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Tesis Doctoral

DIAGNÓSTICO NUTRICIONAL NITROGENADO EN TRIGO MEDIANTE SENSORES Y APORTE DE SUBPRODUCTOS ORGÁNICOS

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Resumen

El nitrógeno (N), debido a su baja disponibilidad en los ecosistemas terrestres, es el nutriente que más directamente influye en la producción vegetal y en el contenido de proteína de los cultivos de grano. Para satisfacer la creciente demanda de alimentos, las aplicaciones de fertilizantes nitrogenados son un factor crucial. La síntesis de fertilizantes minerales nitrogenados requiere grandes cantidades de energía, y en ocasiones sus aplicaciones no se hacen en dosis o momentos correctos provocando pérdidas de N en el medioambiente.

Por otro lado, en las últimas décadas el sector ganadero ha experimentado un importante crecimiento, y con ello, ha aumentado la disponibilidad de subproductos orgánicos. Además de otros aspectos positivos para el suelo, la aplicación de subproductos de origen ganadero devuelve parte de los nutrientes extraídos por los cultivos al suelo, lo que contribuye a la fertilidad del mismo y a la consiguiente productividad de los cultivos.

El objetivo general de este trabajo es la mejora de la fertilización nitrogenada del cultivo de trigo en condiciones de clima mediterráneo húmedo, mediante la generación e integración de nuevo conocimiento acerca de: (i) la dinámica de mineralización de varios suelos de Araba; (ii) la disponibilidad de N tras el aporte de distintos subproductos orgánicos de origen ganadero; y (iii) la utilización de sensores ópticos proximales para el ajuste de la dosis de N mineral aportado y para el seguimiento del estado nitrogenado del cultivo en estados fenológicos clave.

Los resultados obtenidos mediante dos ensayos de invernadero y un ensayo de campo de tres años indican que: (i) la heterogeneidad del suelo, incluso en áreas geográficas reducidas, origina una alta variabilidad en la dinámica del N_{min} a lo largo del ciclo de cultivo del trigo, dificultando la estimación de la disponibilidad de N; (ii) las dinámicas de mineralización de la materia orgánica son muy variables, dependiendo de la tipología y composición bioquímica de cada subproducto orgánico de origen ganadero aportado al suelo. Así, la gallinaza y el purín son una fuente importante y muy rápida de N mineral para el cultivo, mientras que la tasa de mineralización del estiércol es baja; (iii) las lecturas normalizadas de los sensores proximales de campo Yara N-Tester™ y RapidScan CS-45 (NDVI y NDRE) permiten el ajuste de la dosis de N al inicio de la etapa de encañado del trigo cuando se aplica purín de vacuno en fondo y cuando no se

aplica fertilización de fondo orgánica; (iv) los valores absolutos de NDVI obtenidos con el sensor proximal RapidScan CS-45 posibilitan el seguimiento del índice nutricional nitrogenado (INN) a lo largo del ciclo de crecimiento del cultivo de trigo; (v) en los estados fenológicos aparición del segundo nudo, hoja bandera y mitad de floración, el rango de valores NDVI umbral obtenidos con el sensor proximal RapidScan CS-45 para la obtención de los mayores rendimientos productivos en el cultivo de trigo es de 0,70 – 0,75; (vi) el sumatorio de los valores NDVI en los estados fenológicos inicio de encañado, aparición del segundo nudo, hoja bandera y mitad de floración, es un indicador de la variabilidad del rendimiento productivo del cultivo de trigo; (vii) cuando el rendimiento de trigo es inferior a 8000 kg ha^{-1}, las lecturas obtenidas con el medidor de clorofila Yara N-Tester^{TM} en la mitad del periodo de floración estiman los valores de proteína en grano.

En conclusión, los sensores proximales permiten determinar el estado nutricional del trigo en distintos momentos de su ciclo productivo y ajustar la dosis de N para optimizar la producción y el contenido de proteína del grano. La planta engloba la variabilidad en el aporte de N por parte del suelo, por parte de los subproductos ganaderos y el efecto de la climatología del año, por lo que este sistema de control es muy apropiado desde un punto de vista productivo y ambiental, contribuyendo así a la sostenibilidad de los sistemas de producción agrícola.

Laburpena

Nitrogenoa (N), lurreko ekosistemetan eskuragarritasun txikia duenez, landare-produkzioan eta ale-laboreen proteina-edukian eragin zuzenena duen nutrientea da. Elikagaien eskari gero eta handiagoari erantzuteko, ongarri nitrogenatuak erabiltzea funtsezkoa da. Ongarri mineral nitrogenatuen ekoizpenak energia-kantitate handia behar izaten du, eta, batzuetan, ongarri hauek ez dira dosi edota une egokietan aplikatzen, eta horrek N galerak eragiten ditu ingurumenean.

Bestalde, azken hamarkadetan abeltzaintza-sektoreak hazkunde handia pairatu izan du, eta, horren ondorioz, azpiproduktu organikoen eskuragarritasuna nabarmenki handitu egin da. Lurzoruarentzat onuragarriak diren beste faktore batzuez gain, abeltzaintza-jatorriko azpiproduktuak aplikatzeak laboreek erauzitako nutrienteen portzentai bat itzultzen dio lurzoruari, eta horrek lurzoruaren emankortasuna eta, ondorioz, laboreen produktibitatea areagotzen ditu.

Lan honen helburu nagusia gari-laborearen ongarritze nitrogenatua klima mediterraneo hezeko baldintzetan hobetzea da, honako gai hauen inguruko ezagutza berriak sortuz eta integratuz: (i) Arabako hainbat lurzoruren mineralizazio dinamika; (ii) N-aren eskuragarritasuna, abeltzaintzatik eratorritako zenbait azpiproduktu organiko aplikatu ostean; eta (iii) gehitutako N mineralaren dosia doitzeko eta funtsezko egoera fenologikoetan laborearen egoera nitrogenatuaren jarraipena egiteko sentsore optiko proximalak erabiltzea.

Bi negutegi entsegu eta hiru urteko landa entseguan lortutako emaitzek adierazten dutenez, (i) eremu geografiko txikietan ere, lurzoruaren heterogeneotasunak eragin handia du N_{min} dinamikan laborantza-zikloan zehar, eta horrek zaildu egiten du N erabilgarriaren kalkulua, (ii) materia organikoaren mineralizazio-dinamikak oso aldakorrak dira, lurzoruari aplikatutako abeltzaintza-jatorriko azpiproduktu organiko bakoitzaren tipologiaren eta konposizio biokimikoaren arabera. Hala, oilo-zirina eta minda laboreentzako N mineral iturri garrantzitsua eta oso azkarra diren bitartean, simaurraren mineralizazio-tasa askoz ere txikiagoa da; (iii) Yara N-Tester™ eta RapidScan CS-45 (NDVI eta NDRE) landa-sentsore proximalen irakurketa-balio normalizatuei esker, nitrogeno-dosia doitu daiteke gariaren zurtoinaren luzapenaren etaparen hasieran, hondoan behi-minda aplikatzen denean eta hondoan ongarri organikorik aplikatzen ez denean; (iv) RapidScan CS-45 sentsore proximalaren bidez

lortutako NDVI balio absolutuek nutrizio-indize nitrogenatuaren (NIN) jarraipena ahalbidetzen dute gari-laborearen hazkuntza-zikloan zehar; (v) bigarren adabegiaren agerpenaren, bandera hostoaren agerpenaren eta loraketa erdiaren egoera fenologikoetan, gari-laborearen errendimendu produktibo handienak lortzeko RapidScan CS-45 sentsore proximalarekin lortutako NDVI muga-balioak 0,70 – 0,75 bitartean aurkitu behar dira; (vi) zurtoinaren luzapenaren etaparen hasieraren, bigarren adabegiaren agerpenaren, bandera hostoaren eta loraketa erdiaren egoera fenologikoetako NDVI balioen batukaria, gari-laborearen errendimendu produktiboaren aldakortasunaren adierazle da; (vii) gariaren errendimendua 8.000 kg ha^{-1} baino txikiagoa denean, Yara N-Tester™ klorofila-neurgailuarekin loraketa erdian egindako irakurketek ale-proteinaren balioak zenbatesten dituzte.

Laburbilduz, sentsore proximalek aukera ematen dute gariaren nutrizio-egoera ekoizpen-zikloaren une desberdinetan zehazteko, N dosia doitzeko eta ekoizpena eta alearen proteina-edukia optimizatzeko. Landareak barneratzen ditu lurzoruak eta abeltzaintzako azpiproduktuek N emateko duten aldakortasuna, eta urteko klimatologiaren eragina. Beraz, kontrol-sistema hau oso egokia da ekoizpenaren eta ingurumenaren ikuspegitik, nekazaritzako ekoizpen-sistemen jasangarritasuna bultzatuz.

Abstract

Given its low availability in terrestrial ecosystems, nitrogen (N) is the nutrient that most directly influences plant production and protein content in grain crops. To meet the growing food demand, the application of N fertilizers is crucial. The production of mineral N fertilizers requires large amounts of energy, and occasionally its application is not made at the right rates or at the right times, thereby causing losses of N to the environment.

On the other hand, in recent decades, the livestock sector has undergone significant growth, resulting in increased availability of livestock organic by-products. Soil application of livestock organic manures, in addition to other benefits, returns part of the nutrients taken by the crops to the soil, contributing to its fertility and, concomitantly, to the productivity of the crops.

The main objective of this study is the improvement of the N fertilization of the wheat crop under humid Mediterranean climate conditions, through the generation and integration of new knowledge regarding: (i) the mineralisation dynamic of several soils in Araba; (ii) the availability of N after the soil application of different livestock organic manures; and (iii) the use of proximal optical sensors to adjust the optimum mineral N rate, as well as to monitor the crop N nutritional status at key growing stages.

The results achieved through two greenhouse experiments and a three-year field experiment suggest that: (i) soil heterogeneity, even in small geographical areas, causes a high variability in N_{min} dynamics throughout the wheat growing cycle, making difficult to estimate the N availability; (ii) the organic matter mineralisation dynamic is highly variable and depends upon the typology and biochemical composition of the livestock organic manures applied to the soil. In this sense, hen manure and slurry constitute an important source of quickly available N for the crops, while the mineralisation rate is much lower in the case of the cattle manure; (iii) the normalised readings of the Yara N-Tester™ and RapidScan CS-45 (NDVI and NDRE) field sensors allow the adjustment of the mineral N rate at the beginning of the stem elongation after the application of cattle slurry as basal fertilizer, as well as when no basal organic fertilisation is applied; (iv) the absolute NDVI values obtained with the RapidScan CS-45 proximal sensor enable the monitoring of the N nutritional index (NNI) throughout the wheat growing cycle; (v) in order to obtain the highest wheat yields, the threshold NDVI values obtained with the RapidScan CS-45 proximal sensor should be 0.70 – 0.75 at the second node appearance,

flag-leaf emergence, and mid-anthesis wheat growing stages; (vi) the sum of the NDVI values at the beginning of stem elongation, second node appearance, flag-leaf emergency, and mid-anthesis growing stages, is an indicator of the variability of the wheat crop yield; (vii) when the wheat grain yield is lower than 8000 kg ha^{-1}, the readings obtained with the Yara N-Tester^{TM} chlorophyll meter at the mid-anthesis growing stage allow an estimation of the grain protein content.

