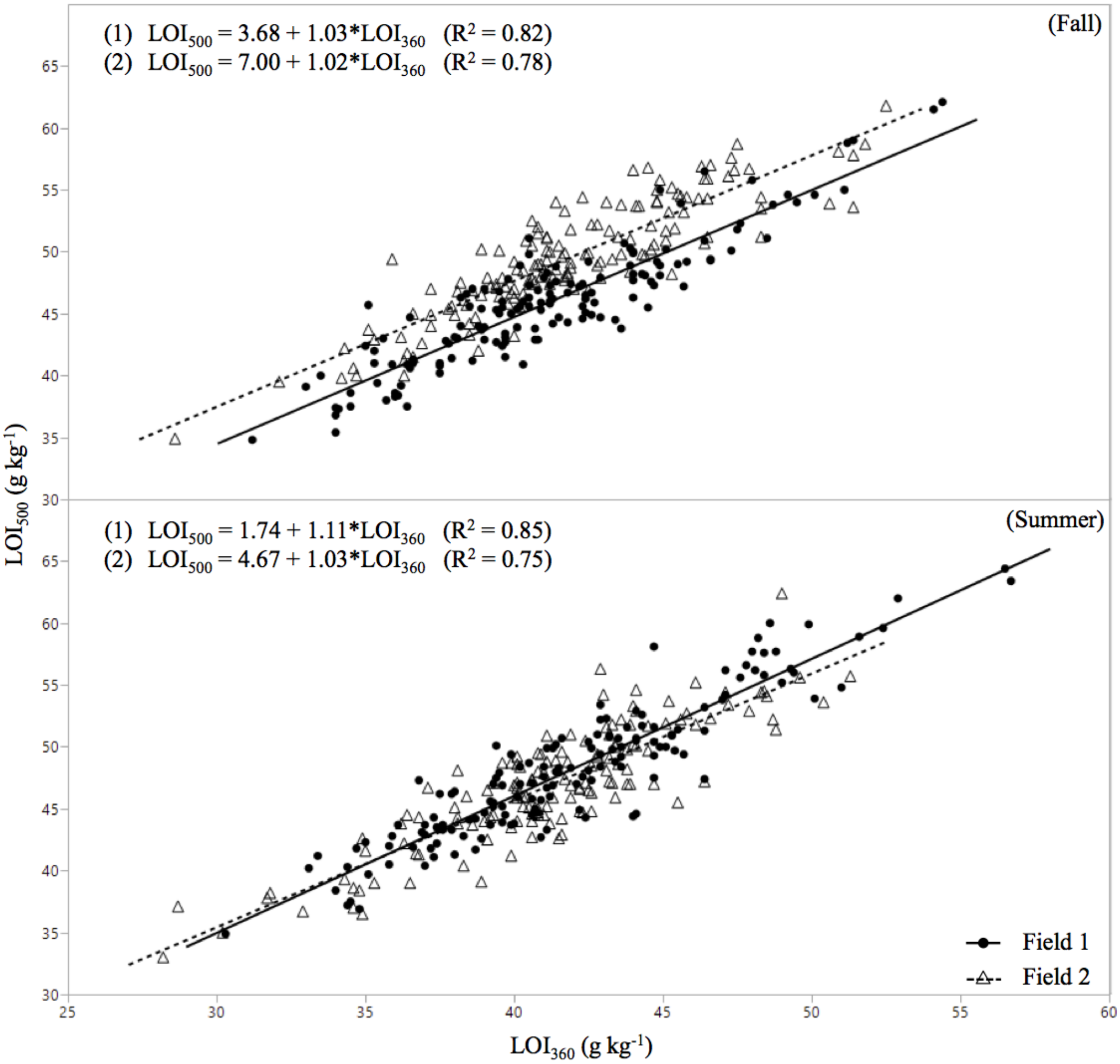

Figure 3. Loss-on-ignition at 360°C (LOI_{360}) values compared to 500°C (LOI_{500}) values for two fields in Aurora, NY, sampled in the summer and fall (150 per sampling round per field). Field 2 received manure in the fall two wk prior to soil sampling.

The prediction of the soil N supply potential for fields using LOI_{500} equivalents derived from LOI_{360} measurements resulted in similar classifications as those obtained using the original LOI_{500} values (Table 5). Across both fields and sampling seasons, LOI_{360} and LOI_{500} were linearly related:

$$LOI_{500} = 1.064\ LOI_{360} + 3.46 \quad (r^2 = 0.78) \qquad [1]$$

Use of this equation to determine LOI_{500} equivalents, resulted in an identical classification for the ISNT-N/Critical ISNT-N ratio for 92% of the samples analyzed for the study (table 5), with slightly better predictions in the fall (94% identical classification) than in the summer (90% identical classification). This is an improvement over use of LOI_{360} values without converting to LOI_{500} equivalents for ISNT-N interpretations; without the conversion only 76% of the samples were classified the same.

Table 5. Percentage of samples for which the soil N supply potential prediction using the Illinois soil nitrogen test (ISNT) results adjusted for loss-on-ignition at 500^{o}C (LOI_{500}) (as done in Ketterings et al., 2013) was identical to predictions made using LOI_{500} equivalents derived from LOI data obtained at 360^{o}C for two fields samples in the summer and the fall. Field 2 received manure after corn silage harvest and two week prior to fall sampling.

	Summer	Fall	Average
Scenario A: one equation†			
Field 1	93.9	95.9	91.8
Field 2	85.3	92.7	
Scenario B: two equations‡			
Field 1	94.6	96.6	92.6
Field 2	83.9	95.3	
Scenario C: four equations§			
Field 1	94.6	98.6	93.2
Field 2	84.6	95.3	

† Scenario A (one equation) refers to a single regression equation for both fields and sampling seasons.

‡ Scenario B (two equations) uses an equation for the fields and sampling rounds not impacted by recent manure additions (Field 1 both sampling rounds and Field 2 summer sampling) and a second equation for the field that had received manure in the fall (Field 2 fall sampling).

§Scenario C (four equations) has a single regression equation for each field and sampling round.

Use of a single conversion equation for each season increased the predictions for fall slightly (to 97%) while not impacting the predictions for the summer sampling rounds. The use of a specific conversion equation for fall for Field 2 only (where manure had been applied) did not improve the accuracy of the prediction based on ISNT-N. These results suggest that a single linear regression conversion equation (Equation [1]) can be used.

4. Conclusions

For the two tilled cropland fields in this study, 15 or more samples per a 4-ha field were needed to obtain a 95% or greater probability of a means that was within the true mean of the field ± CV (6.4%) for LOI_{360}, LOI_{500}, ISNT-N, and ISNT-N/Critical ISNT-N, independent of timing of sampling or manure history. High spatial correlations were found for LOI, ISNT-N and ISNT-N/Critical ISNT-N. Soil nitrate was more variable and less spatially dependent. Despite reduced spatial dependence upon manure application, sampling intensity did not need to increase to obtain reliable field means. Because of a strong relationship between LOI_{500} and LOI_{360} across both fields and sampling times, LOI_{360} data could be transformed to LOI_{500} equivalents and then used to determine soil N supply potential as measure by ISNT-N/Critical ISNT-N. Fall sampling after harvest presented slightly better accuracy in determining the soil N supply potential (based on ISNT-N/Critical ISNT-N) than sampling the summer. Sampling protocols should be adjusted (3.75 samples ha^{-1} or more) where greater accuracy is needed for LOI, ISNT-N and ISNT-N/Critical ISNT-N. Soil nitrate sampling is less practical due to the large number of samples needed to accurately quantify soil nitrate levels of a field.

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Chapter II

Analysis of Vegetation Indices to Determine Nitrogen Application and Yield Prediction in Maize (Zea mays L.) from a Standard UAV Service

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Abstract

The growing use of commercial unmanned aerial vehicles (UAV) and the need to adjust N fertilization rates in maize (*Zea mays* L.) currently constitute a key research issue. In this study, different multispectral vegetation indices (green-band and red-band based indices), SPAD and crop height (derived from a multispectral compact camera mounted on a UAV) were analysed to predict grain yield and determine whether an additional sidedress application of N fertilizer was required just before flowering. Seven different inorganic N rates (0, 100, 150, 200, 250, 300, 400 kg·N·ha^{-1}), two different pig slurry manure rates (Ps) (150 or 250 kg·N·ha^{-1}) and four different inorganic-organic N combinations (N100Ps150, N100Ps250, N200Ps150, N200Ps250) were applied to maize experimental plots. The spectral index that best explained final grain yield for the N treatments was the Wide Dynamic Range Vegetation Index (WDRVI). It identified a key threshold above/below 250–300 kg·N·ha^{-1}. WDRVI, NDVI and crop height showed no significant response to extra N application at the economic optimum rate of fertilization (239.8 kg·N·ha^{-1}), for which a grain yield of 16.12 Mg·ha^{-1} was obtained. This demonstrates their potential as yield predictors at V12 stage. Finally, a ranking of different vegetation indices and crop height is proposed to overcome the uncertainty associated with basing decisions on a single index.

Keywords: maize; nitrogen; multispectral vegetation indices; crop height; UAV

Abbreviations: Chlorophyll meter, CM; Digital surface model, DSM; Digital ground model, DGM; Economic optimum nitrogen rate, EONR; Green normalized difference vegetation index, GNDVI; Nitrogen, N; Normalized difference index, NDVI; Near infrared, NIR; Pig slurry, PS; Unmanned aerial vehicle; UAV; Wide dynamic range vegetation index, WDRVI.

1. Introduction

Nitrogen (N) fertilization of maize (*Zea mays* L.) is an important research topic. Nitrogen, together with genetic improvement, is one of the most important factors affecting production and can account for up to 30% of the total cost of producing maize [1]. N fertilization is universally accepted as a key input for increasing maize grain yields and optimizing economic returns [2]. In many irrigated Mediterranean areas, maize is one of the most important field crops. In the Ebro valley (NE Spain), grain yields commonly range from 12 to 15 Mg·ha^{-1}, with a total plant nitrogen uptake of 250–300 kg·ha^{-1} [3]. Farmers usually decide N application rates on the expected crop N uptake which is, in turn, based on yield goals. However, they do not often consider the possible effect of having high N levels in the soil prior to planting, conditions which are common in many crop-growing areas [4]. In irrigated maize, most of the N fertilizer tends to be applied at planting or during the earliest stages of crop growth, as this simplifies crop management. This may constitute a problem since N uptake does not all occur at the same time. Under favourable soil moisture conditions, approximately one-third of the total N uptake occurs after pollination [5]. Consequently, consideration should be given to N applications via sidedress, applied at or near flowering (VT stage or tasseling, [6]), with appropriate doses of N being applied at planting and/or at lay-by (e.g., V7–8, 7–8 leaves with visible leaf collars) in order to maintain yield potential [7].

Over-fertilization can occur as a consequence of N fertilization in early stages of crop growth. This does not increase grain yield but, instead, wastes fertilizer, increases costs, and can cause nitrate pollution [8]. This problem has led farmers, scientist and politicians to explore how to improve N efficiency, reduce N inputs and prevent water and soil pollution associated with maize production [9–11].

In recent years, the use of chlorophyll meters (CM), which measure leaf chlorophyll content to estimate N nutrition status, has increased among researchers and farmers [10,11]. Hawkings et al. [12] found that the adjusted R^2 of the relationship between CM readings and the nitrogen rate difference (ND) for the economic optimum nitrogen rate (EONR) was 0.76 for a maize-maize rotation. However, despite the good correlations, these methods fail to capture the spatial variability that is often present within plots. Data acquisition also tends to be time-consuming and can be hindered by a series of practical limitations [8,9]. One alternative to ground-based measurements involves image-based (satellite or airborne) remote-sensing. This technique, either using active or passive sensors, has been recognized as a potential tool for both spatial and temporal improvement of N management in field crops [13,14], and can also be used to detect N deficiencies in maize [15]. Cilia et al. [16], using multispectral airborne images, created a variable rate N fertilization map based on the difference between actual and optimal crop N content. This map of maize N content also related well to the real maize N content obtained using traditional destructive measurement techniques (R^2 = 0.70). In this respect, multispectral satellite images, such as Aster and QuickBird, and manned-flight airborne images have been used to assess irrigated maize N status at V12 (12 leaves with visible leaf collars) and later stages of crop growth, as well as to determine field variability for in-season N management in order to complement ground-based measurements [9,10,17]. In fact, most previous studies have shown that both real and false colour images acquired between growth stages V7 and VT could be used to predict N deficiencies and N requirements in maize [18]. Combining CM readings and aerial and/or satellite remote sensing images would therefore seem to offer a practical solution to in-season site-specific N applications in large fields [19].

Image acquisition at stage V12 is generally preferred to other alternatives because the observed maize N uptake at this stage tends to be about 40% of the total [6], and crop response to N fertilizer is high if N deficiencies are detected. In addition, when taking aerial images at stage V12, strong background reflectance from the soil is minimized. This is one of the most challenging obstacles to detecting maize N deficiencies in the early stages of crop growth [18]. At the same time, tassel colour interference, which can occur if the images are taken at later stages, is also avoided. Readings for earlier vegetation stages can usually be discarded since only weak correlations are found ($R^2 \leq 0.29$) for the prediction of optimum N rates [20].

Despite the apparent advantages offered by remote sensing from satellites and manned aircraft, the cost of obtaining high-resolution multispectral images for relatively small areas is considered an important drawback [10,21]. At present, this can be overcome by using unmanned aerial vehicles (UAV), mounted with multispectral cameras. Image acquisition with UAV can be deployed quickly and repeatedly, meaning lower costs, greater flexibility in terms of flying heights and mission timing, and higher spatial resolutions [22]. In recent years, UAV-based research has been carried out to monitor vegetation for agricultural purposes [16]. Most of these applications have been possible due to the miniaturization of multispectral and thermal cameras. However, radiometric and geometric calibrations are required to provide images that are similar to those available from traditional satellite-mounted sensors [23].

Several studies on the use of UAV in the assessment of N status in maize have been published. These have mainly focused on standardizing NDVI (Normalized Difference Vegetation Index) values for their use as part of an N sufficiency index (the NDVI reading divided by the NDVI value of a corresponding well-fertilized N field) [24], and on comparing ground-sensor measurements with hyperspectral images [11]. In [11], indices based on UAV hyperspectral imagery were used to calculate greenness, chlorophyll and photochemical indices. These indices were found to be as reliable as ground-level measurements for assessing crop nitrogen (N) status. This finding was also in line with the work of Isla et al. [17], who investigated the N nutritional status of maize using multispectral data acquired from an aircraft. Scharf and Lory [14] also showed that maize colour measured using aerial photography could be used to predict N sidedress requirements. Correlations between colour and the EONR ranged from 0.60 to 0.79 after the removal of soil pixels.

Other multispectral indices have been reported as being particularly useful for assessing maize N status. McMurtrey et al. [25] reported that as N deficiencies increased, leaf reflectance increased in the green band (0.55 µm), decreased in the NIR (Near-Infrared) (0.70 µm) and remained almost unvaried in the red band (0.67 µm). Along the same lines, Bausch and Duke [8] proposed an N reflectance index for maize based on the Green Ratio Vegetation Index (GRVI), which correlated highly with the N sufficiency index (average SPAD reading for a given treatment divided by the average SPAD reading for a well-fertilized N field; with SPAD being a device for indirect chlorophyll measurement) after stage V11 (11 leaves with visible leaf collars). More recently, other authors have reported the greater sensitivity of the GRVI than red-based indices for assessing N deficiencies in maize [26]. Other green-based vegetation indices have also been qualified as particularly useful for assessing maize N status at V12 or later growth stages [9]. For example, Schlemmer et al. [27] and Li et al. [28] showed the significance of the green and red-edge bands for estimating chlorophyll and N content in maize. The GNDVI (Green Normalized Difference Vegetation Index) has also been considered useful for assessing leaf chlorophyll variability when the leaf area index is moderately high [29]. In other cases, green-based indices have shown high correlations with maize grain yield, explaining 86% of the observed variance [17].

In view of the growing application of UAV services and of the importance of N fertilization in maize production, the purpose of the present study was to analyse the potential utility of different multispectral vegetation indices in conjunction with crop height to support decisions regarding the need to apply N fertilizer just before flowering (V12). The study involved the use of a standard UAV service for acquiring multispectral images with a broad-band compact camera, from which crop height data were automatically derived. It explores the possibilities of using this type of service in commercial farms on a day-to-day basis.

2. Materials and methods

2.1. Study Area

The experimental field was located at the IRTA Research Station in Gimenells (Lleida, NE Spain, 41°65′N, 0°39′E) and had an area of 110 × 130 m^2 (Figure 1). Soils were well-drained, had no salinity problems, and were characterized by the presence of a petrocalcic horizon at a depth of 80–100 cm, being classified as Petrocalcic Calcixerept [30]. The area has a semi-arid climate with a mean temperature of 19.1 °C and low precipitation during the maize growing season (192 mm) [31]. Irrigation is therefore required to achieve high grain yields. The field was irrigated using a sprinkler irrigation system, which provided approximately 750 mm of water (with no appreciable nitrate content) over the maize growing season. Conventional tillage was applied, which included disc ploughing and cultivation to a depth of 25–30 cm. The study field was divided into 45 experimental plots, each of 10 × 15 m^2. These plots were sown with maize of the variety PR33Y72 (FAO cycle 600) on 10 April 2014.

A pre-emergence herbicide (S-Metolachlor 40% and Terbutilazine 18.75%) was applied at 3 L ha^{-1} to control weeds. At post-emergence, 1 L ha^{-1} of Dimethylamine salt of dicamba 48.2% (3.6-dichloro-o-anisic acid) and 0.75 L·ha^{-1} Nicosulfuron 6% were applied to control *Abutilon theophrasti* M. and *Sorghum halepense* L., respectively.

The maize was harvested on 29 October 2014. Grain yield was determined by harvesting two complete central rows (1.5 × 10 m^2) using a small-plot combine. A grain sample of 250 g was taken from each plot to determine moisture content (GAC II, Dickey-John, Auburn, IL, USA) and adjust grain yield to 14% moisture.

It should be noted that the plots had been continually used throughout the previous 12-year period as an experimental field for studies related to maize N fertilization. It should also be noted, in this respect, that the same treatments were applied to each individual plot as in the previous years. As a result, the effect of the different N treatments would have been expected to be more evident.

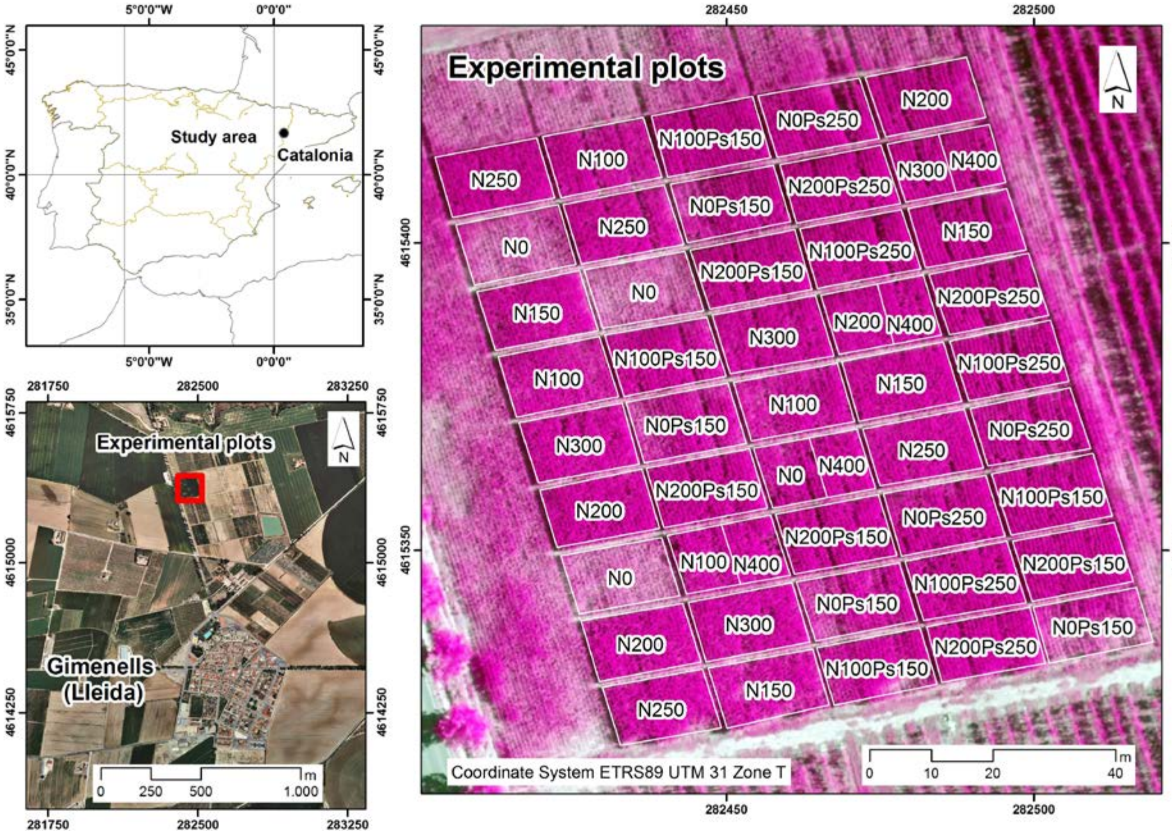

Figure 1. Location of the study area and detail of the experimental plot layout for the maize nitrogen treatments. The background picture is a false colour composition (NIR, Red, and Green) of the image acquired by the UAV on 30 June2014. The label "N" refers to inorganic nitrogen, "Ps" refers to pig slurry manure, and the numerals express the rate of N applied in kg·ha^{-1} for each N source.

2.2. Fertilizer Treatments

Seven different inorganic N rates were applied: 0, 100, 150, 200, 250, 300 and 400 kg·N·ha^{-1}. Two different pig slurry manure rates (Ps): 150 and 250 kg of organic N·ha^{-1} were used in the study (Figure 1) and four different inorganic-organic combinations were also applied: N100Ps150, N100Ps250, N200Ps150, and N200Ps250.

Each N treatment included four replications (except for the treatments with Ps250, with three replications) under a split plot design. Pig slurry (Ps) applications were done before sowing and the slurry was then ploughed into the soil between 3–5 hours after application to reduce ammonia (NH_3) volatilization losses. Inorganic N fertilization (33.5% N, as ammonium nitrate) was applied by hand, in two equal parts: 50% at the first sidedress (stage V3–V4: 3–4 leaves with visible leaf collars) and 50% at the second sidedress (stage V6–V7: 6–7 leaves with visible leaf collars) [31]. In the case of the N400 treatment, a third sidedress was applied at stage V12 (when the UAV images were acquired). In this case, N application was distributed differently (37.5% at stage V3–V4, 37.5% at V6–V7 and 25% at V12) with a view to reducing the risk of pollution by nitrate leaching, which is associated with high rates of N application. Additionally, phosphorus and potassium fertilizations were applied before planting, at rates of 150 $kg{\cdot}P_2O_5{\cdot}ha^{-1}$ and 250 $kg{\cdot}K_2O{\cdot}ha^{-1}$, to ensure no deficit of either of these elements. This type of fertilization had been performed each 2 years based on previous soil analysis.

