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COORDINATORE Prof. Giacomo Pietramellara

*Agronomic techniques for precision management of field crops*

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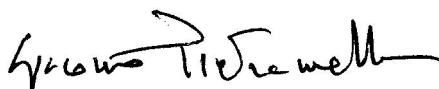


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Integrating satellite data with a Nitrogen Nutrition Curve for precision top-dress fertilization of durum wheat. Fabbri C., Mancini M., Dalla Marta A., Orlandini S., Napoli M., (2020). *European Journal of Agronomy*. DOI: 10.1016/j.eja.2020.126148

Effect of soil available P and N on wheat production in P-deficient soil conditions. Napoli M., Fabbri C.\*, Guerrini L., Orlandini S., Mancini M. Not yet submitted

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## Introduzione

Nel panorama agricolo mondiale l'Unione Europea sta varando misure straordinarie in termini di ambizione e risorse economiche allocate. Nella visione del Green Deal ritroviamo la necessità di coniugare produttività, qualità delle produzioni e rispetto dell'ambiente delle coltivazioni (EU, 2019). In questa ottica uno dei principali ruoli attuali della ricerca agronomica è quello di utilizzare i moderni sistemi di analisi, monitoraggio e intervento, legati all'agricoltura di precisione per migliorare gli aspetti produttivi, qualitativi e ambientali delle coltivazioni.

Con l'avvento della meccanizzazione, a partire dalla metà del secolo scorso, l'agricoltura ha infatti perso gran parte della sua capacità di gestire la variabilità spaziale e temporale che caratterizza la coltura all'interno delle singole unità produttive. In un contesto di elevata variabilità pedologica e meteorologica gli agricoltori somministrano fertilizzanti basandosi sul principio che questi non devono essere fattori limitanti per le aree del campo con maggiori aspettative produttive. Con una distribuzione uniforme degli input si opera spesso un sovradosaggio nelle aree dell'appezzamento meno produttive. La ricerca di razionalizzazione dei costi di produzione, unita alla crescente consapevolezza ambientale, sta guidando la ricerca scientifica verso lo sviluppo di macchine agricole "intelligenti" in grado di eseguire rapidamente una gestione sito specifica delle colture. Questa svolta deve tenere conto della conoscenza della variabilità dello stato fenologico e vegetazionale delle colture in campo (Basso et al., 2012; Diacono et al., 2012) e della variabilità del suolo nei suoi principali aspetti di contenuto di elementi nutritivi disponibili e di caratteristiche fisiche.

In questo nuovo scenario produttivo la gestione delle concimazioni diviene uno strumento fondamentale per perseguire il miglioramento della produttività, della qualità delle produzioni sia per gli aspetti tecnologici di trasformazione sia per quelli nutraceutici e salutistici, e dell'efficienza d'impiego degli input volta al controllo dell'impatto ambientale.

### **Aspetti produttivi del frumento legati alla concimazione azotata di precisione**

Per il monitoraggio dei parametri qualitativi e quantitativi della vegetazione, tradizionalmente effettuato attraverso rilievi visivi e/o manuali le tecniche di rilievo remoto possono ora essere applicate attraverso lo sviluppo di un'ampia varietà di indici di vegetazione specifici. Gli indici vegetazionali sono in grado di descrivere con buona attendibilità statistica le differenze vegetazionali all'interno delle unità produttive ma hanno maggiore difficoltà ad essere associate ai fattori della crescita e dello sviluppo causa delle differenze stesse. La maggior parte delle pubblicazioni che analizzano la variabilità spaziale delle produzioni frumenticole si basa sul rapporto della pianta con il fertilizzante azotato. L'azoto è il principale macronutriente limitante o scarsamente disponibile nei suoli (Guignard et al., 2017). La disponibilità di N è limitata dalla sua elevata mobilità nel suolo ed è regolata principalmente dalla quantità di sostanza organica e dal tasso di mineralizzazione (Witing et al., 2019). Il tasso di mineralizzazione è a sua volta influenzato da diversi fattori quali umidità, contenuto di sostanza organica, pratiche agronomiche adottate (Selles et al., 2011; Johnston et al., 2014; Sun et al., 2015), e da una complessa dinamica di interazioni con molte caratteristiche chimico-

fisiche del suolo quali temperatura, pH, tessitura, ecc. (Lindsay et al., 1989; Sato et al., 2005; Widdig et al., 2019; Brucker et al., 2020).

La fertilizzazione azotata ha lo scopo di integrare le carenze di azoto disponibile nel suolo e di consentire al frumento una piena espressione delle potenzialità produttive in funzione delle altre caratteristiche pedoclimatiche. Le strategie di fertilizzazione azotata sono molteplici e tutte hanno lo scopo di soddisfare le esigenze nutrizionali della pianta e di contenere la distribuzione di fertilizzante entro i termini di convenienza economica dell'attività. In vaste aree collinari del centro Italia caratterizzate da produttività di frumento attorno a 4000 kg ha<sup>-1</sup> si effettuano 2 o 3 distribuzioni di copertura nelle fasi fenologiche che vanno dall'accestimento agli stadi avanzati della levata. La distribuzione temporale del fertilizzante è fortemente condizionata dal peculiare andamento meteorologico che caratterizza ogni singola stagione. La distribuzione spaziale viene effettuata sempre più frequentemente sulla base di monitoraggio remoto basato su differenti indici vegetazionali. Gli indici di vegetazione hanno mostrato essere strettamente correlati a una serie di proprietà biofisiche e processi vegetali, tra cui la produzione primaria, la percentuale di copertura vegetale, la biomassa delle foglie verdi e i flussi di anidride carbonica. Il Normalized Difference Vegetation Index (NDVI) è probabilmente l'indice più conosciuto e più utilizzato. È direttamente correlato alla capacità fotosintetica, e quindi all'assorbimento di energia delle chiome delle piante (Inoue et al., 2008). Le osservazioni NDVI sono state frequentemente applicate per comprendere le caratteristiche e la variabilità della vegetazione da scala locale a scala globale. Questo indice mostra correlazioni significative con i cambiamenti nella concentrazione di pigmenti fogliari, l'accumulo di sostanza secca aerea, il LAI e contenuto di acqua (Ceccato et al., 2001; Aparicio et al., 2002; Sims e Gamon, 2003, Dalla Marta et al., 2019), la risposta della coltura all'apporto di fertilizzante azotato (Tilling et al., 2007).

Tuttavia, nel descrivere la *canopy*, l'adozione dell'NDVI e di altri indici è principalmente limitata dagli effetti della trasparenza dell'atmosfera, all'effetto del suolo nelle prime fasi vegetative e dall'effetto della saturazione dell'indice quando la vegetazione diventa molto densa ed il LAI supera valori critici di 2,5-3,0 (Dalla Marta et al., 2015). Buoni risultati sono stati ottenuti anche con indici basati su peculiari bande, in particolare la banda red-edge per l'NDVI Red Edge, che hanno mostrato minore sensibilità al fenomeno della saturazione (Delegido et al., 2013) o il Modified Chlorophyll Absorption in Reflectance Index (MCARI). Rispetto all'NDVI, questi indici mostrano in particolari condizioni migliori correlazioni con lo stato nutrizionale delle colture e il contenuto di clorofilla delle foglie delle piante (Perry et al., 2008). Altri indici sono stati messi a punto con lo scopo di ridurre la fonte di variazione dovuta all'effetto del suolo e quindi per avere una buona attendibilità anche nelle prime fasi fenologiche quando la *canopy* non copre completamente il terreno. Tra questi, sono stati ampiamente utilizzati il Soil Adjusted Vegetation Index (SAVI) (Huete, 1988) e la versione "Optimized" (OSAVI) (Rondeaux et al., 1996). Alcuni studi hanno mostrato come l'indice di Greenness, calcolato come LAI \* SPAD, possa essere utilizzato insieme agli indici di vegetazione telerilevati per valutare il livello di azoto nella chioma e la sua risposta alla fertilizzazione azotata (Camarano et al., 2011).

Per molti anni uno dei limiti di impiego del telerilevamento nei sistemi di supporto alla gestione agronomica a livello di azienda agricola è stata la mancanza di un'adeguata risoluzione spaziale e

temporale delle immagini in grado anche di seguire lo sviluppo delle colture in piccole aree. Fino a pochi anni fa le immagini a risoluzione spaziale moderata-alta (10–30 m) avevano una bassa frequenza di rivisitazione (es. Landsat/ETM), mentre una risoluzione temporale più elevata era spesso caratterizzata da una bassa risoluzione spaziale (250 – 1000 m) (es. MODIS). Successivamente alcuni satelliti commerciali quali il Quikbird, il WorldView, il RapidEye hanno permesso di arrivare a risoluzioni idonee al contesto operativo delle colture estensive, quali il frumento, oltre che avere una frequenza di rivisitazione tale da ovviare ai problemi di acquisizione causati dalla trasparenza dell'atmosfera (Vuolo et al., 2010; Beerli e Peled, 2009).

Con i recenti satelliti, come WorldView, RapidEye, Sentinel 2, il tempo che trascorre dall'acquisizione delle immagini alla restituzione dei dati è breve. Ciò rende possibile l'utilizzo di immagini spettrali nella gestione operativa grazie all'utilizzo di specifici modelli. Tali modelli tengono conto di diverse esigenze e parametri relativi alla coltivazione delle colture, come lo stress idrico (Altenbach, 2012) e lo stato nutrizionale rispetto all'azoto (Cammarano et al., 2011; Eitel et al., 2011), il vigore delle piante e la crescita della biomassa (Filella and Penuelas, 1994; Broge and Leblanc, 2001), la previsione della resa dei cereali (Labus et al., 2002), la presenza di malattie (Hervè, 2004) ed il controllo delle infestanti (Yan et al., 2012), le condizioni del suolo ed i *trend* meteorologici (Dalla Marta et al., 2011; Guasconi et al., 2011; Moldestad et al., 2011). Altri studi hanno integrato i dati del telerilevamento con modelli di simulazione delle colture per valutare la resa delle colture su scala regionale (Mkhabela et al., 2011).

### **Aspetti produttivi del frumento legati alla variabilità del fosforo nel terreno e alla concimazione fosfatica sito-specifica**

La variabilità spaziale della vegetazione all'interno delle unità produttive può essere ricondotta a differenti fattori associati alla crescita e allo sviluppo. Fra questi i macronutrienti quali l'azoto e il fosforo rappresentano sicuramente dei fattori chiave in molti ambienti pedoclimatici. Il fosforo è un macronutriente che presenta notevole variabilità spaziale in molti terreni coltivati, sia a grande sia a piccola scala (Roger et al., 2014; Delgado, 2019).

Differenti autori hanno messo in luce come la produttività del frumento sia condizionata, in tutti quei casi in cui gli apporti di fertilizzante non soddisfano le esigenze della pianta, dal livello di fosforo disponibile nel suolo. In tutti questi casi si evidenzia come i contenuti di fosforo disponibile compresi fra 16 e 20 mg kg<sup>-1</sup> terreno rappresentino soglie critiche in differenti condizioni pedoclimatiche e come la produzione di frumento cali proporzionalmente al contenuto di fosforo disponibile (Bai et al., 2013; Tang et al., 2009; Shatar e McBratney, 1999; Senthilkumar et al., 2012; FU et al., 2012).

L'impiego di telerilevamento per il monitoraggio dello stato vegetativo della coltura può spesso trarre in inganno circa la corretta concimazione da effettuare. Nella maggior parte dei casi dove si ricorre all'agricoltura di precisione per le fertilizzazioni si cerca di compensare o accompagnare gli squilibri vegetativi solamente con l'impiego di fertilizzante azotato. La ricerca delle cause degli squilibri spaziali della vegetazione, entro le unità produttive, deve essere estesa ai molteplici fattori di crescita e produzione. Fra questi il fosforo gioca, per il frumento, un ruolo chiave in molti terreni coltivati. La gestione della fertilizzazione di precisione deve quindi comprendere anche la variabilità degli

elementi nutritivi “immobili” nel terreno al fine di compensare gli squilibri nutritivi secondo la legge di “Liebig”. La gestione della variabilità di gli elementi nutritivi deve infatti basarsi sul principio secondo il quale le prestazioni delle colture possono essere ottimizzate applicando nutrienti in quantità bilanciate su terreni che non sono in grado di fornire questi nutrienti per soddisfare la domanda delle colture. La nutrizione equilibrata delle colture mira a un equilibrio dinamico tra il fabbisogno delle colture e l'assorbimento dei nutrienti da parte delle colture ed è una chiave per migliorare l'efficienza nell'uso dei fertilizzanti. Un apporto di nutrienti secondo il fabbisogno potenziale della coltura, nello specifico contesto pedoclimatico, ha l'obiettivo di ottenere uniformità spaziale delle produzioni e non di gestirne la variabilità.

L'apporto sito-specifico di fertilizzante fosforico sito-specifica è pertanto uno degli aspetti più interessanti per l'evoluzione dell'agricoltura di precisione per molti terreni ove le richieste della coltura non sono soddisfatte dalla dotazione dei suoli. La metodologia per la realizzazione di mappe di prescrizione richiede però un oneroso costo per le analisi chimiche dell'elemento nel suolo in un numero di punti rappresentativo della variabilità dell'elemento. Fra le nuove tecniche per il monitoraggio delle variabilità spaziale può essere impiegato l'*electrical resistivity profiling* eventualmente combinato con le mappe di vegetazione. Tali metodologie sono già state utilizzate per il rilievo di differenti caratteristiche del suolo quali la vulnerabilità ai nitrati, la presenza di sostanza organica, ecc. (Pezzuolo et al., 2016; Cillis et al., 2019). Il loro impiego riduce in maniera importante il numero di campioni di terreno su cui eseguire analisi di laboratorio e può inoltre costituire una base per la realizzazione di mappe di variabilità spaziale di altri elementi nutritivi del suolo.

### **La concimazione solfatica quale elemento condizionante la qualità tecnologica delle produzioni di frumento**

La qualità tecnologica delle farine panificabili è strettamente legata alla quantità e alla qualità del glutine contenuto nelle cariossidi. Molte filiere locali hanno difficoltà nel mantenimento degli standard qualitativi dei prodotti finali riconducibili al contenuto di glutine delle farine e conseguentemente alla qualità reologica degli impasti. Fra queste possiamo citare tutte le filiere basate sulle vecchie varietà di frumento, antecedenti quel miglioramento genetico che ha consentito l'incremento del glutine e tutte le produzioni di beni alimentari derivanti da disciplinari che legano la produzione frumenticola a specifici territori. Quest'ultime sono affette molto spesso dall'influenza della variabilità meteo-climatica si manifesta anche sui parametri qualitativi quali il contenuto proteico (Dalla Marta et al., 2011, Don et al., 2005). Fra le tecniche sperimentali studiate per migliorare la qualità tecnologica dei semilavorati del frumento ritroviamo il condizionamento del frumento (Kweon et al., 2009), le soluzioni legate alla macinazione (Cappelli et al., 2020; Hackenberg et al., 2018); i miglioramenti al processo di impasto (Bayramov & Nabiev, 2019); l'impiego di composti atti a migliorare l'impasto (Farbo et al., 2020; Tebben et al., 2018).

Il condizionamento delle proprietà reologiche degli impasti può essere effettuato anche con tecniche agronomiche adottate in fase di coltivazione, in un'ottica di agricoltura di precisione volta ad un elevato controllo del processo produttivo (Maignant et al., 2021).

Fra le principali tecniche agronomiche adottate ritroviamo le fertilizzazioni azotate. Queste hanno molti vantaggi in quanto l'N è un elemento essenziale per la crescita della pianta e la biosintesi delle proteine (Shewry et al., 2013). In tal senso gli agricoltori sono soliti applicare input elevati di N per soddisfare la domanda della coltura ed ottemperare ai requisiti premiali nella vendita del frumento, ma questa strategia presenta importanti rischi ambientali legati alla lisciviazione e volatilizzazione dell'azoto (Rasheed et al., 2014; Novoa R, & Loomis R.S., 1981; Triboni et al., 2000). Nella ricerca della uniformità del contenuto di glutine all'interno delle unità produttive ritroviamo anche tecniche di telerilevamento e gestione sitospecifica del fertilizzante azotato finalizzate al miglioramento delle proprietà reologiche (De Santis et al., 2021). Anche l'impiego di fertilizzanti a rilascio controllato e la fertilizzazione azotata fogliare sono tecniche che hanno mostrato la loro validità nel favorire l'accumulo di azoto post-antesi e il contenuto proteico della granella (Zhang et al., 2021).

La disponibilità di zolfo, al pari di quella dell'azoto, ha un effetto sulla resa del frumento (Withers et al. 1995; Zhao et al. 1999). Salvagiotti e Miralles (2008) per un solo sito e per una sola varietà trovano un effetto della fertilizzazione con zolfo sull'aumento del numero di cariossidi per unità di superficie ma non una variazione del peso delle cariossidi. In particolare l'aumento del numero di cariossidi è legato all'incremento del numero di spighe per unità di superficie, senza variazioni del numero di cariossidi per spiga. La fertilizzazione solfatica ha un effetto sinergico sull'impiego dell'azoto da parte della pianta. È stato dimostrato che questo effetto è dovuto a un maggiore capacità di assimilazione dell'azoto dal terreno, senza cambiamenti nell'efficienza interna alla pianta (Salvagiotti et al. 2009; Gülüt et al., 2021).

La fertilizzazione azotata del frumento modula l'espressione del genotipo variando anche la composizione quantitativa del glutine, definita dai pesi relativi delle diverse frazioni proteiche. Un'elevata disponibilità di azoto aumenta la frazione delle gliadine e l'estensibilità dell'impasto (Godfrey et al. 2010). Anche la fertilizzazione con zolfo svolge un ruolo chiave nella formazione dei legami disolfuro, definendo le proprietà viscoelastiche dell'impasto. In abbondanza di zolfo le frazioni proteiche ricche di zolfo ( $\alpha$ ,  $\beta$ ,  $\gamma$  gliadine) del frumento risultano aumentate rispetto alle componenti proteiche povere di S ( $\omega$ -gliadine) (Shewry et al. 2009).

### **L'impatto ambientale delle fertilizzazioni nella coltivazione del frumento**

Un aspetto importante da considerare per la programmazione delle scelte strategiche a medio termine dell'azienda agricola è l'indirizzo della politica dell'Unione Europea in materia di agricoltura. Su tali indirizzi si attueranno le future politiche locali di sostegno.

In tal senso la Commissione Europea ha presentato nel 2019 un piano d'azione, denominato Green Deal, con l'obiettivo di riduzione delle emissioni di gas a effetto serra per il 2030 di almeno il 50 % rispetto ai livelli del 1990, e di raggiungere la neutralità emissiva entro il 2050.

Per raggiungere gli obiettivi del Green Deal europeo sarà necessario ridisegnare le politiche per l'approvvigionamento di energia pulita in tutti i settori dell'economia ed elaborare una serie di politiche profondamente trasformative in materia di agricoltura ed ambiente. In particolare due ulteriori Comunicazioni chiariscono il ruolo dell'agricoltura rispetto agli obiettivi del Green Deal:

- Comunicazione della Commissione Europea "Strategia dell'UE sulla biodiversità per il 2030 - Ripartire la natura nella nostra vita" (Bruxelles, 20.5.2020 COM (2020) 380);
- Comunicazione della Commissione Europea "Dal produttore al consumatore - per un sistema alimentare equo, sano e rispettoso dell'ambiente" (Bruxelles, 20.5.2020 COM (2020) 381).

Nelle due comunicazioni alcuni temi si sovrappongono e/o si completano a vicenda e non si distinguono ambiente e agricoltura come due entità separate.

Nella comunicazione “Strategia dell’UE sulla biodiversità” fra gli obiettivi principali che riguardano l’agricoltura ritroviamo la riduzione dell’inquinamento causato dai flussi di azoto e fosforo legati all’inefficienza d’uso dei fertilizzanti come minimo dimezzando le perdite d’inefficienza dei nutrienti. Nella comunicazione “Dal produttore al consumatore” viene riportato che il settore agricolo è responsabile del 10,3 % delle emissioni di gas a effetto serra dell’UE. Quasi il 70 % di esse proviene dal settore dell’allevamento e consiste in gas a effetto serra diversi dalla CO<sub>2</sub> (metano e protossido di azoto principali responsabili). In tale scenario fra gli obiettivi che vengono posti per 2030 ritroviamo:

- riduzione delle perdite di nutrienti (in particolare azoto e fosforo) di almeno il 50% garantendo, nel contempo, che non si verifichi un deterioramento della fertilità del suolo;
- raggiungimento di almeno il 25 % della superficie agricola dell’UE investita a agricoltura biologica; gli agricoltori dovranno avere accesso a una gamma di sementi di qualità di varietà vegetali adattate alle pressioni esercitate dai cambiamenti climatici.

Per realizzare molti di questi obiettivi viene indicato come strumento operativo i finanziamenti distribuiti attraverso la Politica Agricola Comunitaria (PAC).

In tale contesto l’analisi degli impatti ambientali legati alla coltivazione del frumento, sia in conduzione biologica che convenzionale, rappresenta l’elemento fondamentale per definire le fasi e gli input causa di inquinamento (Maisterling et al., 2009; Tuomisto et al., 2012; Van Stappen et al., 2015).

Il Life Cycle Assessment (LCA) è una metodologia standardizzata per la valutazione degli impatti legati anche alle produzioni agricole. LCA esamina le diverse categorie di impatto, tra cui il riscaldamento globale, l’acidificazione, l’eutrofizzazione, ecc. consentendo così un’indagine completa sui diversi processi di produzione. In questo senso, utilizzando l’LCA, è possibile valutare l’impatto globale di uno specifico alimento durante l’intero processo produttivo e confrontare diversi processi produttivi (Brentrup et al., 2004; Fallahpour et al., 2012; Van Stappen et al., 2015).

L’impiego dei fertilizzanti in agricoltura risulta un elemento critico per la sostenibilità ambientale delle coltivazioni. Fra i fertilizzanti sotto accusa ritroviamo l’azoto sintetico e il fosforo (Guignard et al., 2017). Contemporaneamente le vecchie varietà di frumento, con genotipi molto variabili rispondenti ad una maggiore adattabilità alla variabilità pedologica e meteorologica, sembrano rispondere bene produttivamente anche con minori input di coltivazione (Migliorini et al., 2016; Fatholahi et al., 2020). La “Farm to fork” propone l’adozione di soluzioni tecnologiche innovative per sostenere questa trasformazione si cita ricorrentemente l’agricoltura di precisione ed i sistemi di monitoraggio remoti, primo fra tutti l’osservazione dallo spazio. In tal senso l’efficienza d’uso dei fertilizzanti nell’agricoltura convenzionale e l’efficienza d’uso di tutti gli input di coltivazione sia in agricoltura convenzionale che nella biologica, ottenibile attraverso la gestione della variabilità spaziale della componente colturale e pedologica, sembra essere la strada per il miglioramento delle prestazioni ambientali della coltivazione del frumento.

## Obiettivi

Nel frumento, rispetto alla tecnica agronomica utilizzata negli ultimi anni, si può perseguire un obiettivo di elevata produttività senza ricorrere a elevati sovradosaggi di input quali i fertilizzanti.

In un contesto di elevata variabilità pedologica e meteorologica gli agricoltori somministrano fertilizzanti basandosi sul principio che questi non debbano essere fattori limitanti per le aree del campo con maggiori aspettative produttive. Con una distribuzione uniforme degli input si opera così un sovradosaggio nelle aree dell'appezzamento meno produttive. Fra gli input somministrati il fertilizzante azotato è il principale elemento nutritivo su cui si basano le strategie produttive. Minore importanza viene data al fertilizzante fosfatico e la dose somministrata difficilmente è valutata sulle risposte produttive del frumento. Allo stesso tempo molte produzioni tipiche richiedono la standardizzazione della qualità della granella riconducibile alla trasformazione tecnologica. In tale contesto operativo risulta necessario comprendere quali "strumenti agronomici" possano essere utilizzati per garantire le proprietà reologiche degli impasti.

Sulla base di queste considerazioni l'obiettivo della ricerca è stato quello di analizzare differenti set di dati raccolti su alcuni campi sperimentali realizzati in aree collinari del centro Toscana al fine di valutare l'effetto delle concimazioni azotate, fosfatiche e solfatiche sulla quantità e qualità delle produzioni frumenticole.

In particolare gli obiettivi specifici sono stati:

- valutare l'effetto delle fertilizzazioni azotate sulla crescita e produzione del frumento al fine di ottimizzare l'impiego di immagini satellitari per la fertilizzazione sito-specifica;
- valutare l'effetto della variabilità del fosforo disponibile sulla quantità e qualità del raccolto al fine di considerare l'impiego di mappe di fosforo disponibile per la concimazione sito-specifica;
- valutare l'effetto della fertilizzazione solfatica sulla qualità reologica degli impasti.

Per completare il quadro conoscitivo si è posto l'ulteriore obiettivo di valutare l'effetto di differenti modelli agronomici di coltivazione sugli aspetti di impatto ambientale della coltivazione del frumento. Particolare attenzione è stata posta nella valutazione dell'impiego dei fertilizzanti azotati e fosfatici, utilizzati in agricoltura convenzionale, su differenti indicatori di impatto ambientale.

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# Integrating satellite data with a Nitrogen Nutrition Curve for precision top-dress fertilization of durum wheat

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## Abstract

The present study developed a method to use RapidEye satellite information for N management in durum wheat cultivation. The estimation of the N status was based on the development of Nitrogen Nutrition Index (NNI), referred to as the ratio between actual N concentration (Nac) and the minimum N content required to obtain maximum biomass (critical N concentration (Nc)). Nc was then calculated by means of a Nc dilution curve, which was calibrated through the use of durum wheat experimental fields (the number varying per year) in Val d'Orcia area (Tuscany) over three consecutive growing seasons from 2009/2010 to 2011/2012, respectively. The data (Nac and biomass) to produce estimated NNI were obtained by matching the available field samples with information obtained from remote sensing. Statistical analysis indicated that both modified chlorophyll absorption in reflectance index (MCARI) and enhanced vegetation index (EVI2) were the best vegetation indices (VIs) for estimating Nac and biomass, respectively. The validation, attained using the 2012/2013 experimental field data, showed that the proposed model was very reliable. This could be an effective method in advising farmers on the application of midseason N fertilization treatments, through precision farming operations.

## Keywords

Precision farming, vegetation indices, NNI, remote sensing, N requirement

## 1. Introduction

On the 1 June 2018, the European Commission set goals for the new Common agricultural policy (CAP) for beyond 2020, focusing on the contribution of innovation and sustainability of crop production in Italy (through Regional Agricultural Policies), as for the rest of Europe (EIP-AGRI

partnership). One of the key-points reported was the necessity of effective nutrient management, more specifically, avoiding environmental losses and preserving high quality crop yields (Guerrini et al., 2020; Meredith, 2019). The use of precision farming techniques was proposed as a solution to the reported aims, and these techniques are shown to be supported by the use of different technologies (Balafoutis et al., 2017; Rogovska et al., 2019; Rütting et al., 2018). Precision N management is aimed at supplying the correct fertilization rate for satisfying the specific requirements of crops, both in space and time, as a tool to improve N use efficiency (Cao et al., 2017; Rütting et al., 2018). The relationship between N accumulation and both plant growth and production are crucial in assisting farmers to reduce the yield gap (Wang et al., 2019), whilst avoiding either the surplus or insufficient use of nutrients.

Justes et al. (1994) described N trends related to biomass for common wheat by assessing the “critical N concentration ( $N_c$ ) dilution curve”. The assumption made by this curve is that N content decreases during crop growth cycle, whilst simultaneously giving rise to increasing biomass (Greenwood et al., 1990, 1986). Over the last thirty years,  $N_c$  dilution curves have been estimated for many crops, including winter wheat (Justes et al., 1994), spring wheat (Ziadi et al., 2010), japonica rice (Ata-Ul-Karim et al., 2013), indica rice (Sheehy et al., 1998) and corn (Ziadi et al., 2008). The construction of  $N_c$  dilution curves for wheat have already been developed, but as genotypes and different environments influence crop growth cycles, it is essential to compute environment-specific  $N_c$  dilution curves to correctly quantify the N demand (Tahir Ata-Ul-Karim et al., 2016). One method that was implemented to improve the N fertilization efficiency, without yield loss, is the Nitrogen Nutrition Index (NNI), that is calculated starting from the  $N_c$  dilution curve and the actual N concentration ( $N_{ac}$ ) of crops (Ata-Ul-Karim et al., 2017; Chen, 2015; Lemaire et al., 2008). This index was used as a decision support tool to calculate the amount of N required to match crop requirements (Li et al., 2014; Mistele and Schmidhalter, 2008). The principle constraint in the application of the NNI calculation is that it requires both crop N content and biomass information. Obtaining both information is usually performed by direct sampling, which is time consuming, costly and requires the sampling of numerous plants to be representative. For this reason, research has been carried out on the effectiveness of alternative strategies to that of direct field sampling. Of these strategies, some have successfully used to estimate  $N_{ac}$  by means of proximal sensing data for both wheat (Debaeke et al., 2006; Rodriguez et al., 2006) and maize (Ziadi et al., 2008), whereas others have successfully used remote sensing data for rice (Huang et al., 2015), wheat (Chen, 2015; Quemada et al., 2019), maize (Xia et al., 2016; Zhao et al., 2018), as well as red fescue and perennial ryegrass (Wang et al., 2019). From remote sensing, NNI was indirectly estimated using spatially-distributed derived DM and  $N_{ac}$  from satellite information, and assessing their relationships with both vegetation indices (VIs) and N concentration values (Huang et al., 2015; Nutini et al., 2018).

Certain satellites have been considered more adapt for regional scale agricultural studies than others, such as the optical platforms Landsat (Croft et al., 2019; Leslie et al., 2017) and MODIS (Alarcon et al., 2010). New satellites have been suggested to provide more information for the detection of crop parameters, attributable to the support of both the red-edge (RE) region and a higher resolution. One such example is Sentinel-2, launched by the European programme Copernicus and the European

Space Agency (ESA), showing a high-resolution power and a shorter revisit time (Clevers and Gitelson, 2013; Fabbri et al., 2019). In addition to Sentinel-2, RapidEye is an excellent predictor of crop parameters, possessing a higher spatial resolution (5 meters) and a revisit time of only one day. In recent years, some studies suggested that RapidEye was one of the most suitable in evaluating both biomass variation in vegetation (Ramoelo et al., 2015) and N content detection (Basso et al., 2016; Eitel et al., 2007; Magney et al., 2017). However, published results using VIs from RapidEye imagery to assess vegetation status and crops parameters are still limited.