In conclusion, proximal sensors allow both the determination of the wheat crop nutritional status at different growing stages, and the adjustment of the mineral N rate, to optimise crop yield and grain protein content. Plants comprise the variability arising from the N supply from the soil and from the applied livestock manure, as well as from the effect of the seasonal climate. Thus, this control system is highly appropriate both from a productive and environmental point of view, contributing to the sustainability of the agricultural production systems.

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Capítulo 1

Introducción

1 Introducción general

1.1 La producción de alimentos y el medioambiente

1.1.1 Perspectivas de la producción de alimentos

La percepción de los límites de la producción de alimentos para una población mundial en crecimiento ha sido fuente de preocupaciones y de debate durante años. La agricultura de todo el planeta se encuentra bajo la presión de producir suficientes alimentos saludables y de calidad para una población en crecimiento (FAO, 2020). Se estima que para 2050 la población mundial alcance los 10.000 millones de habitantes, lo que llevará a un incremento del 50% en la demanda de productos agrícolas (FAO, 2017). Durante los últimos 50 años, las dinámicas globales de producción y consumo de alimentos han evolucionado rápidamente. En buena parte gracias a los cambios en las prácticas agrícolas, la capacidad del planeta para proporcionar alimentos a su población ha aumentado notablemente, incrementándose la productividad y la diversidad de alimentos con una menor dependencia estacional (Alexandratos y Bruinsma, 2012).

La demanda de productos de origen animal en 2050 también aumentará un 70% respecto a 2005 (Alexandratos y Bruinsma, 2012), por lo que se incrementará la demanda de materias primas para su alimentación y la cantidad de subproductos generados. Estos incrementos se darán especialmente en países con economías emergentes, por la combinación de distintos factores como el crecimiento poblacional, el aumento de los ingresos y la rápida urbanización (Delgado *et al.,* 2001). Respecto a Europa, se estima que la demanda de carne se vaya reduciendo paulatinamente debido a cambios en los hábitos de consumo impulsados por los crecientes problemas de salud, y cuestiones éticas y ecológicas (Vanhonacker *et al.,* 2013).

Del aumento necesario en la producción de alimentos para el consumo de la población mundial en 2050, se prevé que el 90% provenga de un incremento de los rendimientos de los cultivos, y el 10% restante de la ampliación de la superficie de cultivo (FAO, 2009). La intensificación agrícola en algunas ocasiones se interpreta sólo como un aumento de los rendimientos por unidad de tierra, sin tener en cuenta otros factores medioambientales (Ickowitz *et al.,* 2019). Sin embargo, es de vital importancia que esta intensificación se produzca de una manera sostenible, aumentando entre otros factores la eficiencia del uso de los fertilizantes con el objetivo de reducir pérdidas económicas y daños medioambientales. La intensificación sostenible es un modelo emergente de agricultura, diseñado para conciliar la acelerada demanda mundial de productos agrícolas con la gestión ambiental a largo plazo (Spiegal *et al.*, 2018).Es necesario que se dé una transición hacia sistemas de producción más sostenibles, promoviendo un cambio desde los modelos actuales de producción lineales, donde los recursos se convierten en producto y desecho, a modelos circulares que en cierto modo intentan imitar los principios y el funcionamiento de los sistemas ecológicos naturales (Chojnacka *et al.*, 2020). La economía circular se basa en modelos de bucle cerrados, en los que los desechos y los subproductos se integran efectivamente en el sistema como activos valiosos, reduciendo así la utilización de los recursos naturales y la producción de desechos.

1.1.2 Políticas europeas para la producción agroalimentaria

En Europa se han puesto en marcha importantes políticas para garantizar un suministro estable de alimentos asequibles y de calidad sin comprometer el medio ambiente. En este sentido, y con el objetivo de reducir la liberación de nitratos de las actividades agrícolas a las aguas superficiales y subterráneas, ya en 1991 la Unión Europea publicó la **Directiva de Nitratos** (1991/676/EC), con regulaciones específicas que los países miembros deben cumplir.

La **Política Agrícola Común (PAC)**, puesta en marcha en 1962, contempla la sostenibilidad de la agricultura y de las zonas rurales de la UE. La PAC tiene como objetivo apoyar a los agricultores, y entre sus objetivos específicos se encuentran: a) mejorar la productividad agrícola, asegurando el suministro de alimentos y garantizando un nivel de vida razonable a los agricultores, b) conservar los paisajes y zonas rurales mediante la gestión sostenible de recursos naturales, y c) mantener viva la economía rural. La PAC ha ido evolucionando a lo largo de los años, a través de distintas reformas, para dar respuesta a las circunstancias cambiantes. El 1 de junio de 2018, la Comisión Europea presentó las propuestas legislativas sobre el futuro de la PAC (MAPA, 2020) para el período posterior a 2020 (PAC post-2020). Esta reforma de la PAC tiene una mayor ambición medioambiental y acción por el clima, por lo que es coherente con el Pacto Verde Europeo que se mencionará más adelante. El objetivo fundamental de la nueva propuesta es conseguir una agricultura sostenible como modelo diferenciado en los mercados internacionales, logrando cumplir el objetivo de producir suficiente para la creciente demanda poblacional, con menos recursos y preservando los compromisos medioambientales. En este sentido, aparecen los eco-esquemas, basados en prácticas agrícolas beneficiosas para el clima y el medio ambiente. Los eco-esquemas serán obligatorios para los Estados miembros y voluntarios para agricultores y ganaderos. Supondrían un 20% del presupuesto del primer pilar, que son los fondos que los estados reciben directamente desde Europa sin necesidad de cofinanciación, a diferencia del presupuesto del segundo pilar que los estados tienen que cofinanciar y que está destinado al desarrollo rural. Estas ayudas se abonarían a los agricultores y ganaderos que cumpliesen condiciones medioambientales que vayan más allá de la condicionalidad. Los eco-esquemas que actualmente propone el Ministerio de Agricultura, Pesca y Alimentación (MAPA, 2020) y que están en fase de borrador son ocho, y engloban el pastoreo extensivo, la implantación y mantenimiento de cobertura vegetal viva en cultivos, incorporación al suelo de restos de poda leñosos, fomento de rotaciones con cultivos mejorantes, fomento de aplicación de planes individuales de fertilización y de uso sostenible de productos fitosanitarios, implantación y conservación de márgenes, islas de vegetación y corredores multifuncionales, y participación en programas individuales o colectivos de valorización energética de estiércoles de rumiantes y equino y biomasa de origen vegetal.

El **Pacto Verde Europeo** (EC, 2019a), presentado el 11 de noviembre de 2019, establece una hoja de ruta para impulsar el uso eficiente de recursos mediante el paso a una economía limpia y circular, con objeto de restaurar la biodiversidad y reducir la contaminación. El Pacto Verde Europeo presenta distintos ámbitos de actuación, siendo uno de ellos la estrategia **de la granja a la mesa**, en que uno de los problemas que se resalta es el exceso de nutrientes en el medio ambiente, considerándolo una fuente importante de contaminación del aire, del suelo y del agua, lo que repercute negativamente en la biodiversidad y en el clima. Por ello, se establecen medidas para hacer frente a este exceso de nutrientes, estableciendo como objetivo una reducción de las pérdidas de nutrientes en al menos un 50% sin alterar la fertilidad del suelo, y una reducción en el uso de fertilizantes de al menos un 20% para el año 2030. Para conseguir estos objetivos, se establece el desarrollo de un plan de acción de gestión integrada de nutrientes para abordar la contaminación por nutrientes en origen y aumentar la sostenibilidad del sector agroganadero. Se indica la importancia de trabajar en la aplicación de técnicas precisas de fertilización y prácticas agrícolas sostenibles, especialmente en la ganadería intensiva, utilizando los subproductos orgánicos como fertilizantes. La Comisión también impulsará el desarrollo de cultivos ecológicos con la finalidad de que el 25% de todas las tierras agrícolas dediquen su producción a la agricultura ecológica para el año 2030. En este tipo de producción no está permitida la utilización de fertilizantes de síntesis, por lo que la única fuente de N estaría en los productos orgánicos. Por otro lado, se espera acortar las cadenas de suministro para reducir la dependencia del transporte de larga distancia, disminuyendo las emisiones de gases de efecto invernadero asociadas al transporte y promoviendo el consumo de productos locales y regionales. El consumo de productos locales diferenciados en origen otorga un valor añadido al producto y promueve el aumento de la diversidad de semillas y variedades.

1.1.3 Sistemas de producción agrícola y ganadera

Una de las fuentes de alimento más importantes del mundo son los cereales ya que aportan el 63% de las calorías y el 56% de las proteínas en la dieta (FAO, 2017). Dada su importancia en la alimentación se estima que para el año 2050 la demanda de cereales aumente un 60% (respecto a 2005), alcanzando los 3.000 millones de toneladas (FAO, 2017). Ya en 2018, los cereales ocuparon la mayor superficie de cultivo a nivel mundial (728 millones de hectáreas) y se cosecharon 2.100 millones de toneladas (FAOSTAT, 2020). Los cereales se destinan para consumo humano directo o como pienso para la alimentación del ganado. El consumo humano directo varía considerablemente de unos continentes a otros, aportando en algunos lugares de África y Asia el 70% de la ingesta de energía, mientras que en algunos países de Europa aporta aproximadamente el 30% (Kearney, 2010). Se estima que un tercio del cereal producido a nivel mundial es destinado a la alimentación ganadera, lo que supone que se destinen a este fin aproximadamente 210,5 millones de hectáreas (Mottet *et al.,* 2017). Aún más, un tercio de la tierra cultivable total está dedicada a la producción de piensos para consumo animal, por lo que la ganadería utiliza una parte importante de los 200 millones de toneladas de fertilizantes nitrogenados, fosfatados y potásicos que se aplican anualmente (FAO, 2017).