2.3. Remote and Proximal Sensing Data Acquisition and Analysis

An aerial survey was carried out with the Atmos-6 UAV (CATUAV, Moià, Catalonia, Spain) (Figure 2). This drone has a wingspan of 1.80 m, a length of 1.29 m and a payload of 500 g. The survey was conducted on 30 June 2014, at 10 h (GMT), with maize at V12 stage. The flight height was 180 m above ground, with a speed of 38 km h^{-1}. The time of flight was very short since the area to capture was only about 0.8 ha, and irradiance conditions did not vary during image acquisition. The images were acquired using a VEGCAM-Pro camera, with a 14 Mp Foveon X3 image sensor. This camera has a total weight of 307 g and a size of 110 × 85 × 78 mm. It works in three wide spectral bands: green (525–575 nm), red (615–685 nm) and near infrared (755–805 nm), with a radiometric resolution of 8 bits/pixel (with a pixel value range of 0–255). Other characteristics of the camera include: objective 16.6 mm / F4, sensor size 20.7 × 13.8 mm^2, effective pixels 2650 × 1768 × 3 layers, instantaneous field of view (IFOV) 0.0265 × 0.0265 deg.

The photos were acquired with a horizontal overlap of at least 60% to allow the use of stereoscopy to compute the elevation in each pixel. The images were then rectified and mosaicked with the aid of Pix4D software (Pix4D SA, Lausanne, Switzerland) to produce images at a spatial resolution of 0.15 m. The geometry of the camera was accurately calibrated using a calibration panel and the RapidCal software (PIEneering, Helsinki, Finland), yielding standard errors for the principal point of ±0.0122 mm and ±0.0098 mm in X and Y respectively. The photos were georeferenced on the basis of ground control points selected on a 1:2500 scale orthophoto, produced by the Cartographic and Geologic Institute of Catalonia. Due to the small size of the experimental field (0.8 ha), only three sequential photos were needed to produce the mosaic, and artifacts were not produced. Reflectance values were computed for each band by dividing the pixel values by those of a calibrated diffuse Spectralon reflectance target (Labsphere, North Sutton, NH, USA).

Figure 2. The Atmos-6 UAV operated by CATUAV (Moià, Catalonia, Spain), which was used as the platform for multispectral data acquisition.

Three vegetation indices were computed based on the reflectance values: NDVI (Normalized Difference Vegetation Index, Equation (1), [32]), GRVI (Green Ratio Vegetation Index, Equation (2), [20]), and WDRVI (Wide Dynamic Range Vegetation Index, Equation (3), [33]).

$$NDVI = \frac{NIR - Red}{NIR + Red} \tag{1}$$

$$GRVI = \frac{NIR}{Green} \tag{2}$$

$$WDRVI = \frac{(\alpha \cdot NIR - Red)}{(\alpha \cdot NIR - Red)} \tag{3}$$

where *NIR* is the reflectance of the near infrared light, *Red* is the reflectance of the red light, *Green* is the reflectance of the green light and α a weighting coefficient that can vary from 0.1 to 0.2. The *WDRVI* was created to increase correlations with the vegetation fraction for crops such as wheat, soybean and maize, thereby enabling a more robust characterization of the physiological and phenological characteristics of the crop [33]. In the present study, we used $\alpha = 0.1$ because of its better fit to N dose and maize yield.

In addition, the crop height in each pixel was calculated based on an intensive photogrammetric analysis of stereopairs with Pix4D software. This made possible the creation of a digital surface model (DSM) of the experimental plots. A digital ground model (DGM) based on these data was also built by selecting bare soil pixels around the experimental plots and capturing their height from the DSM. By connecting the different bare soil pixels, it was then possible to create a triangulated irregular network representing ground elevation. Finally, maize plant height was calculated by subtracting DGM from DSM.

In-field crop height measurements were taken at the VT stage (tasseling) (10 to 15 days after the UAV flight). These measurements were then used to calculate their correlation with those obtained via the photogrammetric process. For this, the heights of 5 plants were measured in each experimental plot using a tape measure. These height measurements were repeated until tassel insertion, in order to avoid the possibility of differences in height caused by the length of the tassel. According to Duncan et al. [34], tassel size varies with both plant population and variety. Linear regression analysis was performed between the image and in-field height, yielding an R^2 coefficient of 0.82 (RMSE = 0.15 m and p-value < 0.001). This indicates a good correlation between the two types of measurement and that the height values obtained from the digital height model could be used as a good estimator of crop height at the moment of image acquisition.

Non-destructive chlorophyll readings were also taken from plant leaves in the experimental plots at VT stage, with a view to comparing the measurements with the spectral indices and correlate them with yield. These measurements were taken using a small, lightweight, portable, hand-held meter (SPAD-502 indirect chlorophyll meter; Minolta Corp, Ramsey, NJ, USA). SPAD values calculate relative chlorophyll content based on the amount of light transmitted by the leaves at two different wavelengths: red (650 nm) and near infrared (940 nm). In agriculture, the SPAD meter is often used to improve N management and increase yields by predicting N status and determining fertilization requirements [35]. In this case, 5 plants were sampled from each plot and three readings were taken from each selected ear leaf: from the base, middle and top of each plant. The 15 measurements taken from each experimental plot were then averaged to obtain the plot SPAD value.

2.4. Statistical Analysis

To analyse the spectral indices and crop height according to the different N fertilizer treatments, fifty points were randomly sampled within each individual plot, excluding a 1 m buffer from the borders. In addition, the points that lay on bare soil were moved to a nearby plant to avoid measurements in areas without crop. A multiple comparison analysis using Tukey's Honest Significant Difference (HSD) test at a significance level of 0.05 was then carried out. For each N treatment, this analysis compared the mean values of the spectral indices (included SPAD) and crop height, distinguishing different significant groups. JMP Pro 12 (SAS Institute, Cary, NC, USA) statistical package was used for the statistical analysis.

Linear-plateau models were fitted for spectral indices, crop height and yield with the N fertilization treatments. In this way, the economic optimum rates of fertilization, as well as the saturation point for N fertilization of spectral indices and crop height were identified by locating the intersection of the two lines [36].

Linear and quadratic regression analyses were also carried out between the mean values for the spectral indices and crop height from the different treatments and yield. These were used to determine the best variable to estimate yield at the V12 stage.

3. Results and discussion

3.1. Multiple-Rank Analysis of Spectral Indices, Crop Height and Yield

Table 1 shows the mean values of the vegetation indices (n = 50 for each treatment replication), crop height (n = 50), SPAD (n = 15 for each treatment replication) and yield (n = 4) for the different N treatments applied on the experimental plots. The table also presents the mean values of the variables analysed. The treatments are sorted according to the total amount of N applied until stage V12. Treatments not connected by the same letter are significantly different at a p-value of <0.05. Figure 3 provides a visual comparison of the vegetation indices and crop heights in the experimental fields.

For all the considered variables, the N fertilizer treatments were grouped in three to nine homogeneous groups. Of these, N0 (the control treatment) presented the lowest values for all of the variables and also for grain yield (3.16 $\mathrm{Mg \cdot ha^{-1}}$). The N0 treatment was clearly identified as being totally different from the other N treatments, with no nitrogen applications in these plots over the 12 continuous years of the experiment. The treatments involving applications between 0 and 150 $\mathrm{kg \cdot N \cdot ha^{-1}}$ were separated into several different homogeneous groups, which in most cases exhibited clear N deficiencies in their spectral indices. The N100 and N0Ps150 treatments were associated with the lowest grain yields and the lowest index values after the N0 treatment. The N150 treatment resulted in higher grain yield than the N0Ps150 treatment due to the greater efficiency of applying N at sidedress rather than at planting [37,38].

Table 1. Mean values of the vegetation indices and crop height (n = 50 for each treatment replication), SPAD (n = 15 for each treatment replication) and yield (n = 4) for the different nitrogen (N) treatments applied on the experimental plots. Tukey's HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of < 0.05. The total amount of N (kg·ha^{-1}) applied up to vegetation stage V12 is given in brackets.

N treatment	**NDVI**	**WDRVI**	**GRVI**	**SPAD**	**Crop Height (m)**	**Yield (Mg·ha^{-1})**
N0	0.451 d	−0.574 f	1.253 f	30.06 b	0.81 i	3.16 e
N100 (100)	0.942 c	0.619 e	1.355 e	48.68 a	1.65 h	9.79 cd
N150 (150)	0.963 b	0.747 d	1.366 cde	51.76 a	1.85 fg	12.02 bcd
N0Ps150 (150)	0.926 c	0.584 e	1.362 cde	46.30 a	1.88 fg	8.70 de
N200 (200)	0.974 ab	0.809 bcd	1.367 cde	56.30 a	1.88 fg	15.04 abc
N250 (250)	0.967 ab	0.781 cd	1.363 de	56.71 a	1.86 fg	16.17 ab
N0Ps250 (250)	0.971 ab	0.808 bcd	1.373 abcd	54.26 a	1.99 cde	15.14 abc
N100Ps150 (250)	0.975 ab	0.834 abcd	1.377 abcd	56.30 a	1.99 cd	14.65 abc
N300 (300)	0.971 ab	0.793 cd	1.366 cde	56.23 a	1.82 g	15.40 ab
N400 (300) *	0.979 ab	0.846 abc	1.367 bcde	**	1.93 def	17.64 a
N100Ps250 (350)	0.986 a	0.894 ab	1.385 a	56.48 a	2.11 ab	17.00 ab
N200Ps150 (350)	0.987 a	0.900 a	1.383 ab	56.89 a	2.03 bc	17.56 a
N200Ps250 (450)	0.987 a	0.899 ab	1.382 abc	57.36 a	2.16 a	16.98 ab

* A total of 300 kg·N·ha^{-1} was applied up to stage V12 (image acquisition). ** Not measured

The treatments that combined inorganic and organic N, with a total amount of applied N of between 350 and 450 Kg·N·ha^{-1} (N100Ps250, N200Ps150 and N200Ps250), were at the top of the ranking. This seems to indicate that up to V12 stage, the plots fertilized with a combination of organic and inorganic N performed better in terms of plant vigour than those only fertilized with inorganic N. The reason for this may be a progressive mineralization of organic N, which would have facilitated N availability during all development stages [39,40]. The experimental plots in which these treatments were applied also produced the highest grain yields, although not exclusively, as the N200, N250, N0Ps250, N100Ps150, N300 and N400 were also classified in homogeneous group “a”, the one with the highest yields.

The results obtained for the N400 treatment require particular attention. Based on the total amount of N applied, this treatment might have been expected to be in the upper part of the homogeneous group classification (group “a” or “ab”) for the different variables considered. However, it was not, even though the N400 treatment did produce the highest grain yield (17.64 Mg·ha^{-1}). The explanation for this lack of correspondence is related to the total amount of N applied on the experimental plots before image acquisition. Sidedress applications were completed at stage V6-V7 for all treatments except the N400, with the final sidedress for the N400 plots being administered at stage V12. As result, only 75% of the N fertilizer (300 kg·N·ha^{-1}) was applied in those plots before the image acquisition date.

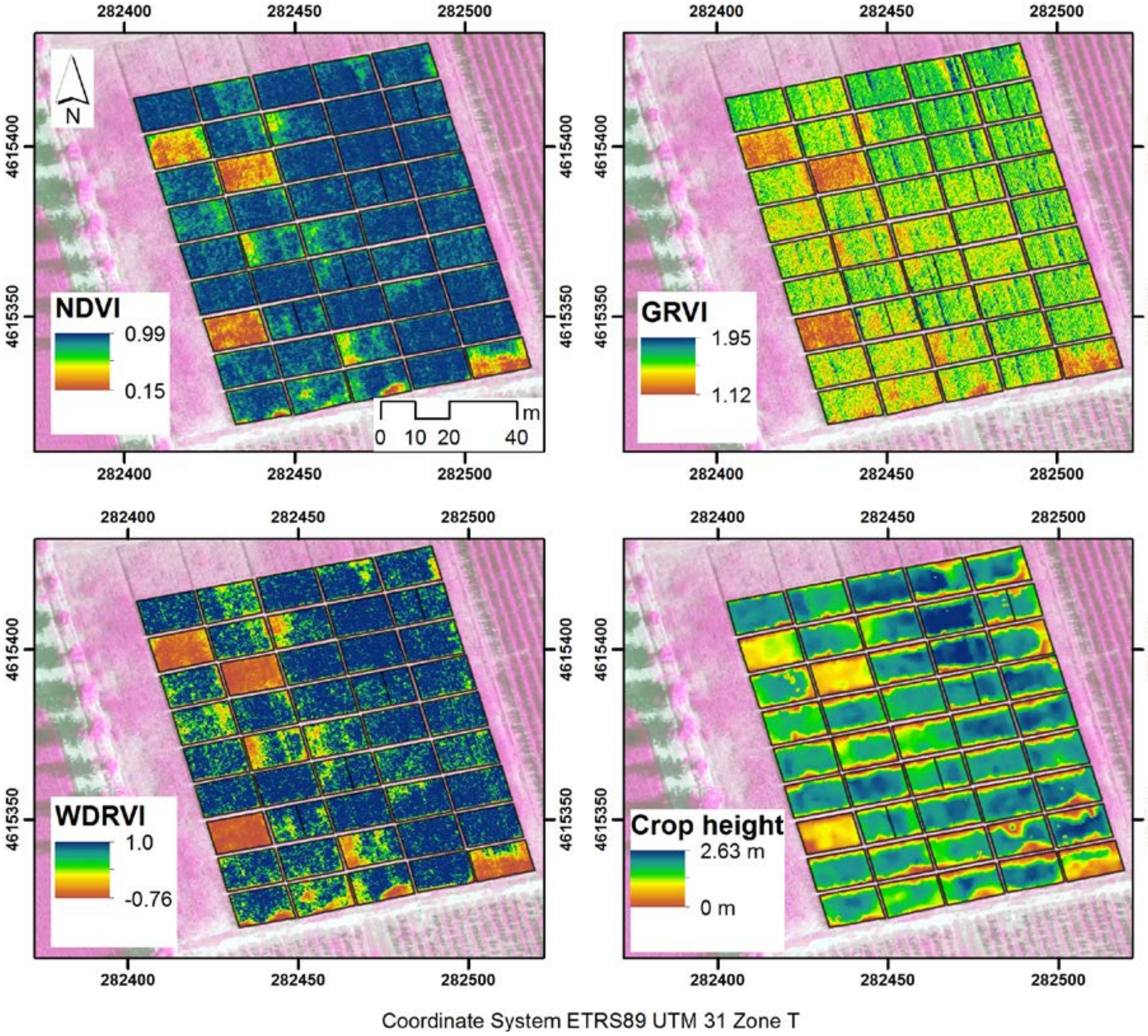

Figure 3. Vegetation indices and crop height for each experimental field (see the labels of the different nitrogen fertilizer treatments in Figure 1).

According to the vegetation indices and crop height (Table 1), a total application of 250–300 kg·N·ha^{-1} corresponds to the threshold value. Above this quantity, the mean values of the analysed variables were significantly higher than for the other N treatments (except for the SPAD, which showed a homogeneous response to the different treatments). This, together with the final performance of the N400 treatment, seems to indicate the possibility at V12 stage of determining whether a supplementary N sidedress application is required to achieve a higher yield.

Among the variables analysed (Table 1), the GRVI performed better than the NDVI at identifying the treatments equal to or below 300 kg·N·ha^{-1}. The latter showed an increase in values that was in line with the total amount of applied N. However, this response was either more homogeneous or greater than for applications of 200 kg·N·ha^{-1}, yet without showing a clear discrimination between the treatments occupying the highest positions in the ranking. Nevertheless, GRVI did not clearly differentiate between the N150 and N300 treatments, and this created a certain degree of ambiguity. In addition, GRVI values for the N250 and N300 treatments did not directly correlate with the total amount of applied N. These results were in line with those published in other research work. Isla et al. [17] reported a better performance of green-based vegetation indices in determining maize vigour due to problems of saturation associated with NDVI for some types of vegetation during their later stages of growth. For example, indices such as the GNDVI have been considered more useful for assessing leaf chlorophyll variability when the leaf area index is moderately high [29]. Xiang and Tian [22] also reported that the GNDVI and GRVI offered the best ways to identify three different N treatments over the whole maize growing season, with the greatest differences in index values being observed during the V6–V8 stages.

The WDRVI was the spectral index that best distinguished between N applications above 300 kg·N·ha^{-1}, but also displayed a certain degree of ambiguity when it came to distinguishing between treatments within the range between N200 and N300. Only one exception to this general rule was observed; this occurred with the N100Ps150 treatment. This was included in the group of treatments classified as “a”, although its results also overlapped with those of other groups. According to this finding, mean WDRVI values for maize fields of less than 0.89 at V12 would be associated with an improvement in grain yield following the application of a third N sidedress around the VT stage.

In the case of crop height measured from the UAV photogrammetric survey, it was observed that the combined inorganic and organic N treatments at doses of 350 and 450 kg·N·ha^{-1} were clearly associated with the largest crop heights. Nine significant different responses were identified among the 13 N treatments. The treatments with total N rates of between 150 and 300 kg·N·ha^{-1} were not classified in ascending order. Nevertheless, a clear differentiation was achieved between treatments that included organic N and those that only contained inorganic N. This was probably due to the effect of higher N availability in the organic plots during the earlier vegetative stages, prior to the sidedress application, which resulted in faster maize plant growth.

With respect to grain yield, the fertilizer treatments N100Ps250, N200Ps150 and N400 showed the greatest response to N (with grain yields equal to or above 17 Mg·ha^{-1}), and were classified in groups "ab" (N100Ps250) and "a" (N200Ps150 and N400). The N treatments with total N content of between 200 and 300 kg·ha^{-1} and the treatment with the highest N application rate (N200Ps250) were classified as either "ab" or "abc" and produced grain yields of between 15 and 17 Mg·ha^{-1}.

To clarify the type of relationships between the N rates and the analysed variables, linear-plateau models were fitted (Figure 4). In this way, it was possible to identify the economic optimum rates of fertilization, as well as the saturation point for N fertilization of vegetation indices and crop height at V12, SPAD at VT and grain yield.

The predicted economic optimum rate of fertilization was determined at 239.8 kg·N·ha^{-1} (Figure 4F). Except for the GRVI, the other vegetation indices and crop height showed no response to higher N rates above the economic optimum rate. Particularly useful to predict yield response in relation to N rates at V12 stage were the NDVI, WDRVI and crop height, with no response to N rates higher than 247.5, 243.1 and 243.0 kg·N·ha^{-1}, respectively. For SPAD, saturation was determined at a lower N rate (203.8 kg·N·ha^{-1}). This could imply an underestimation of the N requirements of maize, which could be translated into a reduction in final grain yield. The opposite was observed for the GRVI, where N requirements may well be overestimated, with an increase in GRVI values at higher N rates (up to 362.2 kg·N·ha^{-1}). This was probably due to an increase in greenness as observed through the index, which was not translated into final grain yield (Figure 4B).

As for grain yield (Figure 4F), there would be no interest in increasing N application above 239.8 kg·N·ha^{-1}, partly because of potential environmental problems but also because of the subsequent reduction in the economic margin. However, higher N rates did present higher yields, suggesting the potential to increase grain yield, since yield values for N treatments N100Ps250, N200Ps150 N200Ps250 were above the linear-plateau which was reached at 16.12 Mg·ha^{-1}. This suggests the potential possibility of increasing yield by increasing N fertilization, something which would be of particular interest in maize-growing areas like the Ebro valley which have the appropriate environmental conditions and irrigation facilities to attain such high yields.

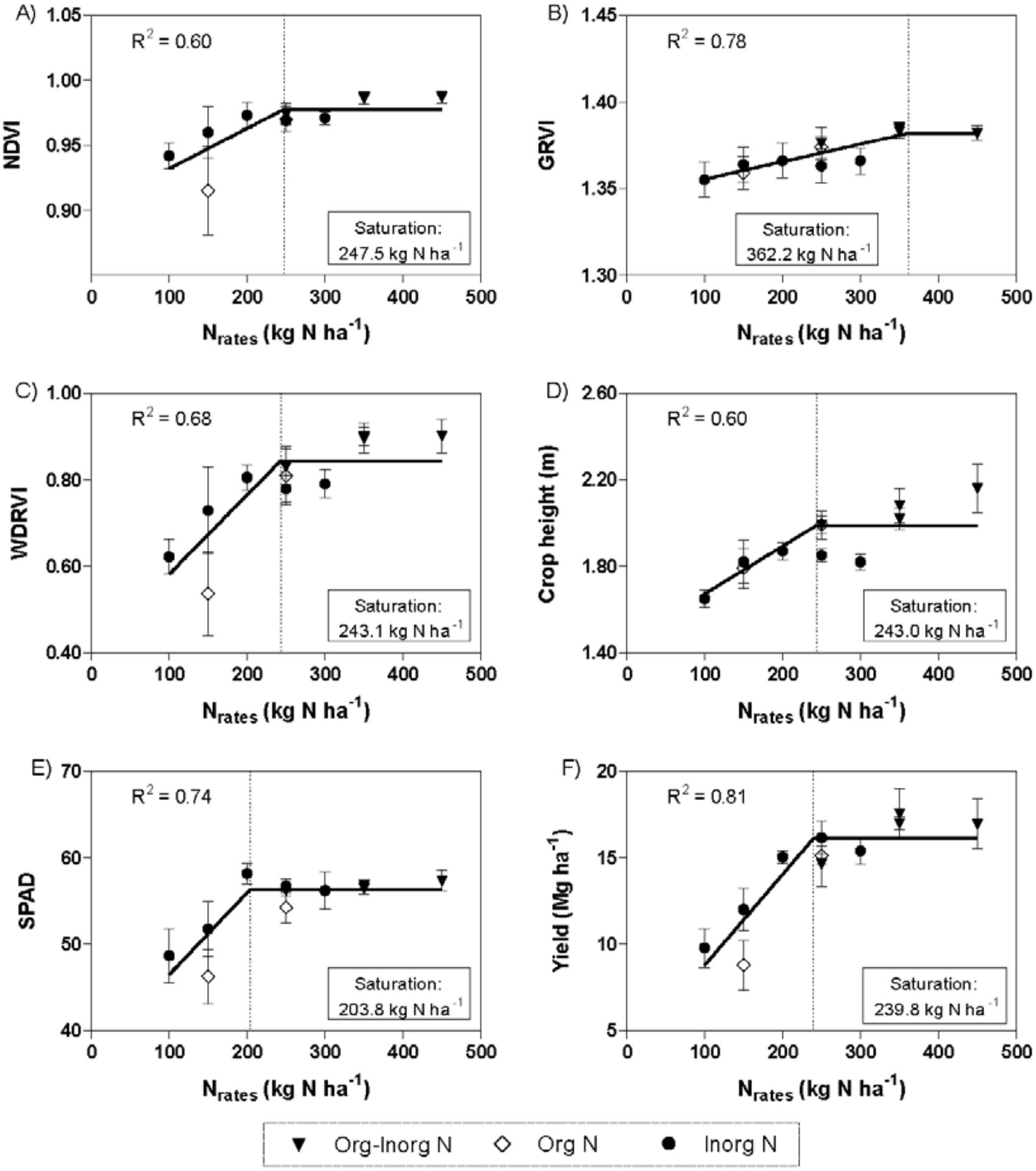

Figure 4. Response of (A) NDVI, (B) GRVI, (C) WDRVI vegetation indices and (D) crop height at V12, (E) SPAD at VT, and (F) grain yield to different N fertilization treatments. Saturation N dose is determined when there is no response to higher N fertilization (predicted economic optimum rate of fertilization). Organic, inorganic and combined organic-inorganic treatments are represented with different symbols. N0 and N400 treatments were not included in the analysis.