The aim of this study was monitoring the vegetative and N requirement status of durum wheat in order to supply site specific N rates for the 2nd top-dress fertilization. The steps to reach the main objective were as follow. These included firstly, developing a specific Nc dilution curve for durum wheat cultivated under heavy-textured soils, interannual climate variability and different varieties. The second step was to identify the most sensitive/appropriate VIs from RapidEye to estimate aboveground biomass and actual N concentration in durum wheat from the tillering stage to milk kernel development. Thirdly, the present research was aimed at evaluating the possibility of using satellite estimated NNI as a tool to calculate precise N crop requirements for top-dress fertilization, and lastly at validating the model by comparing data estimated from the satellite with those measured in experimental fields over four growing seasons.

## 2. Materials and Methods

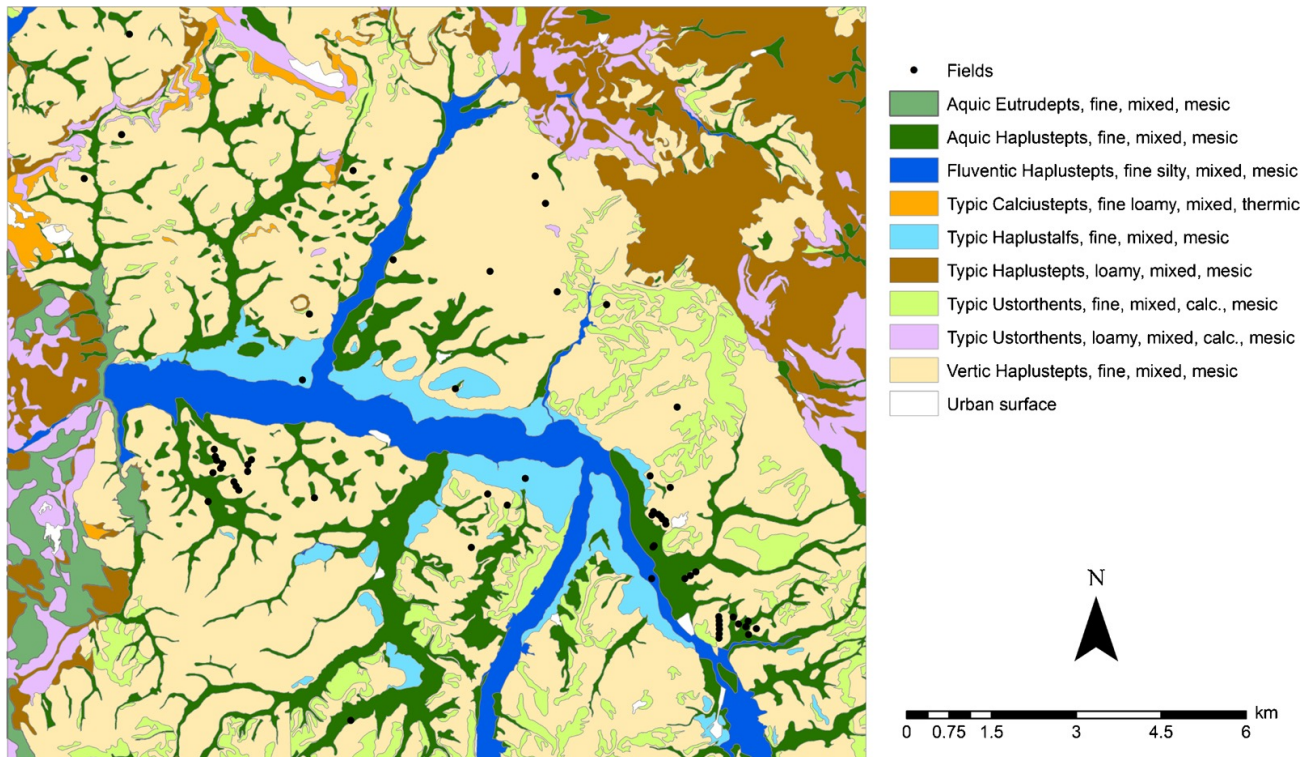
### 2.1. Description of the study area

The Nc dilution curve was developed and tested, starting with a dataset of field durum wheat experiments, carried out on different farms realized in the hilly area of the Siena province (Tuscany, Italy). Soils from the experimental fields along the Orcia stream valley floor (Fluventic Haplustepts, fine silty, mixed, mesic) and those characterizing the valleys of the Orcia tributaries (Aquic Eutrudepts, fine, mixed, mesic; Aquic Haplustepts, fine, mixed, mesic) were mainly comprised of deep and calcareous silty-clay soils (Gardin and Vinci, 2016). Experimental field realized on the foot-hill along the Orcia stream had mainly silty clay loam soil (Typic Haplustalfs, fine, mixed, mesic), while those on the hillside had mainly deep vertic silty-clay calcareous soils (Vertic Haplustepts, fine, mixed, mesic) (Fig. 1).

The area was typically characterized by a Mediterranean climate, with rainy and relatively cold winters and springs, and dry, warm summers. Locally, the period of maximum vegetative growth of durum wheat, from stem elongation to anthesis, is respectively from February to mid-May. An evaluation of the climatic conditions for this period was, therefore, performed. The rainfall-related water supplies were considered by calculating the cumulative monthly rainfall, specifically for the February – May period, corresponding to the analysis period for plant N concentration.

In order to characterize the temperature pattern during the 4 vegetative cycles, the growing degree day (GDD) (Salazar-Gutierrez et al., 2013) data were computed and analysed. The use of GDD to describe the timing of biological processes is well established (Grassi et al., 2020; Li et al., 2012;

Undersander and Christiansen, 1986). The GDD value was daily calculated as the difference between daily average temperature ( $T_{avg}$ ; °C) and a base temperature ( $T_b$ ; °C). In this work, a  $T_b$  of 4 °C was used during the whole crop cycle in accordance with several studies (Porter and Gawith, 1999; Saiyed et al., 2009). The cumulated GDD values were calculated as the accumulation of daily GDD on a monthly basis and for the February – May period.



*Fig. 1. Pedological map of the study area. Field positions are also reported.*

## 2.2. Experimental fields, treatments and sampling

Two durum wheat varieties (*Triticum durum* L. var. Miradoux and var. Claudio) were cultivated in 3 to 6 private farms, each including from 3 to 4 experimental fields of more than 2 ha (the number of both farm and fields varying per year). At each farm, the treatments were arranged in a total randomized design with three replicates per treatment. The inter-row spacing of 0.13 m and a seeding density of 600 seed  $m^{-2}$  were used. Different N top-dress fertilization rates from 70 to 150  $kg N ha^{-1}$  were proposed to farmers on an annual basis. The seeding and fertilization dates were site specific (Table 1). Fertilizers were broadcasted in one or two rates to attain the proposed annual N rate. Ammonium nitrate (AN) was used for top-dress fertilization in most fields (except one treatment consisting of urea (U) application at 110  $kg N ha^{-1}$  in 2013).

Growing season	Farm	1 <sup>st</sup> top-dress fertilization date	2 <sup>nd</sup> top-dress fertilization date	Total applied N (kg N ha <sup>-1</sup> )							
				70 NA	90 NA	100 NA	110 NA	110 U	120 NA	130 NA	140 NA
				N distributed at 1 <sup>st</sup> and 2 <sup>nd</sup> top-dress fertilization (kg N ha <sup>-1</sup> )							
2009-2010	Farm_01	Feb, 05	Mar, 30	0 - 90		40 - 70				50 - 90	80 - 70
2009-2010	Farm_02	Feb, 05	Mar, 30		0 - 100	50 - 60			70 - 60		50 - 100
2009-2010	Farm_03	Feb, 05	Mar, 30		0 - 100			50 - 70		70 - 70	
2010-2011	Farm_01	Feb, 08	Mar, 28	50 - 40				50 - 70		50 - 80	
2010-2011	Farm_02	Feb, 08	Mar, 28	40 - 50						50 - 80	40 - 100
2010-2011	Farm_03	Feb, 08	Mar, 28		50 - 50			50 - 70		40 - 90	70 - 80
2010-2011	Farm_04	Feb, 08	Mar, 28		50 - 50					60 - 70	50 - 100
2011-2012	Farm_01	Jan, 27	Apr, 02			50 - 60				60 - 70	60 - 90
2011-2012	Farm_02	Jan, 27	Apr, 02			60 - 50				70 - 60	90 - 60
2011-2012	Farm_03	Jan, 27	Apr, 02	40 - 50				70 - 50			60 - 80
2011-2012	Farm_04	Jan, 27	Apr, 02	40 - 50				70 - 50			60 - 80
2011-2012	Farm_05	Jan, 27	Apr, 02		50 - 40			50 - 70			80 - 60
2011-2012	Farm_06	Jan, 27	Apr, 02		50 - 40			50 - 70			80 - 60
2012-2013	Farm_01	Mar, 01	Apr, 19	30 - 40		60 - 50	60 - 50				
2012-2013	Farm_02	Mar, 01	Apr, 19	30 - 40		50 - 60	50 - 60			60 - 70	
2012-2013	Farm_03	Mar, 01	Apr, 19	40 - 30		50 - 60	50 - 60			60 - 70	
2012-2013	Farm_04	Mar, 01	Apr, 19	40 - 30		60 - 50				60 - 70	

**Table 1.** Details and dates of the experimental treatments during the four growing seasons; nitrogen fertilization treatments were performed by using ammonium nitrate (AN) and urea (U).

For all fields, phosphorus was broadcasted and incorporated into the soil before seeding as triple superphosphate at the rate of 92 kg ha<sup>-1</sup> (P<sub>2</sub>O<sub>5</sub>). To monitor the dry weight of above-ground biomass (DM; Mg ha<sup>-1</sup>) and the actual nitrogen (N<sub>ac</sub>) concentration in the aboveground biomass (g kg<sup>-1</sup>) of the crop, plants were sampled at different phenological stages (BBCH) during the growth cycle (Table 2).

Growing season	Sampling date	Sampling stage (bbch)	DM (Mg ha <sup>-1</sup> )			N (g kg <sup>-1</sup> of DM)		
			Avg ± SD	Min	Max	Avg ± SD	Min	Max
2009-2010	Apr, 20	45	3.4 ± 1.7	1.7	7.6	28.4 ± 7.5	15.7	36
	May, 11	55	6.1 ± 1.3	4.2	9.1	17.3 ± 4.5	10.4	23.5
	May, 25	65	10.6 ± 2.7	7.7	15.7	17.7 ± 3.2	13.4	22.9
2010-2011	Apr, 07	37	3.0 ± 1.4	0.6	4.8	24.7 ± 6.46	17	38.6
	Apr, 20	45	5.1 ± 2.2	1.2	7.8	23.0 ± 6.6	15.7	39.7
	May, 03	55	7.4 ± 2.1	2.9	11.2	20.8 ± 2.2	17	23.8
2011-2012	May, 17	65	9.2 ± 2.1	6.2	13.4	17.4 ± 3.8	12.4	24.5
	Apr, 12	37	1.5 ± 0.4	1.21	2.71	38.2 ± 3.5	29.3	44.6
	May, 06	55	6.3 ± 1.0	4.7	8.9	28.5 ± 3.1	22.1	35.2
2012-2013	May, 20	65	12.5 ± 1.5	9.5	16.6	9.8 ± 2.3	7.4	17.2
	Apr, 12	37	1.3 ± 0.6	0.4	2.5	23.0 ± 6.6	15.7	39.7
	May, 15	65	5.5 ± 1.5	2.8	8.1	19.7 ± 3.1	13.8	25.9

**Table 2.** Descriptive statistics of durum wheat samples collected in experimental fields within Val d'Orcia, during different phenological stages (BBCH) during the growing seasons from 2009/2010 to 2012/2013, respectively. Dry matter (DM) and nitrogen (N) concentration was represented by the means with standard deviations (Avg ± SD), as well as the minimum (Min) and maximum (Max) values.

For each sampling, within each field, three areas of 5 m × 5 m, corresponding to single RapidEye pixel, were randomly selected. Then, within each area, destructive samplings were performed in five replicates by randomly collecting aboveground biomass in a sampling area of 0.4 m<sup>2</sup>. The DM was determined by drying the fresh samples in an oven at 105°C until constant weight according to Ceotto et al. (2013). The N content of the samples was determined with a CHNS analyser (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA).

### 2.3. Dilution curve development

DM and  $N_{ac}$  data determined in the 2010-2012 season were used to develop the dilution curve by following the statistical methodology proposed by Justes et al. (1994). For each sampling date, the amount of shoot DM varied with N treatments. The corresponding  $N_{ac}$  were subjected to analysis of variance (ANOVA) using R studio statistical program for the determination of critical N points. The latter are representative of points in which N supply is not limiting for biomass growth. The DM and corresponding  $N_{ac}$  data for N-limiting treatments were fitted with simple linear regression, and the biomass data of non-N-limiting treatments were averaged to calculate maximum biomass.

The N concentration during the growing period was computed using a  $N_c$  dilution curve as proposed in Justes et al. (1994) (Equation 1).

$$N_c = aW^{-b} \quad (\text{Eq. 1})$$

where  $W$  is the total biomass expressed in  $\text{Mg DM ha}^{-1}$  concentration in aboveground biomass expressed in  $\text{g kg}^{-1}$  of DM. Parameter  $a$  represents the N content in the total aboveground biomass for  $1 \text{ Mg DM ha}^{-1}$ , and parameter  $b$  represents the dilution coefficient describing the allometric relationship between the N concentration and aboveground biomass (Ziadi et al., 2010).

In earlier growth stages, a constant  $N_c$  value for aboveground biomass values lower than  $1 \text{ Mg DM ha}^{-1}$  was established, as suggested by Ziadi et al. (2010). Under these conditions,  $N_c$  in the shoot material was calculated by averaging the minimum N concentration of non-limiting N points, as well as the maximum N concentration of limiting N points (Justes et al., 1994). Thereafter, the lowest DM threshold of the curve was defined by intersecting the N dilution curve and the constant critical N concentration.

### 2.4. Remote sensing for estimating $N_{ac}$ , DM and NNI

RapidEye images were purchased from the official dealer for Italy (Ipsat s.r.l., Rome, Italy). The images were provided with orthorectification, radiometric and atmospheric correction. RapidEye satellite carries a multispectral sensor recording radiance in 5 spectral bands: blue (B;  $0.440\text{-}0.510 \mu\text{m}$ ), green (G;  $0.520\text{-}0.590 \mu\text{m}$ ), red (R;  $0.630\text{-}0.685 \mu\text{m}$ ), red-edge (RE;  $0.690\text{-}0.730 \mu\text{m}$ ) and near infra-red (NIR;  $0.760\text{-}0.850 \mu\text{m}$ ), with a pixel resolution of 5 m (ESA, 2020). RapidEye images, spanning the dates corresponding to the destructive field samplings, from 2010 to 2013, were processed with ArcGIS 10.3 in order to calculate some of the most commonly used VIs for the estimation of biomass and N concentration of crops (Table 3). Statistical analysis was performed to identify the VIs with the highest correlation to wheat biomass and N content. For each index, the linear regression fit was computed as predictive model for estimating the  $N_{ac}$  and DM. Most suitable indices were selected based on the highest determination coefficient ( $R^2$ ). The NNI was computed as the ratio between  $N_{ac}$  and  $N_c$  and the resulting values were used to quantify the crop N status. According to Ziadi et al.

(2010), when NNI = 1, N nutrition can be considered optimal, whereas NNI > 1 is indicative of N overdose, and NNI < 1 of N deficiency for optimal growth, respectively.

Vegetation Index	Acronym	Equation	References
Canopy Chlorophyll Content Index	CCCI	$\frac{[(NIR - RE) \cdot (NIR + RE)]^{-1}}{[(NIR - R) \cdot (NIR + R)]^{-1}}$	(Barnes et al., 2000)
Enhanced Vegetation Index	EVI	$2.5 \cdot [(NIR - RE) \cdot ((NIR + 6 \cdot RE - 7.5 \cdot B) + 1)^{-1}]$	(Huete, 1988)
Enhanced Vegetation Index 2	EVI2	$2.4 \cdot [(NIR - RE) \cdot (NIR + RE + 1)^{-1}]$	(Miura et al., 2008)
Green Normalized Difference Vegetation Index	GNDVI	$(NIR - G) \cdot (NIR + G)^{-1}$	(Gitelson et al., 1996)
Modified Chlorophyll Absorbtion Ratio Index	MCARI	$\{(RE - R) - [0.2 \cdot (RE - G)]\} \cdot (RE \cdot R^{-1})$	(Daughtry et al., 2000)
Modified Soil Adjusted Vegetation Index	MSAVI	$\{2 \cdot NIR + 1 - \sqrt{2 \cdot (2 \cdot NIR + 1)^2 - [8 \cdot (NIR - RE)]}\} \cdot 2^{-1}$	(Rondeaux et al., 1996)
Normalized Difference Red-Edge	NDRE	$(NIR - RE) \cdot (NIR + RE)^{-1}$	(Barnes et al., 2000)
Normalized Difference Vegetation Index	NDVI	$(NIR - R) \cdot (NIR + R)^{-1}$	(Tucker, 1979)
Optimized Soil Adjusted Vegetation Index	OSAVI	$(1 + 0.16) \cdot [(NIR - R) \cdot (NIR + R + 0.16)^{-1}]$	(Rondeaux et al., 1996)
Soil Adjusted Vegetation Index	SAVI	$[(NIR - R) \cdot (NIR + R + 0.5)^{-1}] \cdot (1 + 0.5)$	(Huete, 1988)
Transformed Chlorophyll Absorbtion Ratio Index	TCARI	$3 \cdot \{(RE - R) - [0.2 \cdot (RE - G)] \cdot (RE \cdot R^{-1})\}$	(Haboudane et al., 2002)
Ratio of MCARI and OSAVI	MCARI/OSAVI	$\frac{\{(RE - R) - [0.2 \cdot (RE - G)] \cdot (RE \cdot R^{-1})\}}{\{(1 + 0.16) \cdot [(NIR - R) \cdot (NIR + R + 0.16)^{-1}]}}$	(Daughtry et al., 2000)
Ratio of TCARI and OSAVI	TCARI/OSAVI	$\frac{3 \cdot \{(RE - R) - [0.2 \cdot (RE - G)] \cdot (RE \cdot R^{-1})\}}{\{(1 + 0.16) \cdot [(NIR - R) \cdot (NIR + R + 0.16)^{-1}]}}$	(Haboudane et al., 2002)

**Table 3.** Vegetation indices (Vis) evaluated for estimating durum wheat biomass and N content indicators as reported in IDB (2020)

## 2.5. Evaluation of crop Nac, DM and NNI by remote sensing

The Nac and DM determined on samples collected in 2013 were used to validate the data on remote sensing for Nac and DM, and to calculate the actual NNI. In particular, Nac and DM estimated by satellite imaging on 14 April and 12 May, 2013, were compared to the measured Nac and DM of samples harvested on 12 April and 15 May, 2013. Then, the actual NNI was compared with that calculated from remote sensed Nac and DM in order to evaluate whether remote sensing of NNI was suitable in monitoring the durum wheat status. The performances of the parameters determined by remote sensing were assessed with the percent bias (PBIAS) (Equation 2), the ratio of the root mean square error to observation standard deviation (RSR) (Equation 3) and the Nash-Suitcliffe coefficient (NSE) (Equation 4), as suggested by Moriasi et al. (2007).

$$PBIAS = \frac{\sum_{i=1}^n (Y_i^{obs} - Y_i^{sim})}{\sum_{i=1}^n (Y_i^{obs})} \cdot 100 \quad (\text{Eq. 2})$$

$$RSR = \frac{\sqrt{\sum_{i=1}^n (Y_i^{obs} - Y_i^{sim})^2}}{\sqrt{\sum_{i=1}^n (Y_i^{obs} - Y^{mean})^2}} \quad (\text{Eq. 3})$$

$$NSE = 1 - \frac{\sum_{i=1}^n (Y_i^{obs} - Y_i^{sim})^2}{\sum_{i=1}^n (Y_i^{obs} - Y^{mean})^2} \quad (\text{Eq. 4})$$

where  $Y_{iobs}$  and  $Y_{isim}$  were the  $i$ th observed and simulated value, respectively, for the parameter being evaluated,  $Y_{mean}$  was the mean of observed data for the parameter being evaluated, and  $n$  was the total number of observations.

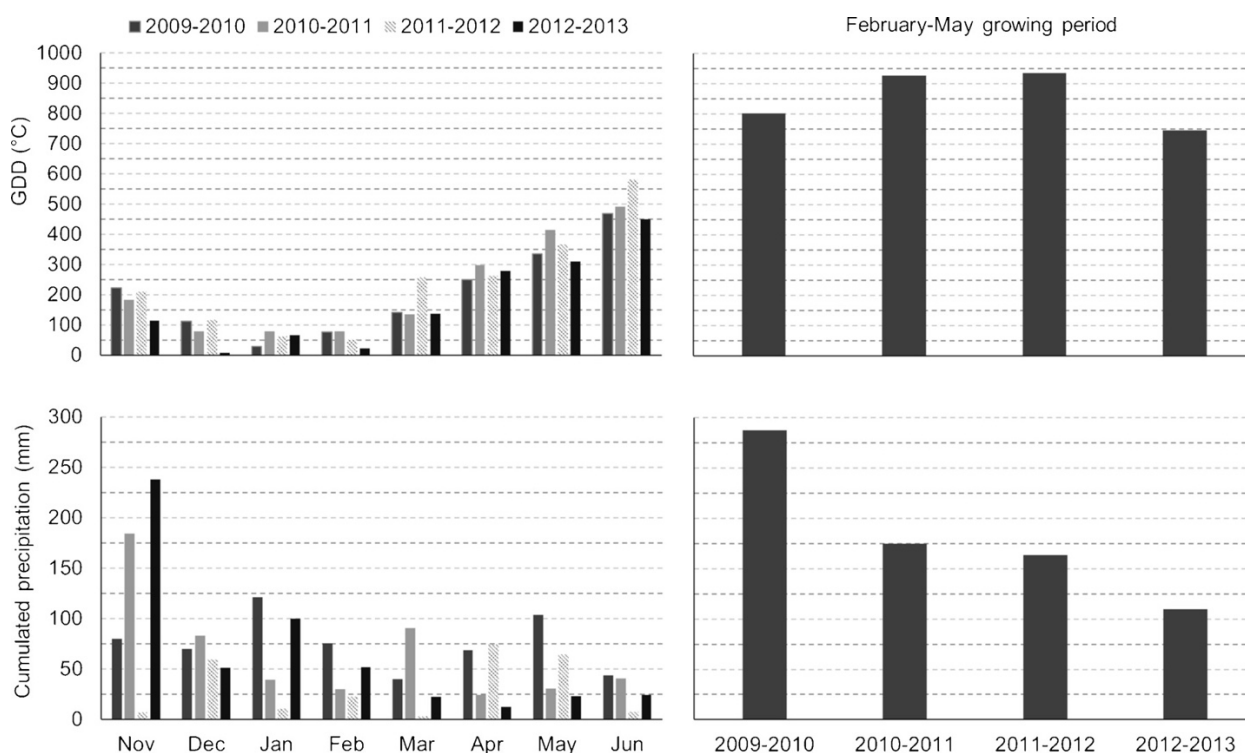
Model performance can thus be classified, as function of the afore-mentioned statistical indices into four respectively classes, namely, “very good”, “good”, “satisfactory”, and “unsatisfactory” (Moriasi et al., 2007; Pulighe et al., 2020). In particular, a PBIAS value lower than 10%, between 10-15% and 15 - 25%, and over 25% was considered “very good”, “good”, “satisfactory” and “unsatisfactory”, respectively. Moreover, a RSR was considered “very good”, “good”, “satisfactory”, and “unsatisfactory” when values were lower than 0.5, between 0.5 - 0.6 and 0.6 - 0.7, and over 0.7, respectively. Further, a NSE was considered “very good”, “good”, “satisfactory”, and “unsatisfactory” when values were higher than 0.75, between 0.75 - 0.65, 0.65 - 0.5, and less than 0.5, respectively. Further, the parameter performances were also evaluated by using the root mean square percent error (RMS%E) (Equation 4). RMS%E values equal or higher than 1.0 were considered “unsatisfactory”, while values lower than 0.3 were considered “good”, as proposed by Veerasamy et al. (2011).

$$RMS\%E = \frac{\sqrt{\frac{\sum_{i=1}^n (Y_i^{obs} - Y_i^{sim})^2}{n}}}{Y_{mean}} \cdot 100 \quad (\text{Eq. 5})$$

### 3. Results and discussion

#### 3.1. Meteorological conditions

Weather conditions are summarized in Fig. 2. The sum of the active temperatures during the growing season showed a large variability in thermal data. The monthly GDD ranged from 28.2 to 636, from 77.4 to 538.7, from 51.1 to 637.1, and from 8.2 to 598.6, during the 1<sup>st</sup>, the 2<sup>nd</sup>, the 3<sup>rd</sup>, and the 4<sup>th</sup> growing season, respectively. In particular, the 2011-2012 growing season was characterized by above average temperature with an unexpected increase in March. The 2012-2013 growing season, from February to May, was the coldest with 1382 GDD, while the 2011-2012 vegetative season was the warmest, with 1902 GDD. In the present study, the cumulated rainfall of the entire growing season (November-May) varied from 249 mm in 2011-2012 to 601 mm in 2009-2010. Further, during the 4 growing seasons, a high variability in monthly rainfall was recorded. For example, from February to May, rainfall showed a maximum of 287 mm in 2010 and a minimum of 110 mm in 2013. It is likely that the variability would have impacted on both the vegetative productive response and the N assimilation capacity along the four growing seasons.



**Fig. 2.** The cumulated growing degree days (GDD; °C) (top row) and the cumulated precipitation (mm) (bottom row). Results were calculated on a monthly basis during the growing season (left panel) and during the February-May period, corresponding to the growing season comprised between tillering to anthesis (right panel).

### 3.2. Determining the critical nitrogen dilution curve

Of the 143 field samplings, carried out between 2010 and 2012, 12 met the statistical criteria defined for the determination of the critical nitrogen dilution curve. These 12 are presented in Table 4.

Growing season	Sampling date	Phenological stage (BBCH)	N fertilization (kg ha <sup>-1</sup> )	DM (Mg ha <sup>-1</sup> )	Nac (g kg <sup>-1</sup> of DM)
2009-2010	May, 11	45	100	1.7	35.8
2009-2010	May, 11	45	140	4.1	26.5
2010-2011	May, 03	55	100	7.1	17.4
2009-2010	May, 25	55	140	5.8	21.5
2011-2012	May, 20	65	120	5.9	19.7
2010-2011	May, 03	55	100	1.6	30.9
2010-2011	May, 03	55	120	7.4	19.2
2011-2012	May, 20	65	150	8.7	19.9
2010-2011	Apr, 20	45	90	2.9	26.9
2010-2011	May, 17	65	140	8.5	19
2010-2011	Apr, 07	37	90	1.2	35.5
2011-2012	Apr, 12	37	150	1.6	40.1

**Table 4.** Sampling data used for determining the critical nitrogen dilution curve; Dry matter (DM) and nitrogen (N) concentration average are reported.

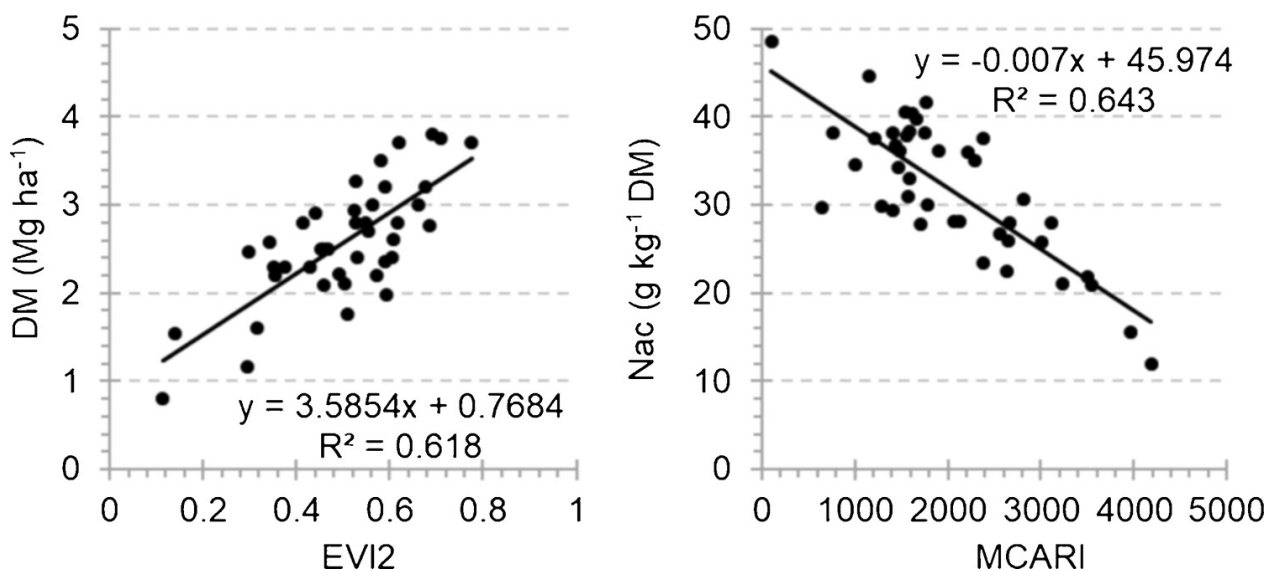
The estimated values for the a and b parameters of the critical N dilution curve were 41.808 and 0.381, respectively. The determination coefficient indicated that the model was able to account for the 90.6% of the total variance. The constant Nc (42.6 g N kg<sup>-1</sup> DM) and the lower DM threshold (0.96

Mg DM ha<sup>-1</sup>) were calculated using four sampled data, with dry matter values ranging from 0.61 to 0.99 Mg DM ha<sup>-1</sup>.

Almost all sampling data measured from 2010 to 2012 were distributed under the critical N dilution curve of Justes et al. (1994), even those treatments with the highest N fertilization rates (Fig. 3). Furthermore, the values of the constant  $N_c$  and of the DM threshold were lower than those determined by Justes et al. (1994) for winter wheat, corresponding to 44 g N kg<sup>-1</sup> DM and 1.55 Mg DM ha<sup>-1</sup>, respectively. This suggested that the curve developed by Justes et al. (1994) for winter wheat was not suitable for durum wheat, at least under the conditions of the present study. It was shown that the critical N dilution curve can be affected by different climatic conditions (Greenwood et al., 1991; Yue et al., 2012). The precipitation variability, previously showed in Fig. 2, may have influenced the effectiveness of N fertilization during the study period. Higher temperatures can reduce the length of growing season, allowing less N to be accumulated into the above-ground biomass (Panozzo and Eagles, 1999). Moreover, different species, within the genus *Triticum*, could also be characterized by different critical N dilution curves (Hoogmoed and Sadras, 2016; Sadras and Lemaire, 2014; Zhao et al., 2016).