El nitrógeno (N), debido a su baja disponibilidad en los ecosistemas terrestres, es el nutriente que más directamente influye en la producción vegetal y en el contenido de proteína de los cultivos de grano. Hay otros dos macronutrientes primarios imprescindibles en la nutrición vegetal, el fósforo y el potasio, que tienen que encontrarse también en cantidades suficientes para no limitar el crecimiento vegetal (Roy *et al.,* 2006). El descubrimiento del proceso de Haber-Bosch para la producción de N mineral a principios del siglo XX, y la extracción de roca fosfórica y minerales de sales de potasio desde finales del siglo XIX, para su utilización como fertilizantes permitieron mejorar la fertilidad del suelo, aumentando la producción de biomasa por parte de las plantas y, por lo tanto, el rendimiento de las mismas (Smil, 2002). Los fertilizantes minerales (*ver apartado 1.3.1 Fertilizantes minerales*) comenzaron a utilizarse de forma generalizada en torno a 1950, lo que, entre otros factores, permitió un rápido crecimiento de la población mundial (Figura 1.1), siendo la disponibilidad de alimentos un factor clave para este crecimiento.

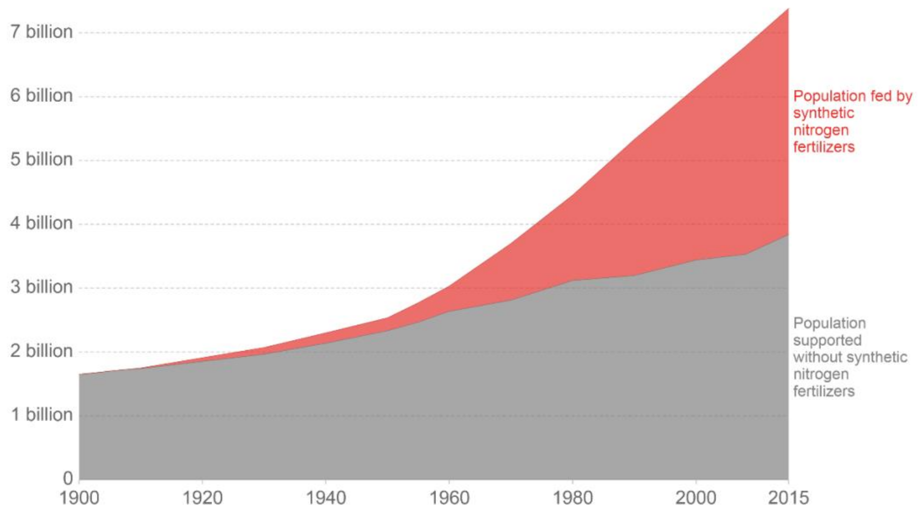


Figura 1.1. Estimación de la parte de la población mundial que puede alimentarse con la aplicación de fertilizantes nitrogenados minerales (rojo) y sin su aplicación (gris). *Fuente: Our World in Data (2019, https://ourworldindata.org/world-population-growth).*

A nivel mundial, en los últimos 60 años, la utilización de fertilizantes nitrogenados, fosfatados y potásicos ha aumentado en un 954%, 375%, y 451%, respectivamente (FAOSTAT, 2020). Mediante la aplicación de fertilizantes se influye en los ciclos biogeoquímicos de los nutrientes, aumentando la cantidad de nutrientes suministrados por el suelo y, por tanto, su disponibilidad para los cultivos (Roy *et al.*, 2006). Sin embargo, este aumento en el uso de fertilizantes ha hecho que los límites planetarios propuestos por Rockström *et al.* (2009), y revisados por Steffen *et al.* (2015), hayan sido superados en el caso del N reactivo antropogénico (Zhang *et al.*, 2015), y que en el caso del fósforo, este elemento se encuentre ya por encima de la zona de incertidumbre (Campbell *et al.*, 2017), siendo probable que la creciente demanda de alimentos incremente aún más el desequilibrio en el balance de ambos nutrientes (Zhang *et al.*, 2015).

Los sistemas de producción ganadera siguen distintos modelos, y actualmente en algunas zonas del mundo los sistemas de producción intensiva (“landless production systems", Figura 1.2), con grandes explotaciones ganaderas para incrementar la eficiencia productiva (Steinfeld *et al.*, 2006), son los más habituales.

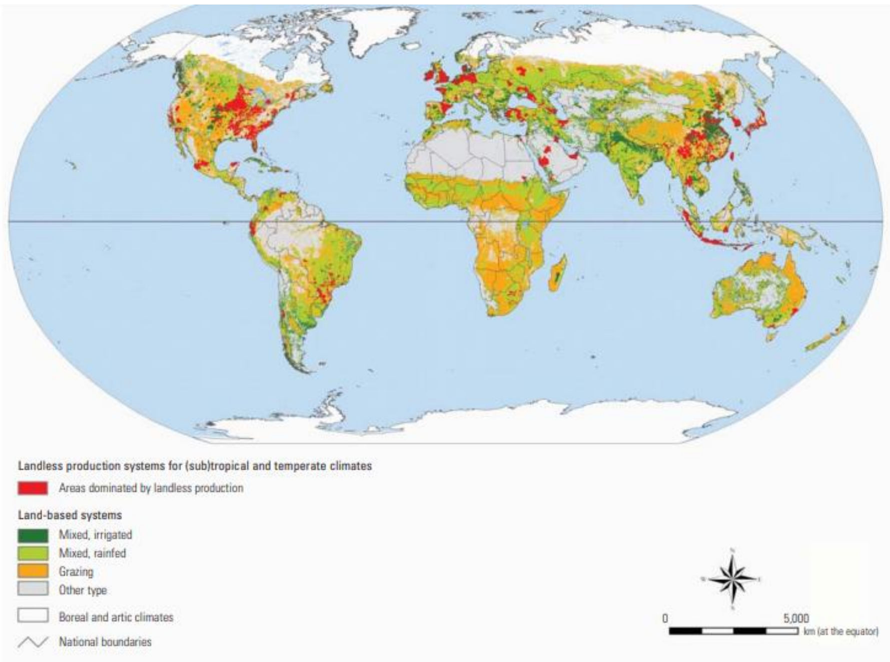


Figura 1.2. Distintos sistemas de producción ganadera en el mundo. *Fuente: Steinfeld et al. (2006).*

Como consecuencia de esta intensificación ganadera se genera una gran cantidad de subproductos orgánicos (*ver apartado 1.3.2 Subproductos de origen ganadero*) en zonas donde la tierra agrícola disponible para el aporte de estiércoles o purines es limitada. Esto puede derivar en un mayor uso de energía en el transporte de estos subproductos a campos de cultivo lejanos, o en un exceso de nutrientes por su aplicación excesiva en los campos de cultivo cercanos (Petersen *et al.*, 2007). Por el contrario, la ganadería tradicional, no presenta problemas relacionados con la acumulación de estiércol, ya que hay una integración adecuada entre ganadería y agricultura (FAO, 2008).

No obstante, a través de los subproductos de origen ganadero, se devuelven parte de los nutrientes extraídos por los cultivos al suelo, lo que contribuye a la fertilidad del mismo y a la consiguiente productividad de los cultivos (Xia *et al.*, 2017). En términos generales, entre el 55% y el 95% del nitrógeno, y alrededor del 70% del fósforo ingerido por el ganado, se excreta en forma de orina o heces (Menzi *et al.*, 2010). Hasta fechas recientes alrededor del 50% del N y el fósforo aplicados como fertilizante en los cultivos del mundo provenían de fertilizantes minerales (Erisman *et al.*, 2008). Sin embargo, dada la estimación ya mencionada de aumento de la demanda mundial de productos de origen ganadero, así como la tendencia a incluir granos más ricos en proteína en las dietas del ganado, se estima que para el año 2050 los subproductos de origen animal utilizados como fertilizantes aportarán aproximadamente 130-153 Tg de N por año, mientras que los fertilizantes minerales aportarán 83-109 Tg de N (Bouwman *et al.,* 2013). La ganadería europea genera 1.400 millones de toneladas de subproductos por año, conteniendo 7 millones de toneladas de N (Leip *et al.,* 2011). Por otra parte, en 2015, en el sector agroganadero europeo se utilizaban 11 millones de toneladas de N mediante su aplicación como fertilizantes minerales (Bernal *et al.*, 2015). Oenema *et al.* (2007) indicaron que en la Unión Europea en el año 2000 los subproductos de origen animal aportaban 100 Tg de N, recuperándose únicamente el 20-40% de este N por los cultivos y disipándose el 60-80% restante al medioambiente. Teniendo en cuenta todo lo anterior, es necesario promover iniciativas y realizar trabajos orientados al reciclaje de los nutrientes provenientes de la ganadería para la fertilización de los cultivos, reduciendo así el uso de fertilizantes minerales y la acumulación de estiércoles en zonas de ganadería muy intensificada.

En general, la eficiencia del N aplicado mediante los fertilizantes, tanto inorgánicos como orgánicos, es un tema muy importante en los sistemas de producción agroganadera. Entre los años 1990/92 y 2002/04 la eficiencia en el uso del N (EUN) mejoró notablemente en la Unión Europea, aumentado del 51% al 59% (OECD, 2008). Sin embargo, el 40% del nitrógeno aplicado de forma orgánica o mineral (*ver apartado 1.3.3 Transformaciones del N en el suelo*) no era utilizado por los cultivos, lo que podía suponer una pérdida económica significativa en el sector agrícola y también efectos negativos sobre el medioambiente, tales como la eutrofización de las aguas, la pérdida de biodiversidad, el calentamiento global y el agotamiento del ozono estratosférico (Campbell *et al.*, 2017). Quemada *et al.* (2020) observaron que la EUN varía dependiendo del tipo de explotación, teniendo las explotaciones agrícolas una EUN media mayor (60%) que las explotaciones ganaderas (54% en porcino, 30% en vacuno de leche). Estos autores observaron que un factor que tiene una gran influencia en la EUN de las explotaciones agrícolas es la aplicación de subproductos de origen ganadero, ya que al aplicar estos subproductos la eficiencia en el uso del N disminuye. Sin embargo, los elevados excedentes de N estimados en las explotaciones en las que se aplican subproductos de origen ganadero (producción ecológica y también producción convencional), se podrían explicar de forma parcial por el almacenamiento de N en la materia orgánica del suelo, ya que la materia orgánica del suelo suele aumentar en estos sistemas (Maillard y Angers, 2014; Gattinger *et al.,* 2012).