Table 2 was created to offer a summary of the results presented above, and shows the treatments ordered according to the mean values of the indices and crop height. These values are ranked (from lowest to highest mean value) for the different N treatments. For each variable, the lowest rank is equal to 1 and the highest rank is equal to 13. The "Sum" column in Table 2 indicates the sum of the ranks in each row (or for each N treatment). In this case, the sum of ranks represents an order of magnitude of each N treatment with respect to its spectral response (vegetation index) and crop height. It is useful to compare the N treatments not only according to the values assigned to each vegetation index or crop height, but considering them all together. The "Rank" column shows the final ranking according to the Sum from the lowest to the highest value.

The ranks shown in Table 2 indicate that the treatments with the highest responses in the spectral indices and in terms of crop height were those that combined inorganic and organic N, with total application rates of 350 or 450 kg·N·ha^{-1} (N200Ps150, N100Ps250 and N200Ps250). These were followed, in descending order, by the treatments containing either only inorganic N or only organic N; the exceptions were treatments N0Ps250 and N100Ps150, which respectively occupied the 8th and 10th positions in the ranking. The control treatment (N0) occupied the lowest position.

Table 2. Nitrogen fertilization treatments ordered by the mean values for each vegetation index and crop height at stage V12. The values are ordered from 1 to 13 (1 = lowest vegetation index and 13 = highest vegetation index). The treatments are ordered according to the "Rank" column, which orders the sum of the ranks. The total amount of N (kg ha^{-1}) applied up to vegetation stage V12 is given in brackets.

N Treatment	NDVI	GRVI	WDRVI	Crop Height	Sum	Rank
N0 (0)	1	1	1	1	4	1
N100 (100)	3	2	3	2	10	2
N0Ps150 (150)	2	3	2	6	13	3
N150 (150)	4	5	4	4	17	4
N250 (250)	5	4	5	5	19	5
N300 (300)	7	6	6	3	22	6
N200 (200)	8	8	8	7	31	7
N0Ps250 (250)	6	9	7	9	31	8
N400 (300)	10	7	10	8	35	9
N100Ps150 (250)	9	10	9	10	38	10
N100Ps250 (350)	11	13	11	12	47	11
N200Ps150 (350)	12	12	13	11	48	12
N200Ps250 (450)	13	11	12	13	49	13

Two treatments providing N from pig slurry manure (N0Ps250 and N100Ps150) were ranked in high positions, but did not finally achieve the corresponding yield rank (7th and 5th respectively). This could have been due to differences in the timings of the N applications. The organic applications were applied before the maize was sown and the inorganic applications at sidedress. In addition, there were differences in the amount of N applied with respect to that applied in other treatments at the same time. These pig slurry applications would have conferred optimum conditions for crop development until V12, the stage at which the images were acquired. However, the reduced amount of total N applied (250 kg·N·ha^{-1}) and/or the lack of a third sidedress had a determining influence on the final yields associated with these treatments.

3.2. Relationship between the SPECTRAL Indices and Crop Height with Yield

Table 3 presents the results of linear and quadratic regression analysis comparing the vegetation indices, SPAD and crop height with grain yield. In general, quadratic regression models did not significantly improve yield prediction with respect to the linear models, at the same time adding complexity. The best correlation was obtained with WDRVI (R^2 = 0.92 and RMSE = 0.87 Mg·ha^{-1}), followed by NDVI and SPAD (R^2 = 0.90 and 0.88, respectively). It should be noted that SPAD was measured at VT and did not show significant differences with respect to the different N treatments (except for the N0 treatment, which was statistically different from the other N treatments, Table 1).

Table 3. Linear and quadratic regression adjustments between the spectral indices and crop height with yield, considering all the total amounts of N applied in all the different treatments, except N0 and N400.

Vegetation Index	**Linear Regression with Yield**			**Quadratic Regression with Yield**		
	R^2	**RMSE Mg·ha^{-1}**	**p-value**	**R^2**	**RMSE Mg·ha^{-1}**	**p-value**
SPAD	0.88	0.82	<0.001	0.89	1.10	0.0002
NDVI	0.90	0.98	<0.001	0.92	0.92	<0.001
GRVI	0.64	1.86	0.003	0.71	1.78	0.0075
WDRVI	0.92	0.87	<0.001	0.92	0.93	<0.001
Crop height	0.60	1.97	<0.001	0.64	1.98	0.0174

The GRVI performed worse than the red-based indices (R^2 = 0.64 and RMSE = 1.86 Mg·ha^{-1}). These results differed from those obtained in other studies, in which green-based indices at V15 (e.g., GNDVI) were highly correlated with maize yield and explained 86% of the variance [17]. Other authors have also pointed to green-based vegetation indices being particularly useful for assessing N status at stage V12 or at later stages of maize growth [9]. Crop height at V12 was not a good indicator of grain yield (R^2 = 0.60 and RMSE = 1.97 Mg·ha^{-1}). These results partially agree with those of Yin et al. [41], who presented crop height as a good predictor of yield in irrigated maize, varying between R^2 = 0.52 and 0.86, depending on the year in a three-year experiment.

The WDRVI was the best spectral index obtained from the VEGCAM-Pro camera mounted on the drone in estimating yield at the V12 stage of maize development. This was in line with the outcomes of multiple rank analysis, which identified WDRVI as the best index for discriminating between total N applications at applications above 250–300 kg·ha^{-1}. The results agree with those from other studies, although those were conducted with data at a very different spatial resolution using the Moderate Resolution Imaging Spectroradiometer (MODIS, 250 m pixel resolution). For example, Sakamoto et al. developed a practical method for near real-time prediction of U.S. maize yield based on the WDRVI taken 7–10 days before the corn silking stage [42,43]. These authors found a strong linear correlation with maize grain yield at both field and regional scales. Wang et al. derived phenology-adjusted spectral indices from MODIS data to be used in developing linear regression models with maize yield data [44]. In this case, the peak correlation between the WDRVI and yield was detected 85 days after green-up date (R^2 = 0.506). The correlation was generally low for NDVI (R^2 = 0.385) and no obvious peak correlation existed. In other cases, the WDRVI also performed better than the NDVI with MODIS based data, and has been shown to be useful for assessing early stages of plant stress in maize and soybeans [45]. As far as we are aware, the WDRVI has been mainly applied in maize studies using MODIS data with 250 m pixel resolution. In the present study, it has been shown that good results in estimation of N nutritional state and grain yield can also be obtained at a very high resolution with data from UAV-mounted cameras.

4. Conclusions

This study was motivated by the growing demand for commercial UAV services in agriculture and the need to adjust N fertilization rates applied to maize crops. With this in mind, one green-based (GRVI) and two red-based (NDVI and WDRVI) vegetation indices, as well as SPAD and crop height (derived from a photogrammetric process), were analysed in order to determine crop status at stage V12 (12 leaves with visible leaf collars, just before flowering), associated with different amounts of supplied N.

The results obtained led us to conclude that the spectral index derived from the VEGCAM-Pro camera that explained the greatest variability between treatments was the WDRVI. This index had previously only been applied in maize studies at moderate resolution (250 m per pixel) with MODIS data on phenological characterization and yield prediction. In the present case study, at very high spatial resolutions, WDRVI was the best index for distinguishing between treatments with applications above or below 250–300 kg·N·ha^{-1} and at grain yield prediction at the V12 stage. NDVI and crop height also showed no significant response to extra N application at the economic optimum rate of fertilization, demonstrating their potential as yield predictors at V12 stage. However, SPAD and GRVI either underestimated or overestimated the optimum N rate.

Although there would theoretically be little interest in increasing N application above 239.8 kg·N·ha^{-1}, the study does show a tendency for increased grain yield with higher N rates. This could be of particular interest in maize-growing areas such as the Ebro valley which have the appropriate environmental conditions and irrigation facilities to attain such high yields.

The ranking of the spectral indices and crop height revealed that the treatments that conferred the greatest responses to N fertilization were those which combined inorganic and organic N with applications of 350 or 450 kg·N·ha^{-1}. The proposed ranking system, which is based on the response of the crop to different multispectral indices and crop height, may help to overcome the uncertainty of decision-making based on a single index, such as the NDVI, which is the approach most frequently used in association with data obtained from multispectral cameras.

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Chapter III

Multispectral airborne images to improve in-season nitrogen management, predict grain yield and estimate economic return of maize in high-yielding environments

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Abstract

Vegetation indices (VIs) derived from active or passive sensors have been used for maize growth monitoring and real-time nitrogen (N) management at field scale. In the present multi-location two-year study, multispectral VIs (green and red-based indices), SPAD and plant height (PltH) measured at V12-VT stage of maize development, were used to determine in-season N fertilization, and to predict grain yield in high yielding environments. Evaluation of the available N (in the top-soil layer, 0-30 cm) slightly improved the relationship between the VIs, SPAD and PltH with grain yield or with applied N rates. Green-based VIs were the most accurate indices to predict grain yield and to estimate the grain yield optimum N rate ($GYON_r$) (216.8 kg N ha^{-1}), but under-estimated the grain yield optimum N available ($GYON_a$) (248.6 kg N ha^{-1}). Red-based VIs slightly overestimated the $GYON_r$ and $GYON_a$, while SPAD highly underestimated them. The green chlorophyll index (GCI) was particularly interesting due to its ability to distinguish maize that will yield less than 84% of the maximum yield. Hence, N deficiencies could be detected and corrected at least up to 84% of the maximum yield. The economic optimum nitrogen rate (EON_r) and economic optimum nitrogen available (EON_a) were determined below the $GYON_r$ and the $GYON_a$, demonstrating that maximum grain yield strategies in maize are not normally the most profit-earning for farmers. Despite the usefulness of multispectral images to aid N management, the adoption of remote sensing technologies by farmers is still limited. Further research is needed to fine-tune the response of maize to N applications when deficiencies are detected at V12 stage. Airborne images could be useful for practical farming implementation because of their high potential to cover large cropping surfaces at a moderately low cost.

Keywords: Vegetation index, airborne images, maize, N fertilization, economic return

Abbreviations: Nitrogen, N; OM, organic matter; N rate, N_{rate}; available N, $N_{available}$; optimum N, N_{opt}; Nitrogen use efficiency, NUE; Unmanned aerial vehicles, UAV; Digital Multi-Spectral Camera, DMSC; Grain yield optimum N rate, $GYON_r$; Grain yield optimum N available, $GYON_a$; Economic optimum nitrogen rate, EON_r; Economic optimum nitrogen available, EON_a.

1. Introduction

Productivity and resource-use efficiency are desirable agronomic, economic and environmental goals in high-demanding resource crops such as maize (*Zea mays* L.) (Cardwell, 1982; Liang and Mackenzie, 1993). Nitrogen (N) is the most limiting nutrient for crop production in many of the world's agricultural areas (Fageria and Baligar, 2005). Nitrogen efficient use is important for economic and environmental sustainability, especially in high N demanding crops such as maize (Stanger and Lauer, 2008). Insufficient application of N can have important economic consequences, whereas an excessive fertilization implies wasting resources while increasing the environmental pollution risk (Khan et al., 2001). Nitrogen fertilization can account for up to 30% of the total maize production cost (Lloveras and Cabases, 2015), having a huge impact on the crop economic returns. Hence, N fertilization clearly impacts the economic return of maize in high yielding environments (>16 Mg ha^{-1} of grain yield) such as those of the Ebro Valley (NE Spain). In our area, maize N extractions are about 250-300 kg ha^{-1} (Berenguer et al., 2008; Yagüe and Quílez, 2010; Cela et al., 2011), therefore the N rates applied are high in order to cover maize N requirements.

Temporal and spatial variation of crop N requirements (Varvel et al., 1997), together with within-field soil N supply variability, can influence the assessment of N-field availability (Maresma and Ketterings, 2017). Nitrogen fertilization of extensive crops, such as maize, is mostly underpinned by the simplification of fieldwork management and by avoiding under-fertilization risk. The practice of applying high N rates (N_{rates}) at early crop stages can trigger over-fertilization. This occurs because, under favourable soil moisture conditions, approximately one-third of the total N uptake occurs after pollination (Hanway, 1962). In fact, the worldwide N recovery in crops is usually less than 50% (Fageria and Baligar, 2005), with the impact that this supposes on N resource efficiency and the pollution of agroecosystems (Bausch and Duke, 1996).

Determination of within-field soil spatial patterns seems to be a useful tool for N management but requires the collection and analysis of a large number of samples. Soil (Magdoff et al., 1984), plant or chlorophyll concentration (Wood et al., 1992) sampling is costly, time-consuming and barely captures the spatial variability that is often present within plots. However, while plant analysis and chlorophyll meter readings have been used for confirmation of N responsive sites, soil tests for available N ($N_{available}$) successfully predicted N crop needs in arid regions, but had less success in humid regions (Wood et al., 1993). Therefore, there is a need to develop a faster, more accurate and possibly more economical method to gather crop information and to estimate and adjust N requirements (Sripada et al., 2005).

Due to the link between net photosynthesis and steady-state fluorescence at airborne spectral level, field-spectral imaging is a very useful technology for crop growth monitoring and real-time management at field scale (Daughtry et al., 2000; Zhang et al., 2011; Zarco et al., 2013). Vegetation indices (VIs) derived from active or passive sensors have been used to distinguish temporal patterns in crop development (Strachan et al., 2002; Quemada et al., 2014). For example, VIs have been used for detecting N stress in maize at early development stages (V4-V7 stage, 4-7 leaves with visible leaf collar) (Sripada et al., 2006; Ma et al., 2014; Jones et al., 2015), though they have not been successfully implemented as yet in farming practices. This was attributed to the strong background soil reflectance. Hence, maize ground cover is vital if the pixel resolution does not allow removal of soil pixels (Scharf and Lory, 2002). However, image acquisition after V8 stage (8 leaves with visible leaf collar) seems to be consistently useful to determine maize N status and to predict yield (Bausch et al., 2008; Bausch and Khosla, 2010; Isla et al., 2011; Cilia et al., 2014; Quemada et al., 2014; Maresma et al., 2016), increasing progressively its accuracy up to VT stage (tasseling). Image acquisition at V12 stage (12 leaves with visible leaf collar) is generally preferred to other alternatives, because maize has already taken up about 40% of the total N (Ritchie et al. 1997) and crop response to N fertilizer is still high if N deficiencies are detected at this stage. Image acquisition after VT stage is affected by colour disturbance of the tassels, reducing the correlation between VIs and N status or yield (Solari et al., 2008). Moreover, after VT the maize response to extra N application is more limited because the crop has less time than from the V8 to V12 stage to absorb the applied N (Shanahan et al., 2008).

Despite the usefulness of multispectral images to aid N management, the adoption of remote sensing technologies by farmers is limited (Robert, 2002). Different technologies have been used for this purpose. Satellite images normally have lower spatial and temporal resolution and, in some areas, satellite images could be disturbed by cloud cover and/or sprinkler irrigation during the period of interest (Hunt et al., 2005). Unmanned aerial vehicles (UAV) have tremendous potential for high-resolution requirements, as for example for detailed site-specific weed control treatments in early post-emergence (Peña et al., 2013). Such high-resolution is probably not necessary for determining maize N status at V12, and airborne images could be useful for practical farming implementation because of their high potential to cover large cropping areas.

While numerous studies have been conducted using remote sensing techniques to determine N status and predict maize grain yield (Daughtry et al., 2000; Bausch et al., 2008; Bausch and Khosla, 2010; Isla et al., 2011; Cilia et al., 2014; Quemada et al., 2014), no studies have been made comparing the effectiveness of these technologies in high-yielding irrigated environments where, even if N stress is not clearly manifested, a reduction in yield can occur.

The objectives of the present study were to use VIs derived from multispectral aerial images: 1) to distinguish among N status of maize at V12 stage, 2) to predict grain yield and economic return, and 3) to determine the amount of N needed to achieve maximum grain yields and economic return in high-yielding environments.

2. Materials and methods

2.1. Study area

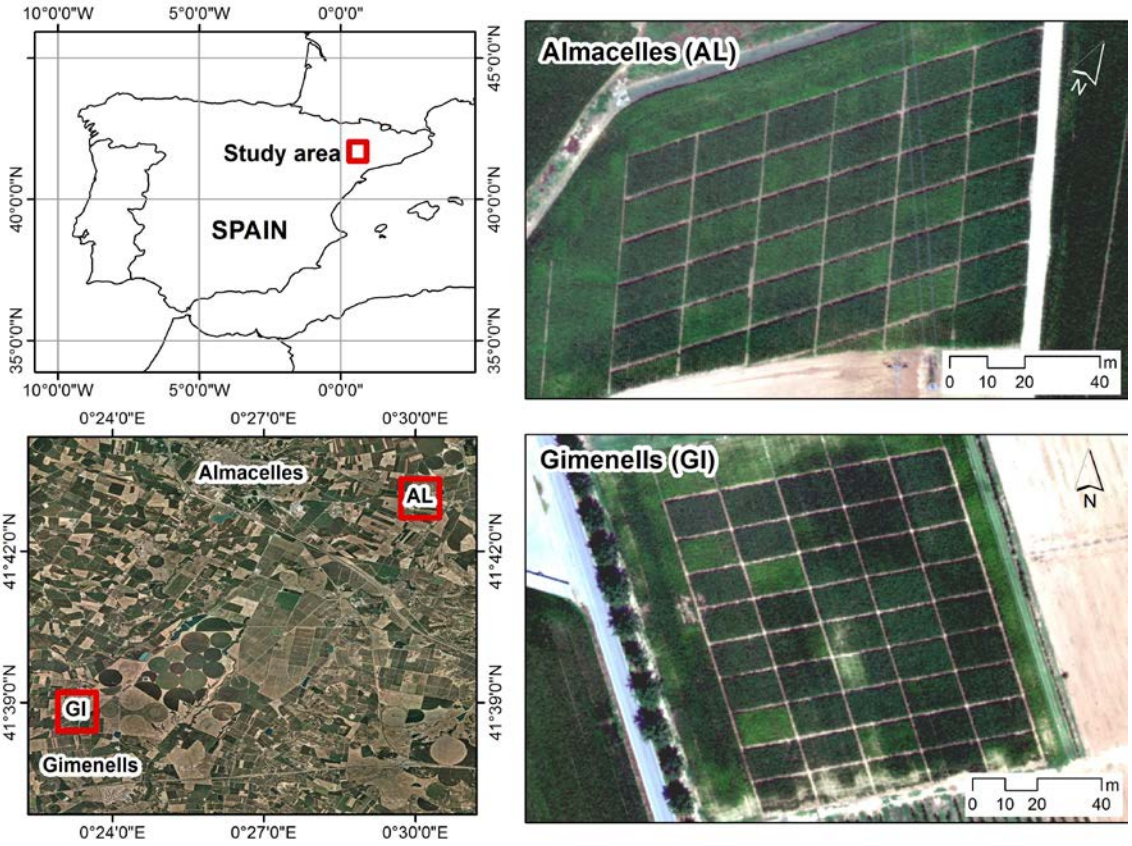

Figure 1. Location of the study area and detail of the experimental fields: Almacelles (AL) and Gimenells (GI).

A two-year experiment (2014-2015) was carried out in two long-term N fertilization trial fields located in Lleida (NE Spain): Almacelles (AL) (41°73’ N, 0°50’ E) and Gimenells (GI) (41°65’ N, 0°39’ E) (Figure. 1).

Table 1. Chemical and physical soil properties of the experimental fields (2014).

Soil properties	**Almacelles (AL)**			**Gimenells (GI)**		
Depth, cm	0-30	30-60	60-110	0-30	30-60	60-110
Sand, %	42	43	17	39	38	45
Silt, %	33	36	63	40	42	38
Clay, %	25	21	20	21	20	17
pH	8.2	8.4	8.4	8.3	8.3	8.3
Organic matter, %	3.3	-	-	2.2	1.40	0.60
Bulk density, g cm^{-3}	1.64	-	-	1.40	1.56	1.63
EC, dS m^{-1}	0.19	0.17	0.22	0.20	-	-
P (Olsen), mg kg^{-1}	90	-	-	31	-	-
K (NH_4Ac), mg kg^{-1}	383	-	-	217	-	-
Soil class†	Typic Calcixerept			Petrocalcic Calcixerept		
Precedent crop	maize			maize		

† Soil Survey Staff (2014)

In both locations and years conventional tillage was applied, including disc ploughing and cultivation to a depth of 30 cm to incorporate maize stover and to prepare the soil for the next sowing. Before planting maize in 2015, GI received three extra cultivation passes to mitigate an irrigation system fault that cause flooding during the previous winter. This did not affect the normal planting date in early April. The hybrid used in both fields was PR33Y72 (FAO cycle 600), at a rate of 90,000 plants ha^{-1}, with 71 cm between rows. Two herbicides treatments were applied: one at pre-emergence to control the majority of weeds (S-Metolachlor 40% and Terbuthylazine 18.75%, at 3 L ha^{-1}) and the other at post-emergence to control *Abutilon theophrasti M.* and *Sorghum halepense L.* (Dimethylamine salt of dicamba 48.2% at 1 L ha^{-1} and Nicosulfuron 6% at 0.75 L ha^{-1}).

Maize was harvested the last week of October with an experimental small-plot combine. Grain yield was determined in the two central rows (1.5×10 m) of each plot. To adjust grain yield to 14% moisture content, moisture content was determined in a 250 g sample (GAC II, Dickey-John, Auburn, IL, USA).

The economic return of each plot was calculated as the difference between the income produced by the selling of the grain yield and the cost of the N fertilizer applied. The N:Maize price ratio is defined as the price per kilogram of N divided by the price per kilogram of maize (price ratio = price of fertilizer N, € kg^{-1} N/price of maize, € kg^{-1} maize) (Sripada et al. 2005). In the present study, the N:Maize price ratio was 5.3:1, considering a N price (N fertilizer plus application cost) of 0.90 € kg^{-1} and a maize grain price of 0.17 € kg^{-1}.

2.2. Fertilizer treatment

Five different inorganic fertilizer N_{rates}: 0, 100, 200, 300 and 400 kg N ha^{-1} (N0, N100, N200, N300 and N400, respectively), with four replications under a randomized block design were considered. The N fertilization treatments were randomized at the beginning of the experiments (2002 and 2010, in GI and AL, respectively) and applied in the same plots the following seasons. The N fertilizer (33.5% N, as ammonium nitrate) was manually applied and split into two equal sidedress applications (50% at V3-V4 and 50% at V6-V7 stage) for all plots except for N400 treatment, where a third sidedress was applied at V10 stage. Thus, the N distribution in the N400 treatment was 37.5% at V3-V4, 37.5% at V6-V7 and 20% at V10 stage. The different N application of N400 was done in order to increase N use efficiency (NUE) and to reduce the risk of pollution by nitrate leaching associated with high N_{rates} at early stages. Phosphorus and potassium were also applied every two years in both locations before planting, at rates of 150 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1}, to avoid deficiencies of those elements.