### 3.3. Analysis of the most suitable Vis for estimating DM and Nac

Regression analyses permitted the evaluation of the most suitable VIs for estimating the Nac and DM (Table 5). Seven VIs, from a total of thirteen, showed a good performance in describing DM, and correlations were highly significant ( $p < 0.001$ ). Indices such as NDRE and EVI2 were significantly correlated with DM ( $p < 0.001$ ), but were not correlated with Nac. The present results corroborated those of Lukas et al. (2016), showing a significant correlation between EVI2 and wheat biomass, and a low correlation with Nac. Both EVI and EVI2 indices were previously shown to be correlated to crop DM, while NDVI was more related to chlorophyll content and Nac (Gao et al., 2000). In the present study, similar to that reported previously by Gao et al. (2000) and Lukas et al. (2016), the best correlation for the VIs was made when using the two bands over 0.690  $\mu\text{m}$  wavelengths. No significant correlation between the EVI and DM was observed in this study, probably due to an influence of the blue band. By performing measurements with different sensors, a higher sensibility of EVI to variations in atmospheric conditions was found previously. This effect was suggested to be attributable to differences in absorption coefficients of the blue band (Fensholt et al., 2006). Moreover, Kang et al. (2016) and Dalla Marta et al. (2015) found respectively that EVI2 and NDRE were saturated in the presence of high canopy cover and this lead to a reduced capacity to describe crop status. In our case, the results were not subject to saturation problems with these VIs. This is probably due to the larger data-set used, comprised of a total of three years and four phenological stages, respectively. Since EVI2 showed the highest  $R^2$  value, it was chosen as the best index for estimating the DM (Fig. 4 left).



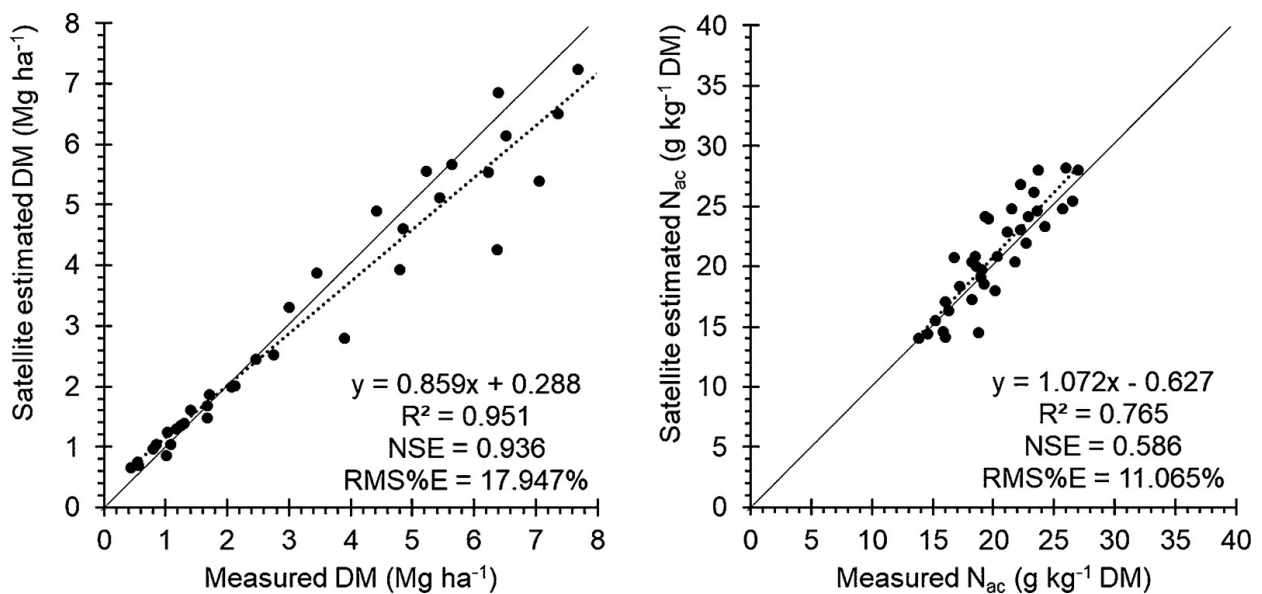
**Fig. 4.** Linear correlation between the EVI2 and the dry matter (DM) (left) and between the MCARI and the actual nitrogen content in DM (Nac) (right). Crop data (DM and Nac) were measured between 2010 and 2012. The two vegetation indices were computed using the RapidEye satellite bands for the selected periods.

Regarding the correlations between the tested VIs and Nac, nine from the total of thirteen were highly significant ( $p < 0.001$ ). The best correlation was found for MCARI (Fig. 4 right), followed by TCARI. The latter were representative of the VIs calculated without the use of the NIR band. The worst results were obtained for the simple indices, EVI2 and NDRE, respectively, which were the only VIs calculated without the use of the Red Band. MCARI is representative of a modified version of the chlorophyll absorption in reflectance index (CARI), that was developed by Kim (1994) to reduce the influence of other materials on photosynthetically active radiation. Significant correlations were also attained for Red band indices, similar to that shown previously for rice by Nutini et al. (2018), even if that part of the spectrum is the one representing the maximum absorption of chlorophylls. Similar results, exhibiting a strong correlation of TCARI and MCARI with the Nac values of the flag leaf (estimated by using SPAD) were shown by Eitel et al. (2007). Both indices have been reported as good chlorophyll content indicators and consequently, N accumulation predictors (Herrmann et al., 2010). The suitability of using MCARI in estimating N plant concentration, during the wheat growth, was shown by Eitel et al. (2007). Since MCARI showed the highest  $R^2$  value, it was chosen as the best index for estimating the N concentration.

#### Validation of the DM and Nac data from remote sensing

Both regression models were validated by using the 2013 data. The DM and Nac values determined on 12 April and 15 May, were compared with those estimated by applying the regression models on the RapidEye bands on 14 April and 14 May, respectively (Fig. 5). The determination coefficient

analysis indicated that the models based on MCARI and EVI2 were able to explain 76.5% and 95.1% of the total variance in April, respectively. These results indicated that the models performed better in validation than in calibration, suggesting that models were not over-calibrated. Moreover, the RMS%E values were good for both DM and Nac, while the PBIAS values were very good, and had scores of 5.6% and -4.1% for DM and Nac, respectively. These results suggested that both models were accurate, and only slightly underestimated the measured values for DM, while slightly overestimating the measured values for Nac. Furthermore, according to the RSR and NSE values, the performance of the model was very good for DM (RSR = 0.25; NSE = 0.94) and satisfactory for Nac (RSR = 0.64; NSE = 0.59). The comparison of the results of PBIAS and RSR revealed that the Nac predicting model performed slightly better in terms of accuracy than in terms of precision. Overall, the models were considered suitable in determining both the DM and Nac in durum wheat. The potential of satellite images for the determination of the N status in cereals was similarly investigated and validated by Huang et al. (2017). These authors found that RapidEye performed better than FORMOSAT – 2, probably due to the RE band. The results of the present study on wheat corroborate those obtained by Kross et al. (2015), who found a good correlation between different VIs calculated from RapidEye images and the biomass parameter for corn and soybean cultivation.

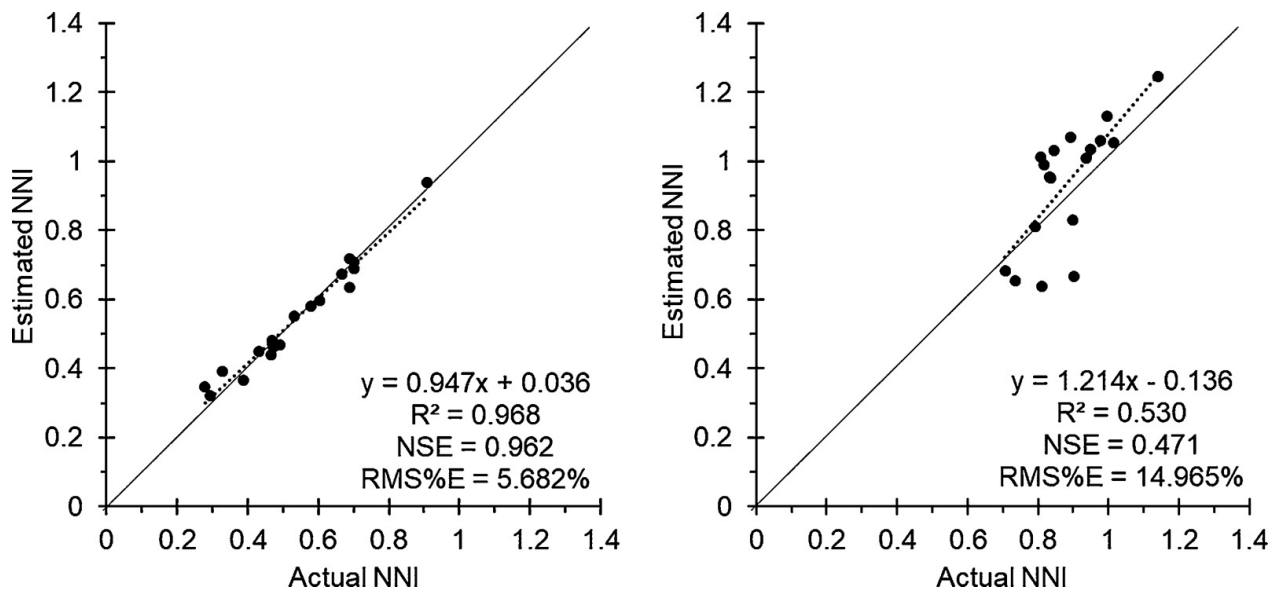


*Fig. 5. Relationship between observed and estimated DM (on the left) and Nac (on the right) using EVI2-estimated biomass and MCARI-estimated nitrogen content in Val D’Orcia, Tuscany (2013). The solid and dotted lines represent the 1:1 reference line and regression fit line, respectively.*

### 3.4. NNI evaluation

The actual NNI calculated from field samples collected on 12 April and 15 May, were compared with those estimated by satellite data on 14 April and 14 May, respectively. Fig. 6 (left) showed a strong

( $R = 0.968$ ;  $p < 0.05$ ) linear relationship between the actual and estimated NNI in April. In particular, the scatter about the regression line was small and the points were clustered along the 1:1 line (NSE = 0.962). Instead, regression fit did not lie close to the 1:1 line (NSE = 0.471) in May, despite the scatter about the regression line was still reasonably acceptable ( $R^2 = 0.530$ ;  $p < 0.05$ ) (Fig. 6 - right).

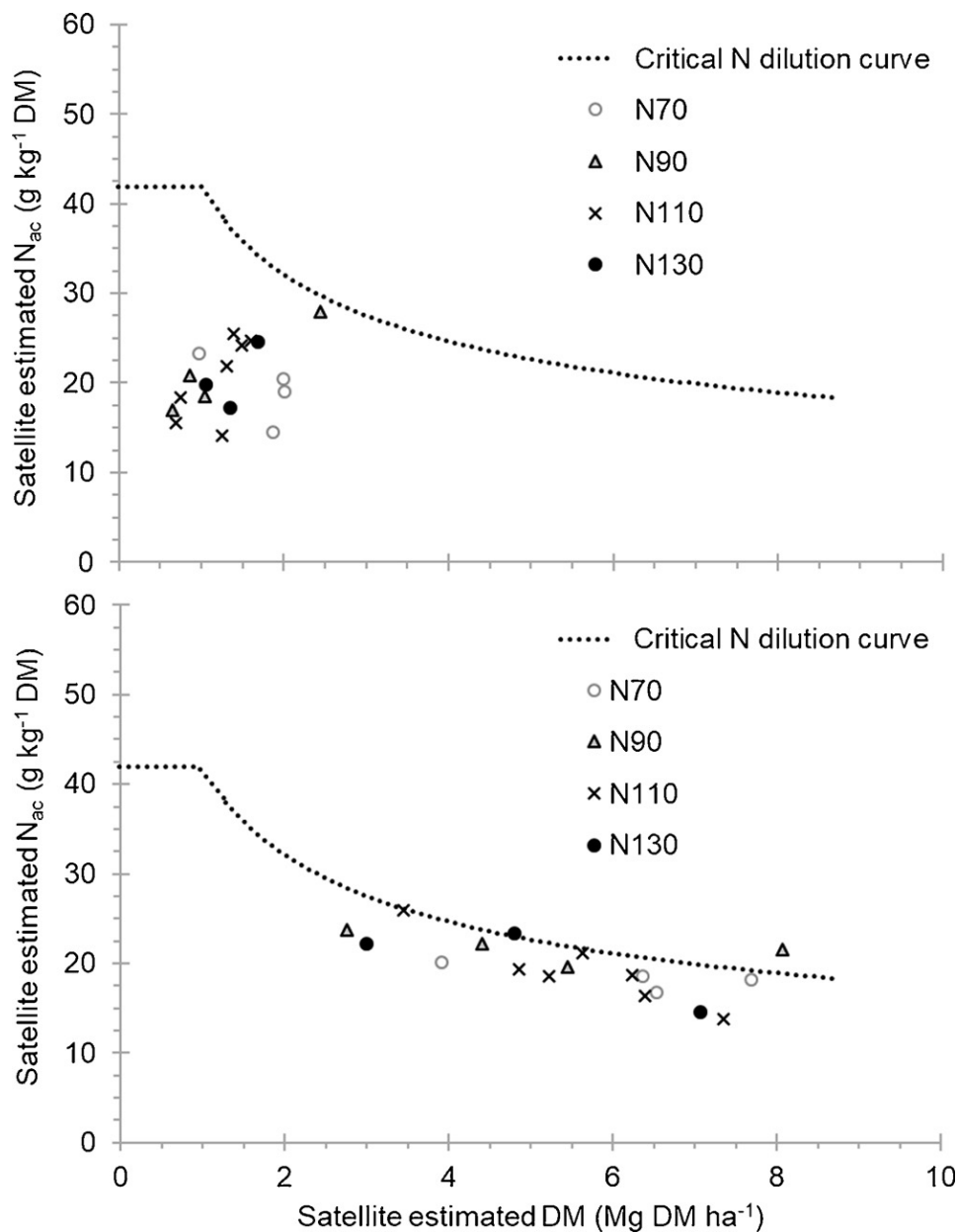


**Fig. 6.** Relationship between actual and estimated NNI. The solid and dotted lines represent the 1:1 reference line and regression fit line, respectively.

The PBIAS values were 1.4% and 5.9% for the data of April and May, respectively, and 4.2% when calculated over the entire period. The RMS% E values were 5.68% and 14.96% for the data of April and May, respectively, and 13.49% when calculated over the entire period. The RSR results scored 0.19 and 1.27 for the data of April and May, respectively, and 0.44 when calculated over the entire period. In April, the PBIAS, the RMS%E and RSR values indicated that NNI model was both accurate and precise. To the contrary, in May, NNI performed better on accuracy than precision. This may be explained by the saturation effect, that occurs when LAI values exceed 2 for wheat (Cabrera-Bosquet et al., 2011; Dalla Marta et al., 2015). In that case, the analysis from RapidEye data is less sensitive to the biomass variations within the field. Moreover, it is also more difficult to distinguish the N dilution within that biomass.

At stem elongation (14 April), NNI data (estimated by satellite) were located under the critical N dilution curve (Fig. 7 top). Despite the different fertilization treatments, the results after the 1st top-dress fertilization (01 March) were very similar, indicating that the adopted N rates were not able to satisfy the respective crop requirements in each treatment. The maximum N rate in the 1st top-dressing fertilization was 65 kg N ha<sup>-1</sup>. At flowering (12 May), NNI results (Fig. 7 bottom) indicated that the N scheduled with the 2nd top-dress fertilization (19 April) treatment was sufficient to match optimal durum wheat requirements for all treatments and that the field data were well-suited on the critical N dilution curve. Therefore, the relationship between biomass and N concentration seemed

to be quite robust over a large spectrum of experimental conditions. The only limitation may have been attributable to the presence of severe stress conditions that reduced regular N uptake, assimilation and translocation, as reported by Justes et al. (1994).



*Fig. 7. (top) Distribution of wheat samples one month after the first fertilization (12 April, 2013) and (bottom) distribution of wheat samples one month after the second fertilization (14 May 2013).*

#### 4. Conclusion

This study demonstrated that VIs calculated from RapidEye satellite spectral measurements were suitable to estimate the nutritional condition of durum wheat. In particular, MCARI and EVI2 were shown to be the most suitable indices for discriminating N content and biomass, respectively. The same approach used by Justes et al. (1994), to develop a dilution curve for common wheat, was used

to develop a dilution curve for durum wheat in Central Italy. The Nc dilution curve, that was calibrated starting from field data showed lower N concentration estimates compared to the curves developed by other authors for wheat (Justes et al., 1994). However, the N dilution during the biomass accumulation showed the same trend to that shown by Justes et al. (1994). Further investigations are required to understand the influence of climate-soil-plant interaction on this result. The proposed method is a reliable solution for applying N mid-season, using site-specific fertilization techniques, thereby avoiding inefficient distribution. The pedoclimatic characteristics of the Val d'Orcia area are consistent with those characterizing the Central Italy. Hence, it is possible to make the proposed methodology extensible to a wider area. The use of the proposed methodology, therefore, also has potential for application in other crops, which can become the focus of additional studies. The present study showed that, satellite images can be used to produce spatially detailed NNI maps for large-scale applications. Moreover, the elaboration of prescription maps accounting for the in-fields variability is reliable thanks to high spatial resolution of satellite images. Recently, Sentinel 2 satellites, have been proposed as free and open access instruments, that offer a cost-effective solution, in providing lower resolution coverage of extensive areas. Therefore, further studies are recommended to calibrate the proposed model for this satellite platform. The proposed methodology can contribute to improve the durum wheat production competitiveness in Central Italy according with the innovation and environmental sustainability policies of European Union.

## CRediT authorship contribution statement

**Carolina Fabbri:** Investigation, Data curation, Writing - original draft. **Marco Mancini:** Conceptualization, Methodology, Investigation, Resources, Writing - original draft. **Anna dalla Marta:** Conceptualization, Methodology, Funding acquisition, Writing - review & editing. **Simone Orlandini:** Conceptualization, Funding acquisition, Supervision. **Marco Napoli:** Conceptualization, Investigation, Methodology, Formal analysis, Writing - original draft.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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# Effect of soil available P and N on wheat production in P-deficient soil conditions

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## Abstract

Available nitrogen (Ns) and phosphorus (Ps) in soil influence the wheat quality and quantity production in many environments where fertilization rates don't match crops requirement. Nutrients availability in the soil is highly variable, even within small fields. A field experiment was conducted in Monteroni d'Arbia during two growing seasons. The experiment included 24 treatments, which were the combinations of four bread wheat cultivars, three nitrogen fertilization levels (35, 85 and 135 kg N ha<sup>-1</sup>), two seeding rates (90 and 180 kg ha<sup>-1</sup>) and one phosphorus level (46 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). In addition, soil samples were collected to determine Ns and Ps. At harvesting, aboveground biomass was collected for each treatment to determinate grain yield, straw and N concentration. ANOVA analysis was performed and two full factorial models were compared: Agronomical Input Model (AIM) and Agronomical and Soil Input Model (ASIM). Results showed the high spatial variability of Ps within the fields, Ns varied between 101.0 and 149.9 mg kg<sup>-1</sup> and Ps between 13.7 and 17.4 mg kg<sup>-1</sup>. ASIM improved AIM explanation to predict yield thanks to soil information (Ns and Ps variables) of about 11% and protein concentration of about 17.3%.

This study demonstrated that soil conditions have to be considered for nutrient management in Tuscany due to their great variability. This study suggests that the within field phosphorus variability need to be managed by means of site-specific fertilization to optimize the wheat production.

## Keywords

Precision agriculture, nutrients spatial variability, phosphorus, nitrogen

## 1. Introduction

Nitrogen (N) and phosphorus (P) are essential and often limiting nutrients for plant growth and crop yield. N and P influence tissues components, protein content and regulate all the biochemical processes occurring in crops, have structural function in macromolecules, participate to metabolic pathways and energy exchange, resulting essential for the growth of wheat (Tisdale et al., 1993; Kutman et al., 2011; Boukhalfa-Deraoui et al., 2015). N and P affect also many aspects of plant physiology like photosynthesis, flowering, seed maturity and seed development (Malhotra et al., 2018).

The interaction between macronutrients is important to optimize production. Grain yield might be increased adding N fertilizer when crop growth is restricted by N deficiencies, but yield may plateau if a critical factor other than N is limiting (Wilson et al., 2020). Johnston et al., 2014 reported that critical soil P levels are related to the N availability. In agricultural ecosystems, soil available N and P are the main determinants of soil fertility, influencing soil productivity. The optimization of soil management has to be pursued through the study of the heterogeneity of soil properties, especially soil nutrients (Długosz et al., 2016). Irfan et al. (2018) found a positive production response increasing the N / P ratio from 4:1 to 4:3, regardless the N fertilization level. P availability balances the effect of excessive N by accelerating crop maturity and retarding excessive vegetative growth and the response of applied N in crops is reduced under P deficiency (Irfan et al., 2018). Xin-Kai et al. (2012) reported that increasing phosphate fertilization levels directly affect wheat yield in conditions that are not N limiting ( $180 \text{ kg N ha}^{-1}$ ). The highest wheat yield of about  $6856 \text{ kg ha}^{-1}$  was reached with  $108 \text{ kg ha}^{-1}$  of  $\text{P}_2\text{O}_5$ . Panayotova et al. (2018) found that N-P interactions in wheat were able to enhance aerial biomass production up to  $11797 \text{ kg ha}^{-1}$  than fertilization with only P up to  $6687 \text{ kg ha}^{-1}$ . Khan et al., 2018 studied the interaction between N and P fertilization on a wheat variety in Pakistan, showing that the interaction significantly affected plant height, number of grains and grain weight, with the highest yield reached with  $120 \text{ kg N ha}^{-1}$  and  $90 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ . Chen et al. (2020) found that only P fertilization was not able to increase significantly yield for wheat cultivated in North China, in contrast to P and N fertilizers interaction. The optimal regime was N  $240 \text{ kg ha}^{-1}$  and P  $150 \text{ kg ha}^{-1}$  fertilization rates.

Crop performance can be optimized by applying nutrients in balanced amounts on soils to meet crop demand through the analysis of soil conditions. However, soils contain usually high pools of P and crops can benefit from only a small fraction, called monocalcium phosphate. The latter availability is often influenced by a complex dynamic of interactions with many chemical-physical characteristics of the soil. Three primary factors control the availability of soil phosphorus: soil pH, amount of organic matter and microbiological activity. In basic soils, available monocalcium phosphate is shortly immobilized into tricalcium phosphate, while in acid soils the phosphate binds to Fe and Al hydroxides becoming insoluble (Lindsay et al., 1989; Sato et al., 2005). In these conditions, crops production

losses related to the unavailability of phosphorus often occur (Penn et Camberato, 2019; Devau et al. 2010; Fageria, 2009). Some soil microorganisms are able to mobilize P in soil by solubilizing P-containing minerals and by mineralizing organic P from organic matter (Widdig et al., 2019; Brucker et al., 2020). The mineralized organic matter in the soil allows organic phosphorus compounds to become available to plants, leading to an increase of mineral available phosphorus in the soil (Wierzbowska et al., 2020). In fact, Sun et al. (2015) and Thompson et al. (1954) reported that organic P shows a linear and positive relation with available phosphorus forms in soil.

The spatial variability of soil chemical-physical-biological properties influences the availability of P in soil, making it extremely variable (Roger et al., 2014; Delgado, 2019). The soil properties spatial variability might be analyzed through geostatistical analysis (Fu et al., 2013). The interpolation technique allows to extend the chemical and physical characteristics from the punctual to the entire surface (Liu et al., 2016; Chen et al., 2020). Spatial variability of soil P has been found at various scales ranging from a few meters to a regional scale (Senthilkumar et al., 2012; FU et al., 2013). Juang et al. (2002) reported the heterogeneity of soil P content even at field level in agricultural soils. In practice, farmers are mainly interested in variability within field and across fields of a farm FU et al (2013). Complex spatial variability of soil P in fields with long histories of cropping and fertilization has long been recognized (Cline, 1944; Peterson and Calvin, 1965; Peck and Melsted, 1973). The conventional nutrient application is considered inefficient, due to the high spatial variability of nutrients. As a solution, several studies suggest the study of spatial variability and application of nutrients site-specifically to optimize production avoiding environmental losses (Argento et al., 2020; Karaman et al., 2001).

The spatial variability of available soil N and P might be influenced by agronomic practices over time, in particular phosphate fertilizer addition (Selles et al., 2011; Johnston et al., 2014; Sun et al., 2015). Sun et al. (2015) showed that application of P fertilizer, organic input, and soil incorporation of crop residues and conversion of soil use from 1982 to 2002, increased P Olsen soil concentration in large regions of Rugao Country, China.

In conditions where P fertilization doesn't meet the crop nutrient demand, the presence of soil available phosphorus influence the growth, the development and the yield (Johnston et al., 2014; Johnston et al., 2019; Chen et al., 2019). In soil phosphorus-deficient conditions, growth is generally more reduced than the rate of photosynthesis per unit of leaf area, affecting crop growth (Rodriguez et al., 1998). Deng et al. (2018) reported that under deficient soil P, the overall plant growth was depressed due to limited photosynthesis, therefore affecting yield. Rodriguez et al., 2000 found that applying phosphorous in deficient P soil conditions, the control P level ( $0 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ) showed an aboveground biomass reduced of about 70% as compared to the highest level ( $200 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ ).

Many researches showed that wheat productivity is strongly influenced by the amount of available P. In low application of P fertilizer, literature reports that P amount less than  $20 \text{ mg kg}^{-1}$  soil appear to influence production, even if other nutrients are widely available (Lichtfouse, 2011). Johnston et

al. (2014) found that three Olsen P levels, 10, 12 and 8 mg kg<sup>-1</sup> soil, were able to influence significantly wheat yield, resulting in 3.87, 6.59 and 7.79 t ha<sup>-1</sup> of grain production, respectively.

The aim of this research was to verify the effect of small soil available P variations on wheat production parameters in soil P deficient. Moreover, the aim was to assess the influence of field spatial variability on yield spatial variability. Different levels of N fertilization were supplied to verify the interaction with N.

## 2 Materials and Methods

### 2.1 Description of the study area

The study was carried out in Monteroni d'Arbia, in a farm located in a hilly area of Tuscany region, Italy. Soils from the experimental fields along the Arbia stream valley floor (Fluventic Haplustepts, fine silty, mixed, mesic) and those characterizing the valleys of the Orcia tributaries (Aquic Eutrudepts, fine, mixed, mesic; Aquic Haplustepts, fine, mixed, mesic) were mainly comprised of deep and calcareous silty-clay soils (Gardin and Vinci, 2016). The experimental fields were set up on sub-alkaline (pH 8.2) loamy-clay soils. The weather conditions are typically of Mediterranean climate, with winters that are rainy and relatively cold; summers that are warm and dry. The climate is affected mainly by Azores and Russian anticyclones and by Mediterranean depressions, with a mean annual temperature of about 13.6°C and cumulative precipitation of 715 mm. In spring rainfall conditions are variable and depends on North Atlantic Oscillation (Dalu et al., 2013; Dalla Marta et al., 2011).

The vegetative growth from tillering to anthesis occur between February and mid-May, while the grain filling occurs from mid-May to the end of June.

During the two-growing season, 2016-2017 and 2017-2018, the monthly cumulated rainfall and the growing degree day (GDD) (Salazar et al., 2013) data were computed and analyzed. Data were collected by a meteorological station located in Monteroni d'Arbia (E 1696252; N 4790654 ).

The GDD value was daily calculated as the difference between daily average temperature (Tavg; °C) and a base temperature (Tb; °C). A thermal threshold of 4 °C for the characterization of the whole crop cycle was considered as reported in other studies (Porter and Gawith, 1999; Saiyed et al., 2008). The cumulated GDD ( $\Sigma$ GDD) values were calculated as the accumulation of daily GDD on a monthly basis and for the February – May period.

### 2.2 Experimental fields, treatments and sampling

The experiment was a combination of 24 treatments (1250 m<sup>2</sup> each), replicated three times in a strip-strip-plot design. The treatments consisted of 4 wheat varieties (Var) (one improved and three local ancient varieties), 3 N fertilization levels (Nf: 35, 85 and 135 kg N ha<sup>-1</sup>), two seeding rates (Sd: 90 and 180 kg of seed ha<sup>-1</sup>) and one phosphorus (Pf) level (46 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>). Bologna was the improved

variety, while Verna, Andriolo and Sieve were the local ancient varieties. Each strip was sown with a different variety and divided longitudinally in the two different seeding density and, transversally, in three N fertilization levels. N fertilizer was applied in the form of ammonium nitrate (N 26%) in two rates at tillering and in the middle of stem elongation. P fertilizer was applied in the form of triple superphosphate ( $P_2O_5$  46%) and distributed in one application at sowing. The wheat was harvested on July 10th in 2017 and on July 13th in 2018. At maturity stage, three random samples of aboveground biomass were collected for each plot in order to determine wheat biomass. The samples were oven-dried at 105 °C until constant weight, to determine the dry weight according to Ceotto et al. (2013). The measured parameters were: grain yield (GY), straw yield (SY), grain N content (GNC), straw N content (SNC). N content was determined using a CHNS analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA). Grain protein content (GPC) was determined as reported in Guerrini et al. (2020). A total of 3 soil samples for each plot and year were collected from 0 – 30 depths in tilled soil before sowing. The soil samples were air-dried, grounded and sieved (2 mm), and then analyzed for determination of soil total nitrogen (Ns) and available phosphorus (Ps). As soil was collected only before sowing, we analyzed the total N as it is more related to the production parameters (grain and straw yield) than mineral N. In fact, the available soil N is subjected to high variability during the growing season, therefore it is representative only of a specific moment. The spatialization of Ns and Ps were created using Arc-GIS10.3

## 2.3 Statistical analysis

We conducted statistical analyses of variance using R 4.1.1 version. Two Full Factorial models were compared: (i) Agronomical Input Model (AIM) which include as agronomic inputs Var, Nf, SD and Y to predict GY, SY, GNC, SNC and GPC; (ii) Agronomical and Soil Input Model (ASIM) which include as agronomic inputs Var, Nf, SD and Y, adding Ns and Ps factors to predict GY, SY, GNC, SNC and GPC.