Para lograr una mejora en la EUN, el ajuste de la dosis de fertilizante (*ver apartado 1.4 Ajuste de la fertilización nitrogenada)*, la correcta elección de los momentos de aplicación (Ravier *et al.,* 2017a), y el tipo y forma de aplicación (Omara *et al.,* 2019) son de vital importancia. El uso óptimo de los fertilizantes nitrogenados debería basarse en el equilibrio entre la demanda de N por parte del cultivo y la disponibilidad de N en el suelo. Sin embargo, tanto esta demanda como disponibilidad de N no son sencillas de predecir. La demanda de N debe responder tanto a la cantidad como a la calidad esperada del producto agrícola. El N disponible depende del tipo de fertilizante aportado, del tipo de suelo y de las condiciones climáticas (Colaço y Bramley, 2018). Por otro lado, las recomendaciones de fertilización históricamente se han basado en el uso fertilizantes minerales, por lo que es necesario estimar el valor fertilizante de los subproductos orgánicos para predecir cuál será su contribución a los cultivos (Whalen *et al.,* 2019). La capacidad de englobar la variabilidad de la demanda de N por parte de los cultivos y la disponibilidad de N en el suelo es un aspecto clave de la agricultura de precisión (*ver apartado 1.4.1 Agricultura de Precisión*). En el contexto de la agricultura de precisión, los sensores proximales se han identificado como herramientas valiosas (*ver apartado 1.4.2.3.3.1 Sensores proximales*) para proporcionar diagnósticos específicos para cada zona y para ajustar la demanda de N en los cultivos (Mulla, 2013).

1.2 El cultivo de trigo

El trigo blando (*Triticum aestivum* L.) es un cereal que se cultiva bajo diversas condiciones climáticas y ha sido un alimento básico de las principales civilizaciones de Europa, Asia y el Norte de África. Su producción también está destinada a la alimentación animal y a la producción de almidón y etanol. El N es el nutriente que mayor influencia tiene en el rendimiento y en la calidad del grano, por lo que un correcto estado nutricional nitrogenado del cultivo es imprescindible para alcanzar altos rendimientos y granos de calidad. Desde su domesticación hace unos 12.000 años, los agricultores han influido profundamente en su evolución para mejorar algunos rasgos deseables (Kiszonas y Morris, 2017). En el pasado más reciente, a su mejora ha contribuido un enfoque basado en las nuevas técnicas de mejora genética y, además, se han hecho grandes avances para conseguir que los cereales expresen las proteínas del sistema nitrogenasa, con el objetivo de que fijen el N atmosférico. Sin embargo, la complejidad de este último enfoque hace que esta solución deba esperar un largo periodo antes de su implementación en las nuevas variedades de cereales. Por el contrario, los inóculos microbianos fijadores de nitrógeno podrían tener un impacto más inmediato tanto en los sistemas industriales como en los agrícolas (Bloch *et al.*, 2020).

En 2018, el trigo blando supuso el 25% de la producción de cereal a nivel mundial con 734 millones de toneladas (FAOSTAT, 2020). En la Unión Europa, en 2020, la producción de trigo blando fue de 116,7 millones de toneladas y la superficie fue de 20,6 millones de hectáreas (EC, 2020), siendo el primer productor mundial seguido de China, India, Rusia EUA y Canadá (MAPA, 2020). Dentro de la Unión Europea, Alemania es el principal productor de trigo blando (29 millones de toneladas), seguido de Francia (21 millones de toneladas), de Polonia (12 millones de toneladas) y de España (7,1 millones de toneladas) (EC, 2020). En España, la superficie cultivada de trigo blando fue de 1,6 millones de hectáreas, siendo Castilla y León la comunidad autónoma con mayor producción (1,4 millones de toneladas), seguida por Castilla La Mancha (0,5 millones de toneladas) (MAPA, 2020). Las producciones medias anuales en Europa son de alrededor de 127 millones de toneladas, y en España de 5,7 millones de toneladas (EC, 2020).

1.2.1 Cultivo de trigo en Araba

En Araba el cultivo de cereal en 2019 ocupó 43.776 ha, siendo el trigo blando el cereal con mayor superficie (24.019 ha) y producción (162.000 t), seguido de la cebada con 13.690 ha y 96.000 t, la avena con 6.063 ha y 33.000 t y, finalmente, el maíz con 4 ha (Eustat, 2020). En Araba, la mitad del trigo cultivado es destinado a la alimentación animal como pienso, y la otra mitad a la alimentación humana en forma de harina (José Luis Fresno, comunicación personal). Sin embargo, esta harina no suele presentar las características de calidad necesarias para elaborar un pan de calidad, ya que su contenido de proteína no siempre es suficiente para ello. La harina de trigo con un contenido de proteína por encima del 9% es considerada harina panificable, y por encima del 12,5% es considerada harina de fuerza (RD 677/2016). En los últimos años, la demanda de un pan diferenciado en origen y en calidad se ha visto incrementada, por lo que producir harinas locales con calidad harino-panadera podría darles un valor añadido en el mercado. No es sencillo incrementar la calidad del grano al mismo tiempo que el rendimiento, debido a que existe una relación negativa entre ambos (Simmonds, 1995). En Araba los rendimientos de trigo suelen ser altos, por lo que supone un reto conseguir un grano de alta calidad. En este sentido, la cantidad y el momento de aplicación del fertilizante tienen que ser cuidadosamente establecidos y ajustados a los requerimientos nutricionales del cultivo para alcanzar el equilibrio entre el rendimiento, la calidad del grano y la sostenibilidad ambiental (Diacono *et al.,* 2013).

En Araba las prácticas de fertilización más comunes son las siguientes: a) en el caso de que los agricultores apliquen fertilizantes orgánicos en fondo (antes de la siembra del trigo), este aporte suele combinarse con la aplicación de fertilizantes minerales; b) además en las parcelas donde se aplican fertilizantes orgánicos, éstos suelen aplicarse cada dos o tres años, y únicamente en fondo debido a las habituales condiciones húmedas del invierno y de la primavera en la zona, que no permiten la entrada de la maquinaria necesaria para aportar los subproductos orgánicos a las parcelas agrícolas; c) lo más frecuente es aplicar 400-450 kg ha^{-1} en forma de complejo (15-15-15) o en forma de "blending" (10-20-10 + 3 MgO + 16 SO_3) en el inicio del ahijado y posteriormente en el encañado una cobertera de 400-450 kg ha^{-1} en forma de Nitrato Amónico Cálcico (NAC) 27%, sin que haya aplicación de fertilizantes orgánicos.

En este territorio, parte de las tierras agrícolas donde se produce cereal habitualmente, son zonas declaradas vulnerables a la contaminación por nitratos (Figura 1.3). Esta declaración supone tener que cumplir las limitaciones establecidas por el "Plan de actuación sobre zonas declaradas vulnerables a la contaminación de las aguas por nitratos procedentes de la actividad agraria", aprobado en la Orden del 15 de octubre de 2008. Según esta orden, la cantidad de nitrógeno aplicado nunca debe sobrepasar los 170 kg N ha^{-1}.

Por otro lado, la superficie ocupada por la producción agrícola en ecológico ha aumentado, al igual que la superficie de cereal en ecológico, ocupando casi 500 ha en 2017 (ENEEK, 2020). En este tipo de producción, es de especial interés el conocimiento de la dinámica de mineralización del N de los distintos subproductos orgánicos, ya que éstos son el único medio para aportar N al cultivo, al estar prohibidos los fertilizantes nitrogenados minerales. En la producción ecológica, el tipo de fertilizante orgánico aplicado y la aplicación en el momento adecuado pueden mejorar el rendimiento y la calidad del grano de trigo. Los fertilizantes que pueden ser utilizados en la producción ecológica están recogidos en el Anexo I del Reglamento (EC) 889/2008.

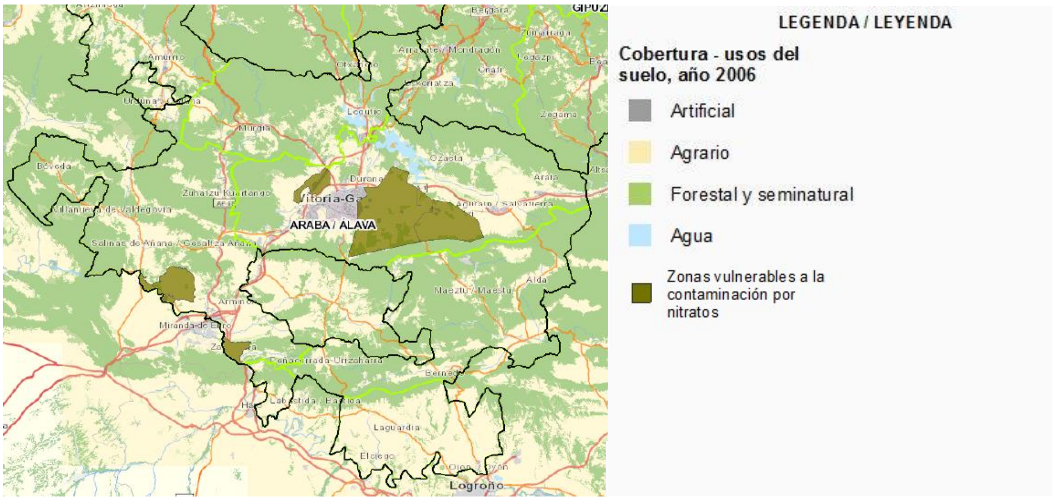


Figura 1.3. Usos del suelo (artificial, agrario, forestal y seminatural, y agua) y zonas vulnerables a la contaminación por nitratos en la provincia de Araba. *Fuente: Visor GeoEuskadi (2020, https://www.geo.euskadi.eus).*

1.2.2 Fenología del trigo y necesidades de nitrógeno

Las decisiones agronómicas en cereales se implementan utilizando escalas que hacen referencia al crecimiento y desarrollo del cultivo. En la fenología del trigo existen etapas en las que un correcto estado nutricional es determinante para alcanzar altos rendimientos o valores de proteína suficientes para obtener granos de trigo de calidad.

La fenología del trigo presenta tres etapas diferenciadas: la fase de crecimiento vegetativo, que comprende el ahijado y el encañado del trigo; la fase de reproducción, que comprende la floración o antesis, y la fase de formación del grano y maduración. Existen diferentes escalas para describir el desarrollo del cultivo de trigo, como BBCH (Meier, 2001), Feekes (Feekes, 1941) o Zadoks (Zadoks *et al.*, 1974), siendo la más utilizada la de Zadoks (Figura 1.4).

Durante la **etapa de ahijado (GS20-GS29)** el cultivo de trigo es capaz de desarrollar hijuelos. La cantidad de hijuelos que desarrolle dependerá del estado nutricional, especialmente de la nutrición nitrogenada, y de las condiciones climáticas. La cantidad de N absorbido por parte del cultivo durante este periodo suele considerarse bastante baja, sin embargo, un buen desarrollo del ahijado confiere un mayor potencial de acumulación de biomasa, por lo que se considera relevante para alcanzar rendimientos de trigo adecuados (Magney *et al.*, 2016). La etapa de ahijado puede verse retrasada debido a las bajas temperaturas del suelo y a las condiciones anaeróbicas del mismo, ambos factores estando afectados por las precipitaciones (Lindstrom *et al.,* 1976). Esta etapa está relacionada con la determinación del número de espigas (Borràs-Gelonch *et al.,* 2012).