2.3. Remote and proximal sensing: data acquisition and analysis

A Digital Multi-Spectral Camera (DMSC) with a DMSC-2k System sensor (Specterra-Services, Australia), mounted on an aeroplane (operated by RS Servicios de Teledetección, Lleida, Spain), was used to acquire multispectral aerial images of the experimental fields at V12 stage. The surveys were conducted under optimum flight conditions 880 m over the experimental plots on 30th and 25th of June 2014 and 2015, respectively. The time of flight was less than 1 hour between 12:30 and 1:30 h (GTM) on sunny days without cloud disturbance. The DMSC-2k System sensor consists of a 4 interline transfer, a 2048×2048 pixel charge-coupled device (CCD) with a Nikon F mount, and 24-28 mm fixed focal length lenses. The spatial and radiometric resolutions were 0.25 m and 14 bit (recorded as 16 bit), respectively. The camera spectral resolution included four independent and replaceable narrow bandwidth spectral filters, which were used to capture four spectral bands of 20 nm range width and centred at 450 nm (blue), 550 nm (green), 675 nm (red) and 780 nm (near infrared). The spectral bands were pre-processed by the provider to compensate for mis-registration due to lens distortion (less than 0.2 pixels) and for scene brightness due to the Bi-directional Reflectance Distribution Function (BRDF).

Seven different VIs reported in the literature (NDVI, GNDVI, GCI, SAVI, GSAVI, WDRVI and EVI) were computed (Table 2). Then, to summarize the response of the different N treatments to each VI, fifty points within each individual experimental plot (excluding 1m buffers from the borders), were sampled and the VI values extracted. Soil distortion did not affect the VIs because soil pixels were avoided in the analysis.

In addition to the VIs, the heights of five plants were manually measured in each experimental plot at VT stage (10 to 15 days after image acquisition). Plant height (PltH) was considered until the last leaf to avoid differences caused by tassel size and type, which varies with both plant population and variety (Duncan et al., 1967). Non-destructive chlorophyll readings were also taken on plant leaves at the VT stage in order to verify the results provided by the VIs. The measurements were taken using a small, lightweight, portable, hand-held meter (SPAD-502, indirect chlorophyll meter; MinoltaCorp, Ramsey, NJ). In agriculture, the SPAD meter is widely accepted to improve N management by predicting N status and determining fertilization requirements (Bonneville and Fyles, 2006). SPAD values indirectly evaluate the leaf chlorophyll content based on the amount of light transmitted by the leaves at two different wavelengths: red (650 nm) and near infrared (940 nm).

In the present study, SPAD values were determined by sampling three different parts of the ear leaf (base, middle and top) in five central row plants in each experimental plot (15 measurements per plot). The measures were then averaged to obtain a SPAD value per plot.

Soil NO_3^-- N was determined before planting at a depth of 0-30 cm by a composite of five individual cores. Soil nitrates were extracted using deionized water and measured using test strips with a Nitrachek® device calibrated according to the standard procedure (Bischoff et al., 1996). The $N_{available}$ for maize during the growing season was determined by the sum of the N_{rate} applied in the experimental plots and the NO_3^-N determined at planting: $N_{available} = N_{soil\ at\ planting} + N_{rate}$.

Table 2. Vegetation indices (VIs) computed in the study to test the usefulness of multispectral aerial images to determine maize N status and predict grain yield[†].

	Vegetation index	Formula	Reference
1)	NDVI (Normalized Difference Vegetation Index)	$\frac{NIR - Red}{NIR + Red}$	Rouse et al., 1974
2)	GNDVI (Green NDVI)	$\frac{NIR - Green}{NIR + Green}$	Gitelson and Merzlyak, 1998
3)	GCI (Green Chlorophyll Index)	$\frac{NIR}{Green} - 1$	Gitelson et al., 2003; Gitelson et al., 2005
4)	[‡]SAVI (Soil Adjusted Vegetation Index)	$\frac{NIR - Red}{(NIR + Red + L)} \cdot (1 + L)$	Huete, 1988
5)	[‡]GSAVI (Green SAVI)	$\frac{NIR - Green}{(NIR + Green + L)} \cdot (1 + L)$	Sripada et al., 2006
6)	WDRVI (Wide Dynamic Range Vegetation Index)	$\frac{(\propto \cdot\ NIR - Red)}{(\propto \cdot\ NIR +\ Red)}$	Gitelson, 2004
7)	EVI (Enhanced Vegetation Index)	$G\,\frac{NIR - Red}{NIR + C_1 Red - C_2 Blue + L_2}$	Huete et al., 2002

[‡] $L = 1 - \frac{2 \cdot s \cdot (NIR - Red) \cdot (NIR - s \cdot Red)}{(NIR + Red)}$

[†] *Green*, *Red*, *Blue* and *NIR* are the reflectance of the Green, Red, Blue and Near Infrared light. L is a correction factor (calculated by the formula presented at the bottom of the table), where s is the slope of the soil line. α is a weighting coefficient (α=0.2). In the EVI calculation, G is a gain factor, C_1, C_2 are the coefficients of the aerosol resistance term and L_2 functions as the soil-adjustment factor (L_2=1; C_1=6; C_2=7.5 and G=2.5).

In order to compare the results of the different N_{rates} and $N_{available}$ among fields and years, indices ratios were calculated dividing the plot average value of VIs, SPAD, PltH, grain yield and economic return by the maximum value in each field and year, which was normally obtained in the over-fertilized plots (400 kg N ha^{-1}). Furthermore, the calculated ratios of VIs, SPAD and PltH were fitted to linear-plateau models with the N_{rates} and $N_{available}$, and with grain yield to determine the accuracy of these variables to determine maize N status and to predict grain yield.

2.4. Statistical analysis

The experiment was analysed as a split-plot in time with completely randomized blocks and four replications. The location (GI or AL) was the main plot, and the N_{rates}

(N0, N100, N200, N300 and N400) were subplots. The ratios calculated from the extracted values in the sampling points were subjected to analysis of variance using the Mixed Model of the Statistical Analysis System (JMP Pro 12, SAS Institute, Cary, USA), considering N_{rates}, the year and the location as fixed factors and the replication as random effect. In addition, the grain yield and economic return means were separated by the LSMeans Tukey HSD test ($p<0.05$), with levels not connected by the same letter considered significantly different.

Linear-plateau regression analyses were also carried out: 1) between the ratios of the VIs, SPAD, PltH and grain yield with the N_{rates} or $N_{available}$ to estimate the grain yield optimum N rate ($GYON_r$) and the grain yield optimum N available ($GYON_a$) (Cerrato and Blackmer, 1990), 2) between the ratios of the economic return with the N_{rates} or $N_{available}$ to determine the economic optimum nitrogen rate (EON_r) and the economic optimum nitrogen available (EON_a), and 3) between the ratios of the VIs, SPAD and PltH with the ratios of grain yield to determine their usefulness to predict grain yield at the V12 stage. Analysing together locations and years, the $GYON_r$, the $GYON_a$, the EON_r and the EON_a were determined where the plateau was reached, determining the non-responsiveness amount of N (saturation point) to extra N application.

3. Results and discussion

3.1. Vegetation indices, SPAD and plant height responses to N rates and available N

Table 3 presents the mean values and the ANOVA of the VIs at V12 stage (n=50 for each plot), PltH and SPAD at VT stage (n=15 for each plot), grain yield and economic return (n=4) for the different N_{rates} applied in the experimental plots. The rates are sorted according to the total amount of applied N.

The control treatment (0 kg N ha^{-1}) presented the lowest VIs, SPAD and PltH values in both locations (p-value<0.05), which were more accentuated in GI probably because of the longer period of time (13 years) without any N application and its lower soil organic matter (OM) content producing less soil N mineralization. Higher VIs values and PltH in 2015 were observed in the N0 treatment in GI, probably because of a higher availability of N during this growing season. The higher summer temperatures in 2015 together with the extra ploughing work done before planting could have contributed to mineralization of the OM (Kirschbaum, 1995; Katterer et al., 1998) and, consequently, to N availability.

Table 3. Analysis of variance and mean values of the vegetation indices (n = 50 for each plot), crop height (n = 15 for each plot), SPAD (n = 15 for each plot), yield and economic return (ECO-return) (n = 4) for the different nitrogen rates (N_{rates}) in the experiments of Almacelles (AL) and Gimenells (GI) during the 2014 and 2015 growing seasons. The results are presented as ratios dividing the indices values or the yield by the maximum observed in each field and year.

Statistics	NDVI		GNDVI		GCI		SAVI		GSAVI		WDRVI$_2$		EVI		SPAD‡		HEIGHT‡		YIELD		ECO-return	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
R^2 model	0.85		0.94		0.94		0.56		0.94		0.94		0.91		0.96		0.90		0.99		0.99	
Almacelles (AL)																						
0	0.92	0.91	0.80	0.77	0.63	0.64	0.95	0.94	0.82	0.79	0.51	0.35	0.89	0.92	0.62	0.69	0.84	0.90	0.42	0.53	0.46	0.58
100	0.95	0.94	0.90	0.88	0.79	0.80	0.97	0.96	0.92	0.90	0.67	0.54	0.93	0.96	0.88	0.80	0.90	0.97	0.75	0.79	0.80	0.83
200	0.96	0.95	0.93	0.91	0.85	0.85	0.98	0.96	0.94	0.92	0.76	0.62	0.96	0.97	0.94	0.94	0.93	0.98	0.92	0.94	0.96	0.97
300	0.97	0.96	0.94	0.93	0.88	0.88	0.98	0.97	0.95	0.94	0.78	0.69	0.96	0.98	0.95	0.96	0.95	0.98	0.94	0.96	0.95	0.96
400	0.99	0.97	0.97	0.95	0.94	0.91	0.99	0.98	0.98	0.96	0.90	0.78	0.99	0.98	0.95	0.96	0.99	0.99	0.96	0.97	0.94	0.93
Gimenells (GI)																						
0	0.71	0.88	0.49	0.65	0.32	0.53	0.83	0.91	0.55	0.67	-1.49	-0.31	0.61	0.80	0.49	0.59	0.63	0.83	0.19	0.34	0.22	0.37
100	0.90	0.88	0.79	0.81	0.73	0.78	0.90	0.88	0.80	0.83	0.49	0.47	0.86	0.87	0.85	0.86	0.95	0.91	0.50	0.61	0.53	0.63
200	0.96	0.96	0.97	0.94	0.94	0.91	0.99	0.97	0.97	0.95	0.59	0.52	0.90	0.94	0.96	0.95	0.88	0.98	0.82	0.84	0.87	0.86
300	0.98	0.98	0.98	0.98	0.96	0.97	0.98	0.99	0.94	0.99	0.85	0.77	0.97	0.96	0.93	0.93	0.94	0.96	0.88	0.93	0.89	0.92
400	0.98	0.98	0.98	0.98	0.96	0.96	0.98	0.99	0.95	0.98	0.80	0.78	0.94	0.96	-	0.97	-	0.95	0.93	0.91	0.92	0.86
ANOVA over locations and years																						
Location (L)	**		**		NS		**		**		**		**		**		*		**		**	
N rates (N)	**		**		**		**		**		**		**		**		**		**		**	
L × N	**		**		**		**		**		**		**		*		**		**		**	
Error a	-		-		-		-		-		-		-		-		-		-		-	
Year (Y)	*		NS		**		NS		NS		NS		**		NS		**		**		**	
Y × L	**		**		**		NS		**		**		*		NS		NS		NS		NS	
Y × N	**		**		**		*		*		**		**		NS		**		**		**	
Y × N × L	**		**		**		NS		**		**		**		NS		**		NS		NS	
Error b	-		-		-		-		-		-		-		-		-		-		-	

NS No significance * Significant at p-value < 0.05 ** Significant at p-value < 0.01

‡ N400 values in Gimenells 2014 were missing. ANOVA was calculated without N400 treatment for these variables.

The N100 treatment presented significantly higher VIs, SPAD and PltH values than the control treatment (N0), but lower than the N_{rates} above 200 kg N ha^{-1}. Indeed, there were very little and non-significant differences for most VIs, SPAD and PltH at N_{rates} between 200-400 kg N ha^{-1}. Previous studies in the area determined the need for 290 kg $N_{available}$ ha^{-1} (0-90 cm) to achieve maximum maize grain yields (Berenguer et al., 2008), with extractions up to 386 kg N ha^{-1} (Biau et al., 2012). The N400, and probably the N300 treatment, could easily provide enough N to cover maize's extractions. Though the N_{rate} of 200 kg N ha^{-1} did not seem to cover maize N requirements, no N deficiencies were detected by the studied indices at V12-VT stage. The 200 kg N ha^{-1} applied, together with the initial soil N content at planting plus the N mineralization, seemed to provide enough N for maize development, at least until V12-VT, when maize has already absorbed around 40-50% of the total N (Hanway, 1962).

All VIs, SPAD and PltH were able to differentiate among different N_{rates} of the experiment (p-value<0.01), showing their usefulness for predicting N maize status at V12 stage (Ma et al., 1996; Solari et al., 2008; Xia et al., 2016). Most of the analysed indices statistically differentiated locations (GI-AL) but not years (2014-2015), except for the GCI, which differentiated years but not locations. This finding is considered very interesting, because GCI could be used for determining maize N status in different locations without changing its distribution.

The interaction between location and N_{rates} (L×N) was significant, probably due to the higher indices values observed in AL with respect to GI in the N100 treatment. These differences between locations could be due to field conditions that contribute to soil fertility (Tremblay et al., 2011), as well as to different soil OM contents in AL than in GI, which would have produced higher N mineralization in Al than GI (Table 1). As mentioned above, the difference in the quantity of N mineralization in the two fields was evident. This can be observed with the grain yields obtained in the control treatments (N0), where AL (9.0 Mg ha^{-1}) nearly doubled those of GI (4.8 Mg ha^{-1}). Moreover, interactions between year and N_{rates} (Y×N) and between year and location (Y×L) were identified for most indices, except for SPAD. These interactions could mostly be explained by the better N status of the N0 treatment in GI in 2015 compared to 2014. The extra ploughing done before planting in 2015 probably developed oxidative conditions that contributed to increasing OM mineralization (Sinsabaugh, 2010), and consequently, the $N_{available}$ during the growing season.

The VIs, PltH and SPAD were highly correlated with the N_{rates} applied (Figure 2) and with the $N_{available}$ (Figure 3). All indices were fitted to a linear-plateau model when compared with the applied N_{rates} or $N_{available}$. The $GYON_r$ and $GYON_a$ were determined when the plateau was reached (saturation point), indicating where the VIs, SPAD and PltH did not increase with additional N inputs.

Green-based VIs obtained better correlations than red-based VIs with the N_{rates} or $N_{available}$ (Figures 2 and 3). Indeed, green-based VIs have been normally considered more useful for assessing leaf chlorophyll variability when the leaf area index is moderately high (Gitelson et al., 1996), as it is in maize. Sripada et al. (2005) showed the usefulness of green-based VIs at predicting the $GYON_r$ (R^2=0.67) with aerial CIR (colour infrared) photographs. Bausch and Khosla (2010) used the GNDVI to determine the $GYON_r$ (R^2=0.91) with QuickBird multi-spectral imagery (satellite). In our study, the highest correlations between N_{rates} and VIs were found for GCI (R^2=0.80), GSAVI and GNDVI (R^2=0.73). However, the best index to differentiate among N_{rates} was SPAD at VT stage (R^2=0.89). Red-based VIs showed low correlation with the N_{rates}. The R^2 coefficient was 0.44 for PltH, 0.45 for EVI, SAVI, WDRVI and 0.51 for NDVI.

The response of the VIs, SPAD and PltH to the $N_{available}$ for the crop in the topsoil layer (0-30 cm) was quite similar to that observed for the N_{rates}. However, a consistent increase in the R^2 coefficient when fitting linear-plateau models was observed due to the extra information (initial soil NO_3^--N) added to the model (Figure 3). The use of the $N_{available}$ explained better some variations of the indices values for the same N_{rates} applied. Thus, in-season nitrate analysis can be used for the fine-tuning of remote sensing prediction of N sufficiency rates (Papadopoulos et al., 2014).

The $GYON_r$ and the $GYON_a$ undoubtedly gives interesting information for N management since they allow to differentiate between N-deficient and N-sufficient plots (Quemada et al., 2014). All VIs and PltH achieved saturation between applied N_{rates} of 202.9 and 235.0 kg N ha^{-1}. The SPAD units were much lower, achieving saturation at 142.1 kg N ha^{-1}. Low $GYON_r$ determined by the SPAD at reproductive stage (R_1) was previously reported in a 5 N_{rates} study with lower yielding conditions by Liu and Wiatrak (2011). In their study, SPAD was able to distinguish between N_{rates} up to 135 kg N ha^{-1}. Likewise, in high-yielding environments, Malek (2015) reported saturation of SPAD at 173 kg N ha^{-1}, an amount of N insufficient to achieve maximum maize grain yields. Therefore, in high-yielding environments, the usefulness of SPAD as a predictor of maize N status is probably limited.

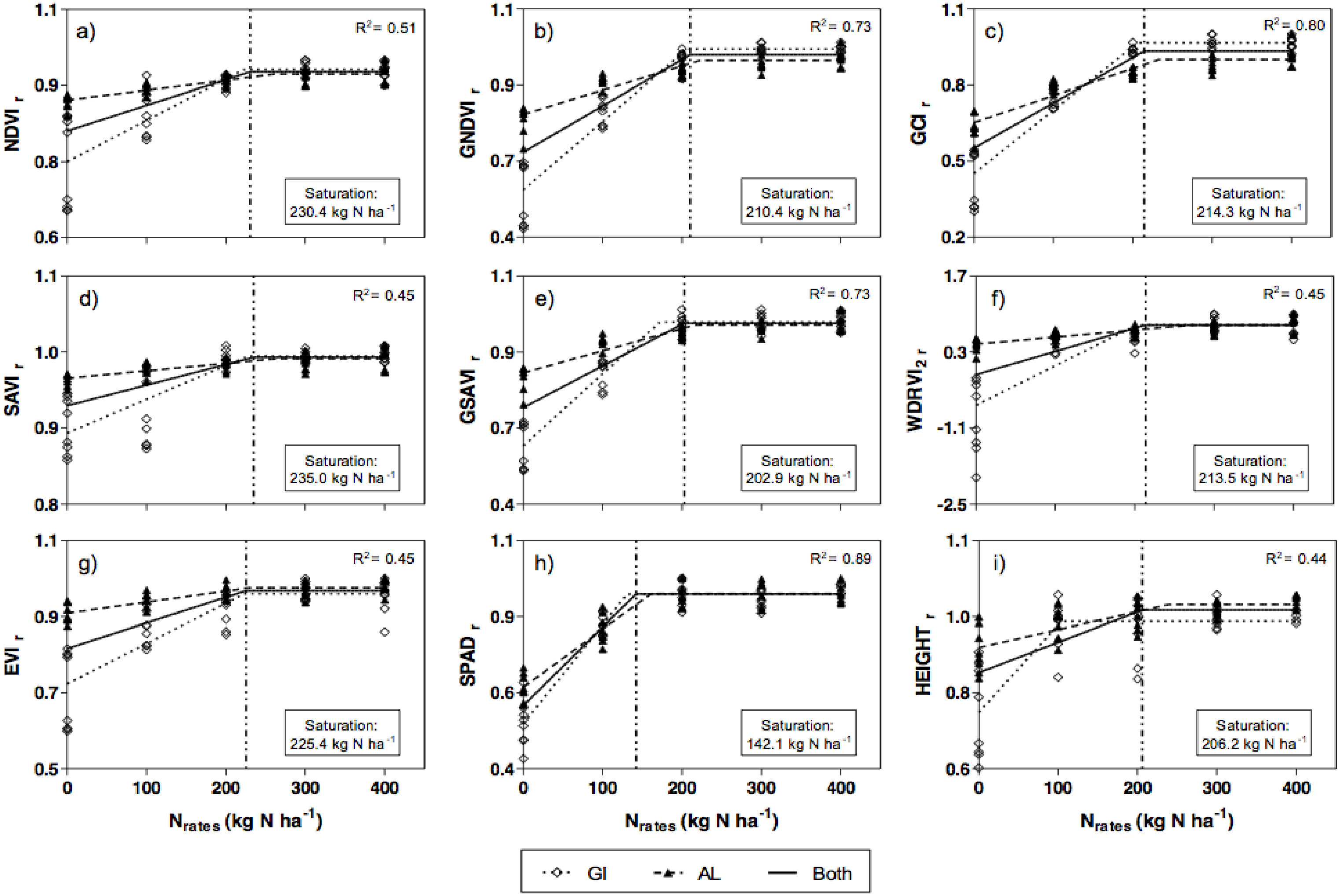

Figure 2. Response of (a) NDVI, (b) GNDVI, (c) GCI, (d) SAVI, (e) GSAVI, (f) WDRVI$_2$, (g) EVI vegetation indices at V12 stage, (h) SPAD and (i) plant height (PltH) at VT stage to different nitrogen rates (N$_{rates}$) tested in the experiments. Saturation N dose was determined when there was no response to higher N fertilization. The results of Almacelles (AL) and Gimenells (GI) are presented as ratios dividing the indices values by the maximum observed in each year. 'Both' indicates that the two fields were analysed together.