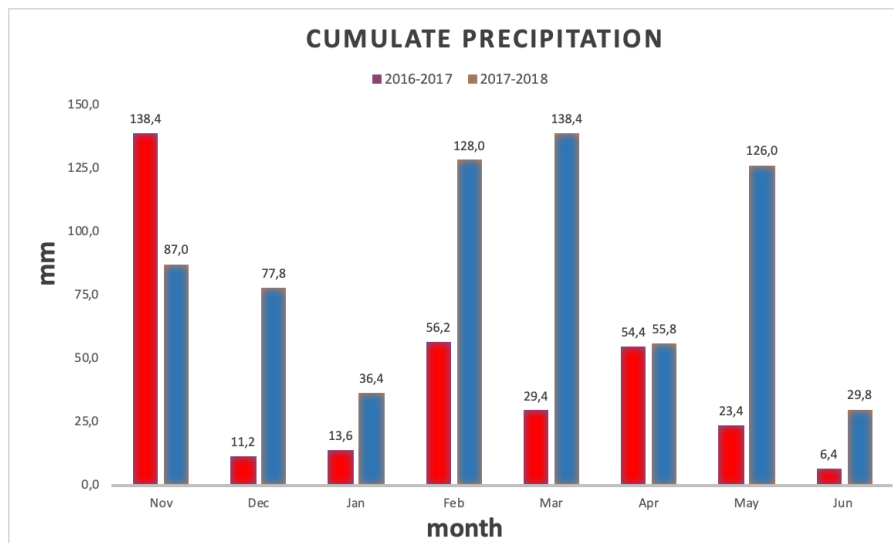
# 3 Results

## 3.1 Growing season weather conditions

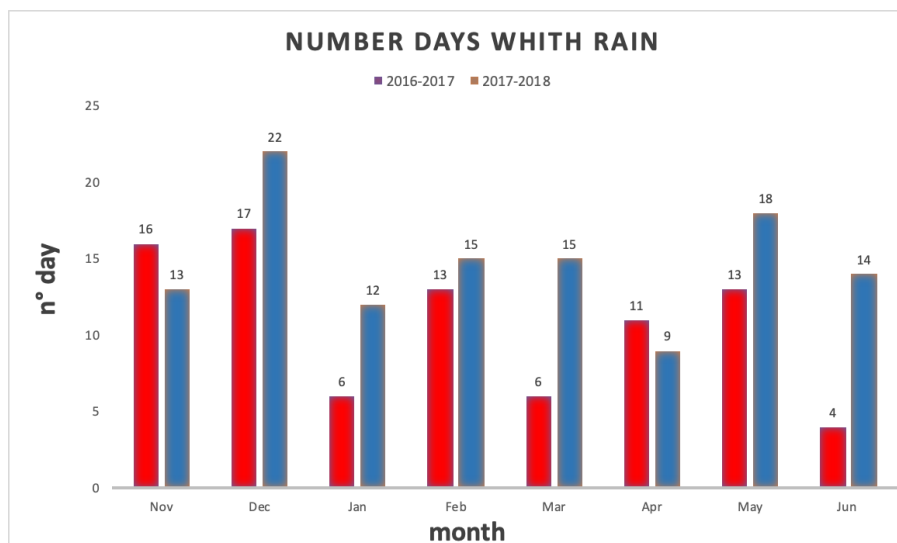
In this study, the cumulated rainfall during the growing season (from November to June) were 333 mm in 2016-2017 and 679 mm in 2017-2018. The variability in the monthly amount of rainfall among the two growing seasons were reported in Figure 1. The highest rainfall amount was showed at the beginning and at March during the growing season 2016-2017 and 2017-2018, respectively. On the other hand, the lowest rainfall amount was detected in June in both the growing season. It is likely that the high variability would have influenced the growth, development and production of wheat

varieties. The number of rainy days was also different in the two growing season, varying from 86 to 118 during the growing season 2016-2017 and 2017-2018, respectively (Figure 2).

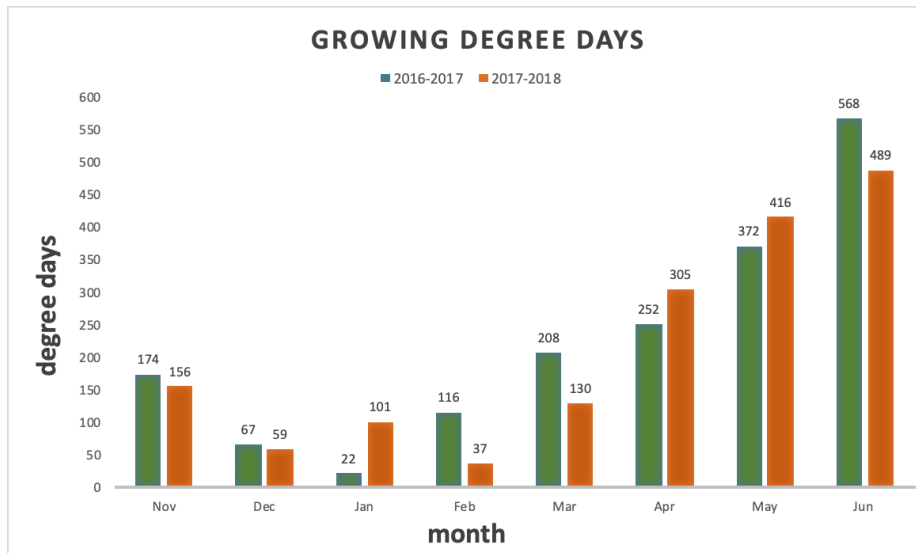
The sum of the active temperatures during the growing season was reported in Figure 3. The monthly GDD ranged from 22 to 568 and from 37 to 489, during the 1st and the 2nd growing season, respectively. In particular, the 1st growing season was warmer than the 2nd. In 2016-2017 growing season it was evident a highest increase of GDD days in February and March in respect to 2017-2018, while lowest in January.



*Figure 1 - Monthly precipitation recorded at Monteroni d'Arbia meteorological station during the experimental period (November to June) in the growing season 2016-2017 and 2017-2018.*



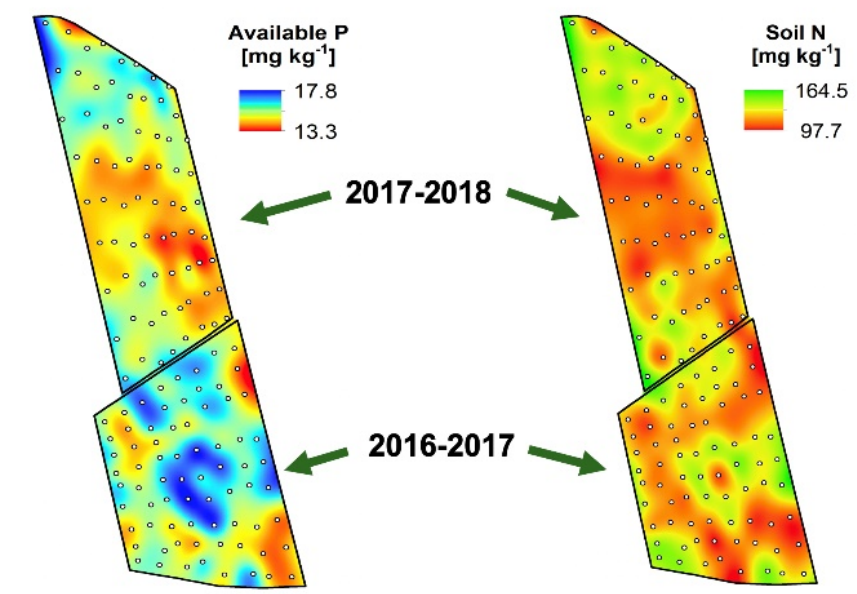
*Figure 2 – Number of rainy days recorded at Monteroni d'Arbia meteorological station during the experimental period (November to June) in the growing season 2016-2017 and 2017-2018.*



*Figure 3 - The cumulated growing degree days (GDD; °C) calculated on a monthly basis during the experimental period (November to June) in the growing season 2016-2017 and 2017-2018.*

### 3.2 Available N and P distribution within the experimental fields

The spatialization map (Figure 4) highlight the variability of Ns and Ps within the two experimental fields. The nutrient distribution resulted discontinuous, with no trends being observed for both in the two fields.



*Figure 4 - Phosphorus and nitrogen spatial distribution within the experimental fields for the two winter wheat growing season.*

Considering both fields, Ns and Ps values varied between 101.0 and 149.9 mg kg<sup>-1</sup> and between 13.7 and 17.4 mg kg<sup>-1</sup>, respectively (Table 1). The variation range for Ps resulted 3.3 mg kg<sup>-1</sup> and 2.7 mg kg<sup>-1</sup> in the first and second field, respectively. On the same time, the variation range for Ns was 42.8 mg kg<sup>-1</sup> and 46.5 mg kg<sup>-1</sup>.

The coefficient of variation highlighted that Ps variability was about the 52% of Ns variability by considering both years.

The Ps values resulted in the range that is commonly reported in bibliography as limiting for the wheat growth. Nevertheless, the limiting effect is visible only when the P fertilization rate does not meet the crop demands. The difference in Ns and Ps soil content in the two fields was minimal and not sufficient to explain the two years production variability, considering the different growing seasons. The difference between two years in the average Ns content is less than 1%, while Ps is 3.3%. Standard deviation (SD) (Table 1) showed that the internal variability of nutrients in the two fields is high and similar.

2016-2017 season					
Available soil nutrient	min	max	mean	SD	CV
Nitrogen	101.0	143.8	121.5	10.88	8.95
Phosphorus	14.1	17.4	15.6	0.78	5.02
2017-2018 season					
Available soil nutrient	min	max	mean	SD	CV
Nitrogen	103.4	149.9	121.7	10.40	8.54
Phosphorus	13.7	16.4	15.1	0.63	4.15

**Table 1** – Available soil nitrogen and phosphorus variability during the two-growing season. SD = standard deviation; CV = coefficient of variability.

### 3.3 Effect of soil available nutrients and agronomical inputs on quality and quantity yield parameters

#### 3.3.1 Grain yield

The full factorial model considering both the agricultural practices (Nf, Sd, Var) and the growing season (Y) showed a highly positive significant correlation with GY ( $R^2 = 0.8063$ ,  $P < 0.01$ ) (Table 2).

Considering the agronomic factors, Nf was the most significant, explaining about 51% of the total variance; followed by Var accounting by about 7% (Figure 5). On the contrary, SD did not significantly affect the GY. Y explained the 28 % of total variance, thus affecting GY less than Nf.

Anyway, ASIM improved AIM explanation thanks to soil information (Ns and Ps variables) of about 11%. The analyzed model showed a greater determination coefficient ( $R^2 = 0.9144$ ,  $P < 0.01$ ) respect to AIM (Table 2).

In ASIM, the agronomic inputs Nf and Var were still highly significant, followed by SD with a lower significance. In order, Nf, Var and SD were able to explain the 40%, 6% and 0.3% of the total variability, respectively (Figure 6). Considering the environmental factors, the Ps distribution showed the largest influence on GY, explaining the 26% of the total variability. Secondly, the Y variable resulted in a highly significant impact on GY detection, explaining the 22% of total variability. Lastly, the Ns was able to explain 2% of total variability, although significant.

In both models it was observed that the first level interaction between Y and Var showed a high significance. The different environment of the two growing seasons showed a highly significantly different effect on yield for each experimental variety.

In literature is widely reported the high influence of Nf on wheat GY. In this work, the large range of the N fertilization levels allowed Nf to be the most incident parameter influencing the results, in both models. The Ps effect in ASIM has been demonstrated to be the second most influent factor on grain yield, even if the variation was low, ranging between 13.7 and 17.4 mg kg<sup>-1</sup>. In phosphorus deficient fertilization conditions, the effect of small variations of Ps has been reported to be an important factor influencing GY (Lichtfouse, 2011; Johnston et al., 2014).

### 3.3.2 Grain protein content

The combined analysis of variance including agricultural practices and growing season (AIM) was also performed for GPC detection. The results showed a highly positive significant correlation, with a  $R^2 = 0.8025$ ,  $P < 0.01$ . Results indicated that Nf was the main agronomic variable influencing the GPC explaining about 50% of total variability. Secondarily, also Var showed a significant effect on GPC but explaining only the 3% of total variability, while SD was no significant. In this model, the growing season effect on GPC was highly significant, accounting for up to 33% of total variability.

Ps and Ns in ASIM allowed to explain a larger part of environmental variability, by 16% and 2%, respectively. The environmental variability explained by the growing season still accounted for a large part of the total variability (27%). In ASIM, all agronomic inputs resulted highly significant, being able

to explain 41%, 3% and 1% of total variability for Nf, Var and SD, respectively. While in AIM the SD effect was shadowed by the unexplained variability, SD in ASIM resulted highly significant thanks to the amount of explained variability adding Ps and Ns. The GPC modelled by ASIM resulted significantly correlated ( $R^2 = 0.9757$ ,  $P < 0.01$ ) with the measured data. These results highlighted that Ps and Ns improved ASIM capacity to explain the GPC variability, by about 17.3% with respect to AIM. The effect of soil available P and N on GPC in P-deficient soil conditions seemed to have a great influence on GPC. Similarly to grain yield models, the two-factors interaction Y and Var showed a high significance both in AIM and ASIM. From the results, the different weather conditions among the two years showed an influence in the protein concentration across the adopted N rates. Culm N accumulation, grain N translocation, the duration and intensity of starch synthesis-accumulation and photosynthesis interruption due to heat stress are factors related to weather condition variability (Orlando et al., 2016; Fabbri et al., 2020). Protein concentration was also affected by the soil available nutrient in the shift from 90 to 180 seeds  $\text{kg ha}^{-1}$ . The fertilization rate, according to Lopez-Bellido et al. (2001), has been demonstrated to be the most influencing factor in determining GPC. The same author found also that the growing season, in particular weather conditions, had a marked effect on GPC. According to Tòth et al. (2020), we found also a great influence of Ns and Ps on GPC. The effect of available soil nutrients resulted more influential where the fertilization rate is not able to match crop requirements. Other researchers have also reported an additive effect of phosphorus on wheat performance if supplied with N in balanced proportion (Brink et al., 2001).

### 3.3.3 Straw yield

The effect of agricultural practices interactions on SY parameter detection was analyzed. Also, in this case, the AIM performed by ANOVA showed a significant positive correlation, slightly less than the others analyzed ( $R^2 = 0.7723$ ,  $P < 0.01$ ) (Table 2). Among all the agronomic variables, Nf was the one that influenced mostly the results, explaining about 60% of the total variability. Var was also highly significant, but was able to explain only the 5% of the total variability, showing a lower effect than fertilization. SD did not show any significant influence on the SY parameter detection inside the model. In this model, the growing season effect on SY was highly significant, accounting for up to 15% of the total variability.

On the other hand, the addition of soil information again allowed the improvement of the model. In this case ASIM showed a greater determination coefficient ( $R^2 = 0.8726$ ,  $P < 0.01$ ) respect to AIM (Table 2).

In ASIM, Nf was still the most influent factor, explaining 45% of the total variability. Analyzing the agronomic factors, Nf was followed by Var, explaining 4% of the total variability, while the SD had no significant correlation. In SY, Ps and Ns showed the highest influence among all the analyzed parameters, explaining 29% and 4% of the total variability, respectively. The environmental variability explained by the growing season still accounted for a large part of the total variability (12%), following Nf. Moreover, Nf level showed a highly significantly different effect on straw yield for each experimental variety.

The effect of Nf on wheat growth and production are widely demonstrated. The P availability in the soil has shown a significant influence on the SY, due to the unfulfilled requirements from fertilization. These results agree with what was found by Irfan et al. (2018), that increasing the P recorded positive productive responses at different levels of N administered. Xin-Kai et al. (2012) found a productive advantage in terms of biomass in different phenological phases.

#### **3.3.4 Grain nitrogen content**

The GNC in both models showed to be mostly influenced by the Y-specific weather conditions. In AIM, Y variable explain the 47% of the total variability, followed by Nf with 42%. The Var showed a significant effect, but explaining only 1% of the total variability, while SD did not show a significantly different influence. In ASIM, Y was the most influent factor with 41% of total variability described, followed by the agronomical input Nf with 37%. The other agro-environmental variables showed a lower influence, in order Ns, Ps and Var with a % on the total variability of 6, 3 and 1, respectively. In this case, both models showed a lower determination coefficient ( $R^2 = 0.6568$  and  $0.7313$ ,  $P < 0.01$ , respectively) respect to the other production and quality parameters (Table 2). In literature it is widely reported that GNC depends on N fertilization and the N use efficiency (Mandic et al., 2015). The different weather conditions during each growing season are able to influence greatly the N remobilization, especially heavy heat stress in final grain filling stage (Akter and Islam, 2017). For that reasons, Y showed a strong influence on GNC.

#### **3.3.5 Straw nitrogen content**

In both models SNC showed to be mostly influenced by the Y-specific weather conditions and Nf. In AIM, Y variable was able to explain the 44% of the total variability, while Nf the 28%. Var resulted highly significant, explaining the 6% of the total variability. SD instead was not significant. In ASIM, Y variable was still able to explain 38% of the total variability, while Nf the 25%. Var showed a significant influence on SNC, explaining the 5% of the total variability. The nutrients available in the soil, Ns and

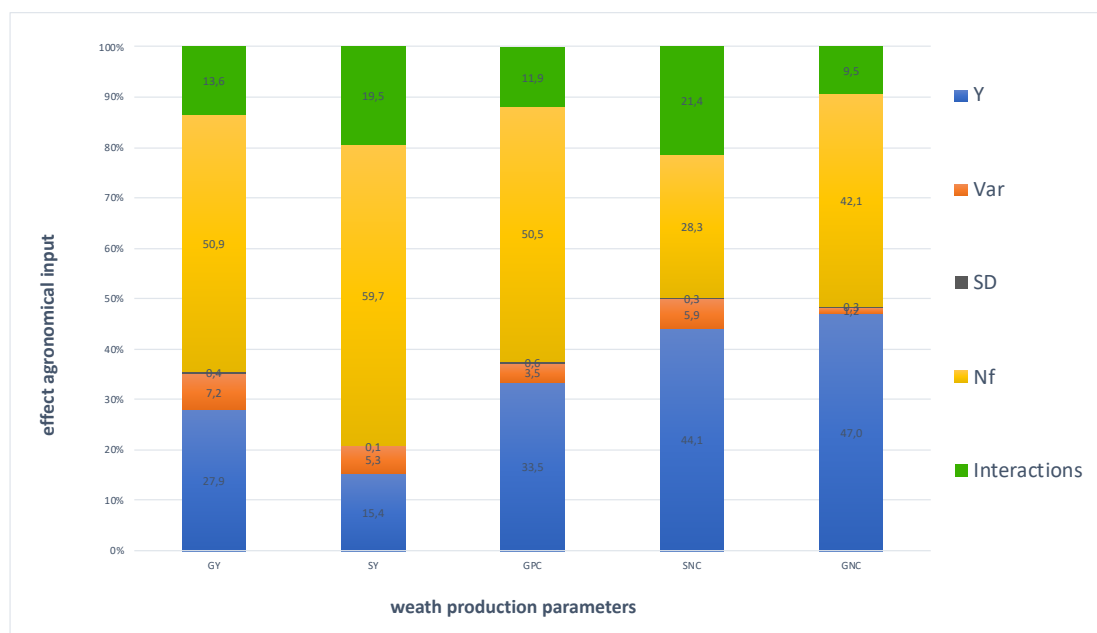
Ps, did not show a significant influence on this wheat parameter. In this case, both models showed the lowest determination coefficient for SNC in comparison to the other parameters ( $R^2 = 0.5505$  and  $0.5950$ ,  $P < 0.01$ , respectively) (Table 2).

The heat waves are cause of stress during grain filling and might interrupt the N translocation (Akter and Islanm, 2017). In this respect, the year has to be considered influent on SNC.

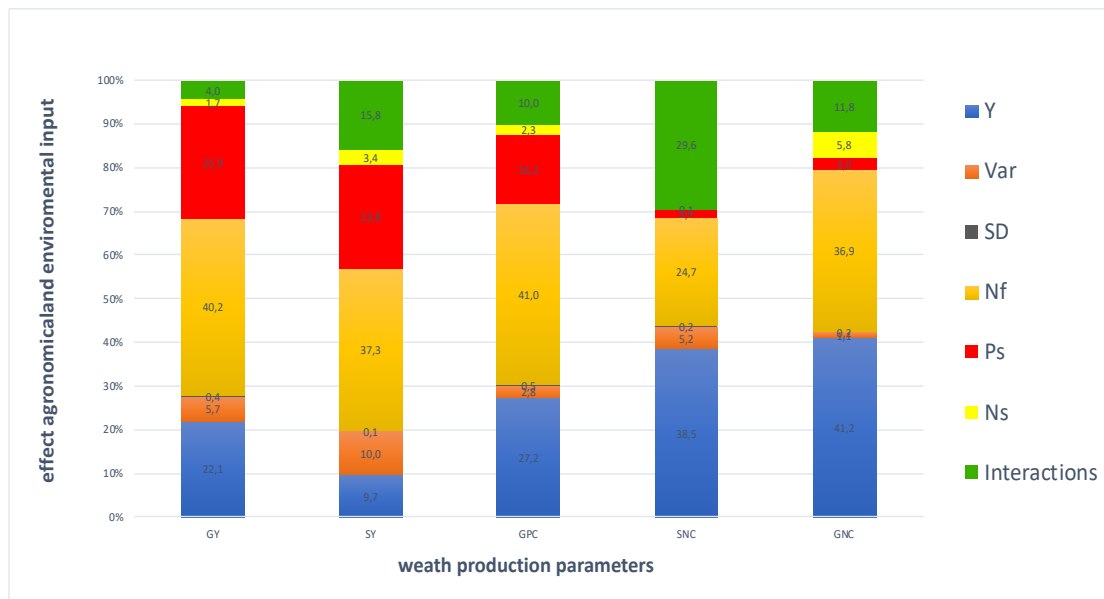
The interactions showed that varieties are significantly influenced by environmental variations (Y).

		Determination coefficient	
		AIM	ASIM
wheat parameter	GY	0,8063	0,9144
	SY	0,7723	0,8726
	GPC	0,8025	0,9757
	SNC	0,5505	0,5950
	GNC	0,6568	0,7313

**Table 2** – Determination coefficient ( $R^2$ ) among collected and modelled grain yield (GY), straw yield (SY), grain protein content (GPC), straw nitrogen content (SNC) and grain nitrogen content (GNC) in wheat for the two models.



**Figure 5** - Agronomical Input Model (AIM) showing the influence (%) of agronomical and environmental inputs on quality and quantity wheat parameters.



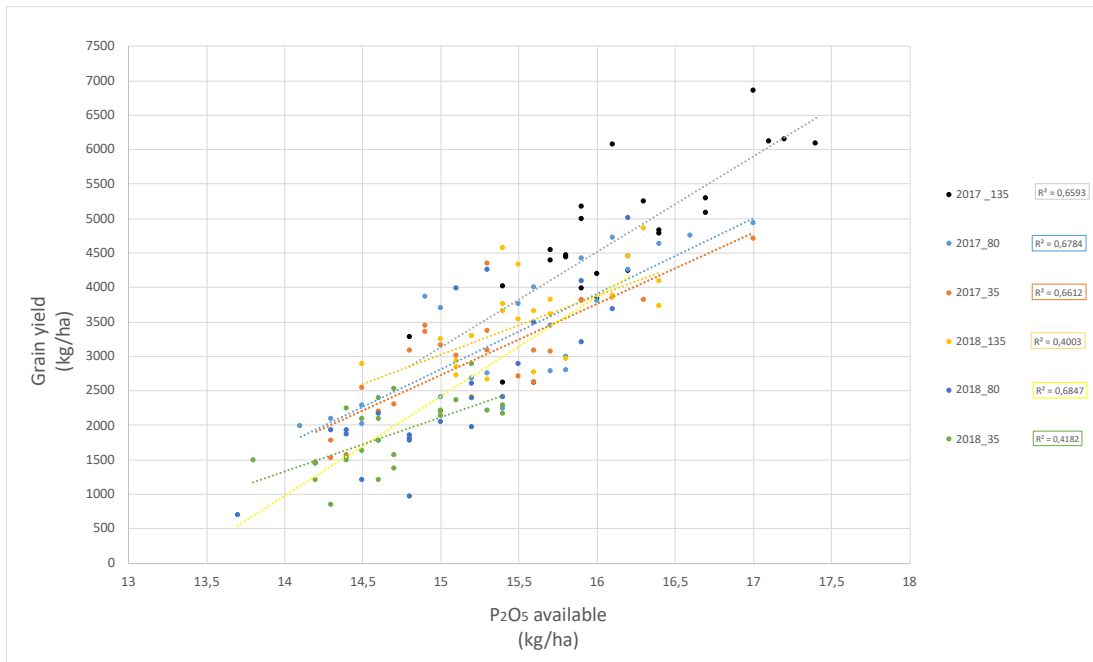
**Figure 6** – Agronomical and Soil Input Model (ASIM) showing the influence (%) of agronomical and environmental inputs on quality and quantity wheat parameters.

### 3.4 Effect of soil P available under different years and N fertilization rates on grain yield

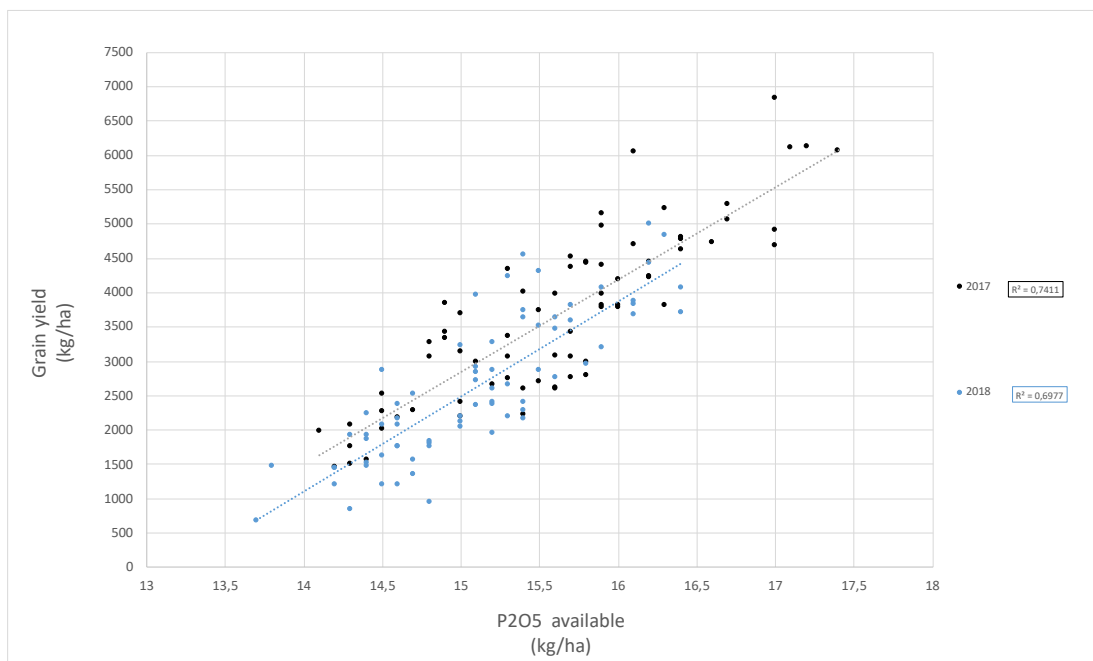
The soil available P showed a significant effect on wheat production across all the N fertilization levels during the two-growing season. All the correlations across the treatments were positive and highly significant (Figure 7). Soil available P was able to influence yield even in low N levels. The P supplied by fertilization was probably not sufficient to satisfy nutrient crop requirements.

Irfam et al. (2018) showed that grain productivity increases from 4:1 to 4:3 N-P<sub>2</sub>O<sub>5</sub> rates using three levels of N fertilization (90-120-150 kg N ha<sup>-1</sup>). In this study the different soil nutrient availability influenced significantly the yield with a ratio higher than 1:1.

In figure 8 the slope and the intercept of the linear regression line were similar in both years. For that reason, the soil available P in both years determined the similar productivity response. The different potential production in the two years was probably influenced by weather conditions.



**Figure 7** – Relationship between  $P_2O_5$  available and grain yield of wheat in two different growing season (2016-2017 and 2017-2018) and for three different nitrogen treatments (35, 80, 135 kg N  $ha^{-1}$ ).



**Figure 8** – Relationship between  $P_2O_5$  available and grain yield of wheat in two different growing season (2016-2017 and 2017-2018).

## Conclusions

This study demonstrated that the N and P soil availability in specific Tuscany areas is limited and variable. Usually, nitrogen is considered as the main element able to influence wheat production, but in the studied area phosphorus demonstrated to have similar influence, considering the low availability in that region soils and the fertilization rate. In fact, in low level P soils, even slight variations have shown a strong impact, underlining the importance of this element. In addition, year variable seemed to have a greater influence on the straw N, grain N and protein content than on the yield. Probably seasonal weather variability has effect on different physiologic processes, as N translocation, photosynthetic activity and starch accumulation, determining quantity and quality of wheat production. Soil nutrients maps represent a useful tool for integrating the information of the vegetation status on which precision nitrogen fertilization is commonly based.

According to “Green Deal” policy, the new CAP after 2020 is mainly focusing on the nutrient optimization, avoiding losses in the environment, source of pollution and economic disadvantages for farmers. The presented study can contribute to improve wheat production through a more efficient management of nutrients according with the new sustainability policies.

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GUERRINI L., NAPOLI M., **MANCINI M.**, MASELLA P., CAPPELLI A., PARENTI A., ORLANDINI S., (2020). Wheat Grain Composition, Dough Rheology and Bread Quality as Affected by Nitrogen and Sulfur Fertilization and Seeding Density. *Agronomy* 2020, 10, 233; doi:10.3390/agronomy10020233  
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## Wheat Grain Composition, Dough Rheology and Bread Quality as Affected by Nitrogen and Sulfur Fertilization and Seeding Density

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### Abstract

Flour from old varieties are usually considered very weak flours, and thus difficult to use in breadmaking especially when processed as Italian “Tipo 2” flour. Hence, the aim of our study was to understand if agronomic treatments can be used to improve flour processability and the quality of three old wheat varieties. An experimental strip-plot scheme was used: three old wheat varieties (Andriolo, Sieve, Verna), two seeding densities, three levels of nitrogen fertilization (N35, N80, and N135), and two levels of foliar sulfur fertilization. Analyzed parameters related to kernel composition, dough rheology and bread quality. Sulfur and nitrogen treatments significantly affected protein composition and dough alveograph strength, which increased by about 34% with nitrogen fertilization, and by about 14% with the sulfur treatment. However, only nitrogen fertilization affected bread characteristics. Crumb density significantly decreased from N35 to N135, while springiness and cohesiveness increased. On the other hand, sulfur did not improve breads. This highlight the importance of performing breadmaking tests in addition to the rheological determinations. The poor technological performance of old wheat flours can be improved with agronomical treatments designed to obtain higher-quality bread.