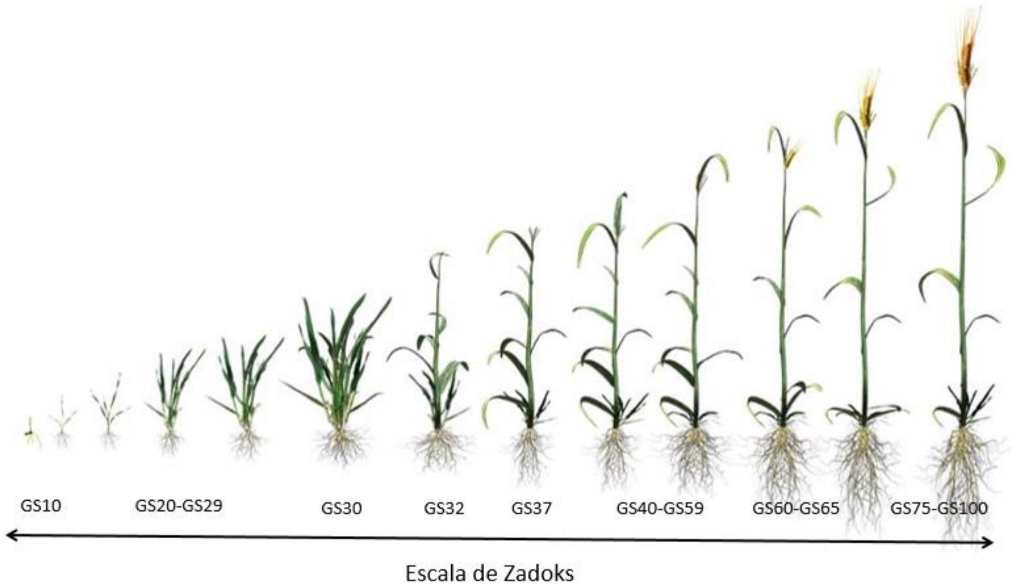


Figura 1.4. Fenología del trigo según la escala de Zadoks (Zadoks *et al.,* 1974).

Durante la **etapa de encañado (GS30-GS39)** una disponibilidad adecuada de N promueve el crecimiento de los hijuelos, aumentando la futura capacidad de almacenamiento de N de la planta, estando relacionada con el número de espigas viables y el número de granos por unidad de superficie (Jeuffroy y Bouchard, 1999). Una deficiencia de N en este periodo lleva a una disminución en el número de granos por unidad de superficie (Jeuffroy y Bouchard, 1999). Este es el periodo en el que la absorción de N es más importante (Figura 1.5).

En la **etapa de floración (GS60-GS69)** el N acumulado en la parte vegetativa del cultivo comienza a removilizarse para el llenado del grano. Hasta el inicio de la floración, la biomasa vegetativa ha sido el sumidero del N absorbido por el cultivo, y puede proporcionar entre el 60 y el 92% del N que hay en el grano en la cosecha (Simpson *et al.,* 1983).

La **etapa de llenado del grano** representa también un periodo importante tanto para el rendimiento como para la calidad del grano. Dependiendo de la duración de esta etapa, y de la tasa de degradación de los fotoasimilados provenientes de la parte vegetativa y de su removilización, el rendimiento y la calidad del grano se verán más o menos incrementados (Barbottin *et al.*, 2005).

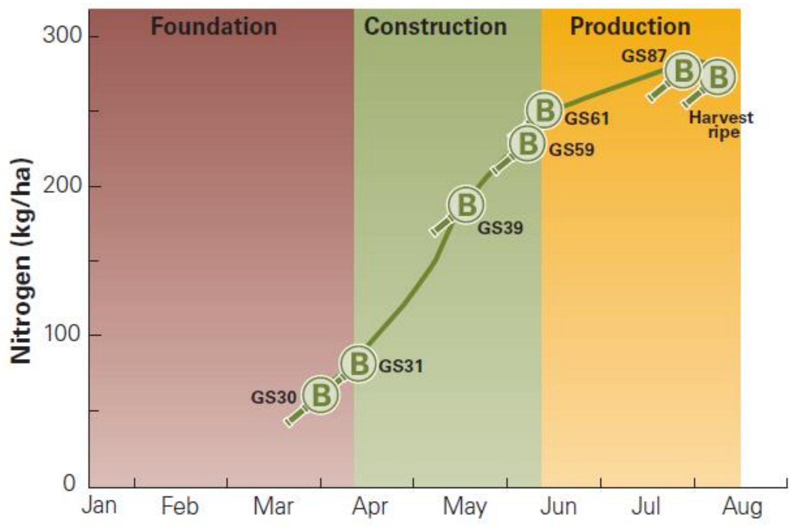


Figura 1.5. Dinámica de la absorción de N (kg ha^{-1}) en el cultivo de trigo desde inicio de encañado hasta la maduración del grano. *Fuente: Sylvester-Bradley et al. (2005).*

1.3 Fertilización mineral y orgánica

1.3.1 Fertilizantes minerales

Los fertilizantes minerales son sustancias producidas por la industria química (como en el caso del N) o bien mediante la explotación de yacimientos naturales (como en el caso del fósforo o del potasio), y su proceso de obtención consiste en la transformación de diferentes materiales presentes en la naturaleza en nutrientes asimilables por las plantas (EC, 2019b). El uso de fertilizantes minerales es muy variable de unas zonas del mundo a otras (Figura 1.6).

La producción de **fertilizantes minerales nitrogenados**, como ya se ha mencionado, se basa en la tecnología Haber-Bosch, en la que se fija el N atmosférico con hidrógeno para producir amoníaco (Erisman *et al.*, 2008). El hidrógeno así como la energía para generar las altas temperaturas y presiones necesarias se obtienen generalmente del gas natural. Hasta 2014, la UE era exportadora neta de nitrato amónico, pero desde 2014 se ha convertido en una importadora neta, alcanzando estas importaciones en 2017 una cantidad de 153.000 toneladas de nitrato amónico. Los principales países productores de fertilizantes nitrogenados cercanos a la EU son Egipto, Argelia, Rusia, Bielorrusia y Ucrania (EC, 2019b).

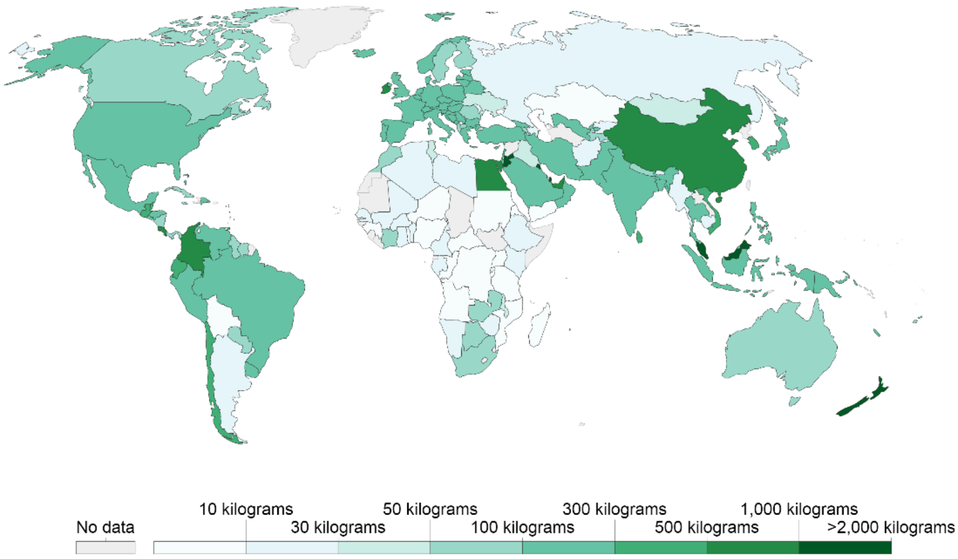


Figura 1.6. Cantidad de productos fertilizantes nitrogenados, fosfatados y potásicos (kg ha^{-1}) utilizados en suelos agrícolas en 2015. *Fuente: One World in Data* (2020, https://ourworldindata.org/fertilizers).

Los **fertilizantes minerales fosfatados** provienen exclusivamente de mineral extraído, la roca fosfórica. Para convertir la roca fosfórica, de muy baja solubilidad, en un producto fertilizante es necesaria una extracción química con un ácido, transformando el mineral en una sal soluble en agua (EC, 2019b). La roca fosfórica fue incluida en la lista de materia prima crítica en mayo de 2014 (EC, 2014). Se estima que para finales del siglo XXI los recursos de fósforo se habrán agotado (Christel *et al.*, 2014). La disponibilidad de roca fosfórica de calidad alta es limitada, ya que sólo se encuentran depósitos en unas pocas regiones del planeta como el Sahara Occidental. Marruecos tiene más del 77% de los recursos fosfatados del mundo y junto con China y EEUU representan dos tercios de la producción mundial. Indicadores a largo plazo prevén una mayor demanda de fósforo, menor calidad y mayores costos de producción (Cooper y Carliell-Marquet, 2013).

Los **fertilizantes minerales potásicos** provienen de distintas fuentes. Las principales fuentes de potasio en el mundo son los minerales de arcilla de los suelos y las rocas, el agua de los océanos y los depósitos de sal de roca de mares secos que contienen minerales cristalizados. La potasa (nombre común otorgado a las sales de potasio) es soluble en agua, por lo que el método de producción de basa principalmente en el proceso de purificación. Las reservas de potasa del mundo son de en torno a 6.072 millones de toneladas, por lo que su disponibilidad no se verá comprometida en varios siglos (Prakash y Verma, 2016). La producción de potasa mundial en 2012 fue de 31,5 millones de toneladas en forma de K_2O (EC, 2019b). Canadá es el mayor productor de potasa, con el 28% de la producción mundial, seguido por Rusia (17%). La demanda global de fertilizantes potásicos se ha incrementado en los últimos 40 años. Al contrario que el fósforo, las necesidades de potasio de Europa están cubiertas por minas localizadas en Alemania, que contienen el 1,6% de los depósitos de potasa del mundo (Prakash y Verma, 2016).

El precio de los fertilizantes minerales se ha visto incrementado con el tiempo debido a distintas razones. El coste del gas natural representa entre el 70 y el 90% de los costes variables para la producción de fertilizante nitrogenado, por lo que su precio depende fuertemente del gas natural (Lécuyer *et al.*, 2014). Los fertilizantes fosfatados también han sufrido aumentos es sus costes, principalmente debido al aumento de la demanda y a la disminución de los depósitos de materias primas de fósforo no renovables (Cooper y Carliell-Marquet, 2013). En el caso del potasio, además de los costes del petróleo, la tendencia en el incremento del precio ha sido impulsada por la demanda de frutas y hortalizas, cuyo cultivo supone el 19% del consumo de potasio a nivel mundial (IFA, 2018). Además, otra de las razones que contribuye a la escalada de los precios de los fertilizantes es el aumento de los precios del petróleo, que hace que aumenten los costes del transporte (Lécuyer *et al.*, 2014). Por otro lado, los fertilizantes minerales representan el 13% del total de las emisiones agrícolas de gases de efecto invernadero (FAO, 2014).