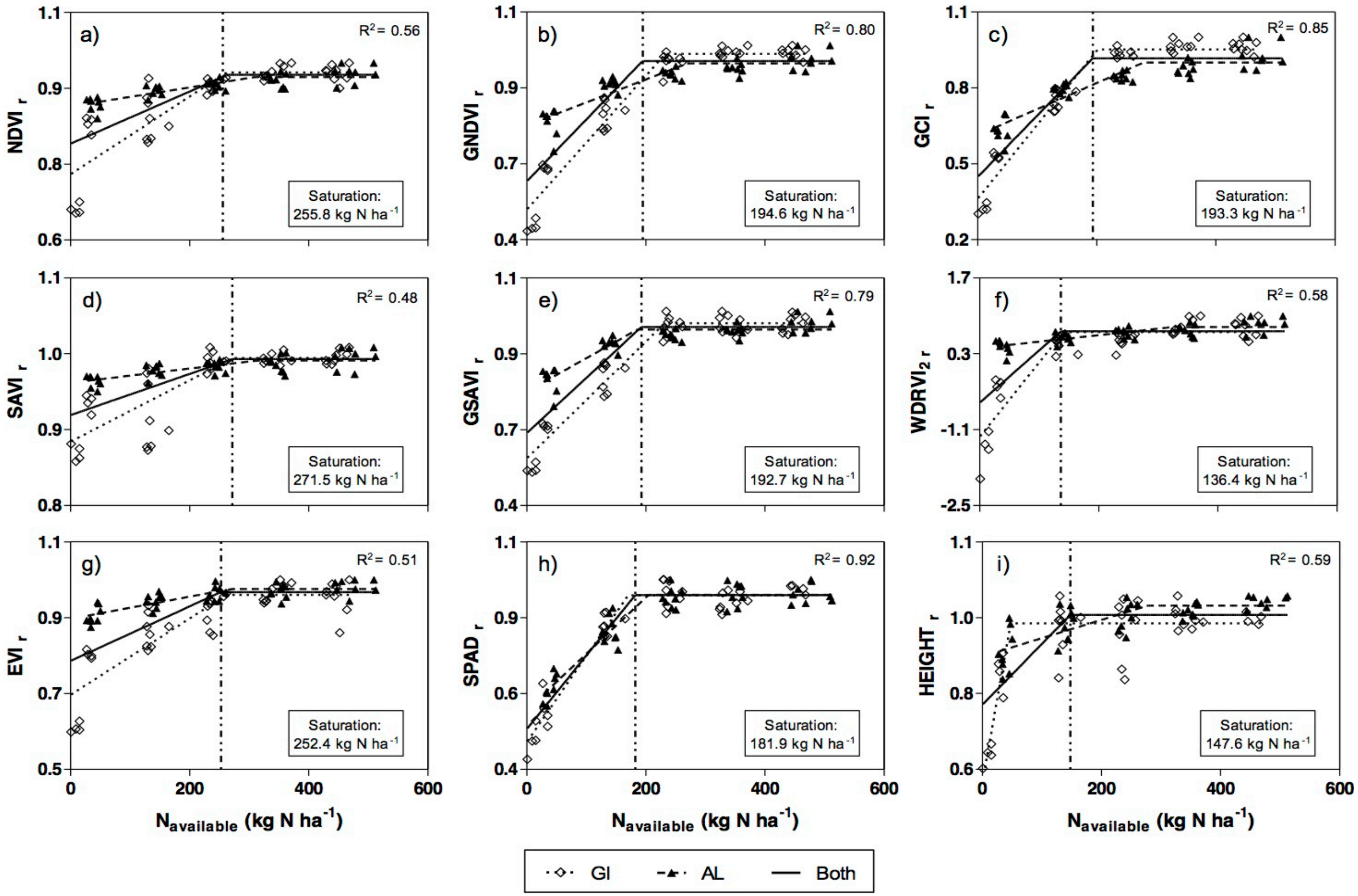

Figure 3. Response of (a) NDVI, (b) GNDVI, (c) GCI, (d) SAVI, (e) GSAVI, (f) WDRVI$_2$, (g) EVI vegetation indices at V12 stage, (h) SPAD and (i) height at VT stage to the available nitrogen in the top soil layer (0-30 cm) (N$_{available}$) determined in the experiments. Saturation N dose was determined when there was no response to higher N fertilization. The results of Almacelles (AL) and Gimenells (GI) are presented as ratios dividing the indices values by the maxim.

Generally, the $GYON_r$ determined by the green-based VIs (≈210 kg N ha^{-1}) was lower than that determined by the red-based VIs (≈230 kg N ha^{-1}). The $GYON_a$ was similar to the $GYON_r$ for the green-based Vis, while there was an increase to 250-270 kg $N_{available}$ ha^{-1} for red-based VIs. SPAD increased the $GYON_a$ by 27% compared with its determined $GYON_r$, but even so the $GYON_a$ was highly underestimated. The relationship between $N_{available}$ and the studied indices could also help to explain the better N status seen in 2015 compared to 2014 in GI, when higher indices values in the N0 treatment were correlated with higher $N_{available}$ (Figure 3).

In our conditions, the $GYON_a$ determined by the VIs for maize production was between 200 and 270 kg N ha^{-1}. These amounts of available N were similar to those reported in other studies in the Ebro Valley (Berenguer et al., 2009; Salmerón et al., 2010; Biau et al., 2013; Cela et al., 2013). Thus, the tested VIs enabled the spatial characterisation of N status and were able to assess crop performance even under low-N stress (Zaman-Allah et al., 2015).

3.2. Grain yield and economic return responses to N rates and available N

Grain yield and economic return varied significantly according to N_{rates} (p-value<0.01), location (p-value<0.01) and year (p-value<0.05) (Table 4). Moreover, interactions between location and N_{rates} (L×N), year and location (Y×L) and year and N_{rates} (Y×N) were detected and can be explained by the reasons described above.

The maximum grain yields were achieved in the N400 treatments in AL and GI (19.96 and 17.0 Mg ha^{-1}, respectively). However, there were small differences in grain yields among the highest N_{rates} (200-400 kg N ha^{-1}) in each location. These findings agree with Maresma et al. (2016) and Yagüe and Quílez (2015), who respectively reported $GYON_r$ of 239.8 and 300 kg N ha^{-1}, in our conditions. This suggests that in high-yielding environments, if N is provided when the crop needs it, maize efficiently uptakes the high N rates and translates them into grain yield. It is important to notice that N_{rates} were split into two equal applications during the growing period, with the aim of increasing N efficiency (Fageria and Baligar, 2005).

Despite the observed tendency of increased grain yield above the N200 treatment, there were no significant differences between treatments. Grain yields increased from 18.8 and 15.1 to 19.4 and 16.8 Mg ha^{-1} in AL and GI, respectively. There was an average difference between the N200 and N400 fertilization treatments of 0.66 Mg ha^{-1} in AL, whereas in GI the difference was 1.75 Mg ha^{-1}. Moreover, the lower grain yields achieved in GI compared with AL, made the differences in yield between N_{rates} more evident. The grain yield increased by 10.5% and 3.5% in GI and AL, respectively between the N400 and N200 treatments. These differences in grain yield between locations could be mainly explained by the lower soil capacity of providing N from OM mineralization of GI, and by other natural fertility conditions (Tremblay et al., 2011).

Table 4. Analysis of variance and mean values of yield and economic return (ECO-return) for the different nitrogen rates (N_{rates}) in Almacelles (AL) and Gimenells (GI) during the 2014 and 2015 growing seasons.

N_{rates} and statistics	YIELD (Mg ha^{-1})			ECO-return (€ ha^{-1} x 1000)		
	2014	2015	Both Years	2014	2015	Both Years
			Almacelles (AL)			
0	8.7 g	9.2 fg	9.0 d	1.5 ef	1.6 ef	1.5 c
100	15.6 de	16.1 de	15.9 bc	2.6 cd	2.7 bcd	2.6 b
200	19.1 a	18.4 abc	18.8 a	3.1 a	3.0 ab	3.0 a
300	19.6 a	18.9 ab	19.2 a	3.1 a	2.9 ab	3.0 a
400	20.0 a	18.9 a	19.4 a	3.0 a	2.9 abc	3.0 a
			Gimenells (GI)			
0	3.5 i	6.1 h	4.8 e	0.6 h	1.0 g	0.8 d
100	9.1 g	11.0 f	10.0 d	1.5 f	1.8 e	1.6 c
200	15.0 e	15.2 de	15.1 bc	2.4 d	2.4 d	2.4 b
300	16.0 de	16.8 cde	16.4 bc	2.5 d	2.6 cd	2.5 b
400	17.2 bcd	16.4 de	16.8 b	2.5 d	2.4 d	2.5 b
			ANOVA over locations and years			
Location (L)		**			**	
N rates (N)		**			**	
L × N		**			**	
Year (Y)		*			*	
Y × L		**			**	
Y × N		**			**	
Y × N × L		NS			NS	

NS No significance * Significant at p-value < 0.05 ** Significant at p-value < 0.01

Consistently, with high grain yields, higher economic returns were found at high N_{rates}. Nevertheless, the maximum economic return was determined in an N200 treatment in AL (3,068 € ha^{-1}) and in an N300 treatment in GI (2,578 € ha^{-1}). The economic return was mostly determined by grain yield at the studied N:Maize price ratio (5.3:1), so a slight reduction in grain yield was drastically penalized by the economic return. It was evident that, at higher N:Maize price ratios (worse relation of prices for farmers), the efficiency of the N will affect more the economic return of the crop. Sripada et al. (2008) tested price ratios from 4:1 to 14:1 (N:Maize), so the 5.3:1 price ratio used in our study was close to the optimum for farmers and highly affected by the grain yield.

However, the economic return is important not only for N management in maize production system. In addition, the reduction in N contamination of groundwater has to be considered (Varvel et al., 1997). As EON_r for maize is usually consistent with good environmental stewardship, it could therefore be used as a tool to determine maize N requirements (Sripada et al., 2008), and could be considered essential for responsible N management of maize crops.

In the present study, when analysing both locations together, the ratios of grain yield and economic return were highly correlated with the N_{rates} (R^2=0.82 and 0.77, respectively) and $N_{available}$ (R^2=0.89 and 0.87, respectively) in linear-plateau models (Figure 4). In that case, as observed for the VIs, knowledge of the $N_{available}$ in the topsoil layer slightly increased the accuracy of the model. In our study, the $GYON_r$ and $GYON_a$ were determined at 216.8 and 248.6 kg N ha^{-1}, respectively, agreeing with the values reported in previous studies in the area (Berenguer et al., 2008, 2009; Biau et al., 2013; Cela et al., 2013; Yagüe and Quílez, 2015). Maize N extractions were probably not completely covered by the determined $GYON_r$ and $GYON_a$ in the linear-plateau model. In similar conditions, Biau et al. (2012) reported maize N uptake of 386 kg N ha^{-1} for grain yields of 16 Mg ha^{-1}. The difference between applied N and N uptake was probably supplied by soil OM mineralization during the growing season. In our conditions, the mineralized N could be around 100 kg N ha^{-1} (Berenguer et al., 2008). Moreover, the practice carried out in the study of incorporating maize stover into the soil over a long-term period contributed to increasing soil OM content which, in specific conditions of temperature and moisture, each year can mineralize and provide a significant amount of N (Biau et al., 2013).

The EON_r was lower than the $GYON_r$ because the NUE is reduced when applying higher N rates. Di Paolo and Rinaldi (2008) reported a reduction of 25 kg of maize per kg of N fertilizer when applying high amounts of N fertilizer (300 kg N ha^{-1}) in irrigated maize in Mediterranean conditions (14 Mg maize grain ha^{-1}). Therefore, the maize yield per kg of applied N is reduced when increasing the N_{rates} and, consequently, the last yield increment normally entails a decrease in crop profitability. This is because the income obtained per kg of grain is lower than the cost of the N fertilizer input. At an

N:Maize price ratio of 5.3:1, and considering both site location and year, the EON_r was 176.6 kg N ha^{-1} while the EON_a was 209.4 kg N ha^{-1}. Rudnick et al. (2016), in a 4-year experiment (2011-2014), determined the EON_r between 196 and 252 kg of N ha^{-1} for non-irrigated maize that yielded 10-15 Mg ha^{-1} of grain. Although EON_r could vary depending on the prices of maize and N (Sripada et al. 2008), Schlegel et al. (1996) concluded that in same fields, the EON_r was relatively insensitive to changes in these prices. In their study, the EON_r was similar for low-, medium-, and high-yielding years, concluding that application of insurance N reduced crop profitability. Therefore, as EON_r and EON_a could be considered stable in same fields, the EON_r (176.6 kg N ha^{-1}) and the EON_a (216.8 kg N ha^{-1}) reported in this study could be useful for N management in maize fields located in high yielding environments.

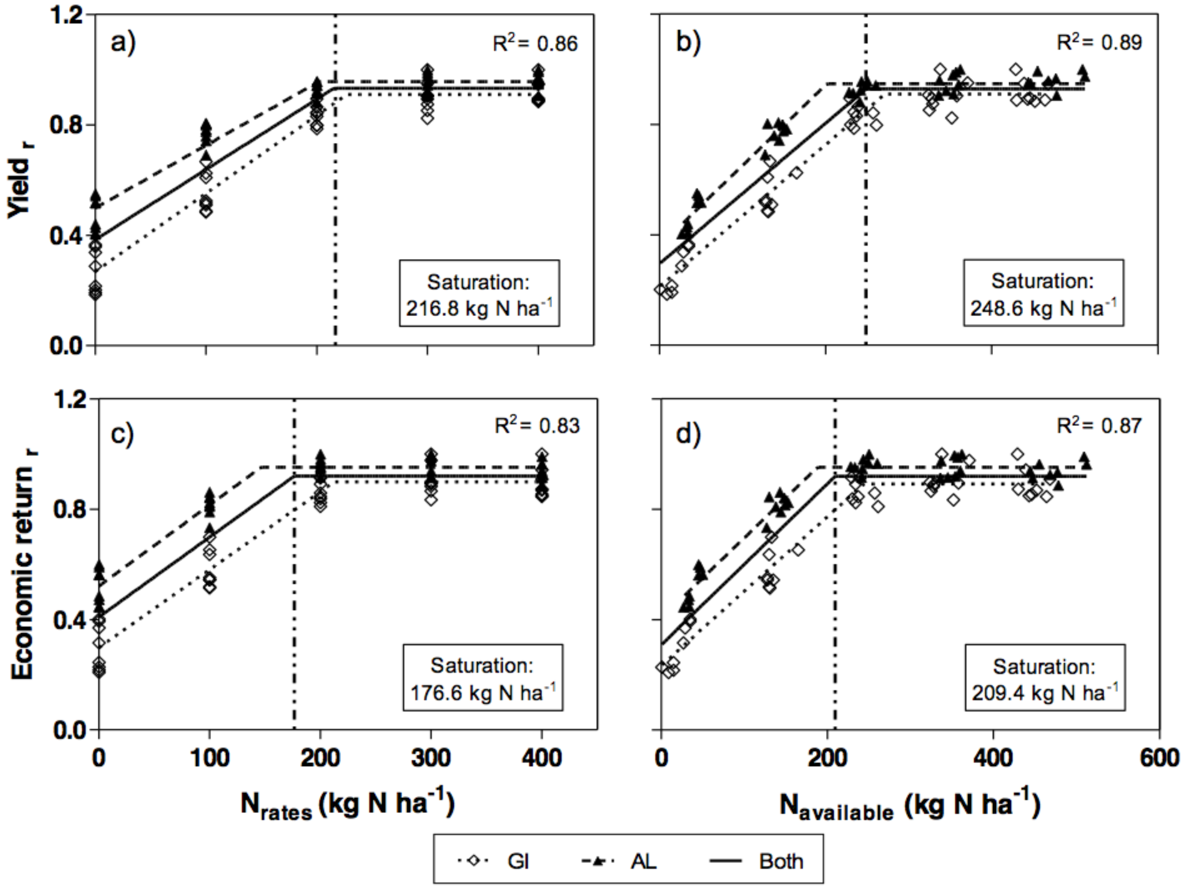

Figure 4. Response of (a) yield to nitrogen rates (N_{rates}), (b) yield to available nitrogen ($N_{available}$), (c) economic return to N_{rates} and (d) economic return to $N_{available}$ as tested and determined in the experiments. Saturation N dose was determined when there was no response to higher N fertilization. The results of Almacelles (AL) and Gimenells (GI) are presented as ratios dividing the yield and economic return values by the maximum observed in each year. 'Both' indicates that the two fields were analysed together.

3.3. Correlation between vegetation indices, SPAD and plant height with grain yield

The calculated ratios of VIs at V12, SPAD and PltH at VT stage showed moderate to high correlations with the grain yield ratios (R^2 between 0.45 and 0.90) (Figure 5). Several studies have proven the usefulness of red-based VIs, as NDVI, for predicting grain yield in different conditions (Sripada et al., 2006; Teal et al., 2006; Inman et al., 2007; Islam et al., 2011; Liu and Wiatrak, 2011; Shaver et al., 2011; Zaman-Allah et al., 2015). However, in the present study the green-based VIs and SPAD showed higher correlation with grain yield than the red-based VIs and PltH. In a two-year study and under similar conditions, Bausch et al. (2008) demonstrated the usefulness of green-based indices at V12 and later growth stages for predicting grain yield (R^2=0.81) and its variability within a field for in-season N management. Isla et al. (2011) also reported better grain yield predictions with GNDVI and SPAD (R^2=0.93) than with red-based VIs (R^2=0.65).

Higher grain yields were achieved when higher indices values were determined by VIs at V12 stage. However, there was variability among the highest indices values that was not clearly translated into grain response. Linear-plateau models were fitted to identify the saturation of the indices for predicting grain yield. This saturation provided information about the percentage of maximum grain yield up to which the index can differentiate. Therefore, the indices that presented saturation at higher grain yields were determined to be more useful due to their capacity to distinguish between nearly optimal maize N statuses.

In this case, green-based VIs performed better than red-based VIs in determining maize vigour. This fact could be explained by problems of saturation associated with red-based indices for some types of vegetation during their later growth stages (Isla et al., 2011).

The highest saturation of VIs for grain yield prediction corresponded to SAVI (at 86% of maximum yield). The usefulness for predicting leaf chlorophyll content and N status in maize in different growth stages of VIs derived from SAVI has been reported by authors such as Zhang et al. (2011) or Naveed Tahir et al. (2013). However, its low correlation with grain yield (R^2=0.48) demonstrated that its usefulness for grain yield prediction is still uncertain.

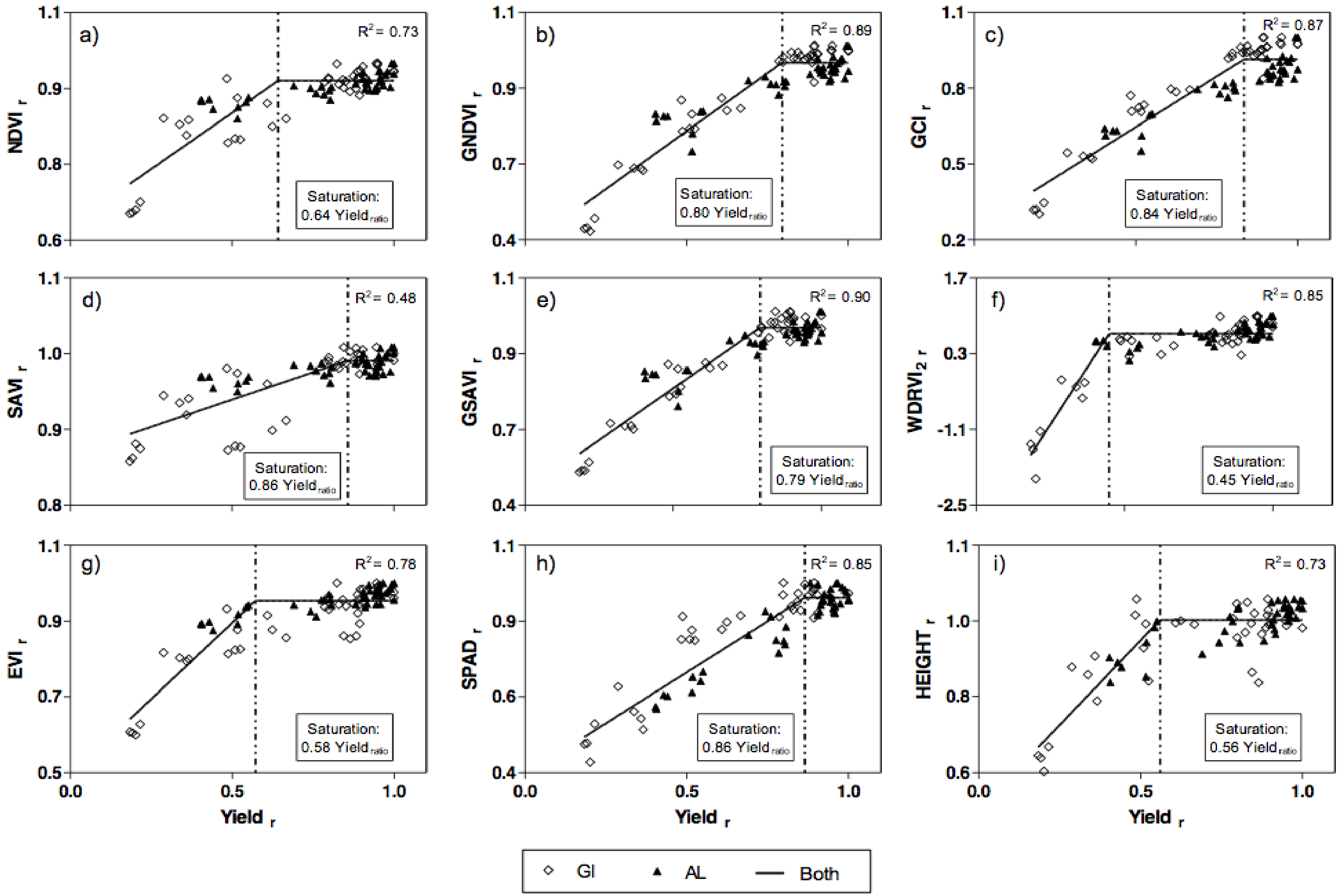

Figure 5. Response of (a) NDVI, (b) GNDVI, (c) GCI, (d) SAVI, (e) GSAVI, (f) WDRVI$_2$, (g) EVI vegetation indices at V12 stage, (h) SPAD and (i) plant height (PltH) at VT stage to yield achieved at harvest in the experiments. Saturation N dose was determined when there was no response to higher N fertilization. The results of Almacelles (AL) and Gimenells (GI) are presented as ratios dividing the indices values by the maximum observed in each year. 'Both' indicates that the two fields were analysed together.

The best VI to predict grain yield was GCI, which was able to differentiate up to 84% of the maximum yield showing high correlation with the linear-plateau model (R^2=0.87). Hence, small N deficiencies in maize at V12 stage could be detected by GCI and solved by an extra N application. It is known that intra-field yield spatial variability can be occasioned by different stress problems (Liu et al., 2004; Pagano and Maddonni, 2007), resulting in a grain yield variability of 15-38% with respect to non-stress conditions. Therefore, under N deficits these differences would probably be higher. Detection of maize N status that would yield less than 84% of maximum yield would improve the average maize grain yields and, consequently, N management. Similarly, the other two green-based VIs tested in the study showed similar trends to GCI at V12. GNDVI and GSAVI saturated at 80% and 79% of the maximum grain yield, respectively.

Higher variability was observed among the red-based VIs. NDVI predicted grain yield accurately (R^2=0.73), but was able to differentiate only up to 64% of the maximum yield. WDRVI and EVI were saturated at 45% and 58%, respectively, of the maximum yield, and, together with the PltH (56%), were the least accurate at differentiating among the highest grain yields. These results partially disagree with those presented by Maresma et al. (2016), who found the WDRVI to be the best grain predictor using a high-resolution UAV service. Nevertheless, although in the present study the plateau was not reached for high relative grain yield, there was a clear tendency of increasing WDRVI values when increasing the yield (Figure 5f). This trend might corroborate the potential of this index to distinguish yield at high N_{rates}.

SPAD and PltH measured at VT proved their potential to predict grain yield. SPAD differentiated up to 86% of the maximum yield, confirming the usefulness of chlorophyll meters for predicting grain yield (Piekielek et al., 1995; Bonneville and Fyles, 2006; Solari et al., 2008). However, PltH was partially useful for grain yield prediction (differentiating up to 56% of the maximum grain yield). Although Liu and Wiatrak (2011) did not find a correlation between PltH and grain yield at V8 stage (R^2=0.03), our results do concur with Yin et al. (2011), who predicted grain yield with PltH at VT stage (R^2 between 0.52 and 0.86, depending on the year).