### Keywords

Old wheat varieties; sulfur fertilization; protein composition; Italian “Tipo 2” flour; baking quality

## 1. Introduction

Wheat cultivated before the 'green revolution' (up until the late 1960s) are currently called 'old wheats', while those registered later are called 'modern wheats' [1,2]. Since the 1970s, the cultivation of old wheats has been progressively abandoned, as they are less productive than modern wheats, with less protein production per hectare, and with a gluten composition characterized by less gliadin and glutenins [3]. The latter is one of the main problems affecting the old wheat varieties, being that wheat end-use is strongly related to the gluten matrix characteristics [4]. In fact, flour from old wheat varieties are reported being very weak flours and not suitable for the industrial baking especially when processed as Italian "Tipo 2" flours [2,5,6]. However, in recent years, old wheats have been re-introduced as a contributing to the safeguarding of germplasm and, consequently, biodiversity [7]. A micro-economy has developed around old wheats, allowing local producers to differentiate their products and increase their remuneration [8]. However, their poor breadmaking performance still remains the biggest obstacle to their popularity.

Italian "Tipo 2" flour made from old varieties has usually poor rheological properties, resulting in a dough that is difficult to work [8]. The resulting breads are usually low volume with a dense crumb structure, two characteristics that are not appreciated by consumers [9]. Moreover, the Italian "Tipo 2" flours were found to result in dough with higher tenacity and lower extensibility than those refined [8]. Hence, several efforts have been made to improve their technological performance. For example, Parenti et al., (2013) [10] added a process control based on a twin arm mixer to find the optimal mixing time, Cappelli et al., (2018) [8] evaluated the amount of water required to optimize the compromise between W and P/L, while bread makers have developed several protocols aimed at improving the quality of the final product [2]. Furthermore, it has been established that also agronomical treatments can significantly affect kernel composition, dough rheology and, ultimately, bread quality. For example, Geleta et al. (2002) [11] observed decreasing the protein concentration in kernel as the seeding rate increased. Gooding et al. (2002) [12] and Zhang et al. (2016) [13] reported the nitrogen availability greatly influencing optimal plant densities for kernel yield and quality traits. Otteson et al. (2008) [14] found that increasing the nitrogen rate increased the protein concentration in kernel and bread loaf volume, while seeding rate did not significant affect both kernel quality baking quality. Salvagiotti et al. (2009) [15] found sulfur fertilization improving the nitrogen uptake rate before anthesis thus increasing the final kernel yield. Tea et al. (2005) [16] and Tea et al. (2007) [17] found that nitrogen and sulfur fertilization at anthesis stage increasing the kernel protein content and improving the gluten network, the dough strength, swelling, and extensibility. Tao et al. (2018) [18] found sulfur fertilization significantly increasing total protein and starch content in kernel, and increasing glutenin and gliadin content, and the ratio of glutenin to gliadin. According to the latter study, the addition of sulfur and nitrogen to the wheat could play an important role in final bread quality, but the idea has received little attention. Particularly, to the best

of the authors knowledge, no work specifically focused on the nitrogen-sulfur interactions while performing breadmaking trials. Furthermore, no study evaluated the effects of seed density, nitrogen fertilization and sulfur addition on dough rheology and bread quality for old wheats. Hence, we tested the effect of three agronomical treatments on three old wheat varieties, namely Verna, Sieve and Andriolo.

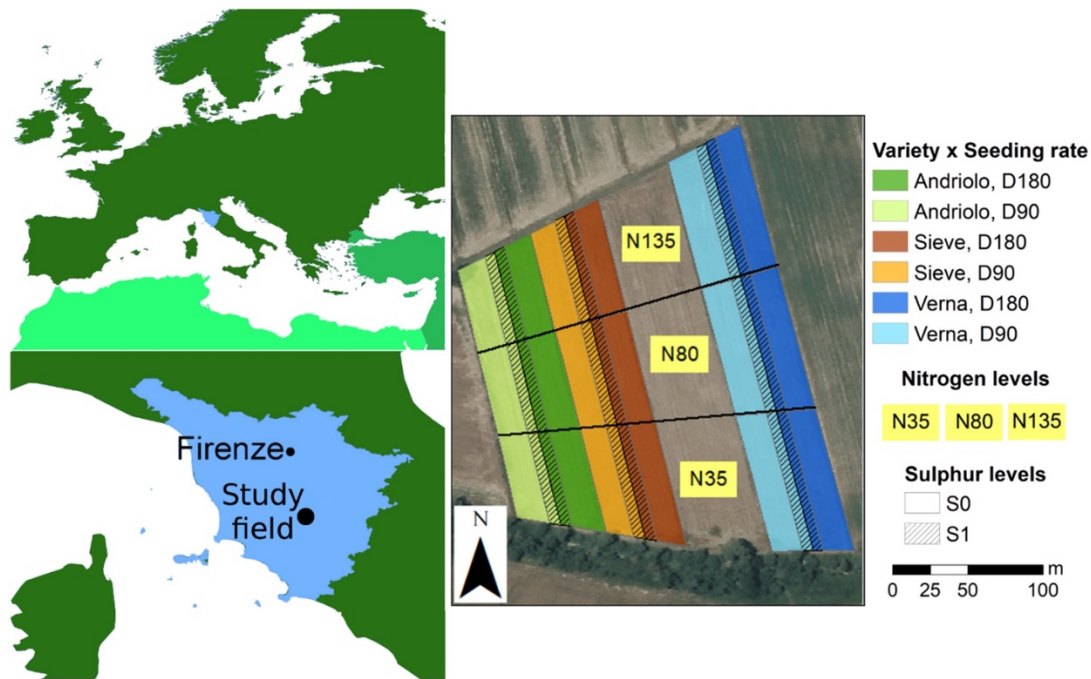
## 2. Material and Methods

### 2.1. Wheat Cultivation

Field experiments were established in October 2016 under rainfed conditions at the Giuseppe Chiarion farm, located in Monteroni d'Arbia, about 20 km south-east of Siena, Tuscany, Italy (43.2007° N, 11.4182° E, 160 m a.s.l.) [19]. Egyptian clover (*Trifolium alexandrinum*, L.) was the previous crop. The soil was silty clay loam, and the 0–0.3 m layer contained 11.4 g kg<sup>-1</sup> total organic carbon, 1620 mg kg<sup>-1</sup> total nitrogen, 14.2 mg kg<sup>-1</sup> available phosphorus, and 273 mg kg<sup>-1</sup> potassium. A meteorological station was placed near the experimental field, and data on temperature and humidity were recorded. Three Italian old genotypes of common wheat (*Triticum aestivum*, L.) were studied, namely Andriolo, Sieve and Verna.

Thirty-six treatments were investigated. These involved combinations of three varieties of common wheat, two seeding rates (90 and 180 kg seed ha<sup>-1</sup>; D90 and D180 respectively), three nitrogen (N) fertilization levels (35, 80 and 135 kg N ha<sup>-1</sup>; N35, N80 and N135 respectively), and two sulfur (S) fertilization treatments (0 and 6.4 S kg ha<sup>-1</sup>; S0 and S1 respectively).

The experimental arrangement was a strip-plot design, with wheat cultivars arranged in vertical strips (main plots). N (nitrogen fertilization) was allocated to horizontal subplots, seeding density was applied to vertical sub-subplots, and S (sulfur fertilization) was applied vertically to sub-sub- subplots (Figure 1).



**Figure 1.** Maps of the study area. (a) Map of Tuscany within Europe. (b) Map of the Chianti region in Tuscany, and the location of the study field. (c) Layout of the study field. Seed densities levels D90 and D180 indicates 90 and 180 kg of seed  $\text{ha}^{-1}$ , respectively. Nitrogen levels N35, N80 and N135 indicate 35, 80 and 135 kg N  $\text{ha}^{-1}$ , respectively. Sulfur levels, S0 and S1 indicate 0 and 6.4 kg S  $\text{ha}^{-1}$ , respectively. The area between the variety Sieve and Verna was cultivated with a 'modern' wheat variety (*Triticum aestivum*, L var. Bologna) with the same cultivation techniques of the 'old' ones (Bologna variety results were not used in this paper).

Seeds were sown on December 19, 2016. A total of 175 kg  $\text{ha}^{-1}$  of triple superphosphate ( $\text{P}_2\text{O}_5$ : 46%) was broadcast on treatments N35, N80, and N135. Nitrogen total dose was scheduled in three different applications: 20% by broadcasting urea (N: 46%) at seeding, 40% by spreading ammonium nitrate (N: 26%) at tillering, and 40% by spreading urea (N: 46%) at stem elongation.

The S1 treatment was performed at booting by spraying a wettable sulfur powder (Thiovit Jet 80 WG<sup>®</sup>, Syngenta, Basel, Switzerland) at a rate of 8 kg  $\text{ha}^{-1}$  (6.4 kg  $\text{ha}^{-1}$  of active ingredient). Although Thiovit is commonly used as a fungicide at a recommended rate of 8 kg  $\text{ha}^{-1}$ , we tested it as an alternative to sulfur fertilizers. At tillering, an herbicide treatment was performed by distributing Axial Pronto 60 (Syngenta, Basel, Switzerland) at a dose of 0.75 L  $\text{ha}^{-1}$  (60 g  $\text{L}^{-1}$  Pinoxaden and 15 g  $\text{L}^{-1}$  and Cloquintocet-mexyl) and Marox SX (Cheminova Agro Italia, Rome, Italy) at a rate of 0.75 L  $\text{ha}^{-1}$  (333 g  $\text{L}^{-1}$  of thifensulfuron-methyl and 167 g  $\text{L}^{-1}$  of tribenuron-methyl). At booting, a fungicide treatment was performed by spraying Amistar Xtra (Syngenta, Basel, Switzerland) at a rate of 0.8 L

ha<sup>-1</sup> (Azoxystrobin 18.2% and Cyproconazole 7.3%) and Sakura (Sumitomo Chemical Co., Tokyo, Japan) at a rate of 1.2 L ha<sup>-1</sup> (Bromuconazole 167 g L<sup>-1</sup> and Tebuconazole pure 107 g L<sup>-1</sup>).

No noticeable crop damage was observed during the growing season due to weeds, insects, or disease. In particular, no fungal attacks were observed either on surfaces treated with sulfur, or on untreated surfaces. Harvesting was performed at wheat commercial maturity (kernel moisture lower than 13%) on 10 July 2017. The three varieties reached the commercial maturity on the same time. For each treatment, three plant samples were randomly collected from an area measuring 0.5 m<sup>2</sup>. Wheat from each treatment was harvested separately using a combine-harvester equipped with Trimble GPS sensors, and yield monitoring sensors designed to measure and record information such as kernel flow and moisture, area covered and location. For each treatment, 5 kg of harvested wheat kernel were sampled for quality and technical analyses.

## 2.2. Analysis of Kernel

For each kernel sample, the following analyses were performed in triplicate. The hectoliter weight (HW; kg hL<sup>-1</sup>) and 1000 kernel weight (KW, g 1000<sup>-1</sup> seeds) were determined according to ISO 7971-1 [20] and ISO 520 [21], respectively. Kernel samples were milled using a grinder with a 0.5 mm screen (Cyclotec 1093 lab mill, FOSS Tecator, Höganäs, Sweden) as reported in Zilic et al. (2011) [22]. Then, wholemeal flour samples (5 mg) were analyzed with a CHNS analyzer (CHN-S Flash E1112, Thermo-Finnigan LLC, San Jose, CA, USA) to determine total nitrogen and total carbon.

## 2.3. Analysis of Proteins

A modified Osborne fractionation [23] was performed to isolate the single protein fractions: water soluble (albumins), 0.5 M sodium chloride soluble (globulins), 70% ethanol soluble (gliadins), 0.1 M acetic acid soluble (glutenin), and insoluble proteins. Two hundred grams of raw wheat kernel were milled using the previously described grinder with a 0.5 mm screen [22]. Then, the resulting wholemeal flour (150 g) was defatted using 600 mL of hexane and vortex for 90 min. This suspension was centrifuged (10000 rpm, 10 min) and extracts discarded. The defatted flour was air-dried under a hood at 20 °C for 24 h. Next, 100 g were sequentially extracted using four solvents (see below). Initially, flour was extracted with deionized water (400 mL) [24] for 30 min, vortexing for 1 min, every 10 min. Then, the mixture was centrifuged for 5 min at 2000 rpm. The supernatant was recovered and stored. The extraction was repeated two more times over the resulting pellet with the same solvent; recovered supernatants were combined, designated as the albumin extract, and stored at 4 °C in the dark. The resulting pellet was extracted with 400 mL 0.5 N NaCl solution [25] for 60 min, vortexed for 2 min, at 10 min intervals. Then the mixture was centrifuged at 3000 rpm for 10 min and the supernatant recovered. This extraction was repeated twice and the supernatants were combined (globulin extract) and stored at 4 °C in the dark. The pellet was then extracted with 400 mL 70% ethanol solution [26] for 60 min, vortexed for 1 min, every 10 min. Then, the mixture was centrifuged

for 10 min at 3000 rpm, and the supernatant recovered. The procedure was repeated three times to remove all of the protein in this fraction, before the three supernatants were combined (gliadin extract) and stored at 4 °C in the dark. Finally, the centrifugate was extracted with 400 mL acetic acid 0.1 M [27] for 90 min, vortexed for 1 min, every 10 min and then centrifuged for 5 min at 3000 rpm, to obtain the supernatant. Acid extraction was repeated three times before the supernatants were combined (glutenin extract) and stored at 4 °C in the dark. Gliadin was precipitated from its extract by adding acetone, following the procedure given in Tecson et al. (1971) [28]. Other proteins were precipitated by adjusting pH to 4.1, 4.3 and 4.8, for albumin, globulin and glutenin, following the procedure reported in Ju et al. (2001) [24]. The precipitate was oven-dried (105 °C, 5 h) and weighed. Remaining, insoluble protein was determined following the procedure given in Bean et al. (1998) [29]. Solvent residues were removed from the pellet resulting from acid extraction by mixing it with 10 mL of acetone, centrifuging (10000 rpm, 5 min) and discarding the extracts. The pellet was crushed with a mortar and pestle, then oven-dried (105 °C, 5 h). Dried pellets were analyzed to determine total Kjeldahl nitrogen (TKN). The resulting TKN values were converted to insoluble protein by multiplying by 5.7 according to ICC Standard 167 (2000) [30]. Total protein was calculated by summing the weight of the five protein groups described above. Protein fractions were expressed as % of total protein on dry weight basis.

#### **2.4. Analysis of Doughs**

The flours were sieved to reach the Italian “Tipo 2” law standard, since their ash content ranged from 0.80% to 0.95%. Dough rheology was assessed with the Chopin alveograph according to ISO 27971 [31] procedures. Briefly, 250 g of flour was weighted and mixed in the alveograph chamber with a NaCl solution (2.5% w/w) for 8 min. No yeasts were added. Then, dough was extruded and rested for 20 min before the measurement. Data are obtained with a Chopin alveograph, which can evaluate the rheological properties of doughs made from these flours. The alveograph measures several parameters, such as: P, which refers to dough tenacity (i.e., resistance to deformation); L, which concerns dough extensibility (i.e., the maximum volume of air that a dough bubble is able to contain); P/L, the ratio between P and L values (i.e., the curve); G, the index of swelling, related to the volume of air required to break the dough bubble; and W, which measures dough strength (i.e., the surface under the curve).

#### **2.5. Breadmaking Process**

To evaluate the effect of agronomical treatments, breads were prepared using the following recipe; 310 g of Italian “Tipo 2” flour was mixed with 180 g of water, and 9 g of NaCl was added. Mixing of ingredients (25 min at room temperature), dough formation, resting, leavening (1 h and 20 min at 40 °C) with 12 g of fresh brewer’s yeast (Lievital, Trecasali, Italy), and baking (55 min at 180 °C) were all carried out with a bread machine (Pain doré, Moulinex, Ecully, France) [32].

## 2.6. Analysis of Breads

The millet displacement method [33] was used to measure bread volume. Specific volume was determined as the ratio between bread total volume and bread weight. Crumb specific volume was determined by cutting 5–10 g of bread crumb and calculating the ratio between its volume measured with the standard millet displacement method and its weight. This method was adapted from [33] as reported in Cappelli et al. (2020) [34] and in Parenti et al. (2019) [32]. Crumb and crust moisture were measured by gravimetry at 105 °C until constant weights were reached.

The Texture Profile Analysis (TPA) of bread samples was carried out by two-bite compression using a Texture Analyzer (TA-XT2, Stable Micro Systems, Godalming, Surrey, England), with a circular flat-plate probe (diameter of 25 mm) according to the procedure described in Kim et al. (2017) [35]. Three slices of about 1 cm thickness were cut at the middle of each bread sample. Thus, for each sample, 3 measurement replicates were performed, and the median value was taken. Mechanical test conditions were as follows: 50% compression rate, 50 N of automatic trigger load, 10 mm of travel distance and 3 mm s<sup>-1</sup> for pre-test, test and post-test speeds. Crumb hardness, cohesiveness, gumminess, chewiness and springiness were measured.

## 2.7. Statistical Analysis

A 3-way ANOVA was used to test the main effect of the three agronomical factors and their interactions. Significance was set at  $p < 0.05$ . When the significance level was reached, a Tukey HSD post-hoc test was run. The inclusion of only one year means that we did not test the varieties, since with different pedo-climatic conditions the varieties response could be different. However, the varieties can still be considered semi-independent replications, which means that the main effects of the agronomical treatments are better defined than if this research had been done on only one variety. The software used was R version 3.6.0.

## 3. Results and Discussion

Our experimental design allowed us to evaluate the effect of the three treatments (N fertilization, seed density, and S fertilization). The effect of the three agronomical treatments was evaluated in terms of kernel quality and, particularly, hectoliter weight, 1000 kernel weight, kernel C and N content, total proteins, and protein composition. Average production for Andriolo, Sieve and Verna varieties (Table 1) was 2925, 4204, and 2955 kg ha<sup>-1</sup>, respectively.

Source of Variation	Kernel Yield (kg ha <sup>-1</sup> )		1000-Kernel Weight (g)		Hectoliter Weight (kg hL <sup>-1</sup> )		Total Nitrogen (%)		Total Carbon (%)	
	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig
<i>Main effect</i>										
<i>Cultivar</i>										
Andriolo	2925.43		44.32		71.83		2.21		45.8	
Sieve	4203.85		40.89		74.05		2.2		45.51	
Verna	2955.07		37.46		72.35		2.28		45.46	
<i>Seeding density</i>										
		ns		**		ns		ns		ns
D90	3395.04	a	41.84	a	72.94	a	2.20	a	45.57	a
D180	3327.86	a	39.94	b	72.54	a	2.26	a	45.61	a
<i>Sulfur</i>										
		ns		ns		ns		ns		*
S0	3357.21	a	41.51	a	72.58	a	2.16	a	45.85	a
S1	3366.16	a	40.27	a	72.89	a	2.3	a	45.33	b
<i>Nitrogen</i>										
		**		ns		ns		**		ns
N35	2667.66	b	41.16	a	72.98	a	2.07	b	45.45	a
N80	3107.08	b	40.81	a	72.12	a	2.12	b	45.71	a
N135	4309.6	a	40.7	a	73.12	a	2.49	a	45.61	a
RSE	564.24		1.84		1.89		0.24		0.36	
<i>Interactions</i>										
<i>Nitrogen × Sulfur</i>		ns		ns		ns		*		ns
<i>Nitrogen × Seed Density</i>		ns		ns		ns		ns		ns
<i>Sulfur × Seed Density</i>		ns		ns		ns		ns		ns

**Table 1.** Mean of kernel quality parameter results by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (\* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error.

Kernel yield was significantly and positively related with nitrogen fertilization. However, differences were found to be related to N135, while no significant differences were found for N35 and N80. On the contrary, seeding density and sulfur treatment resulted not significantly affect final kernel yield. Results indicated 1000 kernel weight significantly decreasing as the seeding density increase from 90 to 180 kg seed ha<sup>-1</sup>. No difference (except those related to cultivars) was found for hectoliter weight. Kernel N was significantly increased by N135, while no significant differences were found for the other fertilization levels. Moreover, results also indicated a significant positive interaction between S and N fertilization in increasing the N accumulation in kernel. Kernel C content was found to be significantly decreased by the sulfur treatment (from 45.82% ± 0.24% to 45.33% ± 0.50% from S0 to S1). However, the decrease in C could not be considered important in terms of kernel quality. These results were consistent with previous studies [12,13,15] reporting nitrogen fertilization increasing the kernel yield. Further, other studies [15,36–38] reported a positive interaction between

N and S fertilization in increasing kernel yield in wheat. Otteson et al. (2008) [14] found that N concentration in kernel being significantly increased by N fertilization, while being not significantly affected by seeding rate. Gooding et al. (2002) [12] and Zhang et al. (2016) [13] found a significant interaction between N fertilization and seeding density in determining the kernel yield. A significant increase in kernel total protein content was due to nitrogen fertilization (Table 2).

Source of Variation	Total Protein (%DW)		Insoluble Proteins (%DW)		Albumins (%DW)		Globulins (%DW)		Gliadins (%DW)		Glutenins (%DW)		Total Gluten (%DW)	
	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig
<i>Main effect</i>														
<i>Cultivar</i>														
Andriolo	12.41		0.35		1.97		1.13		3.92		5.04		8.96	
Sieve	12.37		0.36		1.92		1.10		3.86		5.13		8.99	
Verna	12.84		0.37		1.98		1.16		4.05		5.28		9.33	
<i>Seeding density</i>														
D90	12.18	ns	0.35	ns	1.94	ns	1.12	ns	3.90	ns	5.05	ns	8.95	ns
D180	12.67	a	0.37	a	1.97	a	1.14	a	3.96	a	5.28	a	9.24	a
<i>Sulfur</i>														
S0	12.21	ns	0.35	ns	2.05	**	1.23	**	4.3	*	4.27	*	8.57	**
S1	12.86	a	0.36	a	1.86	b	1.02	b	3.55	b	6.07	a	9.62	a
<i>Nitrogen</i>														
N35	11.7	**	0.34	*	1.81	*	1.04	ns	3.68	ns	4.83	ns	8.51	*
N80	11.98	b	0.35	b	1.85	ab	1.08	a	3.72	a	4.99	a	8.71	ab
N135	13.93	a	0.4	a	2.2	a	1.28	a	4.38	a	5.68	a	10.06	a
RSE	1.34		0.03		0.24		0.31		0.57		0.63		0.40	
<i>Interactions</i>														
<i>Nitrogen × Sulfur</i>		*		ns		ns		ns		ns		ns		ns
<i>Nitrogen × Seed Density</i>		ns		ns		ns		ns		ns		ns		ns
<i>Sulfur × Seed Density</i>		ns		ns		ns		ns		ns		ns		ns

**Table 2.** Mean of kernel protein fraction results by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (\* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error.

Moreover, significant differences were found between N135 level and the two lower levels, while no significant difference was found between N35 and N80. On the contrary, the adopted nitrogen fertilization levels had little effect on the protein composition of these old wheat varieties. Nitrogen fertilization significantly affected albumin content, which increased slightly (from 1.81% to 2.2% of DW), but only for the N135 treatment. Further, N fertilization significantly affected total gluten, which increased by about 18.2% from N35 to N135. In contrast, modern wheat varieties have been reported to be more sensitive to the amount of N, expressed in both their yield potential and protein composition, while it appears to have little effect on albumins and globulins content [39,40]. It has also been reported to positively influence total gliadins and glutenin [41,42].

Results also indicated that the sulfur treatment deeply changed the protein composition. In particular, the sulfur treatment significantly decreased albumin, globulin and gliadin fractions, while

it significantly increased glutenin (from 4.23% to 6.07% of DW). Moreover, also total gluten significantly increased by about 12.3% from S0 to S1. Previous studies have indicated that sulfur availability enhances the activity of enzymes such as nitrate reductase [43] and glutamine synthetase in flag leaves [44], thereby affecting the content of different protein components [45]. In particular, Tao et al., (2018) [18] reported that sulfur availability was positively correlated with glutenin production and negatively correlated with the ratio of gliadin to glutenin. To sum up the results of the impact of our factors on kernel proteins, nitrogen fertilization increased total protein and total gluten content, while the sulfur treatment changed the protein composition, increasing total gluten and glutenin, and decreasing other protein fractions.

Agronomical treatments affected dough rheology and, particularly, dough tenacity (P), dough extensibility (L), and deformation energy (W) (Table 3). On the other hand, the index of swelling (G) showed no significant difference. P increased slightly with sulfur and nitrogen fertilization. Dough extensibility was increased by nitrogen fertilization. Since dough deformation energy is the area under the tenacity and extensibility curve, both an increase in P, and an increase in L increase W. In fact, W increased by about 34% with nitrogen fertilization (from N35 to N135), compared to roughly 14% with the sulfur treatment. Increases due to the agronomical treatment are of particular interest for old wheat flours, especially when processed as Italian “Tipo 2” flours, as they are considered usually very weak in term of deformation energy [1], and any increase in this value has to be considered helpful for the breadmaking process.

Source of Variation	W		P		L		P/L		G	
	Average	sig	Average	sig	Average	sig	Average	Sig	Average	Sig
<i>Main effect</i>										
<i>Cultivar</i>		***		***		ns		***		ns
Andriolo	52	c	33	c	44	a	0.85	c	14.5	a
Sieve	94	a	57	a	40	a	1.53	a	13.9	a
Verna	60	b	40	b	40	a	1.06	b	13.0	a
<i>Seeding density</i>										
		ns		*		ns		ns		ns
D90	72	a	45	a	43	a	1.12	a	14.6	a
D180	65	a	42	b	39	a	1.17	a	13.7	a
<i>Sulfur</i>										
		*		*		ns		ns		ns
S0	64	b	42	b	39	a	1.14	a	13.7	a
S1	73	a	45	a	44	a	1.15	a	14.5	a
<i>Nitrogen</i>										
		**		*		*		ns		ns
N35	58	b	41	b	36	b	1.21	a	13.2	a
N80	69	ab	44	ab	41	ab	1.1	a	14.2	a
N135	78	a	45	a	46	a	1.13	a	14.9	a
RSE	11		4		10		0.29		1.7	
<i>Interactions</i>										
<i>Nitrogen × Sulfur</i>		ns		ns		ns		ns		
<i>Nitrogen × Seed Density</i>		ns		*		ns		ns		
<i>Sulfur × Seed Density</i>		ns		ns		ns		ns		

**Table 3.** Mean of alveograph results by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (\* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error.

Nitrogen fertilization increased the Verna W from 48 to  $67 \times 10^{-4}$  J, the Andriolo W from 49 to  $54 \times 10^{-4}$  J and the Sieve W from 78 to  $111 \times 10^{-4}$  J. These values are consistent with alveograph evaluations in Migliorini et al. (2016) [5] for Verna and Sieve, while they are lower for Andriolo. Overall, W values remain low regardless of the agronomical treatment and, in fact, according to the common classification, flours with W below 90 are not considered suitable for breadmaking. In our study, Sieve, at nitrogen levels N80 and N135, exceeds this threshold and can be considered a weak flour. In this case, nitrogen fertilization was able to change the flour 'class'.

W values were compared to the literature with respect to total protein content [5] and their high molecular weight glutenin content [46]. Our data are consistent with earlier work, as both W and total proteins increased with nitrogen fertilization. Furthermore, a significant relationship between W and total protein was found ( $p = 0.008$ ). Although we only have only data for total glutenin, a significant relationship between glutenin content and W was found ( $p = 0.02$ ). In this case, both W and glutenin were found to be significantly affected by the S treatment. This is consistent with an

effect of S fertilization on dough technological parameters reported in Tea et al. (2005) [16]. On the other hand, neither nitrogen nor sulfur were able to change the P/L ratio. High P/L is another limit of old wheat flours [8], but our data did not highlight any change due to the agronomical treatment. Our agronomical treatments did affect final bread quality (Table 4). Crumb density was significantly decreased by nitrogen fertilization. Crumb density is an important parameter for bread quality, since it can be considered a proxy for bread porosity. Nitrogen fertilization leads to higher protein content in kernel, higher W and, consequently, higher crumb density. Furthermore, nitrogen fertilization also affected crumb texture. Crumb springiness and crumb cohesiveness increased with nitrogen fertilization from N35 to N135 (by 17% and 32% respectively). Springiness describes how the crumb returns to its un-deformed state after a compression force is removed, while cohesiveness describes the amount of effort required to chew, and it is usually seen as a positive characteristic in baked products [47]. Nitrogen fertilization improved both these parameters, resulting in an improvement in bread crumb texture. The tested agronomical treatments, nitrogen and sulfur, significantly increased the total protein content of Italian “Tipo 2” flours; in particular, an enhancement of the storage proteins (i.e., gluten), was obtained. The effect of the agronomic treatments on the gluten proteins was different: nitrogen treatment equally increased glutenin and gliadin fractions, maintaining their ratio roughly unvaried; conversely, sulfur reduced the gliadins and enhanced the glutenins, determining a change in the proportion of the two components of the gluten. In the literature it is largely known that gluten plays a key role for the flour breadmaking performance, since it confers the dough unique visco-elastic properties [48–54]. Hence, sulfur and nitrogen treatments, impacting the gluten quantity, revealed that an agronomic practice could directly affect the most important actor in the breadmaking process [48–54]. This observation was well known for modern refined varieties, and could be extended to Italian “Tipo 2” flours from old varieties. Rheological results showed a significant boost in the dough strength (W) as a consequence of both sulfur and nitrogen treatments. These results could be related to the higher gluten quantity of Italian “Tipo 2” flours, since in the literature it is largely known that gluten proteins significantly improve dough rheological/alveograph properties [48–54]. W represents an important parameter in the evaluation of the flour technological quality: the higher the W index, the higher the dough stability during mixing, gas holding capacity and performance during long fermentation time, since the alveographic test simulates the deformations occurring during the leavening and baking steps [50,52]. Furthermore, in the literature, W values are commonly used to classify flours for their destination use [50,52]. With regard to the evaluation of bread quality, nitrogen treatment produced a significant decrease of bread crumb density, and a significant increase of texture parameters, namely springiness and cohesiveness. These results were consistent with data about the gluten content and alveographic parameters. Indeed, the increased gluten proteins and W value allowed a better gas retention capacity of the dough during the leavening and the baking. In detail, in the leavening, the gluten promotes a better retention of the gas produced during the yeast fermentation, allowing a better

loaf increase; during baking, it allows the creation of a fine-even crumb while water evolves as vapor and gases further expand [50,52]. As a result, bread crumb appeared characterized by a significant lower density and by a porous structure. Moreover, TPA analysis showed that this crumb structure was characterized by a significant increase of springiness and cohesiveness. Both these texture parameters are associated to a better bread quality and are features largely appreciated by consumers [50,52]. Conversely, sulfur fertilization, although producing similar effects of nitrogen in term of W, did not significantly affect bread characteristics. This is probably linked to the observed decrease in the gliadin fraction [54]. The sulfur results highlight the importance of the ratio between gliadin and glutenins to obtain a bread with quality characteristics appreciated by consumers. Furthermore, it is important to point out that all the cited literature referred to breads made from refined flours, while these results allow to evaluate the effect of agronomical treatments on bread quality. Moreover, both chemical and rheological tests showed improvements in the Italian “Tipo 2” flour composition and dough rheology that not resulted in a significant improvement of the bread. Thus, the nitrogen fertilization could be useful to improve the poor technological features of weak flours.