1.3.2 Subproductos de origen ganadero

Aunque este trabajo esté relacionado con los subproductos de origen ganadero, es necesario mencionar que existen otros tipos de subproductos que se generan en grandes cantidades como son los residuos agrícolas (restos de poda, residuos forestales o paja), residuos de la industria alimentaria, de hogares y restaurantes y lodos de depuradora (Chojnacka *et al.*, 2020). Estos residuos se aplican en el suelo, se depositan en vertederos e incluso una pequeña parte de ellos se incinera sin que se recupere su riqueza de nutrientes valiosos (Zabaleta y Rodic, 2015).

Los subproductos de origen ganadero pueden ser un excelente fertilizante ya que aportan nitrógeno, fósforo, potasio, nutrientes secundarios y micronutrientes (Bernal *et al.,* 2015), manteniendo la fertilidad del suelo, reciclando los nutrientes disponibles a nivel local y promoviendo los principios de una agricultura sostenible (Petersen *et al.,* 2007). Por otro lado, son una fuente de materia orgánica, que mejora la estructura del suelo y la estabilidad de los agregados, aumenta la porosidad y reduce la densidad aparente, así como la infiltración del agua (Whalen *et al.,* 2019). Además, su aplicación puede estimular la actividad de los microorganismos del suelo, su biomasa y la composición y diversidad de las comunidades microbianas del suelo (Urra *et al.,* 2019). Bhogal *et al.* (2018) observaron que con la aplicación de subproductos orgánicos se incrementaba el suministro de nutrientes del suelo (nitrógeno total, fósforo, potasio y magnesio) en un corto periodo de tiempo. Sin embargo, para observar beneficios relacionados con la biomasa microbiana y las propiedades físicas del suelo, las aplicaciones tenían que hacerse durante nueve años o más, y estas mejoras dependían de la calidad del material orgánico aplicado. Bhogal *et al.* (2018) determinaron que las aplicaciones de materiales con bajo contenido de materia seca, como los purines, tenían una influencia limitada en los beneficios a largo plazo. Sin embargo, aunque la aplicación de subproductos orgánicos es una importante fuente de beneficios en lo que a la fertilización se refiere, es necesario tener en cuenta que pueden contener residuos de antibióticos, metales pesados y contaminantes orgánicos (Epelde *et al.,* 2018).

Aunque la información expuesta en las siguientes líneas se refiera en algunos casos al fósforo y el potasio, se centrará mayoritariamente en el N, ya que el grueso de este trabajo está dedicado al estudio de la nutrición nitrogenada. Para poder reducir el uso de fertilizantes minerales, utilizando y reciclando los nutrientes provenientes de la ganadería, es necesario estimar el valor fertilizante del N en los distintos tipos de subproductos orgánicos, y predecir su contribución en relación al suministro de N a los cultivos. Sin embargo, la determinación del valor fertilizante de N en los subproductos ganaderos representa un conjunto de desafíos, ya que los distintos subproductos tienen distintos orígenes y distintas características físico-químicas. Los subproductos de origen ganadero pueden proporcionar a los cultivos N disponible como amonio (NH_4^+), y N en forma orgánica, que irá mineralizándose a lo largo del ciclo de cultivo, siendo este aporte dependiente de las características físico-químicas de los subproductos (Chadwick *et al.,* 2001).

1.3.2.1 Características físico-químicas

Los subproductos orgánicos pueden encontrarse en forma sólida (*e.g.* excrementos de animal mezclados con las camas de origen vegetal), semi-sólida (*e.g.* excrementos provenientes de la avicultura como la gallinaza) o líquida (*e.g.* excrementos recogidos en granjas de vacuno o porcino que se limpian con agua) (Whalen *et al.*, 2019)

La composición química de los subproductos orgánicos dependerá de factores como la dieta de los animales y los aditivos (*e.g.* bloques de sal y vitaminas), la cantidad de lecho vegetal y agua mezclada, y la pérdida de nutrientes que se produce durante el almacenamiento y su aplicación en los cultivos (Whalen *et al.,* 2019). El material vegetal asociado a los excrementos sólidos incrementa el contenido de lignina, celulosa, y la relación C:N. La lignina y la celulosa son materiales recalcitrantes que ralentizan la mineralización del subproducto aplicado. El contenido de lignina en los estiércoles sólidos varía de 29 a 86 g de lignina kg^{-1} de estiércol, mientras que en los subproductos líquidos varía de 2,5 a 7 g de lignina kg^{-1} de subproducto orgánico (Chadwick *et al.*, 2001).

Los subproductos sólidos de origen ganadero denominados estiércoles suelen tener bajos contenidos de N amoniacal. En el caso del estiércol de vacuno su contenido suele rondar los 0,6-1,2 kg NH_4^+ t^{-1}, mientras que en los estiércoles de porcino el rango aproximado es de 1,0-1,8 kg NH_4^+ t^{-1} (DEFRA, 2010). Por otro lado, los estiércoles de vacuno suelen tener las proporciones más altas de N orgánico en comparación con otros estiércoles (Whalen *et al.,* 2019), debido a una digestión menos eficiente de las proteínas en los rumiantes que en los no rumiantes (Keys *et al.,* 1969). Respecto al fósforo, el estiércol suele aportar de media unos 5 kg P_2O_5 t^{-1} (AHDB, 2017). El estiércol de porcino aporta 6 kg P_2O_5 t^{-1}, mientras que los de vacuno y ovino aportan 3,2 kg P_2O_5 t^{-1} (AHDB, 2017). En el caso del potasio, los estiércoles de vacuno aportan 9,4 kg K_2O t^{-1}, mientras que los estiércoles de porcino y ovino aportan 8 kg K_2O t^{-1} (AHDB, 2017).

Los purines, y en mayor medida los purines de cerdo, contienen proporciones de nitrógeno amoniacal elevadas (25-80% respecto al N total). Con la aplicación de purines también se aportan cantidades importantes de fósforo (0,6-1,8 kg P_2O_5 t^{-1}) (AHDB, 2017) y potasio (1,7-3,4 kg K_2O t^{-1}) (AHDB, 2017).

Los subproductos de origen avícola, como la gallinaza, contienen cantidades elevadas de ácido úrico, 56-79% respecto al N total (Whalen *et al.,* 2019), así como de N amoniacal, 20% respecto al N total (AHDB, 2017). La cantidad de fósforo aportada mediante estos productos suele oscilar entre 8-21 kg P_2O_5 t^{-1}, y la de potasio entre 8,5-27 kg K_2O t^{-1} (AHDB, 2017).

1.3.2.2 Subproductos más comunes de la Comunidad Autónoma del País Vasco (CAPV)

La mayoría de las explotaciones de vacuno de carne, ovino y caprino en la CAPV tienen un tamaño relativamente pequeño y una orientación ganadera mixta, combinando dentro de la misma explotación vacuno de carne y de leche, y ovino, y/o caprino. El subproducto principal de estas explotaciones es el **estiércol,** teniendo las explotaciones ganaderas de gran tamaño un problema con su almacenamiento (Gobierno Vasco, 2008). El número de efectivos de vacuno para producción de carne es bastante similar en las tres provincias de la CAPV estando comprendido entre 16.600 y 18.600 animales en el año 2019. El número de efectivos de ovino en la CAPV es de 219.020, localizándose el 22% en Araba, el 25% en Bizkaia y el 53% en Gipuzkoa. El número de efectivos de caprino en la CAPV es de 26.058, localizándose en Araba el 15%, en Bizkaia el 55% y en Gipuzkoa el 30%. En 2005, en las explotaciones con este tipo de orientación se generaron 1.470.724 t de estiércol (Gobierno Vasco, 2008).

En las explotaciones ganaderas especializadas de vacuno de leche y de porcino, el principal subproducto es el **purín** (Gobierno Vasco, 2008). Los problemas de falta de capacidad de almacenamiento y de falta de superficie agraria útil para el esparcido son generales en las explotaciones de mayor tamaño o en aquellas que se encuentran en las zonas de mayor carga ganadera (Gobierno Vasco, 2008). El número de efectivos de vacuno para producción de leche es de 21.301 en la CAPV, encontrándose en Araba el 25%, en Bizkaia el 33% y en Gipuzkoa el 42% (Gobierno Vasco, 2019). Respecto al número de efectivos de porcino, en la CAPV hay 35.800 efectivos, encontrándose en Araba el 58%, en Bizkaia el 15% y en Gipuzkoa el 27% (Gobierno Vasco, 2019). En 2005, en explotaciones con este tipo de orientación se recogieron 1.711.445 t de purín (Gobierno Vasco, 2008).

Otro de los subproductos más generados en la CAPV (90.300 t) es el estiércol de las explotaciones avícolas, comúnmente denominado **gallinaza** (Gobierno Vasco, 2008). La mayoría de las explotaciones avícolas de la CAPV no disponen de superficie agraria útil suficiente para el esparcido de la gallinaza. Esto hace que estas explotaciones tengan que transportar la gallinaza a grandes distancias (Gobierno Vasco, 2008). En 2016, en la

CAPV había 1.614.330 aves, encontrándose en Araba el 17%, en Bizkaia el 49% y en Gipuzkoa el 34%. De todos los subproductos y residuos orgánicos generados en el sector primario de la CAPV, el purín supone el 41%, el estiércol supone el 35% y la gallinaza supone el 2% (Gobierno Vasco, 2008).

Tabla 1.1. Contenido medio de materia seca (MS), Ntot (N total), pentaóxido de fósforo (P_2O_5) y óxido de potasio (K_2O) en distintos subproductos ganaderos.

Subproducto	MS (%)	Ntot (kg t^{-1})	P_2O_5 (kg t^{-1})	K_2O (kg t^{-1})
Purín Vacuno	10,5	3,8	1,9	3,7
Estiércol Vacuno	24,0	5,7	3,9	7,4
Estiércol Ovino	33,2	10,2	5,7	15,6

Fuente: NEIKER (comunicación personal). El número de muestras analizadas fue de 272 en purín de vacuno, 275 en estiércol de vacuno, y 45 en estiércol de ovino.