3.4. Vegetation indices, SPAD and plant height to predict $GYON_r$, $GYON_a$, EON_r and EON_a

The VIs, SPAD and PltH were useful at determining the $GYON_r$ (216.8 kg N ha^{-1}) in our study. Linear-plateau models (R^2=0.73-0.80) demonstrated that green-based VIs were saturated at rates of 210 kg N ha^{-1}, which makes them the best predictors of $GYON_r$ at V12 stage. Despite red-based VIs at V12 stage or PltH at VT stage also predicting the $GYON_r$ (206-235 kg N ha^{-1}), the lower correlation with the linear-plateau model (R^2=0.44-0.51) causes uncertainty. Although the SPAD showed its usefulness for predicting grain yield, the $GYON_r$ was widely underestimated (142.1 kg N ha^{-1}) in concurrence with the results of Maresma et al. (2016).

The $GYON_a$ (in the first 30 cm of soil) was 248.6 kg N ha^{-1}. The red-based VIs accurately predicted the $GYON_a$ (252-271 kg N ha^{-1}) and slightly increased their correlation with the linear-plateau model (R^2=0.48-0.58) compared with the $GYON_r$. However, the effectiveness of green-based VIs, WDRVI and PltH at determining the $GYON_a$ was negatively impacted by adding the $N_{available}$ to the model. SPAD, as was observed for the $GYON_r$, highly underestimated the $GYON_a$ (181.9 kg N ha^{-1}).

The EON_r (and EON_a) were determined below the $GYON_r$ (and $GYON_a$). Therefore, since the VIs proved their usefulness for determining the $GYON_r$ and $GYON_a$, they would over-estimate the EON_r (and EON_a). However, at low N:Maize price ratios, EON_r (and EON_a) will be more accurately predicted by the indices since the economic return is mainly affected by the grain yield.

4. Conclusions

Vegetation indices derived from multispectral airborne images showed their usefulness to distinguish maize grain yield potential. Similarly, VIs have proven to be an outstanding tool to accurately determine maize N status at V12 stage, when N deficits can still be corrected by late sidedress applications. Therefore, VIs could help farmers to overcome the uncertainty of N fertilization by determining the N responsive and the N non-responsive areas of the field. Green-based VIs, especially GCI, were the most accurate at predicting the $GYON_r$, whereas Red-based VIs slightly overestimated it. The EON_r was overestimated by the VIs, confirming their higher potential to predict grain yield than economic return.

The determination of the $N_{available}$ did not improve the accuracy of the VIs to predict grain yield. However, in our study N fertilization treatments were maintained during a long-term period and probably the residual N in the soil was stabilized for each N treatment. Commercial fields might have larger differences in N patterns within fields, and $N_{available}$ calculation could be very useful to determine N responsive and N non-responsive areas.

Overall, VIs could contribute to enhancing N management in farming practices. The VIs would reduce the risk of contamination by over-fertilization, improve the economic return and increase the NUE by reducing N inputs while maintaining grain yield. Nevertheless, the adoption of remote sensing technologies by farmers is still limited. Further research is needed to fine-tune the response of maize to N applications when deficiencies are detected at V12 stage. Airborne images could be useful for practical farming implementation because of their high potential to cover large cropping surfaces at a moderately low cost.

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Chapter IV

Nitrogen management in double-annual cropping system (barley-maize) under irrigated Mediterranean environments

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Abstract

Improving nitrogen (N) use efficiency (NUE) is an agricultural necessity, as it can contribute to increasing crop productivity while decreasing environmental degradation. Double-annual cropping systems appear to be a solution to reducing losses of residual N, while increasing productivity and profitability per land unit. In the present three-year N fertilization study (2013-2016), combinations of N fertilization rates (N_{rate}) applied to barley (0 and 100 kg N ha^{-1}) and maize (0, 100, 200 and 300 kg N ha^{-1}) were evaluated. Grain and biomass yields and N content, plant N uptake, residual N, N efficiency, soil organic carbon (SOC) and economic returns of the double-annual barley-maize cropping system were determined. The annual optimum N_{rate} to achieve maximum yields in the barley-maize system was 230-240 kg N ha^{-1} split between maize and barley, with potential total annual yields that could be as high as 20 and 35 Mg ha^{-1} yr^{-1} of grain and biomass, respectively. The longer growing period of these double-cropping systems contributed to promoting higher efficiency of post-harvest residual N in each crop. Barley was especially efficient at using maize residual N, whereas maize was not as clearly affected by barley residual N. After three years of the study, SOC had not changed in any of the N treatments, even in the N treatments with highest N deficiency (0 kg N ha^{-1} yr^{-1} applied). Further research is needed to fine-tune the N fertilization strategy of the double-annual barley-maize cropping system over long-term periods.

Keywords: Double-annual cropping, barley, maize, nitrogen fertilization, NUE

Abbreviations: Nitrogen, N; Organic matter; OM; N rate, N_{rate}; available N, $N_{available}$; optimum N, N_{opt}; Nitrogen use efficiency, NUE; Apparent nitrogen recovery, ANR; Nitrogen recovery efficiency, NRE; Soil organic carbon, SOC; Economic optimum nitrogen rate, EONR.

1. Introduction

World agriculture is currently facing unprecedented challenges. There is a need to increase food production to meet global food demand (Bodirsky et al., 2014), while reducing production and pollution costs. Nowadays, the main method to increase crop yields and to maintain or restore soil nutrients is the application of mineral fertilizers, mainly N (Hirel et al., 2011). However, anthropogenic reactive N input to the biosphere has already exceeded a proposed planetary boundary (Kros, 2013).

The reliable supply of N and other macronutrients, as well as plant breeding improvements, has allowed a large increase in crop production per land unit over the past century. Nitrogen fertilization has promoted economic development, allowing the increase of populations, and sparing forests that would probably otherwise have been converted to agricultural land to meet food demand (Foley et al., 2011). In the most intensive agricultural production systems, over 50% and up to 75% of the N applied to the field is not used by the plant and is leached into the soil (Raun and Johnson, 1999). This means that more than half of the N used for crop fertilization is currently lost into the environment (Lassaletta et al., 2014). Hence, improving nitrogen use efficiency (NUE) in cropping systems across the globe is an absolute necessity, as it is one of the most effective means of increasing crop productivity while decreasing environmental degradation (Cassman et al., 2002; Davidson et al., 2015).

Data from surveys in the Ebro Valley (a semi-arid irrigated area in NE Spain), where irrigated maize is one of the most important and high N-demanding crops (Maresma et al., 2016), indicate that farmers apply rates of 318-453 kg N ha^{-1} yr^{-1} (Cavero et al., 2003; Isidoro et al., 2006; Sisquella et al., 2004). Normal maize grain yields in the area range from 12 to 15 Mg ha^{-1}, with total plant N uptake of 250-300 kg ha^{-1} (Berenguer et al., 2008; Cela et al., 2011; Yagüe and Quílez, 2010). Therefore, when excess N fertilizer is applied, there is a high risk of N leaching during the maize intercrop period (October to April) (Moreno et al., 1996), depending on the rainfall distribution under semi-arid conditions (Salmerón et al., 2011).

To avoid post-harvest leaching of residual N and to increase profitability per land unit, double-annual cropping systems could be implanted. Winter cover crops after summer crops can provide environmental benefits that make them suitable for enhancing NUE in a maize cropping system (Miguez, 2005; Quemada et al., 2013). In double-annual cropping systems, soil is covered during a longer period of the year than with mono-cropping systems. This entails several benefits, including prevention of soil erosion by wind and water (Hirel et al., 2011), an increase of total dry matter production (Lloveras, 1987a, 1987b; Yagüe and Quílez, 2013), increase of land gross margin (Gil, 2013) per land unit, and a reduction of NO_3^--N run-off (Gabriel and Quemada, 2011; Krueger et al., 2012), among others. However, the increased uptake of N and other nutrients with double-cropping systems, coupled with higher productivity, present a significant challenge for maintenance of soil fertility, mandating higher rates of fertilization, and potentially leading to reductions in soil organic C if crop residues are not retained in fields (Heggenstaller et al., 2008).

Double-annual forage cropping strategies (summer crop-winter crop) have been increasingly applied in the NE of Spain during recent years (Ovejero et al., 2016). A summer crop (sorghum or maize) is grown from June to October, and in November a winter cereal such as barley or triticale is subsequently sown as in other forage production areas (Lloveras, 1987a, 1987b; Monaco et al., 2008; Trindade et al., 2001). Indeed, double-annual forage crop production is usually associated with dynamic livestock farming where animals are fed with forages and their faeces, usually mixed with straw, are applied to crops as fertilizer (Lloveras, 1987a; Perramon et al., 2016). Several authors have reported studies of double-annual cropping systems with N organic fertilization in Mediterranean environments (Grignani et al., 2007; Ovejero et al., 2016; Perramon et al., 2016; Yagüe and Quílez, 2010). However, in irrigated Mediterranean environments, there is limited research on the sustainability and economic profitability of a double-annual cropping system (barley-maize), unlinked to livestock farming. Thus, there is a need to evaluate the possible advantages of double-annual cropping systems in irrigated Mediterranean environments unlinked to livestock farming, and if appropriate, encourage farmers to adapt this practice to improve N management and increase the economic benefit.

The objectives of the present research conducted under sprinkler irrigation in a double-cropping system of maize-barley, were i) to determine the effect of annual N fertilization on grain and biomass yields, N uptake, soil NO_3^--N content, N efficiencies, soil organic carbon (SOC) and economic return, and ii) to assess and optimize the N_{rate} and its temporal distribution in a double-annual cropping system (barley-maize) under irrigated Mediterranean environments.

2. Materials and methods

2.1. Study area

A three-year experiment (2013-2016) was conducted in Algerri (Lleida, NE of Spain) under irrigated conditions (41° 46.5' N, 0° 38.7' E). The field experiment comprised an area of approximately 190×18 m^2 with an individual plot size of 60 m^2.

The study area is characterized by a semi-arid climate with low annual precipitation (373 mm) and high annual average temperature (14.3 ºC). Each growing season, around 150 and 700 mm of irrigation water (lacking nitrate) were respectively provided to barley and maize, to achieve maximum yields. Soils were classified as Petrocalcic Calcixerepts (Soil Survey Staff, 2014). Soil quality indicators and physicochemical parameters were analysed in samples using standard methods (MAPA, 1994): soil texture, pH, electrical conductivity (EC), bulk density, available P (Olsen P) and extractable K (NH_4Ac) (Table 1).

Table 1. Chemical and physical soil properties at the beginning of the experiment (2013).

Soil properties	Horizon			
	Ap. 0-27 cm	Bwk_1. 27-48 cm	Bwk_2. 48-82 cm	Bkm. 82-120 cm
Sand, %	35.6	16.3	13.1	-
Silt, %	47.7	53.4	52.0	-
Clay, %	16.7	14.8	15.8	-
pH	8.1	8.2	8.3	-
Organic matter, g kg^{-1}	19.4	9.1	6.2	-
$EC_{1:5}$, dS m^{-1}	0.42	0.29	0.27	-
P (Olsen), mg kg^{-1}	38	20	10	-
K (NH_4Ac), mg kg^{-1}	241	94	59	-
Soil class[†]		Petrocalcic calcixerept		

[†] Soil Survey Staff (2014)

2.2. Experimental design

Eight different N combinations in the double-annual cropping system (barley-maize) were considered in a split-plot design with four replications. The N treatment in barley (winter crop) was the main plot (0 and 100 kg N ha^{-1}) and the N treatments in maize (summer crop) were the subplots (0, 100, 200 and 300 kg N ha^{-1}).

The N fertilization treatments were randomized at the beginning of the experiment (2013) and applied in the same plots thereafter. The N fertilizer (ammonium nitrate, 34.5%) was applied in both crops. In barley, one sidedress was applied in early February (DC 25-27 of the scale of Zadoks et al., 1974), whereas in maize the N treatment was split into two equal sidedresses (50% at V5 and 50% at V10 stage, 5 and 10 leaves with visible leaf collar). Phosphorus and potassium were also applied annually during winter at rates of 150 kg P_2O_5 ha^{-1} and 250 kg K_2O ha^{-1}, to avoid deficiencies of those elements.

2.3. Cropping system

Both crops were managed according to good and normal practices in the area.

- Barley: Conventional tillage was done before planting, after the maize harvest. It included disc ploughing and cultivation to a depth of 30 cm to incorporate previous maize stover and to prepare the soil for the sowing of the barley. The variety Gustav was sown the three years of the experiment at 230 kg ha^{-1} and one herbicide treatment was applied post-emergence to control weeds (Fluroxipir 20%, at 1 L ha^{-1}). The grain and biomass harvests were done between the first and second week of June.

- Maize: The barley stover was removed from the field, and the maize planted with no tillage to reduce the time gap between barley harvest and maize planting. During the three years of the experiment, the hybrid PR32W86 (FAO cycle 600) was sown at a rate of 90,000 plants ha^{-1}, with 71 cm between rows. Two herbicide treatments were applied: one at pre-emergence to control the majority of weeds (S-Metolachlor 40% and Terbuthylazine 18.75%, at 3 L ha^{-1}) and the other at post-emergence to control *Abutilon theophrasti M.* and *Sorghum halepense L.* (Dimethylamine salt of dicamba 48.2% at 1 L ha^{-1} and Nicosulfuron 6% at 0.75 L ha^{-1}). Biomass was evaluated during the first week of October at physiological maturity, and the grain harvest took place between the last week of October and first week of November.

2.4. Sampling and analytical procedures

Barley and maize grain yields were measured in the central part of each plot by harvesting with an experimental plot combine (1.5×10m). Grain moisture was determined using a GAC II grain analysis computer (Dickey-john, Auburn, IL, USA) with a 250g sample, and grain yields were adjusted to 14% moisture. Total biomass yield was measured in the field by harvesting an area of 1.5 and 7 m^2 of barley and maize, respectively. The dry matter content of the aboveground biomass was determined by drying the sample at 60ºC for 48h. As a consequence, the results presented are at constant humidity. Barley and maize sub-samples of grain and biomass were milled and used to determine N concentrations by near infrared (NIR) spectroscopy, using a previously calibrated 500 Infrared Analyser (Bran+Luebbe, Norderstedt, Germany). Total N uptake was calculated for each crop by multiplying plant N content by dry matter at harvest.

Soil NO_3^--N was determined in each plot after the barley and maize harvest at a depth of 0-90 cm from three consecutive layers (30 cm each). Five cores per plot were taken and comprised an individual soil sample. Soil nitrates were extracted using deionized water and measured using test strips with a Nitrachek® device calibrated according to the standard procedure (Bischoff et al., 1996). NH_4^+-N was not measured, because several previous research studies in the area had considered negligible the amount of N when compared to the N present in nitrate form (Villar-Mir et al., 2002; Berenguer et al., 2009). Soil sub-samples were used for determination of SOC content each year after the maize harvest. SOC was measured by dichromate oxidation (Walkley-Black method; Allison, 1965) and consisted of a mix of five soil samples per plot from the 0-30 cm soil layer.

Three N-efficiency parameters were calculated for each fertilized treatment in both crops: the NUE (Quemada and Gabriel, 2016; Zhang et al., 2015; EUNEP, 2015), the N recovery efficiency (NRE) (Ladha et al., 2005) and the apparent nitrogen recovery fraction (ANR) (Fageria and Baligar, 2005; López-Bellido et al., 2005). NUE was determined as the ratio between the total N removed by the aboveground crops divided by the sum of all N inputs to a cropland (kg kg^{-1}). The NRE was calculated as the ratio between aboveground plant N uptake and fertilizer N input. ANR (kg kg^{-1}) was the ratio between aboveground plant N uptake at Nx – aboveground plant N uptake at N0 and the amount of mineral N applied at Nx.

The economic return of each plot was calculated as the difference between the income produced by the selling of the grain yield and the cost of the N fertilizer applied. The N:grain price ratio is defined as the price per kilogram of N divided by the price per kilogram of grain (price ratio = price of fertilizer N, € kg^{-1} N/price of grain, € kg^{-1} grain) (Sripada et al., 2005).

In the present study, the N:grain price ratios used were 5.6:1 and 5.3:1 for barley and maize, respectively. The N price considered was 0.90 € kg^{-1} of N (N fertilizer plus application cost) and the grain prices were 0.16 and 0.17 € kg^{-1} for barley and maize, respectively.

2.5. Statistical analysis

Statistical analyses of data were performed using the JMP Pro 12 software (SAS Institute, Cary, USA). A mixed-design analysis of variance model (ANOVA), taking into account the growing seasons as repeated measurements, was carried out to evaluate the response of the variable measured to mineral N fertilization. In the mixed-design ANOVA model, the N treatment and the growing season were a between-subjects variable (a fixed effects factor) the replicate was a within-subjects variable (a random effects factor). Means were separated by LSMeans Tukey's HSD test ($p<0.05$), where levels not connected by the same letter are significantly different.

Linear-plateau regression analyses were carried out between grain and biomass yields with the total N applied to determine the N_{rate} to achieve maximum yields and economic return (EONR) with the double-annual (barley-maize) cropping system.

3. Results

3.1. Grain and biomass yields

Grain and biomass yields varied over the years with the same N treatments. Nevertheless, the average highest grain and biomass yields were achieved with the highest N_{rate} (Table 2). The barley N rates applied (0 and 100 kg N ha^{-1}) significantly affected barley yields but did not statistically affect maize yields. On average, barley yields achieved in the non-fertilized (0 kg N ha^{-1}) and fertilized (100 kg N ha^{-1}) barley were 3.75 and 6.1 Mg of grain ha^{-1} and 6.4 and 9.5 Mg of biomass ha^{-1}, respectively. Non-fertilized barley had lower grain and biomass yields than fertilized barley, except for the plots where a high N_{rate} was applied to maize (above 200 kg N ha^{-1}) (Table 2). The N_{rate} applied to maize impacted significantly the grain and biomass yields of barley, maize and the annual sum of barley and maize. Maximum maize yields (about 13 and 23 Mg ha^{-1} of grain and biomass, respectively) were achieved with maize N_{rate} above 100 kg N ha^{-1}, independently of the N_{rate} applied to the barley. However, a growing tendency in maize yields was seen when increasing N fertilization to at least 200 kg N ha^{-1}.

Table 2. Average grain and biomass yields, grain and biomass N contents for the different N rates tested in three consecutive growing seasons in Algerri (2013-2016). Tukey's HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. [†]N means N fertilizer treatment.

Treatments (N)		Grain Yield (Mg ha^{-1})			Biomass Yield (Mg ha^{-1})			N grain (g kg^{-1})		N biomass (g kg^{-1})	
Barley	Maize	Barley	Maize	Total	Barley	Maize	Total	Barley	Maize	Barley	Maize
0	0	2.07 c	7.86 b	9.93 d	3.99 c	16.97 c	20.96 e	16.3 abc	11.2 b	12.1 ab	9.0 c
	100	2.77 c	12.73 a	15.50 bc	4.73 c	22.48 ab	27.22 cd	15.4 cd	12.1 ab	11.2 b	9.9 bc
	200	4.37 b	13.34 a	17.71 a	7.48 b	23.08 a	30.56 abc	15.6 bcd	12.4 a	11.4 b	10.8 a
	300	5.79 a	13.25 a	19.04 a	9.41 ab	23.27 a	32.68 ab	16.5 abc	12.6 a	12.4 ab	10.7 ab
100	0	5.70 ab	8.84 b	14.54 c	8.93 ab	18.54 bc	27.47 cd	14.6 d	11.8 ab	11.1 b	9.4 c
	100	5.73 ab	12.45 a	18.18 ab	9.08 ab	22.20 a	31.28 abc	15.7 bcd	12.3 a	12.0 ab	10.0 abc
	200	6.11 a	13.23 a	19.34 a	9.26 ab	23.11 a	32.38 ac	17.1 ab	12.5 a	12.1 ab	10.5 ab
	300	6.71 a	13.42 a	20.13 a	10.72 a	24.06 a	34.77 a	17.6 a	12.7 a	13.9 a	10.5 ab
ANOVA											
Barley [†]N (N_b)		**	NS	**	**	NS	*	NS	NS	NS	NS
Maize [†]N (N_m)		**	**	**	**	**	**	**	**	**	**
N_b x N_m		**	NS	**	**	NS	*	**	NS	*	NS
Error a		-	-	-	-	-	-	-	-	-	-
Season (GS)		**	**	**	**	**	**	**	**	**	**
GS x N_b		**	*	**	**	NS	NS	**	NS	*	NS
GS x N_m		**	**	**	NS	**	*	**	NS	**	NS
GS x N_b x N_m		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Error b		-	-	-	-	-	-	-	-	-	-

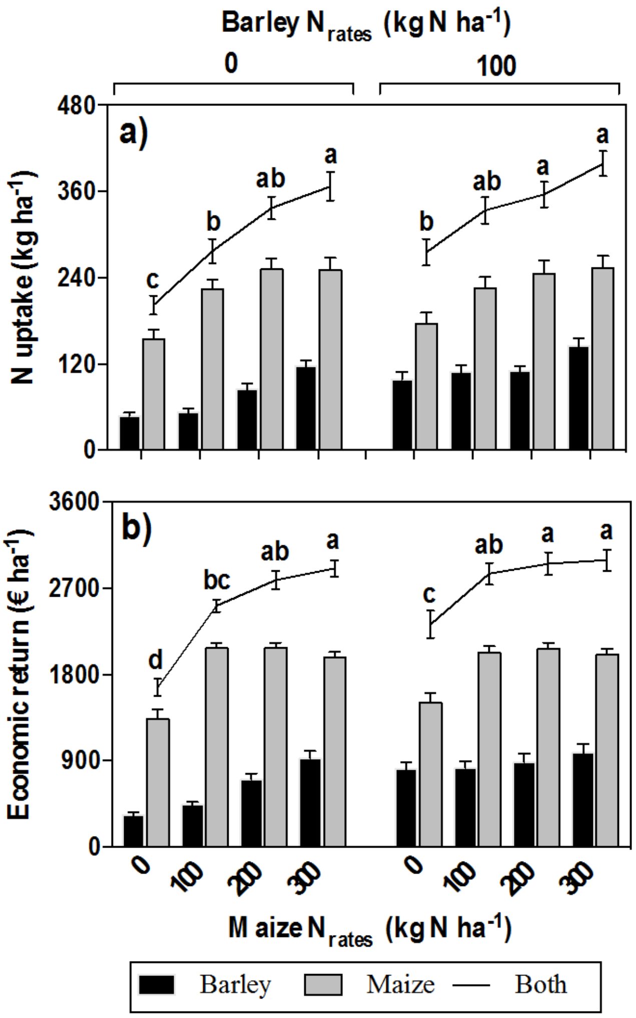
NS No significance   * Significant at p-value < 0.05   ** Significant at p-value < 0.01

The total grain and biomass yields per growing season were significantly affected by the N_{rate} applied in both crops. The control N treatment (0 kg N ha^{-1} yr^{-1} in both crops) obtained the lowest yields for barley and maize. The N_{rate} that totalled 100 kg N ha^{-1} yr^{-1} between both crops yielded more than the control N treatment but less than higher N rates. The N_{rate} that totalled 200 kg N ha^{-1} yr^{-1} between both crops was statistically classified in the same group as the N_{rate} that totalled 300 kg N ha^{-1} yr^{-1}, though an increase in grain and biomass yields was detected when applying higher N rates (Table 2). With applications of 200 kg N ha^{-1} yr^{-1}, 18 Mg ha^{-1} of grain and 31 Mg ha^{-1} of biomass were achieved; yields slightly lower than the ones obtained with 300-400 kg N ha^{-1} (20 and 33 Mg ha^{-1}, respectively).