Source of Variation	Volume (mL)		Crumb Density (g mL <sup>-1</sup> )		Hardness (N)		Springiness (mm)		Cohesiveness		Chewiness (N·mm)	
	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig	Average	sig
<i>Main effect</i>												
<i>Cultivar</i>		***		ns		ns		ns		ns		ns
Andriolo	1043	b	1.42	a	11.7	a	0.74	a	0.32	a	2.57	a
Sieve	1165	a	1.24	a	7.3	a	0.81	a	0.41	a	2.44	a
Verna	993	c	1.36	a	8.6	a	0.75	a	0.39	a	2.34	a
<i>Seeding density</i>												
D90	1052	a	1.26	a	8.3	a	0.80	a	0.39	a	2.51	a
D180	1082	a	1.42	a	10.2	a	0.74	a	0.36	a	2.39	a
<i>Sulfur</i>												
S0	1079	a	1.31	a	10.2	a	0.78	a	0.39	a	2.35	a
S1	1054	a	1.37	a	8.3	a	0.76	a	0.37	a	2.55	a
<i>Nitrogen</i>												
N35	1039	a	1.50	a	11.4	a	0.70	a	0.31	a	2.29	a
N80	1077	a	1.29	b	7.4	a	0.79	b	0.41	b	2.21	a
N135	1085	a	1.23	b	8.9	a	0.82	b	0.41	b	2.85	a
RSE	57		0.26		4.7		0.10		0.09		0.95	
<i>Interactions</i>												
<i>Nitrogen × Sulfur</i>		ns		ns		ns		ns		ns		ns
<i>Nitrogen × Seed Density</i>		ns		ns		ns		ns		ns		ns
<i>Sulfur × Seed Density</i>		ns		ns		ns		ns		ns		ns

**Table 4.** Mean of quality analyses on breads shown by nitrogen fertilization, sulfur fertilization, seed density, cultivar and first order interaction. The sig columns report the ANOVA results (\* = 0.05, \*\* = 0.01, \*\*\* = 0.001, ns = not significant), while lowercase letters represent the Tukey HSD post hoc test results. RSE = residual standard error.

## 4. Conclusions

The aim of the study was to evaluate whether the poor technological bread-making qualities of three old wheat flours could be improved with an agronomical treatment. Thus, we tested the effect of nitrogen fertilization, sulfur fertilization and seed density on kernel composition, dough rheology and bread quality.

Results related to seed density were minor and cannot be used to improve the bread-making properties of the tested varieties. Sulfur fertilization was found to affect protein composition and, particularly, increase gluten content. W values consistently increased with sulfur addition. Since W is a key parameter in the assessment of flour workability, a sulfur foliar application in such weak flour could be a promising strategy to improve their technological performance. However, further studies on a broad range of varieties, with in-depth chemical analyses are still required to fully understand the effect. Finally, nitrogen fertilization was found to be a useful tool to modulate the assessed qualitative parameters as it was able to increase yield, total protein and total gluten content, and protein composition. Furthermore, nitrogen fertilization improved the W value of the dough, and changed bread crumb density and texture. Hence, N fertilization can be successfully used to improve technological parameters of the tested weak flours.

In conclusion, the poor performance of these flours can be improved with agronomical treatments designed to obtain higher-quality bread. These results can be considered of particular interest for old wheats with poor technological performance. However, more work is needed in order to make further improvements to their processability. Moreover, additional trials including more years and different pedo-climatic conditions are required to evaluate the interaction between cultivars and the agronomical treatments.

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# Comparing organic and conventional farming systems: a complete LCA study on wheat

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## Abstract

Assessing the environmental impact of agriculture is a key factor towards reducing human impacts on the food production chain. This study was aimed at assessing and comparing the impacts of organic and conventional farming practices on an ancient soft wheat variety (var. Verna) in Tuscany, Italy. A cradle-to-grave life cycle assessment (LCA) from raw material extraction, through to industrial processing, field utilization, grain harvesting and finally transportation to storage centres was carried out. This study analysed data sampled over a five-year period (2014/2015 to 2018/2019) derived from five organic and conventional farms, respectively. The impact categories considered included: global warming, freshwater ecotoxicity, seawater ecotoxicity, terrestrial ecotoxicity, human toxicity, acidification, eutrophication, photo-oxidant formation, non-renewable energy resource consumption, renewable energy resource consumption, water consumption and land use). In almost all the impact categories, organic farming was shown to perform better environmentally, while conventional farming had a minor impact on land use. However, results relating to acidification, photo-oxidant formation, ozone layer depletion and non-renewable energy resource consumption were considered similar for the two cultivation systems. Normalization of the results showed that seawater ecotoxicity had the greatest impact among all impact categories (> 99%) for both cultivation systems. Moreover, major environmental problems in conventional farming and organic farming were synthetic N fertilizers and low yields, respectively. Results showed that 192 x 10<sup>6</sup> hectares of organic farming would be needed to maintain current wheat production in the EU, compared to just 99 x10<sup>6</sup> hectares cultivated with the conventional method. Accordingly, yield increase in organic farming, and reduction of nutrient losses/emissions from conventional farming, are the two most promising strategies towards maintaining a high agricultural production with concomitant reductions in the related environmental impact.

## Keywords

Carbon footprint; Environmental impact; Sustainable agriculture; Ecotoxicity; Eutrophication; Acidification;

## Highlights

- Organic farming produces 45.9% less wheat (Verna) grains than conventional farming
- Mechanical practices and fertilizers manufacturing are the main sources of impact
- Seawater ecotoxicity represents more than 99% of total impacts
- Land use has a relevant impact on organic farming
- Precision farming contributes to improve environmental performances of wheat

## 1. Introduction

Life Cycle Assessment (LCA) has gradually been adopted in agriculture for the assessment of the environmental impacts on food production processes (Fallahpour et al., 2012; Van Stappen et al., 2015; Krzyzaniak et al. 2018). LCA examines different impact categories, including global warming, acidification, eutrophication, thereby permitting a complete investigation of different production processes and food products. In this sense, by using LCA, it is possible to evaluate the global impact of a specific food during the entire production process and to compare different production processes using a standard functional unit (FU) either as a unit of product or a unit of cultivated area (Brentrup et al., 2004a; Meier et al., 2015).

Wheat is the second most cultivated crop worldwide and is the staple food crop for a significant part of the global population (FAOSTAT, 2014). Modern wheat varieties are required to meet specific technological quality criteria for the transformation industry. Meeting these criteria have been the subject of intense genetic breeding efforts. However, conventional breeding has caused losses in genetic variability with a reduction in crop adaptability to different ecosystems and pedoclimatic variations (Newton et al., 2010; Döring et al., 2015). In the last few years, the use of ancient wheat varieties is increasing, attributable to a higher adaptability to climate variability, lower input requirements and improved nutraceutical properties (Lammerts van Bueren et al., 2011; Migliorini et al., 2016; Boukid et al., 2020; Fatholahi et al., 2020). The latter aspect is a crucial aspect of interest for consumers, where there is an ever-increasing shifting preference towards local and healthy foods, as well as a specific interest in sustainability (Chiriaco et al., 2017). Thus, organic farming is growing significantly on a global level with increases in surface area coverage of approximately 24 millions of hectares from 2006 to 71 millions of hectares in 2018 (Tu et al., 2006; Willer et al., 2020). Consumer preferences for organic food is mainly due to the absence of chemical inputs (synthetic fertilizers and pesticides). Despite a higher use of machinery compared to conventional agriculture, organic farming is more environmentally sustainable in terms of pollution, biodiversity pressure, soil erosion and

energy use, with positive impacts on soil and water quality (Brandão et al., 2010; Tuomisto et al., 2012). Nonetheless, the real impacts of organic farming on global warming and climate change mitigation are currently under discussion (Gomiero et al., 2011; Tuomisto et al., 2012; Chiriaco et al., 2017). On the one hand, organic farming is based on the maintenance of natural soil fertility with a reduced adoption of external inputs. However, on the other hand, the reduced yields per hectare and, consequentially, the higher amount of land required to satisfy food demand, represent a setback in the system. In the last decades, an intensive review of the literature was performed throughout the scientific community in order to assess the real environmental impacts of organic and conventional farming systems (Tuomisto et al., 2012; Meier et al., 2015; Chiriaco et al., 2017). Principal findings reported wide variability, mostly attributable to relevant differences in management practices, as well as differences in the methodological approaches between the two farming systems that render comparisons difficult (Chiriaco et al., 2017). In addition, the zoning of different agricultural aptitude groups may produce different results even when adopting similar agricultural practices. Recent findings affirmed that approximately 84% more land is needed for organic farming compared to conventional farming (Tuomisto et al., 2012). This is principally due to the lower yields (crops and animals) that account for approximately 20 to 34% less than conventional farming (De Ponti et al., 2012; Seufert et al., 2012). Generally, lower yields in organic farming are determined by the lack of nutrients, presence of weeds, pests and diseases (Korsaeth et al., 2008). Moreover, some authors reported that organic farming guarantees a higher soil organic matter (SOM) due to the continuous input of compost, manure and crop residues (Leite et al., 2010; Santos et al., 2012). From the analysis of cumulative greenhouse gas (GHG) emissions, olives, beef and some crops were shown to produce less emissions in organic farming (Casey and Holden, 2006; Tuomisto et al., 2012). In contrast, higher GHGs were produced in certain sectors of organic farming. These included milk production, due to the lower yields and higher CH<sub>4</sub> and N<sub>2</sub>O emissions (Thomassen et al., 2008), and cereal and pig productions, due to higher N<sub>2</sub>O emissions. However, Tuomisto et al. (2012) observed that N<sub>2</sub>O and NH<sub>3</sub> emissions are hugely variable based on the calculation approach. In particular, it was reported that organic farming produced roughly 31% and 18% lower emissions of N<sub>2</sub>O and NH<sub>3</sub>, respectively, per unit of area than conventional farming. However, from calculations based on unit of product, Tuomisto et al. (2012) also reported that organic farming produced 8% and 11% higher emissions of N<sub>2</sub>O and NH<sub>3</sub>, respectively. Lower yields also significantly affect the water footprint. A higher water consumption by approximately 15% was reported in dairy organic farming compared to conventional farming (Palhares et al., 2015). Different impacts between organic and conventional farming on winter wheat production in Belgium were also observed (Van Stappen et al., 2015). A better performance of organic farming in terms of aquatic ecotoxicity, land occupation, water deficiency potential and photo-oxidant formation have been reported (Van Stappen et al., 2015).

Instead, conventional farming has been shown to have a higher impact on terrestrial ecotoxicity, acidification and eutrophication potential. However, this is only true when considering 1 ha as the functional unit. The worst performances in terms of acidification, eutrophication and land occupation were shown in organic farming when the analysis was performed using 1 kg (fresh matter) of wheat grain (Van Stappen et al., 2015). From a meta-analysis on the environmental impacts of organic and conventional farming systems in Europe, lower N leaching losses (approximately 31%) were shown in organic farming compared to conventional farming per unit of area, as a result of the lower N rate normally adopted in organic farming (Tuomisto et al., 2012). In contrast, the same authors affirmed that N leaching losses were roughly 49% higher in organic farming if calculated as a unit of product. According to Aronsson et al. (2007), this is due to the limited N availability in the soil for plant uptake. The use of a non-renewable energy resources in organic farming, as with grasslands, was calculated to be approximately 50% less than conventional farming due to the lower adoption of external inputs (Haas et al., 2001). Similarly, organic farming was reported to represent an effective strategy to reduce the consumption of non-renewable energy resources (with a net reduction of 60%) than conventional farming on barley, when expressed as functional unit of 1 ha (Tricase et al., 2018). In this sense, the energy requirement for the production of 1 kg of urea accounts for 35.1 MJ (Lizarazu et al., 2010). However, from the analysis of terrestrial ecotoxicity, conventional farming was shown to produce only 33% of the impacts produced by organic farming (Tricase et al., 2018). As with other impact categories, this was mainly due to taking into account the higher yields obtained from conventional farming over that of organic farming.

Based on the inconsistencies reported in the literature, there is an important requisite for a comprehensive analysis, considering the most important impact categories. Recent literature has primarily been focused on only a few impact categories. Articles investigating all the available categories are relatively few, and rarely dedicated to the comparison between organic and conventional farming. The present study was aimed at providing a complete evaluation of the environmental impacts between organic and conventional wheat cropping systems in Italy. The significance of the present study is primarily linked to the following aspects: the relevant amount of considered impact categories, the amplitude of the studied area and the time length of the experiment.

## **2. Materials and Methods**

The evaluation of the environmental performance of Verna wheat production comparing organic (ORG) and conventional (CON) farming systems was carried out with LCA. LCA is widely adopted in agriculture for the assessment of the environmental impacts of food production processes (Fallahpour et al., 2012; Van Stappen et al., 2015; Krzyzaniak et al. 2018), and permits a complete investigation of the different production processes or food products.

## 2.1 Goal and scope definition

This study was aimed at performing an environmental sustainability assessment for the comparison of ORG and CON farming on the production chain of an ancient soft wheat variety (var. Verna). From the analysis of the processes, it was possible to identify the critical phases of the processes in order to propose improvement actions to increase the level of sustainability of Verna wheat agricultural systems.

## 2.2 Functional unit and system boundaries

The functional unit was defined as 1 kg of wheat grain. Verna is an ancient soft wheat variety, typically of Tuscany, and is characterized by the following aspects: (i) an effective weed control capacity due to the high plant height; (ii) a sensitive tillering potential that allows Verna to produce a widespread rooting system with a higher nutrient and water use efficiency, compared to modern varieties, and (iii) late ripening. Late ripening may represent a critical factor because of heat stress risks, however, due to the broad rooting system, Verna demonstrates better stress resistance than modern varieties (Lammerts Van Bueren et al., 2011). In recent years, there has been a growing interest in the adoption of this variety in Tuscany, attributable to the low input requirement. In addition, a high level of nutraceutical properties was previously reported for this variety (Dinelli et al., 2008)

The analysis was performed using data acquired from a five-year period, spanning the growing season from 2014/2015 to 2018/2019. In order to obtain a representative overview of the wheat-systems in the Tuscan region, five organic and five conventional farms, homogeneously distributed over the region to include the provinces of Arezzo, Florence, Grosseto and Siena, were included (Fig. 1). The soil and agro-climatic environment can be considered similar in relation to the productivity of Verna, whereas the random distribution of the farms guaranteed data comparability. The climatic conditions of the study were typically Mediterranean, characterized by rainy and cold winters, and dry and hot summers with precipitation concentrated in both autumn and spring (average annual precipitation of 700 mm). Wheat yields are linked to the higher inter-annual meteo-climatic variability (Dalla Marta et al., 2011a; Dalla Marta et al., 2012). The maximum vegetative growth period of winter wheat occurs between stem elongation and anthesis, coinciding with the period between February to mid-May. Verna has a later ripening of 7-10 days, compared to modern varieties, that typically occurs between mid-end July. Early heat waves represent a serious issue causing the interruption of the starch accumulation phase with yield losses. However, given the low sowing densities and deeper rooting systems, plants are better able to overcome short-term periods with anomalous high temperatures.



*Figure 1. Representation of the study area in Tuscany region (Italy) composed of the Arezzo, Florence, Grosseto and Siena provinces, respectively.*

Furthermore, in this study the productive chain with wheat grains as outputs was considered. The straw was not taken into consideration. Straw is used as a source of organic matter for the soil, both directly during harvesting operations and indirectly as manure in livestock farms. The system boundaries encompassed all wheat cultivation activities, from seeds to yields (wheat grains) and transport to the storage centre, including: (i) seed production; (ii) production and consumption of fuels; (iii) production and use of fertilizers; (iv) production and distribution of plant protection products for treatments; (v) transport of cultivation inputs; (vi) water consumption for the dilution of components used for phytosanitary product preparations; (vii) entire life scenarios for cultivation input packaging; (viii) transport of yields from the farm to the storage centre. As Verna is a rainfed crop, water consumption was only necessary for the dilution of phytosanitary products. Farm infrastructures (both agricultural and related factories), production of machinery and tractors for agricultural operations, human labour, and maintenance phases were not considered in the present study. Further, impacts of pesticide use were not considered because an appropriate model is currently unavailable (Naudin et al., 2014).

## 2.3 Impact assessment

In the present study, the SimaPro v8.5 software was used for the impact assessment of ORG and CON production systems of Verna wheat. Through CML vs 3.06 (2016) methodology, the following impact categories were evaluated: global warming, freshwater ecotoxicity, seawater ecotoxicity, terrestrial ecotoxicity, human toxicity, acidification, eutrophication, photo-oxidant formation and ozone layer depletion. Moreover, resource consumption indicators including, non-renewable energy resource consumption and renewable energy resource consumption were calculated using Cumulative Energy Demand (CED) vs. 1.11 (2018). In order to implement the wheat production process impact assessment for Verna (Tab. 1), water consumption and land use were calculated starting with the reporting of water volumes and surfaces used in the life cycle.

	Impact categories	Abbreviations	Reference factor	Units	Reference method	
Environmental Impact Categories	Global Warming	GLW	Carbon Dioxide	kg CO <sub>2</sub> eq	CML vs 3.06 (2016)	
	Freshwater Ecotoxicity	FET	1,4-Dichlorobenzene	kg 1,4-DB eq	"	
	Seawater Ecotoxicity	SET	1,4-Dichlorobenzene	kg 1,4-DB eq	"	
	Terrestrial Ecotoxicity	TET	1,4-Dichlorobenzene	kg 1,4-DB eq	"	
	Human Toxicity	HUT	1,4-Dichlorobenzene	kg 1,4-DB eq	"	
	Acidification	ACD	Sulphur Dioxide	kg SO <sub>2</sub> eq	"	
	Eutrophication	EUT	Phosphates	Kg PO <sub>4</sub> <sup>---</sup> eq	"	
	Photo-Oxidant Formation	POF	Ethylene	kg C <sub>2</sub> H <sub>4</sub> eq	"	
	Ozone Layer Depletion	OLD	Chlorofluorocarbon-11	kg CFC-11 eq	"	
	Resource Consumption Indicator	Non-Renewable Energy Resources Consumption	NRC	Mega Joule	MJ	Cumulative Energy Demand (CED) vs. 1.11 (2018)
		Renewable Energy Resources Consumption	RRC	Mega Joule	MJ	"
		Water Consumption	WAC	Litres	L	Substances Inventory
Land Use		LAU	Square meters	m <sup>2</sup>	"	

**Table 1.** Impact categories

## 2.4 Life cycle inventory

Data collection for the Life Cycle Inventory (LCI) was carried out by means of specific check-lists developed *ad hoc* for wheat cultivation. App. 1 summarized the data collected. Inventories refer to the two farming systems, each with its own phases, consumption and yields, respectively (Fig. 2).

Primary data from the inventories, were analysed with Ecoinvent v.3.4 database processes using geographical and technological analogies

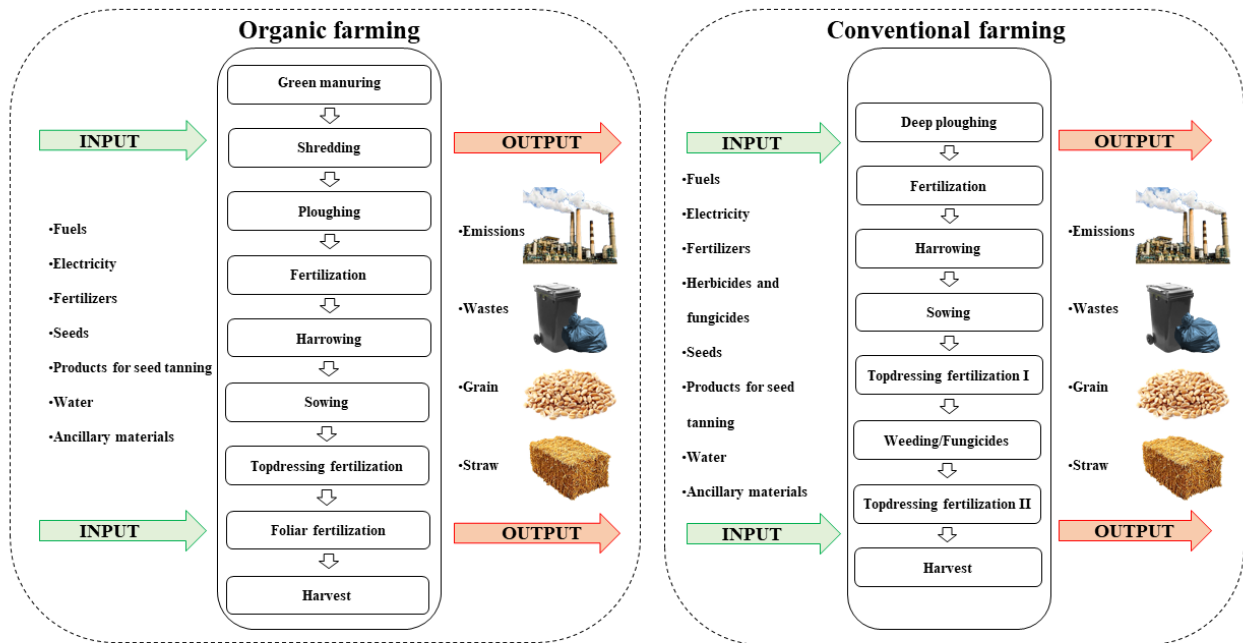


Figure 2. Models of cultivation activities in ORG and CON farming system of Verna wheat

The inventory analysis included all the exploited resources and environmental emissions for wheat cultivation, starting from seed production for sowing to the harvest of yields and subsequent transportation to the storage centre.

The observed data from the different farms showed differences in the used inputs and in the agricultural management strategies for both for ORG and CON. In particular, the most relevant differences were observed between organic farms. Each farm followed the same method throughout the years. In order to define a reference cultivation model for the two systems, all the inputs used by the five farms were calculated by attributing a respective relative weight. For instance, ploughing was carried out by all organic farms and 100% of the “weight” of impacts was assigned. However, three out of the five organic farms performed disc harrowing, to which 60% of impacts was assigned. Average yields throughout five years were 1524 ( $\pm 521$ ) and 2815 ( $\pm 294$ ) kg of wheat grain for ORG and CON, respectively.

Inputs and outputs, relative to the cultivation activity, were divided into 6 different factors in order to investigate the specific contribution of each factor:

- Mechanical Practices (MP). This factor included the use of machinery for tillage, input supplying, as well as harvest and yield transportation (grain and straw) from the farm to the storage centre. Tractor type and power, as well as time and fuel consumption were considered for each mechanical operation (App. 1). A high degree of homogeneity regarding mechanical practices was observed

between the two farming systems, with the exception of fertilization strategies that showed a relevant variability in ORG.

- Fertilizer Manufacturing (FM). This factor considered the impacts generated from the production of fertilizers. In both farming systems, nitrogen-based and phosphorus-based fertilizers were adopted. In ORG, we observed a high variability in agricultural practices and adopted inputs. In particular, for each of the five farms, five different types of fertilizers were used before sowing (Ravel 27, Opengreen; Biosiapor 3.12, Unimer; Endurance N7, Unimer; Endurance N8, Unimer; Siapton, Siapa) (App. 1). In contrast, in CON, a nitrogen-phosphate fertilizer was adopted for the fertilization treatment before sowing (di-ammonium phosphate - Siapor, Unimer). Thereafter, a nitrogen-based fertilizer (ammonium nitrate - Sulfan, Yara) was adopted for two top dressing fertilization treatments, respectively (App. 1).

- Use of Fertilizers (UF). Direct impacts from the use of fertilizers in the field were considered in this category. Both N and P were the elements adopted by both farming systems. However, the dispersion of the fertilizers was carried out differently. N-based fertilizers adopted in ORG can be assumed to be more stable than those adopted in CON (App. 1).

- Manufacturing of Herbicides and Fungicides and Seed tanning (HFS). This factor included the production of seeds for sowing, seed tanning, as well as the manufacturing of herbicides and fungicides (for CON only). Seed tanning was performed using copper-based products for ORG, and a mixture of prothioconazole + fluoxastrobin + tebuconazole for CON (App. 1). Furthermore, in CON, one fungicide (BUMPER P: prochloraz + propiconazole) and different herbicides (MAROX: thifensulfuron methyl + tribenuron methyl; AXIAL PRONTO: pinoxaden + cloquintocet mexyl; MANTA GOLD: fluroxipir + clopiralid + mcpa; ATLANTIS: methyl iodosulfuron + diethyl mefenpir + mesosulfuron methyl) treatments were adopted during the growing season (App. 1).

- Ancillary Materials (AM). In this category, we included the impacts for polypropylene thread production, used for tying straw bales. Furthermore, impacts for transport were considered.

- Waste Materials (WM). This category included all the materials used for the packaging of products. In particular, the analysis was conducted based on both the weight and the type of packaging materials, standardized on FU. In addition, disposal methods, as well as the distances between farms and disposal centres were considered. For both farming systems, an average distance of 50 km between the farms and the disposal centre was considered using the Ecoinvent "Transport, freight, lorry 3.5- 7.5 metric ton, EURO4 {RER}.

- Direct Land Occupation (DLO). For the LAU impact category, the effective cultivated surface was computed.

Within each impact factor, the substances responsible for the impacts were identified.

## 2.5 Life cycle impact assessment (LCIA)

The LCIA (carried out using the SimaPro v8.5 software) highlighted the impacts of each impact category starting from the data collected in the LCI. The combination of inventory data, with specific equivalent factors, permitted the attainment of characterization factors for each impact category. The impacts were reported by specific indicators.

## 3. Results

### 3.1 Global warming

Emissions of greenhouse gases (GHGs) are the driving factor causing the increase in the Earth's surface temperature, known as "Global Warming". The main GHGs from agriculture are principally represented by carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O). GHG emissions are standardized on CO<sub>2</sub> global warming effects and computed as kg CO<sub>2</sub> equivalents (eq) on a 100-year time scale, using IPCC guidelines (IPCC, 2013). In ORG, MP represented the main factor affecting GLW, accounting for 76% of 0.359 kg CO<sub>2</sub> eq FU<sup>-1</sup> (Fig. 3). HFS produced a significant impact, due to the products for seed tanning and the environmental costs involved in seed production for sowing. The FM and UF provided a small contribution due to the limited adoption of synthetic fertilizers. Nevertheless, in CON the main contributors to GLW were FM (39% of the total), MP (29% of the total) and UF (25% of the total) (Fig. 3). Compared to ORG, CON showed higher impacts in terms of GLW, accounting for 0.518 kg CO<sub>2</sub> eq FU<sup>-1</sup> with a net contribution of more than 162% (Tab. 2).

			MP	FM	UF	HFS	AM	WM	DLO	TOTAL
GLW	kg CO <sub>2</sub> eq	ORG	2.70E-01	1.00E-02	2.00E-02	5.00E-02	3.08 E-03	4.20 E-03	-	3.60E-01
		CON	1.70E-01	2.30E-01	1.40E-01	4.00E-02	1.69 E-03	1.31 E-03	-	5.80E-01
FET	kg 1,4-DB eq	ORG	3.03 E-03	5.18 E-03	0.00E+00	4.96 E-03	3.51 E-04	1.31 E-03	-	1.00E-02
		CON	1.88 E-03	4.00E-02	0.00E+00	3.68 E-03	1.91 E-04	4.07 E-04	-	5.00E-02
SET	kg 1,4-DB eq	ORG	1.10E+01	2.62E+01	0.00E+00	1.51E+01	1.30E+00	2.50E+00	-	5.61E+01
		CON	6.85E+00	1.50E+02	0.00E+00	1.23E+01	7.10E-01	7.80E-01	-	1.70E+02
TET	kg 1,4-DB eq	ORG	1.72 E-04	5.56 E-05	0.00E+00	4.66 E-05	1.59 E-06	2.78 E-06	-	2.79 E-04
		CON	1.07 E-04	4.32 E-04	0.00E+00	4.00 E-05	8.57 E-07	8.71 E-07	-	5.80 E-04
HUT	kg 1,4-DB eq	ORG	1.00E-02	9.83 E-03	6.26 E-06	1.00E-02	3.51 E-04	6.69 E-04	-	4.00E-02
		CON	9.47 E-03	9.00E-02	5.61 E-05	7.86 E-03	1.89 E-04	2.09 E-04	-	1.10E-01
ACD	kg SO <sub>2</sub> eq	ORG	2.44 E-03	9.98 E-05	1.00 E-04	4.24 E-04	1.05 E-05	1.13 E-05	-	3.08 E-03
		CON	1.51 E-03	1.22 E-03	8.98 E-04	2.61 E-04	5.72 E-06	3.51 E-06	-	3.90 E-03
EUT	kg PO <sub>4</sub> <sup>3-</sup> eq	ORG	5.51 E-04	3.52 E-05	8.03 E-04	2.20 E-04	2.08 E-06	6.05 E-06	-	1.62 E-03
		CON	3.42 E-04	4.47 E-04	4.26 E-03	3.55 E-04	1.13 E-06	1.88 E-06	-	5.41 E-03
POF	kg C <sub>2</sub> H <sub>4</sub> eq	ORG	4.74 E-05	4.98 E-06	0.00E+00	1.05 E-05	6.62 E-07	2.22 E-06	-	6.58 E-05
		CON	2.94 E-05	3.75 E-05	0.00E+00	5.27 E-06	3.61 E-07	6.88 E-07	-	7.32 E-05
OLD	kg CFC-11 eq	ORG	5.18 E-08	1.04 E-09	0.00E+00	8.55 E-09	5.11 E-11	3.33 E-10	-	6.18 E-08
		CON	3.22 E-08	1.80 E-08	0.00E+00	3.87 E-09	2.61 E-11	1.04 E-10	-	5.41 E-08
NRC	MJ	ORG	4.24E+00	2.06 E-01	0.00E+00	7.70E-01	9.00E-02	2.00E-02	-	5.34E+00
		CON	2.64E+00	2.17E+00	0.00E+00	3.60E-01	5.00E-02	5.56 E-03	-	5.23E+00
RRC	MJ	ORG	8.36 E-03	2.00E-02	0.00E+00	7.34 E-03	2.30 E-03	8.94 E-04	-	3.00E-02
		CON	5.19 E-03	7.00E-02	0.00E+00	6.45 E-03	1.27 E-03	2.78 E-04	-	9.00E-02
WAC	litres	ORG	3.73E-01	7.80E-02	0.00E+00	6.90E-02	4.00E-03	2.00E-03	-	5.26E-01
		CON	2.32 E-01	2.16E-01	0.00E+00	1.55E-01	2.00E-03	1.00E-03	-	6.06E-01
LAU	m <sup>2</sup>	ORG	3.30 E-04	1.09 E-03	0.00E+00	9.60E-01	1.45 E-04	6.89 E-05	6.56E+00	7.53E+00
		CON	2.05 E-04	1.00E-02	0.00E+00	2.50E-01	7.93 E-05	2.17 E-05	3.62E+00	3.89E+00

**Legend:** GLW = Global Warming; FET = Freshwater Ecotoxicity; SET = Seawater Ecotoxicity; TET = Terrestrial Ecotoxicity; HUT = Human Toxicity; ACD = Acidification; EUT = Eutrophication; POF = Photo-oxidant Formation; OLD = Ozone Layer Depletion; NRC = Non-renewable energy Resources Consumption; RRC = Renewable energy Resources Consumption; WAC = Water Consumption; LAU = Land Use. MP = Mechanical Practices; FM = Fertilizers Manufacturing; UF = Use of Fertilizers; HFS = Herbicides and Fungicides manufacturing and Seeds use; AM = Ancillary Materials; WM = Waste Materials; DLO = Direct Land Occupation (only for LAU impact category). DLO refers only to LAU and therefore does not show any correspondence with the other impact categories.