1.3.3 Transformaciones del N en el suelo

Pocos agroecosistemas aportan suficiente N para alcanzar rendimientos satisfactorios, por lo que los aportes de fertilizantes suelen ser esenciales. La fertilización mineral y orgánica constituye la fuente más importante de N en la agricultura, aunque el nitrógeno también se incorpora al suelo mediante la **deposición atmosférica** y por la **fijación** a través de algunos microorganismos de vida libre o que viven en simbiosis asociados con las plantas leguminosas (Figura 1.7). El 90-95% del N total del suelo se encuentra en forma orgánica (Yadav *et al.*, 2017). Al aplicar fertilizantes orgánicos también se aporta N orgánico, y éste se mineraliza liberando N mineral, que es la forma de N preferentemente absorbida por las plantas.

El N orgánico se transforma en N mineral mediante un proceso bioquímico llevado a cabo por enzimas y denominado **mineralización**. La cinética de mineralización dependerá de la recalcitrancia del N orgánico. Los compuestos orgánicos más lábiles se mineralizan más rápido que los más recalcitrantes, siguiendo la siguiente secuencia: urea > aminoácidos > proteínas > ácidos nucleicos > amino azúcares > nitrógeno húmico (Coyne, 1999).

El primer paso de este proceso, denominado **amonificación**, es llevado a cabo exclusivamente por microrganismos heterótrofos que utilizan C como fuente de energía y liberan amonio (Mohanty *et al.*, 2013).

$$C3\text{-}NH_2 \rightarrow C2{=}NH \rightarrow NH_4^+ \qquad (1.1)$$

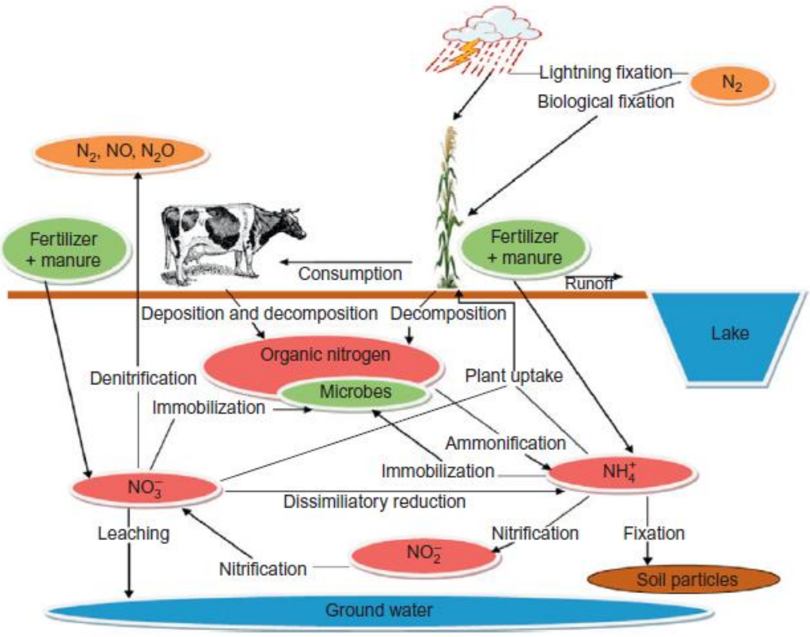


Figura 1.7. Ciclo del N en el suelo. *Fuente: St. Luce et al. (2011).*

En el siguiente paso, llamado **nitrificación**, el amonio pasa a nitrato, proceso mediado por dos grupos de bacterias autótrofas, las bacterias oxidantes de amonio (*e.g. Nitrosomonas*) y bacterias oxidantes de nitrito (*e.g. Nitrobacter*) (Cai *et al.*, 2018).

$$NH_4^+ \rightarrow NH_2OH \rightarrow NO_2^- \rightarrow NO_3^- \qquad (1.2)$$

Ambas formas, al liberarse, quedan disueltas en la solución del suelo (Whalen *et al.*, 2019). Cuando los microorganismos tienen a su disposición una fuente de carbono lábil que pueden utilizar como constituyente y energía inmediata, incorporan a su biomasa el N mineral necesario de la solución del suelo para mantener su equilibrio C:N (Mohanty *et al.*, 2013)

Así, el proceso de mineralización ocurre de forma simultánea con el proceso de **inmovilización** por parte de los microorganismos, y el balance entre ellos, conocido como mineralización neta, determina la cantidad de N mineral que quedará a disposición de las plantas en la solución del suelo (Mohanty *et al.*, 2013).

Tanto el amonio como el nitrato pueden sufrir otros procesos, dependiendo de las características del suelo y de las condiciones ambientales. Uno de esos procesos es la **volatilización** de amoníaco (NH_3) desde la superficie del suelo. Las emisiones de NH_3 se ven favorecidas con temperaturas elevadas, pH altos y concentraciones de amonio altas (Cameron *et al.,* 2013).

El amonio también puede quedar **adsorbido** en el complejo de intercambio catiónico y posteriormente ser desplazado por otros cationes, liberándose así a la solución del suelo (St. Luce *et al.*, 2011). Por otro lado, el amonio puede quedar **fijado** en las arcillas del suelo (Nieder *et al.*, 2011), especialmente en las arcillas del tipo 2:1 (Cavali *et al.,* 2015).

Cuando la concentración de oxígeno es baja en el suelo, el nitrato o nitrito presente en el suelo puede ser reducido a formas gaseosas como los óxidos de N (*e.g.* NO, N_2O y N_2), mediante un proceso microbiano denominado **desnitrificación,** y las emisiones de estos compuestos de N a la atmósfera contribuyen a la reducción de la capa de ozono y al calentamiento global.

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \qquad (1.3)$$

Parte del nitrato presente en la solución del suelo puede ser transportado disuelto en la fase acuosa y arrastrado con el drenaje hasta capas más profundas. Este proceso es conocido como **lixiviación**. El nitrato lixiviado que alcanza los ríos y los lagos puede contribuir a la eutrofización de las aguas cuando se encuentra en concentraciones altas (Bernal *et al.*, 2015).

1.4 Ajuste de la fertilización nitrogenada

Como se ha indicado anteriormente, para mejorar la EUN, el ajuste de la dosis de fertilizante, la correcta elección de los momentos de aplicación, y la cantidad y forma del fertilizante son de vital importancia. Una de las estrategias para lograr este objetivo de mejora se engloba en lo que se conoce como agricultura de precisión.

1.4.1 Agricultura de Precisión

La **agricultura de precisión** es una estrategia de gestión que recoge, procesa y analiza datos temporales, espaciales e individuales y los combina con otras informaciones para respaldar las decisiones de manejo de acuerdo con la variabilidad estimada, y así mejorar la eficiencia en el uso de recursos, la productividad, la calidad, la rentabilidad y la sostenibilidad de la producción agrícola (ISPA, 2020).

Existen numerosas herramientas para recoger, procesar y analizar los datos necesarios para mejorar el diagnóstico nutricional nitrogenado, lo que permite mejorar la fertilización nitrogenada del trigo. Algunas de estas herramientas están basadas en determinaciones en el suelo, otras en mediciones directas del estado nutricional de las plantas, y otras en medidas indirectas de los cultivos basadas en el uso de distintos sensores ópticos.

1.4.2 Herramientas para determinar la fertilización nitrogenada de los cultivos

1.4.2.1 Métodos basados en la evaluación del suelo

El contenido de N mineral del suelo (N_{min}), formado por amonio y nitrato, se ha utilizado regularmente para calcular o corregir la dosis de N necesaria para un rendimiento determinado o potencial. En general, cuando la cantidad de N_{min} en el suelo es baja, será necesario aplicar más fertilizante que cuando la cantidad de N_{min} es más alta (Ryan *et al.,* 2009). En climas con precipitaciones altas en invierno es más adecuado medir el N_{min} a la salida del invierno (St. Luce *et al.,* 2011), porque éste es el momento en el que las necesidades del trigo son máximas, y aunque tuviéramos una muestra analizada previamente nos obligaría a determinar el N_{min} existente en ese momento. La metodología del procedimiento de muestreo (zona de muestreo, profundidad y conservación de la muestra) se considera que es imprecisa, ya que los campos son heterogéneos y la pertinencia de traducir a un único valor toda una parcela es cuestionable (Ravier *et al.*, 2016). De hecho, Meisinger *et al.* (2008) encontraron para este parámetro un 50% de variabilidad en unos pocos metros cuadrados, con coeficientes de variación de 30 al 120%.

Otro problema con este método es que no considera el N que se mineralizará durante el periodo restante del ciclo de cultivo (Villar *et al.*, 2014). Con este fin, Stanford y Smith (1972) establecieron el concepto de **nitrógeno potencialmente mineralizable (N_o)** para estimar el N orgánico que puede ser mineralizado por los microorganismos heterótrofos aerobios a formas inorgánicas como el amonio y el nitrato durante el ciclo de cultivo. Este método está basado en una incubación aerobia a largo plazo en la que el suelo se mantiene en condiciones óptimas, 35 ºC y capacidad de campo. Sin embargo, este método no puede tener en cuenta las condiciones cambiantes de humedad y temperatura que se dan en el campo. Además, requiere largos tiempos de incubación, por lo que no es práctico para establecer recomendaciones de fertilización. Sin embargo, este método es considerado el método estándar para estimar el N puesto a disposición de la planta por el suelo (St. Luce *et al.,* 2011; Ros *et al.,* 2011).

Con el fin de estimar la disponibilidad de N de una forma rápida se han descrito diversas técnicas de **extracción química,** que suelen compararse con la incubación aerobia (Villar *et al.,* 2014). Algunas de ellas utilizan como extractantes soluciones salinas débiles como $CaCl_2$ o $NaHCO_3$, y otras, soluciones salinas fuertes como KCl o hotKCl. Sin embargo, estas técnicas tampoco consideran las condiciones cambiantes del campo, por lo que los resultados de validación varían de un estudio a otro en función de las condiciones ambientales (St. Luce *et al.,* 2011).

En otros casos, se han utilizado las **características físico-químicas** del suelo para poder estimar el N_o. Ros *et al.* (2011) observaron que la materia orgánica del suelo explicaba el 78% de la variación del N_o. Otros estudios concluyen que es preferible usar las fracciones lábiles de la materia orgánica del suelo para estimar el N_o (Wander, 2004). Sin embargo, también se ha indicado que ninguna de esas fracciones de la materia orgánica del suelo es *a priori* un indicador preferible del N_o (Ros *et al.,* 2011; Haynes, 2005). Otros autores (Debosz and Kristensen, 1995; Dessureault-Rompré *et al.,* 2010) encontraron que el Ntot se relaciona positivamente con la mineralización del N. En cualquier caso, dado que las condiciones meteorológicas pueden ser difíciles de predecir, también lo es la mineralización de las distintas formas de N orgánicas presentes en los suelos.