3.2. Biomass and grain N content and total N uptake

Biomass and grain N content (both barley and maize) varied from year to year and were affected by maize N fertilization, but not by barley N fertilization (Table 2). Depending on the N_{rate} applied to barley and maize, barley N content varied from 15.4 to 17.6 g kg^{-1} (grain) and from 11.2 to 13.9 g kg^{-1} (biomass), whereas the N content variation in maize was from 11.2 to 12.7 g kg^{-1} of grain and from 9.0 to 10.7 g kg^{-1} of biomass. The control N treatment (0 kg N ha^{-1}) showed the lowest maize N content in grain and biomass, but did not clearly affect barley N grain or biomass content.

The average total N uptake during a growing season varied from 201.8 to 398.7 kg N ha^{-1} depending on the N_{rate} applied in barley and maize (Figure 1a). Maize N uptake was statistically affected by the growing season and by maize N fertilization, nevertheless, there was not affected by interaction between them. Barley N fertilization affected barley N uptake but not maize N uptake. Though the highest N uptakes per growing season were determined statistically with 200 kg N ha^{-1}, a rising trend was seen of N uptake with higher N_{rate}.



ANOVA	N uptake		
	Barley	Maize	Both
Barley N (N_b)	**	NS	*
Maize N (N_m)	**	**	**
N_b x N_m	NS	NS	NS
Error a	-	-	-
Season (GS)	**	**	**
GS x N_b	NS	NS	NS
GS x N_m	NS	NS	NS
GS x N_b x N_m	NS	NS	NS
Error b	-	-	-

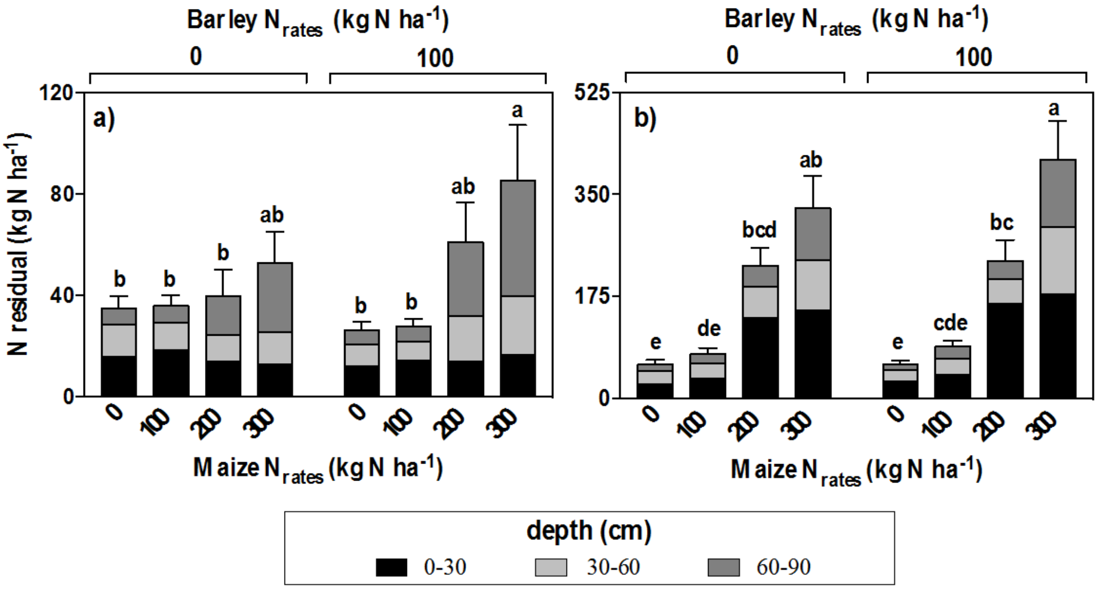
ANOVA	Economic return		
	Barley	Maize	Both
Barley †N (N_b)	**	NS	*
Maize †N (N_m)	**	**	**
N_b x N_m	**	NS	**
Error a	-	-	-
Season (GS)	**	**	**
GS x N_b	**	*	**
GS x N_m	**	**	**
GS x N_b x N_m	NS	NS	NS
Error b	-	-	-

NS: No significance, * significant at 0.05 level, ** significant at 0.01 level

Figure 1. Average barley, maize and the sum of both crops (both) **a) N uptake** and **b) economic return** for the different N rates tested in three consecutive growing seasons (Algerri, 2013-2016). Tukey's HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Error bars indicate the standard error of the mean. †N means N fertilizer treatment.

3.3. Soil NO_3^--N content

Residual soil NO_3^--N content after the barley and maize harvests in the studied depths (0-30, 30-60, 60-90 and 0-90 cm) were statistically affected by growing season and by maize N fertilization (Figure 2). However, barley N fertilization did not affect the amount of N present in soil after the harvest of either barley or maize. A rising tendency in residual soil NO_3^--N was observed when increasing the N_{rate} applied in maize (Figure 2a and Figure 2b). Consequently, with the higher N_{rate} applied in maize (0-300 kg N ha^{-1}) than barley (0-100 kg N ha^{-1}), higher residual soil NO_3^--N contents were determined after the maize harvest than the barley harvest. Especially high was the residual soil NO_3^--N content determined at the highest N_{rate} applied in maize (300 kg N ha^{-1}), between 326 and 410 kg N ha^{-1} in the first 90 cm of soil.



ANOVA	$N_{0\text{-}30}$ residual		$N_{30\text{-}60}$ residual		$N_{60\text{-}90}$ residual		$N_{0\text{-}90}$ residual	
	Barley	Maize	Barley	Maize	Barley	Maize	Barley	Maize
Barley †N (N_b)	NS	NS	NS	NS	NS	NS	NS	NS
Maize †N (N_m)	NS	**	*	**	**	**	**	**
N_b x N_m	*	NS	*	NS	NS	NS	NS	NS
Error a	-	-	-	-	-	-	-	-
Season (GS)	**	**	**	*	**	**	**	**
GS x N_b	**	NS	NS	NS	NS	NS	NS	NS
GS x N_m	NS	**	NS	NS	**	**	**	*
GS x N_b x N_m	**	NS	NS	NS	NS	NS	NS	NS
Error b	-	-	-	-	-	-	-	-

NS: No significance, * significant at 0.05 level, ** significant at 0.01 level

Figure 2. Average of the residual soil NO_3^--N in three consecutive layers (0-30, 30-60 and 60-90 cm depth) after **a) barley** and **b) maize** harvests, in the different N rates tested in three consecutive growing seasons (Algerri 2013-2016). Tukey's HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Error bars indicate the standard error of the mean. †N means N fertilizer treatment.

After the maize harvest, most of the residual soil NO_3^--N was in the first 30 cm of soil (Figure 2b) for all the N treatments tested. However, after the barley harvest, most of the residual soil NO_3^--N was between 60 and 90 cm of depth (Figure 2a).

3.4. N efficiencies

The NUE, the NRE and the ANR were significantly affected by maize N fertilization and growing season, except for barley ANR, which did not depend on the growing season (Table 3). Barley N fertilization did not significantly affect maize N efficiencies except for total (both crops together) NRE. Consistently, N efficiencies were significantly higher with lower N_{rate} applied (Table 3).

Table 3. Average N use efficiency (NUE), N recovery efficiency (NRE) and apparent N recovery (ANR) for the different N rates tested in three consecutive growing seasons (Algerri 2013-2016). Tukey's HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. †N means N fertilizer treatment.

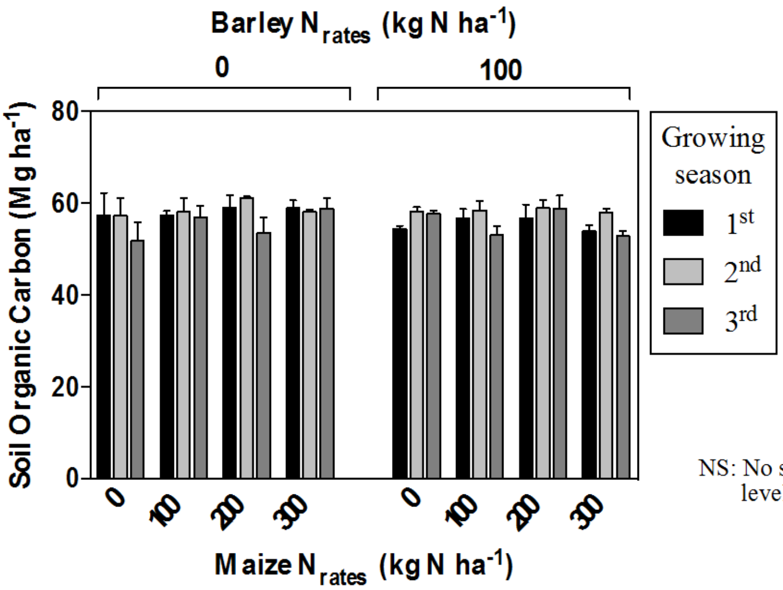
Treatments (N)		NUE (kg kg^{-1})	NRE (kg kg^{-1})			ANR (kg kg^{-1})		
Barley	Maize	Total	Barley	Maize	Total	Barley	Maize	Total
0	0	1.06 a	-	-	-	-	-	-
	100	0.96 ab	-	2.24 a	2.77 a	-	0.69 ab	0.75 a
	200	0.86 bcd	-	1.26 b	1.69 b	-	0.49 bc	0.68 a
	300	0.75 cd	-	0.84 c	1.22 c	-	0.32 c	0.55 a
100	0	0.95 abc	0.99 b	-	-	0.52 b	-	-
	100	0.86 bcd	1.09 ab	2.25 a	1.67 b	0.62 ab	0.70 ab	0.66 a
	200	0.73 d	1.10 ab	1.23 b	1.19 c	0.63 ab	0.46 bc	0.51 a
	300	0.67 d	1.45 a	0.84 c	1.00 c	0.98 a	0.33 c	0.49 a
				ANOVA				
Barley †N (N_b)		NS	-	NS	**	-	NS	NS
Maize †N (N_m)		**	*	**	**	*	**	*
N_b x N_m		NS	-	NS	**	-	NS	NS
Error a		-	-	-	-	-	-	-
Season (GS)		**	**	**	**	NS	**	**
GS x N_b		*	-	NS	NS	-	NS	NS
GS x N_m		**	NS	**	**	NS	NS	NS
GS x N_b x N_m		NS	-	NS	NS	-	NS	NS
Error b		-	-	-	-	-	-	-

NS No significance   * Significant at p-value < 0.05   ** Significant at p-value < 0.01

The highest NUE per growing season was determined for the non-fertilized treatment (1.06 kg biomass kg^{-1} N). Similar results were obtained for barley, maize and both crops together when evaluating the NRE and ANR. For instance, ANR was up to 70% in maize and 80% in both crops together with applications of 100 kg N ha^{-1} yr^{-1}. However, with applications of 300 kg N ha^{-1} yr^{-1} the corresponding values were, respectively, 30% and 60%. Barley NRE and ANR determined in the fertilized barley were higher when the N residual from maize was higher.

3.5. Soil Organic Carbon

The SOC in the first 30 cm of soil did not change over the N treatments analysed in the study (Figure 3). An overall average of 56.9 Mg of C ha^{-1} was determined in the experimental field during the three growing seasons, with small differences among N treatments and growing seasons.



ANOVA	SOC
Barley †N (N_b)	NS
Maize †N (N_m)	NS
N_b x N_m	NS
Error a	-
Season (GS)	**
GS x N_b	NS
GS x N_m	NS
GS x N_b x N_m	*
Error b	-

NS: No significance, * significant at 0.05 level, ** significant at 0.01 level

Figure 3. Average SOC in each growing season among the different N rates tested in the study (Algerri 2013-2016). Tukey's HSD test: different letters indicate homogeneous groups with respect to the mean differences at a p-value of <0.05. Error bars indicate the standard error of the mean. †N means N fertilizer treatment.

3.6. Economic return

The economic return of barley, maize and both crops together was affected by N fertilization of both crops (Figure 1b). The growing season had an impact on the economic return, and showed interaction with barley and maize N fertilization. The 200 kg N ha^{-1} applied in maize obtained the maximum economic return (2,069-2,080 € ha^{-1}), independently of the N_{rate} distribution among crops. However, the maximum economic return in barley was obtained when 300 kg N ha^{-1} were applied in the previous maize (927-984 € ha^{-1}). The economic return of the fertilized barley did not depend on maize N fertilization. However, non-fertilized barley was clearly affected by N fertilization of the previous maize. In that case, the higher the N_{rate} applied in maize, the higher the economic return obtained.

4. Discussion

4.1. Grain and biomass yield

Grain and biomass yields were highly influenced by N fertilization of both crops (barley and maize), showing the large effect of N in increasing cereal yields (Shanahan et al., 2008). Total (barley + maize) grain yield (20 Mg ha^{-1}) and biomass yield (35 Mg ha^{-1}) (Table 2) were slightly higher than those reported in other double-annual cropping system areas, suggesting the high yield potential of a double-annual cropping strategy in irrigated Mediterranean conditions. For example, grain yields in this experiment were higher than those achieved by Iguácel et al. (2010) (17.5 Mg ha^{-1}), by Yagüe and Quílez (2013) (14.9 Mg ha^{-1}) under irrigation in the Ebro Valley, or by Zhang et al. (2006) (11-14 Mg ha^{-1}) with irrigated wheat-maize in China. In Mediterranean environments, biomass yields of 23-26 Mg ha^{-1} were reported in irrigated ryegrass-maize (Grignani et al., 2007), rainfed ryegrass-maize (Perramon et al., 2016), and rainfed triticale-maize (Ovejero et al., 2016). In addition, annual mono-cropping strategies in the Ebro Valley, usually irrigated maize, also achieved lower annual grain and biomass yields than the total annual yields obtained in the present study (Berenguer et al., 2009; Biau et al., 2012; Isla et al., 2015; Maresma et al., 2016; Yagüe and Quílez, 2013).

Normally, double-annual cropping strategies could significantly increase (25-50%) total dry matter production compared to mono-cropping systems (Crookston et al., 1978; Heggenstaller et al., 2008; Lloveras, 1987b; Raphalen, 1980). Despite the variability of grain and biomass yields among growing seasons, double-annual cropping yields seem to be more stable than mono-cropping systems, mainly maize, in irrigated Mediterranean conditions (Berenguer et al., 2009, 2008; Biau et al., 2012; Cela et al., 2013). This higher annual yield stability is probably because the total annual yield comes from two different harvests during a single growing season, which contributes to mitigating the yield variability of a single crop. Indeed, Borrelli et al. (2014) in a maize-based forage system in Northern Italy, concluded that the increase in biodiversity in agricultural systems, as occurs with double-annual cropping systems, has important implications in reducing temporal variability in maize yields. Therefore, as grain and biomass yields are more stable, the N recommendations among years may also be more stable.

The same amount of total annual N fertilizer split between the two crops achieved similar grain and biomass yields (barley + maize), independently of which crop received the N application. When applying a total of 200, 300 and 400 Kg N ha^{-1} per growing season (between both crops), statistically similar yields were obtained, independently of the N distribution between the two crops (Table 2). However, the optimum total N rates to achieve maximum grain and biomass yields were 232.5 and 240.5 kg N ha^{-1} yr^{-1}, respectively (Figure 4a).

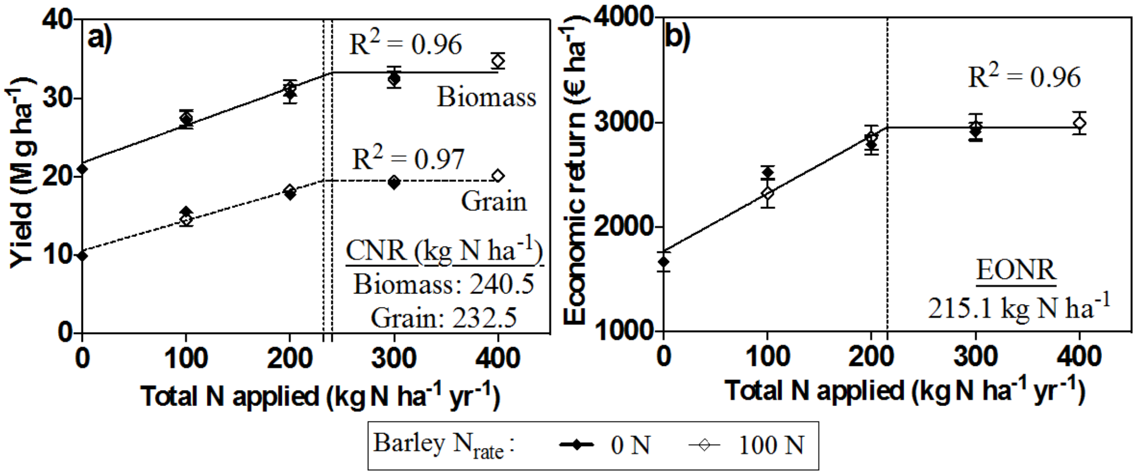


Figure 4. Response curves to total N applied (barley + maize) during a year of **a) total grain and biomass** and **b) Economic-return**. CNR: Critical fertilization N rate to achieve maximum yields. EONR: Economic optimum nitrogen rate. The N:cereal prices ratios were determined at 5.6:1 and 5.3:1 for barley and maize, respectively. Barley N$_{rate}$ indicates if 0 or 100 kg N ha^{-1} were applied to barley. Error bars indicate the standard error of the mean.

Probably, a significant amount of the N applied to each crop (barley or maize) that was not taken up by the crop was available for the following crop. This could be observed mainly in the non-fertilized barley, where the effect of maize residual N was evident in barley yield, with a rising tendency of grain and biomass yields when increasing the N$_{rate}$ applied in maize (Table 2). However, the N residual effect of barley N fertilization in maize yields was not as evident as the N residual effect of maize in barley. Probably, the higher OM mineralization during summer (Magdoff et al., 1984), compared with the other seasons, provided a high amount of N to maize and masked the possible effect of the barley residual N. Moreover, N sequestration of the barley stover (Salmerón et al., 2011) did not occur it because it was removed in the experiment.

4.2. Biomass and grain N content and total N uptake

Crop N uptake was highly influenced by yield because the differences in N content of the crops were lower than the yield differences among N treatments. The N content of both crops reported in this study was similar to that reported by other authors (Berenguer et al., 2008; Delogu et al., 1998; Perramon et al., 2016; Salmerón et al., 2011). Differences in N grain and biomass contents between N treatments were more clearly seen in maize than in barley and in biomass than grain. Non-fertilized maize (independently of barley N fertilization) had the lowest maize N content (grain and biomass), reflecting high N deficit of the crop in these conditions. The biomass N content of non-fertilized maize was clearly lower than the other N treatments (Table 3), probably because there was a high translocation of N from plant to grain to mitigate the N deficit (Cliquet et al., 1990).

The total annual N treatments above 200 kg N ha^{-1} yr^{-1} seem to present similar N grain and biomass contents independently of the total annual N_{rate}. Nevertheless, the higher yields achieved with high total annual N_{rate} contributed to increasing total N uptake with the double-cropping system (Figure 1). As expected, the maximum annual N uptake determined in this study (398.7 kg N ha^{-1}) was higher than that reported in other double-annual cropping studies (Grignani et al., 2007; Ovejero et al., 2016; Perramon et al., 2016). However, N uptake by each crop was in agreement with the reported percentages of total N uptake by the winter crop and summer crop in these previous works, which were 35% and 65%, respectively (Grignani et al., 2007; Perramon et al., 2016).

4.3. Soil NO_3^--N content

The soil NO_3^--N content after the barley or maize harvest showed high variation between the compared maize N treatments. However, barley N fertilization did not seem to have an effect on the amount of residual NO_3^--N after barley or maize harvest. Higher soil NO_3^--N concentrations were determined in the topsoil layer (0-30 cm) than in deeper layers after the maize harvest. This suggests that a major part of maize residual N could probably be used by the barley if this crop is sowed immediately after the maize harvest. The faster the subsequent barley is established, the lower the probability of losing the residual NO_3^--N from maize.

In double-annual cropping systems, the residual soil NO_3^--N from previous crops could potentially be taken up by the next crop thereby partially avoiding N leaching of nitrates (Ovejero et al., 2016). Winter crops mitigate N runoff after maize harvests caused by winter and early spring rains (Gabriel and Quemada, 2011; Hirel et al., 2011; Salmerón et al., 2011). Grignani et al. (2007), Perramon et al. (2016) and Ovejero et al. (2016) found an increase in winter crop yields after high N_{rate} applied to maize in double-annual cropping systems. Heggenstaller et al. (2008) proved that the extended annual growth duration of crops in double-cropping systems would lead to both increased dry matter production and reduced potential for NO_3^--N leaching compared to the mono-cropping corn system.

Though the highest amount of residual N after maize harvest was determined in the topsoil layer (0-30 cm), the residual N after the barley harvest was more concentrated in the deepest soil layer (60-90 cm). This fact suggests that the maize residual N that was not taken up by the barley, was leached to deeper layers and was more likely to be lost. When maize roots reach to explore these layers (60-90 cm), NO_3^--N would probably have been leached out of the system. The high temperatures during maize growing increased soil OM mineralization (Sinsabaugh, 2010). Thus, the low effects of residual N coming from barley N treatments (0 and 100 kg N ha^{-1}) in maize suggest that N provided by OM mineralization could be higher than the effects of barley residual N. Confirming this, in our study the calculated N mineralized from OM during the maize growing season was 160-170 kg N ha^{-1}, much higher than the residual amount of N after the barley harvest, 15 kg N ha^{-1} (Figure 2).

Traditional applications of 100 and 300 kg N ha^{-1} to barley and maize, respectively (Isidoro et al., 2006; Sisquella et al., 2004), seem to be excessive for our double-annual cropping system and could contribute to polluting the agro-ecosystem environment. However, with the lower N_{rate} (200-300 kg N ha^{-1} yr^{-1}) applied in the double-annual cropping system, almost maximum grain and biomass yields were achieved while the build-up of soil NO_3^--N was prevented. Therefore, the risk of off-site N transport is reduced (Krueger et al., 2012).

4.4. N efficiencies

The N efficiencies determined for the overall double-annual cropping system decreased as the N_{rate} increased, agreeing with the trend reported by Fageria and Baligar (2005) for cereal crops. The N efficiencies calculated in this study were similar or higher than the ones reported in similar conditions in mono-cropping maize (Berenguer et al., 2009; Martínez et al., 2016; López-Bellido et al., 2005; Bosch-Serra et al., 2015), or in double-cropping systems (Ovejero et al., 2016) fertilized with organic or inorganic N. Therefore, the increase of NUE contributed to mitigating N leaching while maintaining or increasing yields. Quemada et al. (2013) and Heggenstaller et al. (2008) concluded that replacing a fallow with a non-legume cover crop reduced N leaching by 50% and 34%, respectively. The establishment of two crops in the same year could help promote the higher efficiency of residual N (Yagüe and Quílez, 2013).