**Table 2.** Environmental impacts of Verna wheat production processes between organic (ORG) and conventional (CON) farming systems from each of considered factors expressed as per one kg of wheat grain

### 3.2 Freshwater ecotoxicity

FET involves the impact of toxic substances produced by the various processes on freshwater organisms. As with the other ecotoxicity categories (see sections 4.3 and 4.4), FET was reported as kg 1,4 dichlorobenzene equivalents (1,4-DB eq). FM showed a relevant impact on ORG, accounting

for 35% of the total. The second was represented by HFS, with an impact of 33% of the total. MP characterized 20% of the total impacts in ORG. Progressively, in descending order, the main impacts were shown to be linked to nickel (26%), beryllium (21%), copper (13%) and cobalt (12%). FM accounted for 88% of the total impacts in CON, with HFS amounting to 7% (Fig. 3). Likewise, in ORG, the main impacts were related to heavy metals, such as nickel (29%), beryllium (21%), copper (14%) and cobalt (12%), respectively. The impacts of ORG were approximately 30% ( $0.015 \text{ kg 1,4-DBeq FU}^{-1}$ ) of those of CON ( $0.050 \text{ kg 1,4-DBeq FU}^{-1}$ ) (Tab. 2).

### 3.3 Seawater ecotoxicity

SET refers to the effect of toxic substances, emitted in the environment, on seawater organisms. Similar to FET, SET is highly dependent on FM and HFS. In ORG, we observed an impact of 47% and 27% for FM and HFS, respectively. Emissions of hydrogen fluoride (41%) and beryllium release in the aquifer (33%), due to FM and HFS, were the principle impact factors. To the contrary, in CON the main impacts were primarily from FM, and secondarily from HFS, accounting for 88% and 7% of the total impacts, respectively (Fig. 3). Likewise, in ORG, hydrogen fluoride and beryllium represented the main impact factors contributing to 38% and 37% from FM, respectively. Total impacts from CON ( $170.304 \text{ kg 1,4-DBeq FU}^{-1}$ ) were approximately three times higher than ORG ( $56.069 \text{ kg 1,4-DBeq FU}^{-1}$ ). Furthermore, SET had  $10^3/10^4$  higher impacts compared to FET in both farming systems (Tab. 2).

### 3.4 Terrestrial ecotoxicity

TET considers the effects of toxic substances on land organisms. TET is mainly derived from MP, FM and HFS in both farming systems. In particular, MP contributed to 62%, FM to 20% and HFS to 17% of the total impacts in ORG, respectively. In CON, FM contributed to 74% and MP to 18% of the total impacts, respectively (Fig. 3). Heavy metals (zinc and mercury) had higher impacts in ORG accounting for approximately to 80% of the total. In CON, mercury (35%), cypermethrin (25%), zinc (16%) and nickel (10%) were the main impacts. In total, CON produced 208% ( $5.80E^{-4} \text{ kg 1,4-DBeq FU}^{-1}$ ) higher impacts than ORG ( $2.79E^{-4} \text{ kg 1,4-DBeq FU}^{-1}$ ) (Tab. 2).

### 3.5 Human toxicity

HUT refers to the effects of toxic substances (released from wheat cultivation into the environment) on human health. Similarly, to the ecotoxicity categories, HUT was also expressed as  $\text{kg 1,4-DBeq}$ . In ORG, the main impacts were related to MP, HFS and FM that accounted for 42%, 28% and 27% of the total, respectively. In particular, nitrogen oxides (15%), selenium (12%) and chromium VI (10%) were shown to incur the main impacts. Instead, in CON the main impacts were related to FM that accounted for 84% of the total (Fig. 3). Chromium VI (26%), selenium (17%) and nickel (16%) represented the main source of impact for CON. Cumulative impacts of ORG ( $0.036 \text{ kg 1,4-DBeq FU}^{-1}$ ) were approximately one third of those of CON ( $0.110 \text{ kg 1,4-DBeq FU}^{-1}$ ) (Tab. 2).

### 3.6 Acidification

This impact category is caused by the release of protons into aquatic and terrestrial ecosystems, mainly through rain, with effects on the development of life. The acidification potential is assessed as  $\text{SO}_2$  or  $\text{H}^+$ . In this study  $\text{kg SO}_2 \text{ eq}$  was adopted. Principally, ACD is determined as the release of sulphur dioxide, nitrogen oxides and ammonia. MP embodied the main impact factor from ORG, accounting for 79% of the total impacts. This is mainly attributable to fuel usage that represented a significant source of nitrogen oxide (76%) and sulphur dioxide ( $\text{SO}_2$ ) (20%) emissions. Nevertheless, HFS embodied a relevant impact factor with 14% of the total. In CON, acidification was affected by several factors. In particular, MP showed the higher impact (39%) following fuel use. However, FM and UF represented relevant factors accounting for 31% and 23% of the total impacts (Fig. 3), respectively. In total, ORG and CON contributed to  $0.003 \text{ kg SO}_2 \text{ eq FU}^{-1}$  and  $0.004 \text{ kg SO}_2 \text{ eq FU}^{-1}$ , respectively, with a higher impact of 27% from CON (Tab. 2).

### 3.7 Eutrophication

ETP is the undesired proliferation of biomass in ecosystems following a nutrient enrichment process. In this study, ETP was mainly linked to the following factors: release of nitrates and phosphates (via leaching and erosion) release of  $\text{NH}_3$  following (over) fertilization of fields, and  $\text{NO}_x$  production from the use of tractors. Normally, ETP is referred as  $\text{kg of PO}_4^{3-} \text{ eq}$ .

In ORG, the highest contribution was from UF, with 50% of the total impacts. Nevertheless, MP and HFS showed a relevant impact with 34% and 14% of the total, respectively. Nitrogen oxides represented 37% of the total impacts followed by nitrate (30%) and phosphate (28%). In CON, the main impacts were from UF with 79% of the total. This aspect represented one of the main issues related to the environmental pressures of agriculture. FM (8%), HFS (6%) and MP (6%) all showed a small influence on ETP in CON (Fig. 3). Nitrates characterized the main impact source (74% of the total), with a lower contribution from both phosphates (8%) and nitrogen oxides (8%). Overall, ORG presented an impact of  $0.002 \text{ kg of PO}_4^{3-} \text{ eq FU}^{-1}$  corresponding to 30% of the impact of CON ( $0.005 \text{ kg of PO}_4^{3-} \text{ eq FU}^{-1}$ ) (Tab. 2).

### 3.8 Photo-oxidant formation

POF is an indicator related principally to the formation of tropospheric ozone, caused by the reactions of organic components in the presence of light and heat. Generally, it is formed during hot periods (eg. summer). This impact category is largely affected by air pollutants such as nitrogen oxides ( $\text{NO}_x$ ),  $\text{SO}_2$  and non-methane volatile organic compounds (NMVOC). These compounds are mainly produced by extraction and distribution of fossil fuels, vehicle exhausts and combustion processes (Derwent et al., 2005; Preiss, 2015). Photochemical Ozone Creation Potential (POCP) represents the contribution of a substance on photochemical ozone production and is expressed as  $\text{kg of C}_2\text{H}_4 \text{ eq}$ . In ORG, MP and HFS embodied the main impact factors with 72% and 16% of the total, respectively. The high impact of MP was due to carbon monoxide (43%) and  $\text{SO}_2$  (38%) emissions. In CON, FM showed the

highest impact with 51% of the total, attributable to emissions following fertilizer synthesis. However, MP produced relevant impacts with 40% of the total (Fig. 3). The predominant impact sources were represented by SO<sub>2</sub> (57%) and carbon monoxide (25%) emissions. Higher yields of CON reduced the impacts from MP, but fertilization balanced the total impacts of the two farming systems. Total impacts were 6.58 E<sup>-5</sup> kg C<sub>2</sub>H<sub>4</sub> eq FU<sup>-1</sup> and 7.32E<sup>-5</sup> kg C<sub>2</sub>H<sub>4</sub> eq FU<sup>-1</sup> from ORG and CON respectively. For this impact category, ORG produced 10% lower impacts than CON (Tab. 2).

### 3.9 Ozone layer depletion

Stratospheric ozone depletion causes the increase of ultraviolet ray incidence, harmful to living organisms. For a specific evaluation of this phenomenon, the Ozone Depletion Potential (ODP) index was proposed. This impact category is referred to the amount of ozone depleting substances, and includes chlorofluorocarbons (CFCs) and Hydrofluorocarbons (HFCs), expressed as kg of CFC-11 eq. In ORG, MP embodied 84% of the total impacts, and was followed by HFS with a net contribution of 14%. Methane, bromotrifluoro- and Halon 1301 were found to be responsible for 98% of the total impacts. Even in CON, MP represented the main impact factor, contributing to 59% of the total. However, FM also played an important role with 33% of the total impacts (Fig. 3). Methane, bromotrifluoro-, Halon 1301 and methane, bromochlorodifluoro-, Halon 1211 were responsible for 83% and 13% of the total impacts, respectively. In total, CON showed an impact of 5.41E<sup>-8</sup> kg of CFC-11 eq FU<sup>-1</sup> while ORG produced 6.18E<sup>-8</sup> kg of CFC-11 eq FU<sup>-1</sup>, with a higher impact of 14% (Tab. 2).

### 3.10 Non-renewable energy resources consumption

NRC was assessed as the budget of primary non-renewable energy resources, used for the production processes of wheat. In the present study non-renewable energy resource consumption was reported in mega joules (MJ).

Following the lower yields, a MP, constituting 80% of the total impact was found in ORG. In addition, 14% of the total impact was represented by HFS. Crude oil represented 93% of the impacts. MP (50%) and FM (42%) mainly caused NRC in CON (Fig. 3). This was mainly due to fuel consumption, attributable to both mechanization and processes for the production of chemical fertilizers. Crude oil and natural gas consumption represented 69% and 26% of total impacts, respectively. Nevertheless, higher total impacts were produced by ORG (5.34 MJ FU<sup>-1</sup>) than CON (5.23 MJ FU<sup>-1</sup>) with a net increase of 2% (Tab. 2).

### 3.11 Renewable energy resources consumption

This impact category assesses the consumption of primary renewable energy resources used within the production processes, not calculated thus far. Similar to NRC, this indicator is reported as MJ. In ORG, the highest impact was derived from FM, with 46% of the total. MP and HFS represented 24% and 21% of the total impacts, respectively. The highest use of renewable resources was water (50%). Biomass (35%), as well as wind, solar and geothermic factors (15%) produced a lower impact.

The total consumption of RRC in ORG accounted for 0.035 MJ FU<sup>-1</sup>. In CON, FM represented the predominant part of the impacts (85%), whereas HFS and MP accounted for 7% and 6% (Fig. 3), respectively. Energy from water and biomass resources produced similar impacts (45% and 42%, respectively). Wind, solar, geothermic factors then represented the residual part of the impacts (13%). RRC in CON accounted for 0.087 MJ FU<sup>-1</sup>. Thus, ORG consumed 40% more of renewable energy resources than CON (Tab. 2).

### 3.12 Water consumption

This impact category refers only to the water used for the production processes relating to cultivation inputs. In addition, WAC for herbicide and fungicide dilutions was considered. However, given that wheat is a rainfed crop in Italy, there was no consumption of water for irrigation. The adopted methodology did not consider the water required for the dilution of pollutants to the legal values, as indicated in the water footprint methodology (e.g. Available WATER Remaining, vs 1.02 2016 - AWARE).

MP that accounted for 71% of 0.526 l H<sub>2</sub>O FU<sup>-1</sup> represented the predominant part of WAC in ORG. Instead, FM and HFS characterized 15% and 13%, respectively. In CON, MP represented 38% of the total (0.606 l H<sub>2</sub>O FU<sup>-1</sup>). In this case, FM and HFS were shown to have a higher impact representing 36% and 26%, respectively (Fig. 3). The impact gap between the two farming systems was 115.21% higher in CON (Tab. 2). The relevant impact of MP in ORG was related to the lower yields compared to CON. However, in CON the lower impact of MP was replaced by the higher impacts from both FM and HFS than ORG.

### 3.13 Land Use

This impact category is calculated for land occupation, for both the cultivation phase and the production of adopted external inputs, and is reported as square meters per year (m<sup>2</sup> y).

In both farming systems, approximately the 90% of land occupation was linked to DLO (Fig. 3). The residual part referred to the external input production and logistic services on farms. CON required 52% of LAU than ORG (3.89 and 7.53 m<sup>2</sup> y, respectively) (Tab. 2).

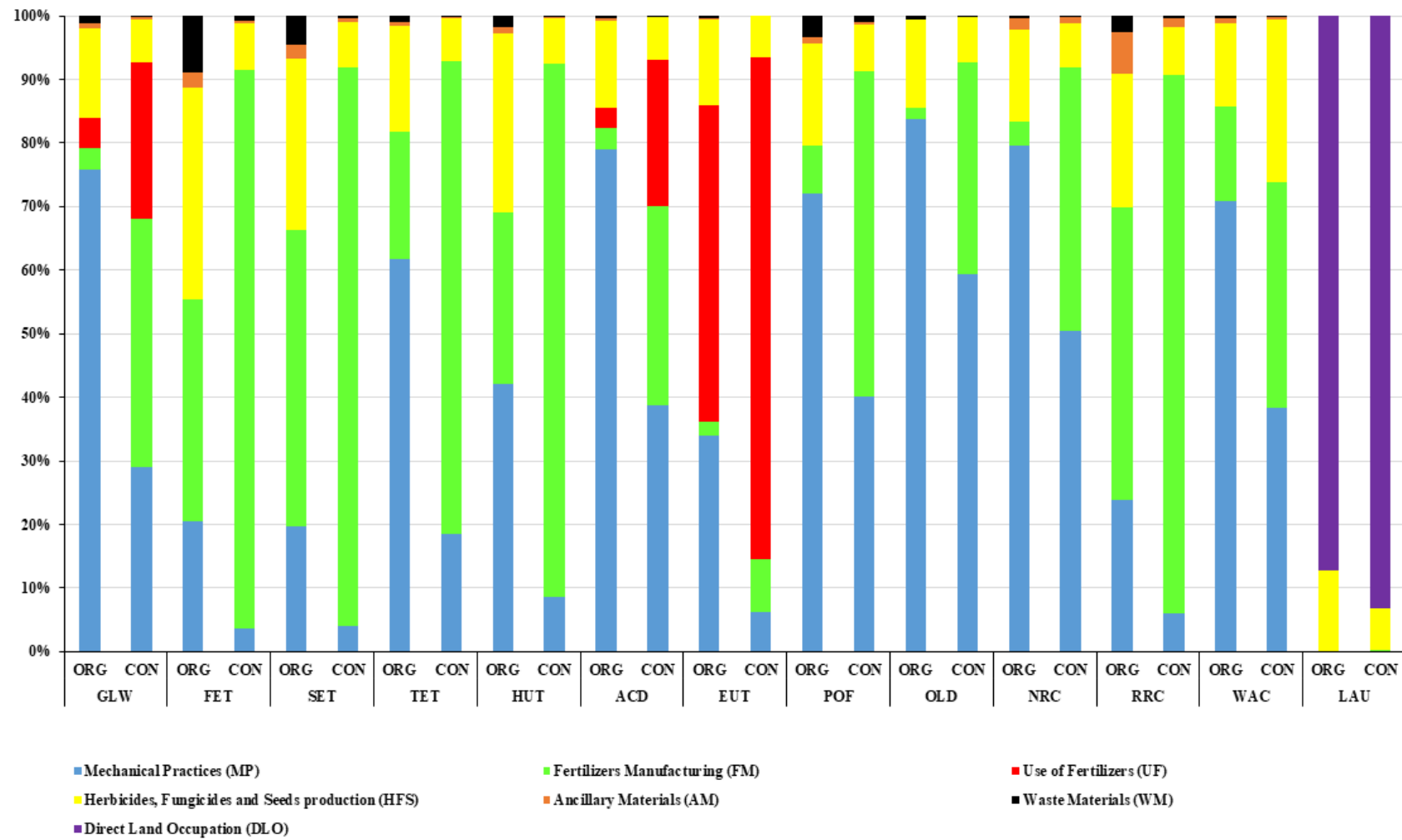


Figure 3. Relative contribution (percentage) of cultivation factors to impact categories in Verna wheat production between organic (ORG) and conventional (CON) farming systems.

### 3.14 Normalization

Results from the LCIA were standardized based on their respective reference scales on the EU25 level (CML vs 3.06, 2016). Therefore, dimensionless values that permitted a comparison between the different impact categories were obtained (Tab. 3). The normalized values allowed for an objective characterization of the phenomenon extent with respect to the amplitude of its reference scale. During normalization, the indicator values per functional unit for impact category were related to specific normalization factors (Brentrup et al., 2004a; Fallahpour et al., 2012; Krzyzaniak et al., 2018). The analysis showed that CON generated a higher impact compared to ORG. From all considered environmental impact categories, in both farming systems, SET had the greatest impact followed by EUT, ACD and GLW, respectively. FET, TET, HUT and POF generated lower impacts. Nevertheless, wheat production showed a negligible impact in both farming systems for OLD.

Impact category	Units	Normalization Factors	Organic farming	Conventional farming
GLW	kg CO <sub>2</sub> eq	1.99E-13	71.5E-15	115.7E-15
FET	kg 1,4-DB eq	1.93E-12	28.6E-15	96.7E-15
SET	kg 1,4-DB eq	8.57E-15	480.5E-15	1459.5E-15
TET	kg 1,4-DB eq	2.06E-11	5.7E-15	11.9E-15
HUT	kg 1,4-DB eq	1.29E-13	4.7E-15	14.2E-15
ACD	kg SO <sub>2</sub> eq	3.55E-11	109.4E-15	138.6E-15
EUT	kg PO <sub>4</sub> <sup>3-</sup> eq	7.58E-11	122.6E-15	410.2E-15
POF	kg C <sub>2</sub> H <sub>4</sub> eq	1.18E-10	7.8E-15	8.6E-15
OLD	kg CFC-11 eq	1.12E-08	0.7E-15	0.6E-15

*Table 3. Normalization values based on the Europe 25 scale (CML vs 3.06, 2016) for the different environmental impact categories*

## 4. Discussion

### 4.1 Global warming

From the present study regarding GLW, the most relevant impact factor was MP, attributable to fuel consumption from agricultural machinery (Fig. 3) (Fallahpour et al., 2012). Of the MP impacts, ploughing was shown to produce the greatest impact, amounting to approximately 29% and 32% of the total fuel consumption for ORG and CON, respectively (App. 1). The present results are

corroborated by recent publications, investigating the environmental impacts of agriculture, in which a relevant effect of mechanization to on-farm emissions were reported (Lovarelli et al., 2017). However, the contribution of fuel consumption to the environmental impacts of agricultural activity was shown to be liable to strong fluctuations based on different factors such as pedo-climatic conditions, production systems (organic or conventional farming), tillage systems (conventional tillage, minimum tillage, no tillage etc.) and yields (Lovarelli et al., 2017; Carranza-Gallego et al. 2018). This latter aspect was shown to represent a critical feature. The reason was that despite the comparable fuel consumption use between ORG and CON (App. 1), the greater impact generated by ORG was related to lower yields (Fig. 3) (Meisterling et al., 2009; Chiriaco et al., 2017; Carranza-Gallego et al. 2018; Tricase et al., 2018). In this sense, we observed an impact of 0.27 and 0.17 kg CO<sub>2</sub>eq FU<sup>-1</sup> for ORG and CON, respectively (Tab. 2). However, when considering a hectare as a functional unit, the net contribution of GLW from fuel consumption appeared similar between the two farming systems (415.62 and 476.67 kg CO<sub>2</sub> eq ha<sup>-1</sup>, for ORG and CON, respectively). The present findings are in line with Ali et al. (2017) when compared to CON (MP=29% of total impacts), but significantly higher when compared to ORG (MP=76% of total impacts) (Fig. 3). This highlighted that in order to ensure comparable yields with CON farming, the ORG system, requiring a greater surface area of land, is at risk in causing significant impacts from the use of machinery. Our results, were shown to be higher than previous observations, that reported an average impact of 67.4 kg C ha<sup>-1</sup> using conventional tillage on wheat in USA (West and Marland, 2002). The relevant impacts of FM and UF are predominantly related to the energy consumption for the production of N-based compounds (e.g. nitrates, ammonium, urea) and to N<sub>2</sub>O emissions in fields by fertilization treatments. Recent studies reported that from 1% to 5% of the N distributed during fertilization is lost as N<sub>2</sub>O, contributing greatly to global warming (Meisterling et al., 2009; Venterea et al., 2012; Verdi et al., 2019). Thus, the impacts related to fertilization represents a serious issue for conventional farming. Previous research supported the present findings, showing a significantly higher contribution of fertilization (fertilizers production and use) to GLW in CON (0.37 kg CO<sub>2</sub>eq FU<sup>-1</sup>) than ORG (0.03 kg CO<sub>2</sub>eq FU<sup>-1</sup>) (Tab. 2). Fallahpour et al. (2017) reported similar findings, with a global warming mitigation potential from organic fertilizer use of approximately 80% compared to chemical fertilizers.

Nowadays, soil organic carbon levels are low and are also considered unchanged. There were no significant changes between the two systems. Therefore, in the balance, it was not possible to consider the greater potential for organic C sequestration in ORG soils. This was attributable to the adoption of non-conservative tillage practices and to the hot, dry summers, which expose soil organic matter to rapid oxidation. In addition, as affirmed by the present study (App. 1), green manure practices had been abandoned, despite ORG protocol suggestions.

## 4.2 Freshwater ecotoxicity

The lower impact on FET in ORG was mainly due to the reduced use of pesticides, fungicides, and fertilizers compared to CON. According to Prechsl et al. (2017), heavy metal emissions into the environment from fertilizers represent the predominant impact. However, copper used for seed tanning, as well as the metals linked to its extraction, all generate a relevant impact on freshwater ecotoxicity in organic farming (Sydow et al., 2020). The direct use of copper was not shown to generate impacts, although recent studies reported the movement of this element from the soil to the aquifer with relevant impacts on the environment (Rocha et al., 2011). Krzyzaniak et al. (2018) showed lower impacts (kg 1,4-DB) on a mallow cultivation in Poland. Similar to our observations, the main impacts were related to the fertilizer sector with a lesser impact attributed to chemical weeding. In an irrigated-rainfed wheat experiment, Taki et al. (2018) observed a relevant impact on FET from chemicals use (averagely 0.05 kg 1,4-DB per kg of wheat grain). The intense adoption of chemicals (pesticides, fungicides and fertilizers) was primarily responsible for the higher impact on FET from wheat cultivated in CON (Tab. 2). Improved N and P-based fertilizers, through site-specific fertilization strategies, in order to reduce the release of heavy metals from FM, may represent an effective strategy to reduce FET impacts in CON.

## 4.3 Seawater ecotoxicity

Corroborating recent literature, SET in the present study represented the major impact category in wheat cultivation accounting for 99.77% and 99.73% of the total impacts for ORG and CON, respectively (Tab. 3). An average impact of 320 kg 1,4-DB per kg wheat grains on 75 farms in Iran was reported recently (Ghasemi-Mobtaker et al., 2020). Those authors reported that N and P-based fertilizers represented the sector with the greatest impact. In over 210 farms in Iran, an average impact on seawater ecotoxicity of 239.5 kg 1,4-DB per kg wheat grain was reported (Taki et al., 2018). The most relevant contribution was due to P-based fertilizers, accounting for over 70% of the total impacts. Monti et al. (2009), comparing four perennial energy crops and wheat-maize rotations in Italy, found a 10 to 30 times higher impact on seawater ecotoxicity compared to other impact categories. Regardless of the system considered, seawater ecotoxicity represented the most affected impact category regardless of the farming system. In the present study, the relevant impact of ORG on SET was related to copper used for seed tanning (Tab. 2). As with other heavy metals, copper has a strong impact on marine ecosystem (Zhang et al., 2017). Similar to FET, the adoption of those fertilization strategies allowing the improvement of efficiency use (precision farming, slow release fertilizers etc.) ensures the reduction of SET impact of wheat cultivation in CON.

#### 4.4 Terrestrial ecotoxicity

Terrestrial ecotoxicity is predominantly related to fertilizer production, fuel combustion and pesticide use (including herbicides and fungicides) (Charles et al., 2006; Alaphilippe et al., 2013; Krzyzaniak et al., 2018; Ghasemi-Mobtaker et al., 2020). Fertilization treatments were reported to have a relevant impact on terrestrial ecotoxicity when comparing British and Swiss wheat production systems (Charles et al., 2006). By comparing the different fertilization strategies, those authors observed that by lowering fertilization, only terrestrial ecotoxicity was shown to decrease. Similarly, in the present study CON showed a relevant impact of FM due to the elevated adoption of fertilizers. The present results were consistent with those presented in the literature, in which total impacts from FEM amounted to 20% and 74% for ORG and CON, respectively. (Krzyzaniak et al., 2018; Ghasemi-Mobtaker et al., 2020). There was a relevant impact of MP in ORG (Fig. 3), mainly due to mercury (Chen et al., 2016) and zinc emissions produced by fuel combustion combined with the lower yields. In the present study, a significant impact from HFS on terrestrial ecotoxicity in ORG was noted (Fig. 3), consistent with previous findings showing that mineral fungicides contaminate soil following copper release (Alaphilippe et al., 2013). Regarding ORG, tillage efficiency improvements represented a key factor in reducing impacts of TET through the decrease of fuel consumption. Additionally, the use of alternative seed tanning products may represent an additional strategy to reduce TET impacts. In CON, similar to FET and SET, environmental performance improvements were strongly linked to nutrient use efficiency.

#### 4.5 Human toxicity

The resultant production of Chromium VI, NO<sub>x</sub>, Nickel (air) and Selenium (water) from FM and HFS, indicated that industrial processes impacted on HUT from both farming systems. Recent literature is consistent with our results, suggesting that the reduction of chemicals (fertilizers, herbicides, pesticides and products for seed tanning) may represent an effective strategy to reduce the HUT impact magnitude of wheat cultivation (Taki et al., 2018; Ghasemi-Mobtaker et al., 2020). MP was shown to represent an additional source of toxic compounds, especially from ORG, thereby highlighting the environmental impact of fuel combustion (Fig. 3). Reducing mechanization (e.g. adopting conservative-tillage practices) was reported to reduce toxic substances in both air and water, and to contribute to the maintenance of SOM levels and the intrinsic fertility of the soil (Ding et al., 2002).

#### 4.6 Acidification

Acidification is primarily attributable to the combustion of fossil fuels at power stations and industrial plants, vehicle exhausts, and agriculture (Van Zelm et al., 2015). The results of the present study corroborated recent literature where acidification impacts from wheat cultivation were shown to

range between 1.95 and 7 g SO<sub>2</sub> kg<sup>-1</sup> wheat grain (Achten and Van Acker, 2016; Holka et al., 2016; Fallahpour et al., 2017; Ghasemi-Mobtaker et al., 2018). More than 60% of acidification from wheat cultivation was shown to be attributable to on-farm emissions from diesel and fertilizers into the air, water and soil, and from heavy metals of fertilizers into the soil, respectively (Ghasemi-Mobtaker et al., 2018). Environmental performance in ORG was improved, as fuel combustion from MP formed approximately 80% of the total impacts (Fig. 3). CON showed more than 90% of ACD due to the combustion of fuels and fertilizers (production and use).

Similar to previous reports, we observed that sulphur oxides (SO<sub>x</sub>) and NO<sub>x</sub> production from exhaust, fertilizers production processes and use constituted the main components of ACD (Holka et al., 2017; Taki et al., 2018). Generally, organic fertilizers have a weak tendency of soil acidification. In contrast, chemical fertilizers used in conventional systems have different properties. Di-ammonium phosphate in a water solution has a weak alkaline reaction due to the hydrolysis of the salt, while ammonium nitrate has an acid reaction (Malquori, 1986). In some fertilizers, such as YARA SULFAN the acidity caused by ammonium nitrate is additive due to the high sulphur (S) content (15%). In similar cultivation conditions, Fallahpour et al. (2017) observed that roughly 48% of the “total acidification gases” were due to NH<sub>4</sub>, whereas 15% and 36% were from N<sub>2</sub>O and SO<sub>2</sub>, respectively. The use of fertilizers in CON did not seem to offset the greater use of diesel per kg of product in ORG. For this reason, the impacts between the two farming systems were shown to be similar. According to Houshyar and Grundmann (2017) reducing the ACD implicated the adoption of minimum tillage strategies under conditions not negatively affecting yield.

#### 4.7 Eutrophication

EUT is directly connected to nutrient dynamics and the intense use of N and P- based fertilizers (Brentrup et al., 2004b; Fallahpour et al., 2017; Huang et al., 2017). Generally, in order to offset the nutrient losses related to different factors (site-specific pedo-climatic conditions, fertilizers dispersion methods etc), fertilizers rate exceed the needs of plants. However, fertilizer use efficiency is highly dependent on the farming system, where anthropic actions drive plant-environment interactions (Fabbri et al., 2020). We observed a positive correlation between N and P rate and EUT, resulting in a higher impact in CON. Intense tillage has a relevant effect on N and P losses, both by increasing N and P organic mineralization and by promoting erosion losses (Pulighe et al., 2020). Thus, minimum tillage may represent an effective strategy to mitigate losses. The opportunity to mitigate organic N and P losses and to protect the soil from erosion renders the adoption of cover crops an additional effective strategy to reduce EUT (Houshyar and Grundmann, 2017; Prechsl et al., 2017; Taki et al., 2018). In ORG, fertilizers have a lower impact. This is attributable both due the lower quantity used and that N-based fertilizers are under organic form. However, as was observed previously, the use of manure and sewage in ORG is a critical factor in increasing the impacts on EUT

due to higher NH<sub>3</sub> emissions (Van Stappen et al., 2015; Prechsl et al., 2017). The adoption of slow release fertilizers may favour N use efficiency by hampering leaching losses that represent the principle impact factor.