The high annual NRE (up to 2.77 kg biomass kg^{-1} N) could be explained by N deposition and biological N fixation that could involve relevant N contributions in Mediterranean and semi-arid areas (Quemada and Gabriel, 2016). Indeed, the total annual N mineralized in our study was estimated at 190 kg N ha^{-1} yr^{-1} (with a soil OM of 19.4 g kg^{-1} %). The increase in the N_{rate} applied provoked a reduction of maize ANR whereas barley ANR was increased. This seems to indicate that maize residual N had more impact on barley yields than barley residual N on maize yields. There were no significant differences in the annual ANR among N treatments, but a decrease was detected when the total amount of N applied to both crops was increased.

The total amount of N applied in a growing season below 200 kg N ha^{-1} could trigger a high risk of soil N mining (NUE > 0.9) (EUNEP, 2015). However, N_{rate} above 200 kg N ha^{-1} yr^{-1} showed NUE > 0.5, with this being classified as desirable for crop production by the EUNEP (2015). Thus, the double-annual barley-maize system seems to require applications above 200 kg N ha^{-1} yr^{-1} to maintain the sustainability of the system. Nevertheless, the highest total annual N_{rate} tested in the study (400 kg N ha^{-1} yr^{-1}) did not achieve as high an NUE as the 300 kg N ha^{-1} yr^{-1} rate, confirming higher N losses when fertilizer management is not optimized according to crop N requirements (Quemada et al., 2013).

4.5. Soil Organic Carbon

The non-variation of total SOC observed in the N treatments after 3 years of the double-annual cropping system (barley-maize) seems to indicate that in the short term the maize-barley system under study is sustainable. However, further trial years are required to confirm this sustainability. Heggenstaller et al. (2008) concluded that the increased extractions with the double-annual cropping system present a significant challenge for the maintenance of soil fertility and could potentially lead to SOC reductions if crop residues are not retained in fields. In a four-year study, Bertora et al. (2009) reported a higher induced C when residues were incorporated, but no SOC reduction when the residues were not incorporated. Therefore, the incorporation of maize stover, as was done during the 3 years in this study, seems to be sufficient to maintain SOC levels over a short-term period.

Other researchers have found an increase in SOC as a result of double-annual cropping systems. Grignani et al. (2007) reported an increase in SOC after 7 years of a continuous double-annual maize-based irrigated cropping system, and Krueger et al.

(2012) reported a 26% increase in SOC concentration from 0 to 5 cm over 3 years in a rye-maize silage system. In both cases, the increase in SOC was attributed to the application of organic fertilizers. In wheat-maize rotation, Fuentes et al. (2009) reported an increase in SOC when the residues were incorporated.

Even in the N treatments with the highest N deficit for crop growing (0 kg N ha^{-1} yr^{-1} applied), the SOC levels were maintained in the 3-year period of the experiment. Hence, further research is needed to guarantee the long-term sustainability of N fertilization strategies in double-annual cropping systems, especially, when a risk has been detected of soil N mining (NUE > 0.9).

4.6. Economic return

The economic return of the double-annual cropping system was strongly determined by the grain yields achieved. High grain yields were translated into high economic returns. The increase in total annual grain yields achieved in double-annual cropping systems compared with mono-cropping systems could entail higher profitability per land unit. However, the cost of field operations should be taken into account to adjust the real profit of each cropping strategy. In the Ebro Valley, and taking into account field operations, Gil (2013) reported a 13-22% gross margin increase of the double-annual cropping system (barley-maize) compared to mono-cropping maize. Therefore, the establishment of two crops in the same year may help to increase economic savings (Yagüe and Quílez, 2013).

Determination of the economic return of different N treatments is of interest because the optimum economic return is consistent with good environmental stewardship and could be used as a tool to determine crop N requirements (Sripada et al., 2008). Although the highest economic return was determined for the highest amount of total annual N applied (400 kg N ha^{-1} yr^{-1}) for the studied N:cereal price ratio, non-significant differences were detected with total N_{rate} above 200 kg N ha^{-1} yr^{-1}. Indeed, the economic return showed similar behaviour to N uptake, increasing with high N_{rate} (Figure 1a and 1b). However, a trend was seen of increasing N uptake in the highest total annual N treatment (400 kg N ha^{-1} yr^{-1}) which was not reflected in the economic return, suggesting luxury N consumption when there is excessive N in the soil. This could reduce the profitability of the system.

It was evident that at higher N:cereal price ratios (worse price relation for farmers), N efficiency will greatly affect the economic return of the cropping system. Sripada et al. (2008) tested price ratios (N:Maize) from 4:1 to 14:1, so the 5.6:1 and 5.3:1 price ratios used in our study were close to the optimum for farmers and were highly affected by grain yield. As the fertilizer to maize price ratio is positively correlated with NUE (Zhang et al., 2015); higher N:cereal price ratios will produce higher efficiencies according to the EONR. Similarly, Sripada et al. (2008) reported EONR variation according to the N:cereal price ratio. However, Schlegel et al. (1996) in an experiment with irrigated continuous maize concluded that in the same fields the EONR was relatively insensitive to cereal prices and the application of insurance N reduced crop profitability.

In irrigated Mediterranean high-yielding maize, Maresma et al. (2017) concluded that a maximum yield strategy is not normally the most profit-earning for farmers. However, low N:cereal price ratios contributed to reducing differences between the EONR (215.1 kg N ha^{-1} yr^{-1}) and the N_{rate} to achieve maximum yields (232.5 kg N ha^{-1} yr^{-1}) (Figure 5b).

5. Conclusions

The double-annual cropping system (barley-maize) has proven its high yield potential and stability in Mediterranean irrigated environments. The total annual sum of grain or biomass yields in the barley-maize system could be up to 20 and 35 Mg ha^{-1} yr^{-1} of grain and biomass, respectively. In Mediterranean conditions, these yields were rarely achieved by mono-cropping systems. In consequence, the double-annual cropping system could be an interesting alternative to increase yields without increasing the cultivated area. Moreover, the extended duration of the cropping season in double-cropping systems contributed to reducing the potential for NO_3^--N leaching, compared to the mono-cropping system. In a double-annual rotation, the following crop could use the residual N of the previous crop, enhancing the NUE of the cropping system. In irrigated Mediterranean environments, barley was especially more efficient in the uptake of maize residual N than maize in the uptake of barley residual N.

The total annual optimum N rates to achieve total annual maximum grain and biomass yields were 232.5 and 240.5 kg N ha^{-1} yr^{-1}, respectively. In concordance, the EONR was determined below the maximum grain yield strategy (215.1 kg N ha^{-1} yr^{-1}). However, at the determined EONR a very high NUE was obtained, suggesting some risk of soil N mining. Indeed, even in the N treatments with the highest N deficit (0 kg N ha^{-1} yr^{-1} applied), the yields were maintained in a three-year period without decreasing SOC levels. Thus, the sustainability of the different N fertilizer rates where there exists a risk of soil N mining (NUE > 0.9) should be tested over a long-term period. Further research is needed to fine-tune the N fertilization strategy of double-annual cropping system (barley-maize) over long-term periods.

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General Discussion

General Discussion

Traditionally, the aim of researchers in the field of nitrogen (N) fertilization has been the development of strategies that contribute to providing plants with enough N to maximize crop yields, while trying to keep N out of other ecosystems where it is harmful. Nitrogen excess has been recognized as a worldwide problem linked to agriculture for at least 40 years (Singh and Sekhon, 1979). Nowhere is N more important than in agricultural systems. The addition of N to sustain and increase crop yields is a pervasive and fundamental feature of modern crop management (Robertson and Vitousek, 2009). Proper N management is essential for the sustainability of agriculture. High-yielding ecosystems, as the studied in this thesis, pose an especially significant challenge to N management. As higher amounts of N are required to reach higher yields, higher N fertilization rates are applied, with the consequent risk of losing part of this nutrient and causing pollution.

The present research is in concordance with some of the proposed approaches of Cherry et al. (2008) for improving the N use efficiency (NUE) in high-yielding agricultural systems:

IV) *Provide farmers with decision support tools that allow them to better predict crop N requirements and avoid overfertilization*: the accuracy of soil sampling protocols, together with the usefulness of nitrate levels to fine-tune N fertilization of crops was tested in this thesis with a view to determining within-field N fertilization responsiveness and non-responsiveness.

V) *Better manage the timing, placement, and formulation of fertilizer N in cropping systems to ensure N is available where and when plant demand for N is greatest*: A relatively new technology for maize N assessment was tested: the use of high resolution multispectral aerial images. The methods developed can be used to determine in-season N requirements and to apply N more accurately where it is needed.

VI) *Adjust crop rotation to add complexity that improves uptake of available N*: The viability of a double-annual cropping system (barley-maize) was tested in order to add complexity that contributes by taking advantage of residual N, and any subsequent increase or decrease in annual yield was determined.

An in-depth discussion of the results obtained in the field experiments of this PhD thesis and their contributions to improving N fertilization of the Mediterranean irrigated agricultural systems is provided in the following sections.

1. Soil sampling protocols for OM and N determination

Determination of soil organic matter (OM) and N, as major determinants and indicators of soil fertility and quality, is important to have real information of the agricultural productivity of the soils (Al-Kaisi et al., 2005; Fageria and Baligar, 2005; Reeves et al., 1997). However, there is normally an important within-field variability that is affected by both temporal and spatial processes (Sogbedji et al., 2001; Wall et al., 2010). Thus, the determination of available N can vary spatially and temporally among fields influencing the N optimal rates to achieve maximum yields.

Although high variability has been observed among samples (spatial) and between seasons (temporal) in nitrate determination (Cambardella et al., 1994), the results of the present research demonstrated that nitrate levels were not impacted by the time of sampling. The higher soil OM levels determined in summer compared to fall, as reported by Wall et al. (2010), were probably the result of higher OM mineralization caused by higher temperatures during this season (Katterer et al., 1998; Kirschbaum, 1995). Soil nitrate distribution presented low-moderate spatial structure, suggesting high within-field variability. Several studies have shown a moderate correlation between nitrate values with ranges of <20 m (Gross et al., 1995; Jackson and Caldwell, 1993). In contrast to soil nitrate, soil OM displayed a high spatial structure (Baxter et al., 2003; Cambardella et al., 1994; Geypens et al., 1999) with correlations for distances of up to 50 m. High soil nitrate heterogeneity results in low levels of accuracy in determining the average field value. Even with high sampling densities (12.5 samples per ha), the probability of obtaining the true average nitrate value of the field was 85%. However, the high within-field homogeneity of soil OM required just 3.75 samples per ha to accurately determine (> 95% of probability) the average soil OM of a field.

The traditional practice in the Ebro Valley of adapting N fertilization based on soil N samples could be risky depending on the sampling intensity. It is undoubtedly better to have some information of soil N content than not to have any. However, accurate determination of the crop N rate to cover its N requirements will need a large number of samples. Grid soil sampling can be an accurate methodology to determine variable rate N application maps in some fields, but it is unlikely to be widely adopted because of the time and expense required (Ferguson et al., 1996).

To reduce the effect of soil nitrate heterogeneity in N fertilization, in this study the Illinois Soil Nitrogen Test (ISNT) was evaluated. The ISNT estimates the soil N supply potential for field specific recommendations (Khan et al., 2001; Mulvaney et al., 2001). In the environments of New York (USA), the ISNT adjusted for soil OM has been successfully used to determine soil N supply potential. This determination has contributed to improving traditional N recommendations in maize (Klapwyk and Ketterings, 2006). Moreover, the ISNT reflects a high spatial structure that contributes to reducing the number of samples needed to fine-tune maize N fertilization. Further research is needed to verify the usefulness of the ISNT for adjusting N fertilization in Mediterranean conditions, but it could be an excellent tool for improving N practices.

2. Use of multispectral aerial images to detect maize N deficiencies

In the Ebro valley (NE Spain), high-yielding maize entails N uptakes of over 300 kg N ha^{-1} yr^{-1} (Berenguer et al., 2008; Biau et al., 2012). These high N uptakes, together with the previously reported temporal and spatial within-field soil N variability, have motivated the development of technologies to accurately determine N fertilization requirements. Multispectral aerial images could be used for this purpose due to their ability to detect maize N deficiencies at growing stages when they can still be corrected. Aerial image acquisition after V8 stage (8 leaves with visible leaf collar) seems to be consistently useful to determine maize N status and to predict yield (Bausch et al., 2008; Bausch and Khosla, 2010; Isla et al., 2011; Cilia et al., 2014; Quemada et al., 2014). Sripada et al. (2005) described this technology as a fast and accurate method to determine in-season maize N requirements, which is needed to provide more precise and economical management and potentially decrease N pollution.

In irrigated Mediterranean conditions, Isla et al. (2011) and Quemada et al. (2014) have also proven the usefulness of multispectral images to improve N management in maize. Nevertheless, in order to verify previous studies, the use of multispectral images to determine N fertilization of maize in high-yielding environments needs to be evaluated. The results of the present research suggest that green-based vegetation indices are highly correlated with maize N status. Moreover, green-based indices were able to predict grain yield and non-responsive maize N status with greater accuracy than red-based indices. This could be due to problems of saturation associated with red-based indices for some types of vegetation during their later growth stages (Isla et al., 2011). Therefore, green-based vegetation indices derived from multispectral aerial images could be used to improve N management. For instance, the Green Chlorophyll Index (GCI) was determined as the most useful index because of its capacity to distinguish among maize N statuses up to 84% of maximum yield. The detection of maize N status that would yield less than 84% of maximum yield and its correction by N fertilization could improve N management and possibly maize grain yields in irrigated Mediterranean conditions. In order to determine grain yield variability that vegetation indices could not distinguish in high-yielding areas (above 84% of maximum yield), the Wide Dynamic Range Vegetation Index (WDRVI) could be used. At very high spatial resolutions, the WDRVI was the best index for distinguishing between treatments with applications above or below 250 kg N ha^{-1}. Although there would theoretically be little interest in increasing N application above 250 kg N ha^{-1}, mainly because many areas have been declared N vulnerable areas (DOGC, 2009), the present research showed a tendency for increased grain yield with higher N rates. This could be of particular interest in maize-growing areas such as those of the Ebro valley, even with the N reduction regulations, which have the appropriate environmental conditions and irrigation facilities to attain such high yields. Probably, in these cases, the determination of the available N at planting or the N supply potential of the soil (by the ISNT method, suggested above) would also contribute to fine-tuning in-season N recommendations.

In this thesis, two different technologies were used to carry the camera to acquire the aerial images: the aircraft and the emerging Unmanned Aerial Vehicles (UAVs). Traditionally, aircrafts and satellites have been used successfully for growth monitoring and real-time management at field scale (Daughtry et al., 2000; Zhang et al., 2011; Zarco et al., 2013) with the capacity to cover large surface areas. However, the growing use of commercial UAVs is seen as another possibility to quickly and repeatedly acquires multispectral images.

Although both methods (aircraft and UAVs) can be used to capture aerial images of maize fields, several differences between UAVs and aircraft were detected in this study. The UAV system allowed higher spatial resolution than the aircraft (0.15 and 0.25 m, respectively), but lower radiometric resolution (8 and 14 bits/pixel, respectively). In addition, the airborne camera used on the aircraft had 4 spectral bands (blue, green red and near infrared), whereas the UAV camera had 3 (green, red and near infrared). Therefore, the UAV image had more detail of the crop surface (higher spatial resolution) than the aircraft camera, but the pixels were able to differentiate the reflectance in fewer categories. These findings can be useful for the implementation of this technology by farmers. UAV services could be used in early stages because soil pixels that distorted the images could be more accurately removed than with the aircraft images. Similarly, the UAV services could help farmers to detect weeds or irrigation defaults at very early stages (Peña et al., 2013). However, to determine sufficient and non-sufficient maize N status, the higher radiometric resolution of the airborne camera will probably contribute to a better assessment of N recommendations.

3. Double-annual cropping systems

The high-yielding conditions of irrigated maize in the Ebro valley have influenced farmers in the application of high N rates to this crop. Indeed, vulnerable N zones have been declared in some areas of the Ebro Valley as a consequence of high N application over the years. Surveys in this area (Cavero et al., 2003; Isidoro et al., 2006) have shown an excess of N fertilizer applied to the agricultural systems. When an excess of N fertilizer is applied, there is a high risk of N leaching during the maize intercrop period (October to April) (Moreno et al., 1996) which contributes to reducing crop profitability. To avoid leaching of residual N and to increase profitability per land unit, double-annual cropping systems can be implanted.

The results of the study undertaken for this thesis confirm that double-annual cropping systems increase the NUE compared to mono-cropping systems. This is mainly due to the clear residual effect of maize N fertilization on the subsequent barley. The winter crop (barley) was able to use the maize residual N and, when maize residual N was high, showed no yield response to N fertilization. However, barley residual N did not significantly affect maize yields. Probably, the high soil OM mineralization during summer (Magdoff et al., 1984), which was around 160 kg N ha^{-1}, provided the maize with a high amount of N and masked the possible effect of barley residual N.

Double-annual cropping strategies could also help farmers to increase total annual yields (Yagüe and Quílez, 2013) and field gross margin (Gil, 2013) per land unit, while reducing NO_3^--N runoff (Gabriel and Quemada, 2011; Krueger et al., 2012). The most efficient N fertilization strategies, in the double cropping systems used in the field trials, were the ones in which N was split between the two crops, avoiding the application of high N rates that account for a higher risk of N leaching. Though the total optimum N rates reported for double-annual cropping systems (230-240 kg N ha^{-1} yr^{-1})

were slightly lower than those determined for achieving maximum maize grain yields in mono-cropping conditions (Iguácel et al., 2010; Maresma et al., 2016), higher annual grain yields and NUE values were determined in the double-annual cropping system used. In this situation, soil sampling could be a useful technique because the N rate of one crop could be adjusted depending on the residual N of the previous crop. Nevertheless, as higher NUE values and annual yield stability were achieved in double-annual cropping system compared to mono-cropping ones, there is a lower risk of N leaching if sustainable N management is continued over time.

4. General overview

There is a global interest in improving agricultural practices to increase yields and NUE at the same time (Robertson and Vitousek, 2009). However, in many agricultural areas, N fertilization (especially of extensive crops) is mostly underpinned by the simplification of fieldwork management and by avoiding the risk of under-fertilization. This practice normally leads to the application of high N rates at early crop stages, which can trigger over-fertilization. To avoid these practices, farmers should be supported with decision tools to predict crop N requirements and to determine its time of application. The present research aims to verify the usefulness of different strategies to improve NUE in Mediterranean irrigated environments.

Despite the usefulness of the described tools to aid N management, their adoption by farmers is still limited. The implementation of an accurate soil sampling strategy as well as the adoption of remote sensing technologies require investment and knowledge that in most cases are seen as economically unrecoverable or difficult to acquire. On the other hand, the popularity of the double-annual cropping strategy is growing among farmers, who see increased annual yields and land profitability. The results of the double-annual cropping system have been especially interesting in new irrigation areas where high investments in efficient irrigation systems have been made. The increase in the gross margin of double-annual cropping systems compared to mono-cropping ones contributes to recovering the investment made in irrigation systems. However, there are still cultural practices that make some farmers reluctant to adapt complex crop rotation systems.

This study has attempted to underline the potential profitability that the implementation of various technologies to better adjust N fertilization could entail. Farmers will see a reduction in N fertilizer applied to the field (which entails a reduction in N fertilizer cost), while maintaining yields. Both farmers and the environment will be taking advantage of technological advances to increase profitability and sustainability, respectively.

5. Future research

Successful achievements of studies can open the door to further research in many directions. By way of example, further studies in this field could focus on:

I) *Soil sampling protocols*:

- The evaluation of different soil N tests to better determine the N supply potential and the total N available for the crop in a growing season. The ISNT method could be tested in Mediterranean conditions to verify its usefulness in other parts of the world.
- Determination of soil N patterns in cultivated fields in Mediterranean environments to determine optimum soil sampling intensity for accurate prediction of N availability for the subsequent crop.

II) *Multispectral aerial images*:

- Maize response to N fertilization when deficits are detected at V12 stage (image acquisition). As maize has absorbed around 40% of the total N at that stage, a high response to N fertilization is expected if N is applied at this stage. However, the efficiency of maize in terms of absorbing N after the V12 stage should be evaluated to fine-tune N management as determined by multispectral aerial images.
- Assessment of maize N fertilization in double-annual cropping systems. In double-annual cropping systems lower maize yields are achieved and faster plant growing and development take place (compared to monocrop maize) due to the more favourable conditions (especially, temperature). Therefore, as less N application is provided to maize in this double-annual cropping system (compared to monocrop maize), there is less capacity for improving maize N fertilization. The usefulness and profitability of multispectral aerial images remain uncertain and should be evaluated in emerging maize-based double-annual cropping systems.

III) *Double-annual cropping systems*:

- Evaluation of the long-term sustainability of the proposed double-annual cropping system (barley-maize) unlinked to livestock farming. In this research, the maize stover was incorporated whereas the barley straw was removed. This management strategy did not entail a reduction in soil OM over a 3-year period (duration of the experiment). However, future long-term experiments could verify whether the high yields achieved in this crop rotation system do not jeopardize the sustainability of the agro-ecosystem over a long-term period.
- Evaluation of alternative crops, such as pulses, to increment the complexity of the system and take advantage of the combination of an N-fixing crop (pulse) with a high N-demanding crop (maize). These alternatives could increase the NUE of the field due to lower N input requirements. However, the yields achieved by the winter crops must be analysed to verify their profitability.

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General Conclusions

General Conclusions

The present research thesis addresses three key aspects in nitrogen (N) management strategies to improve the efficiency of N fertilization in high-yielding irrigated agro-ecosystems: a) the soil sampling strategy to determine the amount of organic matter and nitrates, b) the N status at V12 from multispectral aerial images and its relation to yield, and c) the convenience of a double-crop (barley-maize) system to improve N efficiency. In view of the results obtained, the following conclusions can be drawn.

I) The Illinois soil nitrogen test (ISNT) is recognized as an alternative method to reduce the sampling density required to determine the amount of nitrates available in the soil. Its within-field homogeneity, together with its capacity to estimate soil N supply potential, could be useful to improve N fertilization.

II) Sampling density should be adapted to the object of study. Optimum soil sampling densities of 3.75 and 12.5 samples ha^{-1} were determined for OM and nitrates, respectively.

III) Green-based vegetation indices (VIs) are more accurate than red-based ones in predicting grain yield and determining the optimum N rate for maize at V12 stage. The Green Chlorophyll Index (GCI) was the most notable of the VIs due to its ability to distinguish among maize N status up to 84% of maximum grain yield.

IV) The Wide Dynamic Range Vegetation Index (WDRVI), at V12 stage and at very high spatial resolution, could overcome the uncertainty of fertilizing apparently well-nourished maize areas that do not achieve maximum yields. It was able to distinguish between applications below or above 250 kg N ha^{-1}.

V) The double-annual cropping system (barley-maize) in irrigated Mediterranean environments shows higher yield potential and stability when compared to mono-cropping systems. Average annual grain and biomass yields were as high as 20 and 35 Mg ha^{-1}, with N rates of 230-240 kg N ha^{-1} yr^{-1} split between the two crops.

VI) Barley is able to efficiently use maize residual N, which contributes to increasing N use efficiency (NUE) of the double-annual cropping system compared to mono-cropping strategies.