#### **4.8 Photo-oxidant formation**

The use of fossil energy, fuel combustion and fertilizer manufacturing all constitute the primary factors affecting POF (Taki et al., 2018; Ghasemi-Mobtaker et al. 2020). From the analysis of our results, we observed that fuel consumption and input manufacturing (fertilizers and pesticides) affected POF the most (Fig. 3). ORG has a great impact from fuel consumption following the high impact of MP when compared to the low usage of synthetic inputs (Derwent et al., 2005). According to Queiros et al. (2015), CON is characterized by a relevant impact from FM and MP due to environmental emissions from both industrial plants and exhaust. Recent findings proposed the use of activated charcoal enriched filters in catalytic converters to abate exhaust impacts from diesel engines (Naveenkumar et al., 2020). Conversely, the use of urea to reduce NO<sub>x</sub> emissions produced an insignificant effect since the latter show a negligible contribution on POF impacts. Lessening SO<sub>2</sub>, linked to diesel exhaust, is challenging since available technologies are only used in large-scale facilities (Osaka et al., 2015). SO<sub>2</sub> emissions from the production of P-based fertilizers represent an additional source of impact affecting POF (Salam, 2013). This was emphasized by the global sulphur consumption that accounted for more than 50% with P-based fertilizer manufacturing (Ceccotti et al., 1998). Moreover, climatic conditions strongly affect photo-oxidant formation (Yang et al., 2020). The present study was based on the EU 25 scale (CML vs 3.06, 2016) for average European conditions. Nevertheless, it should be noted that the study area, with a typical Mediterranean climate, has unfavourable irradiation and temperature conditions for the impact of this category. Thus, the adoption of this methodology may underestimate POF impacts than the reality.

Recent findings reported different soil NO<sub>x</sub> emission levels based on N-compounds into the soil (Yang et al., 2020). NO<sub>2</sub>- and NO<sub>3</sub>- compounds shows higher NO<sub>x</sub> emission potentials than NH<sub>4</sub><sup>+</sup> under intense solar radiation condition. Thus, the combined effect of different forms of N (NO<sub>3</sub><sup>-</sup>) derived from fertilizers and solar radiation both represented a key factor on the POF process. In this sense, the adoption of different N fertilizers (e.g. NH<sub>4</sub><sup>+</sup>) would have a direct effect on POF impacts mitigation.

#### **4.9 Ozone layer depletion**

The contribution of agriculture to stratospheric ozone depletion is mainly related to fuel combustion, fertilizers and pesticide production (Queiros et al., 2015). Following the regulation proposed in the Montreal Protocol, the atmospheric concentration of halocarbon compounds was significantly reduced and is under control nowadays. Nevertheless, novel anthropogenic emission sources

represent a critical issue hampering the stratospheric ozone layer recovery (Revell et al., 2012). The WMO Scientific Assessment Panel (WMO, 2011) recently recognized a negative effect of N<sub>2</sub>O on ozone layer recovery. Recent literature reported that approximately 90% of anthropogenic N<sub>2</sub>O reaches the stratosphere, thus contributing to the catalytic destruction of ozone. Nevertheless, Floeming et al. (2011) affirmed that anthropogenic CO<sub>2</sub> and CH<sub>4</sub> emissions hampered negative N<sub>2</sub>O effects, thereby encouraging stratospheric ozone recovery. However, the combustion processes were still shown to represent a serious issue in both farming systems due to the emissions of ozone layer depleting substances such as halocarbons. Despite the reduced application of fertilizers and pesticides in ORG (App. 1), the lower yields contributed to a higher impact compared to CON. However, from comparisons to other impact categories, analysed in the present study, OLD was negligible (Monti et al., 2009) (Tab. 3).

#### **4.10 Non-renewable energy resource consumption**

NRC impacts of wheat cultivation are mainly related to fuel consumption (80% and 50% for ORG and CON respectively) and, in CON, fertilizer production (42%). Tillage had a significant impact, not only in fuel consumption, but also due to effect on yields. In fact, yields were strongly affected by the climatic conditions of the area. In the same location, inter-annual variability is one of the main causes of differing yields from year to year and, consequently, of a differing energy source consumption for FU (Failla et al., 2020). The climatic conditions (temperature and humidity) of different areas have a direct impact on yields, energy consumption and indirect impacts on cultivation practices, such as irrigation, that have to be adopted in order to overcome environmental limits. In this sense, for climatic areas similar to central Italy, Mondani et al. (2017) reported an energy consumption of 8.45 - 9.05 MJ kg<sup>-1</sup> of grain. Such consumption increases when passing into arid and semi-arid regions where irrigation is used. Likewise, because of the variability in climatic conditions and agricultural management strategies, a wide variability of NRC impacts is available in the literature (Achten and Van Acker, 2016; Ghasemi-Mobtaker et al. 2020). Given the continuous growth in food demand, agricultural efforts have been focused on maximizing yields with the increased adoption in fertilizer usage, particularly in conventional farming systems. The production of synthetic fertilizers has caused a direct effect on fossil energy consumption, increasing NRC impacts of conventional farming (Achten and Van Acker, 2016).

#### **4.11 Renewable energy resource consumption**

Renewable energy sources are mainly related to biofuels and electricity (Nguyen et al., 2013). The present results, 0.03 and 0.09 MJ kg wheat grain<sup>-1</sup> for ORG and CON respectively, corroborated those in the literature, reporting an average RRC from 0.03 to 0.15 MJ kg wheat grain<sup>-1</sup> (Achten and Van Acker, 2016; Mondani et al., 2017; Ghasemi-Mobtaker et al. 2020). In wheat production, straw

represents one of the main by-products and its usage to produce electricity is common practice (Nguyen et al., 2013). However, alternative uses of this by-product should be considered, along with the respective repercussions on various impact categories within the LCA. From an agronomical point of view, straw has a significant importance in maintaining the physical, chemical and biological fertility of the soils. Removal would require compensation through the extra input of nutrients, which in turn would necessitate the increased use of fertilizers, with additional impacts in terms of emissions attributable to the production and use of these products. Nguyen et al. (2013), calculated that the removal of straw would necessitate an extra input of fertilizers by 1.5 kg N, 0.77 kg P and 12.8 kg K, respectively. Those authors also reported that the incorporation of 1 t of straw produced a carbon sequestration rate of approximately 80 kg C, thereby demonstrating the relevant contribution towards mitigating climate change. Biogas, represents an effective option for the energetic valorisation of straw and maintenance of soil fertility as the by-product of the process, digestate, is a valuable organic fertilizer to maintain soil fertility. Several studies reported that the appropriate management of digestate produced comparable yields to those generated from the use of mineral fertilizers, but with limited environmental impacts (Albuquerque et al., 2012; Verdi et al., 2018; Verdi et al., 2019a; Verdi et al., 2019b).

#### 4.12 Water consumption

Fertilizer manufacturing is a process that requires a significant amount of water (Tab .2). The N-fertilizer synthesis process requires an energy vector representing water in the form of steam (Madanhire et al., 2015). Moreover, water is also the basis for cooling systems on an industrial scale. Given that water is used for washing processes that dramatically decreases water quality, consumption increases. To date, water is an irreplaceable resource for fertilizer production, as is evident for many other industrial processes. In this sense, water consumption represents a critical environmental issue for CON, given the application of synthetic fertilizers ( $2.16E^{-01}$  litres FU<sup>-1</sup>). Moreover, agriculture exerts a high impact on water consumption for fuel production. In the present study, results on WAC from MP are a relevant amount of total WAC in both farming system (Tab .2). Due to the lower yields, ORG shows a relevant impact on WAC from fuel consumptions in MP. In CON, WAC impacts are related also to FM and HFS due to the higher adoption of chemicals than ORG. Furthermore, oil extraction and the refining of fuel require significant water usage (Carter, 2015). In the year 2010, on a global scale, approximately 66 billion of cubic meters of water were consumed from the energy sector amounting to 15% of global water withdrawal (IEA, 2012). Mielke et al. (2010) reported that water consumption for oil extraction exceeded seven times the volume of oil produced. Fracking processes (U.S. EPA, 2016) represent a relevant amount of water usage. Carter (2015) reported an average consumption of 1.000 to 10.000 litres of water to produce one tonne of fuel from an oil source. Biofuels may represent an alternative to the use of fossil resources. Nevertheless,

it was shown that in order to produce one tonne of biofuel, from irrigated crops, more than 10.000 litres of water were consumed via both direct and indirect uses (irrigation, raw materials extraction, cultivation input production etc.) (Dalla Marta et al., 2015). In this way, production potentials related to pedo-climatic conditions and the adoption of cultivation inputs play a key role on water demand for biofuel production (Dalla Marta et al., 2011b; Dalla Marta et al., 2014). In addition, energy crops for biofuel production were reported to be in conflict with food crops, and the use of marginal fields is not sustainable from an energetic point of view (Dalla Marta et al., 2010).

#### 4.13 Land Use

In the period 2015-2018, European areas cultivated with wheat accounted for approximately 62 million hectares with average yields of 4141 kg ha<sup>-1</sup> grain (FAO, 2020). Consistent with the observations of the present study, recent research reported an average land occupation of 4.5 m<sup>2</sup> kg wheat grain<sup>-1</sup> (Holka et al., 2016; Ridoutt and Garcia, 2020). Yields of Verna wheat are lower than those of modern varieties grown in Tuscany, where average yields are similar to those generally produced in Europe. According to our observations, in order to guarantee European yields, would necessitate a DLO for Verna of about 91 and 168 million ha for CON and ORG, respectively. Therefore, a LAU of roughly 99 and 192 million ha for CON and ORG, respectively, would be needed. Tuomisto et al. (2012) stated that organic farming ensures higher levels of biodiversity (from 30% to 50%) than those observed in conventional systems. However, same authors reported a higher land use of about 84% than that for the conventional system with relative impacts on ecosystems. This is consistent to our observations, reporting 94% more LAU in ORG than CON. Lower yields in ORG would imply a strong increase in DLO for agricultural use with a higher impact on forestry ecosystems and biodiversity.

### 5. Conclusions

The reduction of negative external factors associated with agri-food production is the key factor towards sustainable development. Of the goals of the EU “Farm to Fork Strategy” (COM 381/2020), a 50% reduction in nutrient losses with no deterioration to soil fertility, and a 20% reduction in the use of synthetic fertilizers by 2030 are central to rendering make food systems fair, healthy and environmentally-friendly. The present study has provided confirmation that principle environmental issue of conventional farming is the production and (over) use of nitrogen fertilizers and, to a lesser extent, of phosphate fertilizers. Therefore, the development and adoption of innovative strategies that increase the resource use efficiency play a key role in improving environmental performance of conventional agriculture.

Currently, the use of fixed rates of fertilizer inputs on crops that demonstrate both variable and diversified requirements represents one of the main causes of resource wastage. The adoption of

precision farming for managing variability in space and time, together with the use of slow-release N fertilizers and nitrification/urease inhibitors, may contribute to long-term sustainability of agriculture production. Furthermore, the increase in soil fertility through the adoption of functional cover crops and intercropping, as well as conservative tillage represent additional essential elements. The cultivation of ancient crop varieties, such as Verna, characterized by a high variable genetic heritage and, thus greater resilience, compared to modern varieties, constitutes an efficient adaptation strategy to climate change.

Energy was also identified to be a key factor towards improving the performance of both cultivation systems (especially for tillage and chemicals manufacturing). Raw material extraction and energy consumption, linked to the industrial sector, could potentially adopt natural energy resources (solar, wind and hydroelectric). However, energy crops introduce a new LCA linked to the cultivation activity, which in turn lead to additional burdens on different impact categories, and which also require soil and water consumption creating an unsustainable energy balance. Alternative tillage strategies to reduce fuel consumption, such as minimum tillage, often produce impacts on yields, requiring the adoption of crop inputs (e.g. herbicides) that significantly affect the environmental performance. Furthermore, in order to reduce the energy consumptions, the reduction of N-synthetic fertilizers through site-specific fertilization is crucial.

Climate changes will worsen crop yields and, consequentially, cultivation input use efficiency. Drought effects on the latest phenological phases of wheat growing season play a key role on productivity and crop failure. The optimized management of water resources has also the potential to increase the water-use efficiency of all system inputs, through the adoption of specific storage and irrigation strategies, especially in those areas where precipitation fluctuates.

For almost all the impact categories, conventional farming showed the worse performances due to the production and consumption of non-renewable resources. On the other hands, the EU goal of reaching 25% of agricultural land under organic farming by 2030, must account with the necessity to offset the low yield by increasing cultivation surface areas.

Therefore, it is essential that the programming of EU policy address future investments towards technological and organizational renewal in line with the identified solutions.

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Appendix 1. Scheme of surveys and average results all over the 5 organic (a) and 5 conventional (b) farms.

Tillage operation	Weight (%)	Number	Fuel (l/ha)	Fuel Consumption (l/ha)	Tractor type	Power (hp)	Equipment	Time (min/ha)
Shredding of crop residues	20	1	Diesel	15.0	Wheeled	140.0	Shredder	90
Ploughing	100	1	Diesel	41.1	Wheeled	130.0	Plough	158
Fertilization	40	1	Diesel	10.4	Wheeled	90.0	Centrifugal Fertilizer	25
Disk Harrowing	60	1	Diesel	26.2	Wheeled	105.0	Disk Harrow	90
Drag Harrowing	20	1	Diesel	30.0	Wheeled	120.0	Tooth Harrow	180
Sowing	100	1	Diesel	22.4	Wheeled	132.0	Sower Machine	68
Harrowing	20	1	Diesel	15.0	Wheeled	103.3	Tooth Harrow	56.7
Topdressing Fertilization I	20	1	Diesel	9.0	Wheeled	90.0	Centrifugal Fertilizer	30
Foliar Fertilization I	20	1	Diesel	6.0	Wheeled	90.0	Stream Bars	30
Foliar Fertilization II	20	1	Diesel	6.0	Wheeled	90.0	Stream Bars	30
Harvesting	100	1	Diesel	20.4	Wheeled	136.3	Combine Harvester	82.5

Commercial name of fertilizers	Weight (%)	Rate (kg/ha)	Fertilizer Composition	Packaging Type	Packaging Material	Packaging Weight (kg)	Packaging Capacity (kg or l)	Fertilizer Provider	Distance (km)	Transport	Disposal
Ravel 27 Opengreen	20	350	P <sub>2</sub> O <sub>5</sub> 27%	Bags	Polypropylene	0.5	30	CAPSI***	12	Tractor	Special Waste
Blesiapor 3-12	20	50	N 3%; P <sub>2</sub> O <sub>5</sub> 12%	Bags	Polypropylene	0.5	50	CAPSI	15	Tractor	Special Waste
Endurance N7	20	110	N 7%	Bags	Polypropylene	0.5	50	CAPSI	15	Tractor	Special Waste
Endurance N8	20	180	N 8%; P <sub>2</sub> O <sub>5</sub> 0.5%	Bags	Polypropylene	0.5	50	CAPSI	15	Tractor	Special Waste
Siapton Siapa	20	3	N organic 8.7%; C organic 25%	Bottle	Polypropylene	0.1	1	CAPSI	12	Tractor	Special Waste

Commercial name of pesticides	Weight (%)	Water (l/ha)	Pesticides Composition	Packaging Type	Packaging Material	Packaging Weight (kg)	Packaging Capacity (kg or l)	Pesticides Provider	Distance (km)	Transport	Disposal
Seeds**	100	-	Copper Sulphate 20%	Big Bag	Polypropylene	1.75	600	CAPSI	13.8	Tractor	Recycling
Bordeaux Mixture	100	-	Pseudomonas proradix - Bacillus amyloliquefaciens	Bags	Polypropylene	0.2	30	CAPSI	13.8	Tractor	Special Waste
Ekoseed - pro-ced	20	-		Bags	Polypropylene	0.05	1	CAPSI	13.8	Tractor	Special Waste

Post Harvest Actions	Weight (%)	Fuel Consumption (l/ha)	Time (min/ha)
Grain transportation to storage center	100	7	8

Straw Management	Weight (%)	Fuel Consumption (l/ha)	Time (min/ha)
Bales production	50	23	62.5
Shredding	50	0	0

Other Waste Materials	Weight (%)	Material	Amount (kg/ha)	Disposal
Wire for bales production	50	Polypropylene	4	Recycling

a) Average data for ORG

Tillage Operation	Weight (%)	Number	Fuel consumption (l/ha)	Fuel	Tractor type	Power (hp)	Equipment	Time (min/ha)
Deep Ploughing	100	1	50.5	Diesel	Wheeled	218	Subsoiler Plough	112
Fertilization	100	1	10.8	Diesel	Crawler	121	Centrifugal Fertilizer	31
Disk Harrowing	100	1	21.6	Diesel	Wheeled	176	Disk Harrow	72
Sowing	100	1	17.1	Diesel	Wheeled	111	Sower Machine	52
Topdressing Fertilization I	100	1	8.7	Diesel	Wheeled	111	Centrifugal Fertilizer	24
Weeding and Fungicides treatment	80	1	9.5	Diesel	Wheeled	149	Stream Bars	20
Topdressing Fertilization II	20	1	8.0	Diesel	Wheeled	100	Centrifugal Fertilizer	25
Harvesting	100	1	21.4	Diesel	Wheeled	144	Combine Harvester	98

Commercial Name of Fertilizers	Weight (%)	Rate (kg/ha)	Fertilizer Composition	Packaging Type	Packaging Material	Packaging Weight (kg)	Packaging Capacity (kg or l)	Fertilizer Provider	Distance (km)	Transport	Disposal
Diammonium Phosphate	20	250	N 18%; P <sub>2</sub> O <sub>5</sub> 46%	Big Bag	Polypropylene	1.75	600	CAPSI**	10	Tractor	Special Waste
Siapor Unimer	80	212.5	N 11%; P <sub>2</sub> O <sub>5</sub> 25%	Big Bag	Polypropylene	1.75	500	CAPSI	7.25	Tractor	Special Waste
Ammonium Nitrate	80	187.5	N 26%	Big Bag	Polypropylene	1.75	600	CAPSI	8.5	Tractor	Special Waste
Sulfan	20	200	N 24%; SO <sub>3</sub> 15%	Big Bag	Polypropylene	1.75	600	CAPSI	5	Tractor	Special Waste
Commercial Name of Pesticides	Weight (%)	Water (l/ha)*	Pesticides Composition	Packaging Type	Packaging Material	Packaging Weight (kg)	Packaging Capacity (kg or l)	Pesticides Provider	Distance (km)	Transport	Disposal
Seeds	100	-		Big Bag	Polypropylene	1.75	600	CAPSI	13.8	Tractor	Recycling
Scenic (Seed tanning product)	100	166.7	Prothioconazole 3.35 %; Fluoxastrobin 3.35 %; Tebuconazole 0.45 % Thifensulfuron methyl 33.3%; Tribenuron methyl 16.7%	-	-	-	-	-	-	-	Special Waste
Marox (Herbicide)	60	200.0	Pinoxaden 6.4%; Cloquintocet-methyl 1.55%	Bottle	Polypropylene	0.02	0.1	CAPSI	6	Tractor	Special Waste
Axial Pronto (Herbicide)	20	175.0	Fluroxypyr 6% Clopyralid 2.3% 2-methyl-4- chlorophenoxyacetic acid 26.7%	Bottle	Polypropylene	0.12	1	CAPSI	6	Tractor	Special Waste
Mantia Gold (Herbicide)	40	150.0	Iodosulfuron-methyl- sodium 0.6%; Mefenpir- diethyl 9%; Mesosulfuron- methyl 3%	Bottle	Polypropylene	0.15	3	CAPSI	6	Tractor	Special Waste
Atlantis (Herbicide)	20	166.7	Prochloraz 34.8%; Propiconazole 7.8%	Bottle	Polypropylene	0.03	0.5	CAPSI	6	Tractor	Special Waste
Bumper P (Fungicide)	60			Bottle	Polypropylene	0.12	1	CAPSI	6	Tractor	Special Waste

Post Harvest Actions	Weight (%)	Fuel Consumption (l/ha)	Time (min/ha)
Grain transportation to storage center	100	7	8

Straw Management	Weight (%)	Fuel Consumption (l/ha)	Time (min/ha)
Bales production	50	23	62.5
Shredding	50	0	0

Other Waste Materials	Weight (%)	Material	Amount (kg/ha)	Disposal
Wire for bales production	50	Polypropylene	4	Recycling

### b) Average data for CON

\* water consumptions a related to dilution of products

\*\* for organic farms seed tanning is carried out in CAPSI before provision to farmers

\*\*\* CAPSI: Consorzio Agrario Provinciale di Siena (<https://www.capsi.it/>)

## Conclusioni

Lo studio ha permesso di comprendere alcune dinamiche fondamentali della fertilizzazione sito specifica del frumento coltivato nelle aree collinari del centro della Toscana. L'approccio alla fertilizzazione di precisione non può considerare solamente l'azoto, ma deve almeno considerare tutti e tre i principali macronutrienti.

Da un canto gli indici vegetazionali calcolati a partire dal monitoraggio remoto effettuato con Rapid Eye sono risultati adatti a stimare le condizioni nutrizionali del grano duro rispetto all'azoto. In particolare, MCARI ed EVI2 si sono dimostrati gli indici più adatti per discriminare rispettivamente il contenuto di N e la biomassa. La curva di diluizione, calibrata a partire dai dati di campo, ha mostrato stime di concentrazione di N più basse rispetto alle curve sviluppate da altri autori per il frumento (Justes et al., 1994). Tuttavia, la diluizione di N durante l'accumulo di biomassa ha mostrato la stessa tendenza di quella mostrata da Justes et al. (1994). Il metodo proposto è una soluzione affidabile per l'applicazione sito specifica dell'azoto in fase di copertura. Le caratteristiche pedoclimatiche della Val d'Orcia sono coerenti con quelle che caratterizzano il Centro Italia, pertanto è possibile rendere la metodologia proposta estendibile ad un'area più ampia. Le mappe di prescrizione che tengano conto della variabilità dei campi sono affidabili grazie all'elevata risoluzione spaziale delle immagini satellitari del Rapid Eye. Una valida alternativa può essere rappresentata dall'impiego di acquisizioni Sentinel 2 per il vantaggio dell'accesso libero e gratuito. Tali immagini offrono una soluzione economicamente vantaggiosa, tuttavia avendo una minore risoluzione spaziale necessitano di una validazione volta a definire la differenza di rappresentatività rispetto al Rapid Eye.

Solitamente, l'azoto è considerato il principale elemento in grado di influenzare la produzione di grano, ma nelle aree collinari del centro della Toscana il fosforo ha dimostrato avere un'influenza molto importante sulla quantità e qualità delle produzioni di frumento. Lo studio ha infatti dimostrato che la disponibilità fosforo nel terreno può essere limitata e variabile. Nei suoli ove il contenuto di fosforo disponibile è inferiore a  $20 \text{ mg Kg}^{-1}$  variazioni anche minime mostrano un forte impatto sulla produzione, coerentemente con la bibliografia. In tali contesti la fertilizzazione fosfatica uniforme, basata sulle dosi frequentemente utilizzate dagli agricoltori, ha mostrato essere limitante la produzione. In tali contesti le mappe dei nutrienti del suolo rappresentano uno strumento utile per integrare le informazioni sullo stato della vegetazione su cui si basa comunemente la fertilizzazione azotata di precisione. La gestione della fertilizzazione fosfatica eseguita in presemina con sovradosaggi può essere ugualmente inadatta considerando che le caratteristiche di alcuni suoli rendono indisponibile il fosforo distribuito in breve tempo. In tali contesti la strategia da adottare deve prevedere una distribuzione di precisione sia nello spazio che nel tempo, nel corso della crescita e dello sviluppo della coltura.

In una visione agronomica di concimazione di precisione occorre tenere conto non solo della gestione della variabilità spaziale delle coltivazioni volto a massimizzare le produzioni e l'efficienza d'utilizzo

degli input, ma anche di tutte le tecniche volte ad incrementare e stabilizzare negli anni le caratteristiche qualitative delle produzioni. Per tale motivo lo scopo del terzo studio sulle fertilizzazioni è stato quello di valutare se la qualità tecnologica di farine di frumenti di nuove e vecchie varietà potesse essere migliorata attraverso trattamenti agronomici differenti dall'impiego di fertilizzante azotato e fosfatico. Pertanto è stato valutato l'effetto della concimazione con zolfo e della densità di semina sulla composizione del chicco, sulla reologia dell'impasto e sulla qualità del pane. Dai risultati è emerso che la fertilizzazione solfatica influisce sulla composizione proteica e, in particolare, aumenta il contenuto di glutine. La fertilizzazione con zolfo, effettuata in fase fenologica fra fine levata e botticella è correlata positivamente con la forza dell'impasto (W). Poiché W è un parametro chiave nella valutazione della panificabilità della farina, la concimazione fogliare solfatica costituisce una strategia promettente per migliorarne le prestazioni tecnologiche. Questa tecnica agronomica può essere fondamentale sia nei casi ove si debba ricorrere a produzioni locali la cui qualità risente molto dal peculiare andamento meteorologico annuale, sia nell'impiego di vecchie varietà di frumento ove la frazione glutinica della componente proteica è inferiore a quella delle varietà migliorate. Tuttavia, sono ancora necessari ulteriori studi su una più ampia gamma di varietà, e su tecniche di fertilizzazione solfatica alternative che comprendano differenti quantitativi, applicazioni al suolo, differenti epoche di distribuzione e le interazioni con la fertilizzazione azotata. Si deve sottolineare, però, che la transizione all'agricoltura di precisione deve essere associata alla transizione ecologica. Tra gli obiettivi principali posti nella "Strategia Farm to Fork" dell'UE (COM 381/2020), troviamo la riduzione del 50% delle perdite di nutrienti senza deterioramento della fertilità del suolo e una riduzione del 20% dell'uso di fertilizzanti sintetici (azoto e fosforo in particolare) entro il 2030.

La ricerca svolta ha fornito conferma che il principale problema ambientale legato alle coltivazioni frumenticole in conduzione convenzionale nell'area collinare toscana è la produzione e l'eccesso di somministrazione dei fertilizzanti azotati e, in misura minore, dei fertilizzanti fosfatici. Pertanto, lo sviluppo e l'adozione di strategie innovative che aumentino l'efficienza nell'uso delle risorse svolge un ruolo chiave nel miglioramento delle prestazioni ambientali dell'agricoltura convenzionale. L'utilizzo dell'agricoltura di precisione per la gestione della variabilità nello spazio e nel tempo, insieme all'uso di fertilizzanti azotati a lenta cessione e/o degli inibitori della nitrificazione/ureasi, può contribuire all'incremento dell'efficienza d'uso dei fertilizzanti e alla sostenibilità a lungo termine della produzione agricola. Anche l'impiego di energia è stato identificato come un fattore chiave per migliorare le prestazioni ambientali sia del sistema di coltivazione convenzionale sia biologico. L'impiego di risorse energetiche rinnovabili, solare, eolico e idroelettrico è da considerarsi come strategia principale da perseguire. L'impiego di colture da energia introduce nuovi impatti ambientali dovuti alla coltivazione, oltre a richiedere ulteriori consumi di suolo e acqua. Strategie di lavorazione alternative per ridurre il consumo di carburante, come la minima lavorazione, spesso producono impatti sui raccolti, richiedendo l'adozione di input colturali (ad esempio erbicidi) che influiscono in

modo significativo sulle prestazioni ambientali. I cambiamenti climatici intervengono negativamente sulla produttività delle colture e, di conseguenza, sull'efficienza nell'uso degli input di coltivazione. Nelle aree del Mediterraneo, quale quella studiata, l'effetto di ondate di calore e fenomeni di siccità sulle ultime fasi fenologiche della stagione di crescita del frumento giocano un ruolo chiave sulla produttività. Fra le strategie auspicate per il miglioramento delle prestazioni ambientali possiamo citare la gestione ottimizzata delle risorse idriche con il fine di aumentare l'efficienza nell'uso dell'acqua e di tutti gli input di coltivazione. Infine l'obiettivo UE di raggiungere il 25% dei terreni agricoli coltivati ad agricoltura biologica entro il 2030 sembra essere, per la frumenticoltura, un'azione virtuosa in termini ambientali ma deve tenere conto della necessità di compensare la bassa resa aumentando significativamente le superfici coltivate.

L'analisi svolta, nel suo complesso, può contribuire a migliorare la produzione di frumento attraverso una gestione più efficiente dei nutrienti secondo le nuove politiche di sostenibilità. L'agronomia, dopo i grandi progressi della meccanica e della chimica si trova di fronte alla nuova era dell'agricoltura digitale. Le grandi moli di informazioni puntuali e la mecatronica devono così essere gestite da nuove figure professionali capaci di unire i tanti puntini di cui si compone un puzzle chiamato campo coltivato. Se fino pochi anni fa gestire campi differenti con strategie agronomiche differenti costituiva un traguardo avanzato, ora si parla di ridurre il singolo appezzamento a matrici numeriche ove le sub unità sono rappresentate da "pixel", ciascuno con le sue peculiari caratteristiche pedoclimatiche e vegetazionali. La difficoltà maggiore consiste nell'attuare un approccio agronomico integrato considerando tutte le variabili input e le loro implicazioni nella crescita, nello sviluppo, nella produttività e nella qualità della produzione di ciascun "pixel". Non possiamo più considerare un singolo input, quale la fertilizzazione azotata, ma occorre una visione più ampia che comprenda anche le implicazioni delle pratiche agronomiche adottate sulla qualità della produzione e dell'ambiente.

Per una visione ancora più ampia di questo nuovo modello di agricoltura digitale occorrerà considerare anche le relazioni che intercorrono fra le pratiche agronomiche e gli aspetti nutrizionali, salutistici e eubiotici delle produzioni. In tal senso occorre sempre più affrontare il tema della qualità del frumento non solo in considerazione della trasformazione agroindustriale. I contenuti in elementi minerali funzionali alla dieta, le vitamine, gli antiossidanti, il rapporto fra amilosio e amilopectina, le fibre sono in parte frutto dell'interazione fra il genotipo e l'ambiente pedoclimatico sito specifico, ed in parte della tecnica agronomica adottata. La rivisitazione della tecnica agronomica nel contesto dell'agricoltura digitale si dovrà sicuramente sposare con gli aspetti funzionali alla dieta e alla salute dell'uomo.

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