Desde AHDB (Agriculture and Horticulture Development Board, 2020), han desarrollado tablas para Reino Unido que permiten estimar el N disponible en la salida de invierno para el cultivo teniendo en cuenta el cultivo precedente, el tipo de suelo y el exceso de precipitaciones invernales (EWR). El EWR se define como la cantidad de precipitaciones que recibe un suelo después de que éste haya alcanzado la capacidad de campo en otoño y antes de que finalice el drenaje. Lo óptimo es que también tengan en cuenta la evapotranspiración en ese periodo. La AHDB desarrolla mapas de EWR para tierras sin cultivar, para trigo de invierno, para cebada de invierno y para colza con una precisión de 40 km2 teniendo en cuenta la meteorología y el tipo de suelo.

1.4.2.2 Métodos basados en el análisis químico de la planta

Los cultivos se consideran buenos integradores de los distintos factores que afectan a su nutrición, tales como la presencia del N mineral, las condiciones climáticas, las propiedades del suelo, y la gestión de los cultivos (Schröder *et al.,* 2000). Un método de referencia para cuantificar el estado nutricional nitrogenado del cultivo es el denominado Índice nutricional nitrogenado (INN), que es el cociente entre la concentración de N del cultivo y la concentración crítica de N para un crecimiento no limitado. Este cociente se puede utilizar a lo largo del ciclo de cultivo para determinar si es necesario un aporte adicional de N (Lemaire y Gastal, 1997). En el caso del trigo, los valores de INN en la floración se correlacionan bien con el número relativo de granos (Jeuffroy y Bouchard, 1999) y con el contenido de proteína del grano (Justes *et al.,* 1997). Se considera que valores de INN cercanos a 1 (INN≈1) indican que el cultivo no tiene una carencia de N, valores inferiores a 1 (INN<1) indican deficiencias de N, y valores superiores a 1 (INN>1) indican que la cantidad de N para el crecimiento del cultivo es excesiva. Ravier *et al.* (2017a) concluyeron que los valores INN han de ser revisados para lograr una mayor eficiencia en el uso del N, y determinaron unos valores umbral de INN a lo largo del ciclo de cultivo del trigo, siempre inferiores a 1, que no comprometen el rendimiento del cultivo. Respecto a la utilidad del método INN es necesario mencionar que su uso no es práctico para los agricultores, ya que es necesario determinar la biomasa y concentración de N del cultivo, lo que resulta lento y costoso. Sin embargo, resulta interesante que ofrezcan unos valores de referencia a lo largo de todo el ciclo del cultivo, lo que no es habitual con otro tipo de medidas para el diagnóstico nutricional nitrogenado.

Existen también otros métodos basados en mediciones directas en la planta, como la medición del **jugo de la base del tallo** (Hoel *et al.,* 1999). Este jugo es un extracto acuoso que representa el "stock" de iones nitrato presentes en la planta y es útil para diagnosticar la aparición de una deficiencia de N en la planta.

1.4.2.3 Métodos basados en el análisis de la planta con sensores ópticos

Las propiedades ópticas de la planta están relacionadas con el contenido de agua, la senescencia de las hojas, las enfermedades y el estado de los nutrientes (Muñoz-Huerta *et al.* 2013). El uso de sensores ópticos proporciona una técnica rápida para la medición del estado biofísico y bioquímico de los cultivos (Antille *et al.,* 2018). Las técnicas de detección óptica se consideran indirectas ya que no pueden medir directamente el contenido de N de la planta (Raun *et al.,* 2002). Se basan en la medición de compuestos como la clorofila (Ravier *et al.,* 2017b), los polifenoles (Cartelat *et al.,* 2005) o índices de vegetación como NDVI o NDRE (Antille *et al.,* 2018), que pueden utilizarse como indicadores del estado nitrogenado del cultivo. Los sensores ópticos pueden utilizar la iluminación ambiental existente (sensores pasivos) o utilizar fuentes de luz activas para mejorar las mediciones en condiciones de iluminación variables y permitir el funcionamiento durante la noche (sensores activos).

Algunos estudios (Samborski *et al.,* 2009; Diacono *et al.,* 2013) recomiendan realizar un procedimiento de normalización de las lecturas de los sensores para eliminar la influencia de factores distintos a la fertilización nitrogenada, como la variedad, el estado hídrico, enfermedades o el estado fenológico en el que se encuentre el cultivo, u otras carencias nutricionales como por ejemplo la de azufre, que pueden confundirse con las carencias de N. Para lograr la normalización de los valores existen distintas estrategias como el uso de una parcela sobrefertilizada, rampas de calibración o parcelas sin aporte de N (Samborski *et al.,* 2009). Estas parcelas se considera que tienen un valor de 100, y así el valor de la parcela problema se referencia al valor de la parcela sobrefertilizada o sin aporte de N. Estos procedimientos no son sencillos de llevar a cabo, ya que sería necesario dejar una superficie extra en cada parcela para este fin. En el caso de la teledetección sería más complicado aún, ya que la zona necesaria para la normalización de los datos requeriría superficies mayores.

Los sensores ópticos se clasifican en tres grupos (Figura 1.8) dependiendo de si miden la transmitancia, la fluorescencia o la reflectancia de la luz (Figura 1.9).

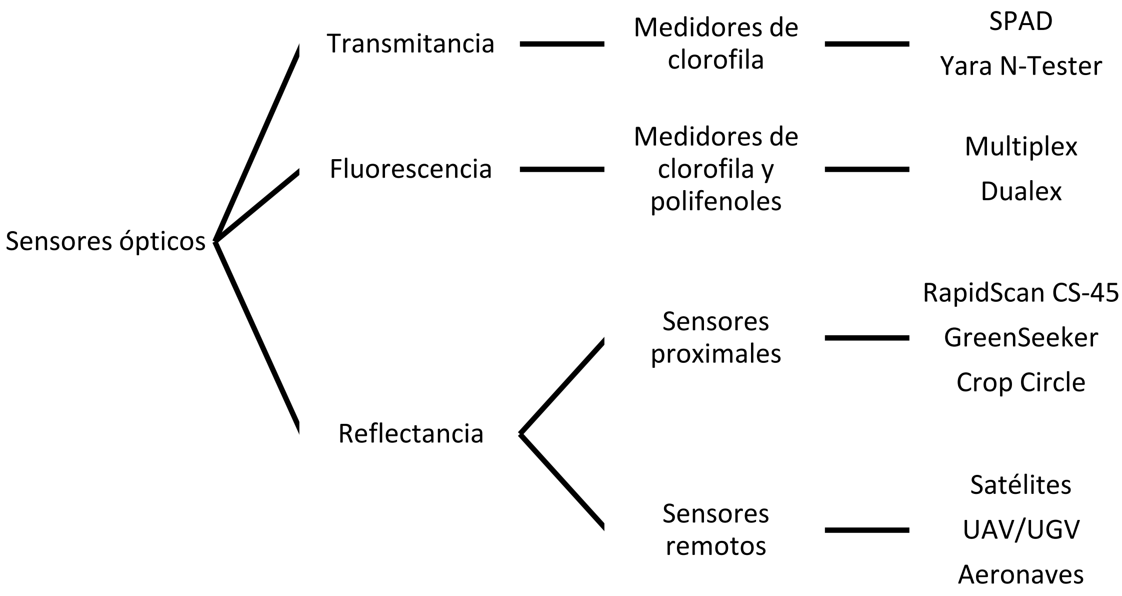


Figura 1.8. Esquema que resume los distintos tipos de sensores ópticos.

1.4.2.3.1 Sensores de transmitancia

Entre los sensores que miden la transmitancia de la luz se encuentran los medidores de clorofila (Figura 1.9B) como Yara N-Tester^{TM} (Yara International ASA, Oslo, Noruega) o SPAD (Konica Minolta, Tokyo, Japón), siendo la primera una de las herramientas utilizada a lo largo de este trabajo. Los medidores de clorofila son herramientas portátiles de mano que miden en dos longitudes de onda, 650 nm (rojo) y 940 nm (infrarrojo cercano o NIR). El ratio de la luz trasmitida en estas longitudes de onda, además del ratio determinado sin muestra, es procesado por la herramienta otorgando un valor sin unidades. Mayor cantidad de rojo absorbida por la hoja significa que hay más clorofila presente. Las medidas son instantáneas, no son destructivas y deben ser tomadas en el centro de la hoja más joven que esté completamente desarrollada pinzándola con la herramienta. Es necesario tomar 30 medidas en cada punto de muestreo para obtener un valor representativo.

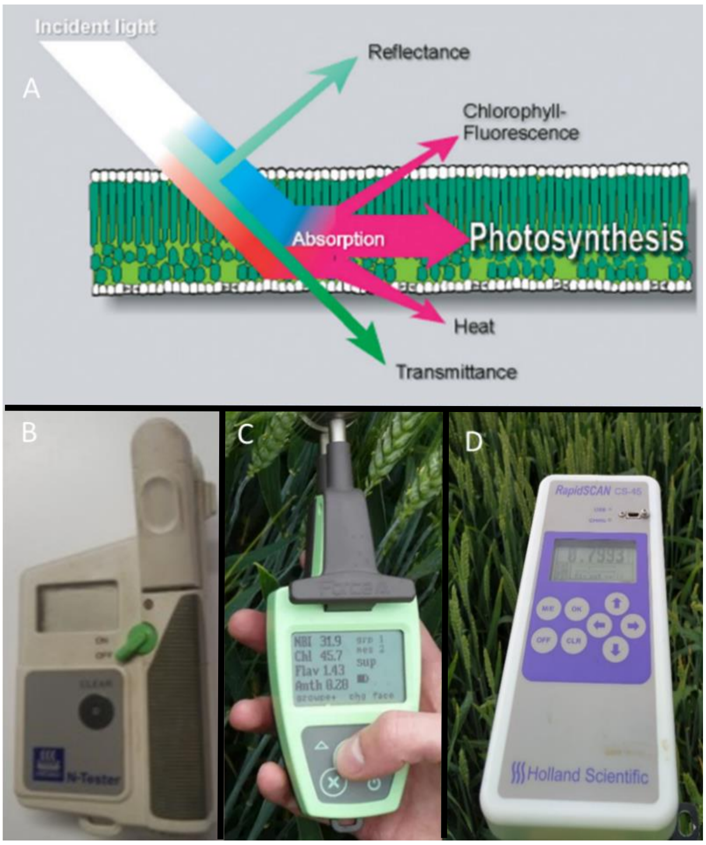


Figura 1.9. A) División de la luz al caer sobre una hoja. Fuente: ESA, 2003, B) Yara N-Tester^{TM} (Yara International ASA, Oslo, Noruega); C) Dualex (ForceA, Orsay, Francia); D) RapidScan CS-45 (Holland Scientific, Lincoln, NE, EUA)

1.4.2.3.2 Sensores de fluorescencia

Dentro de los sensores que miden la fluorescencia, se encuentran los fluorímetros Multiplex y Dualex (ForceA, Orsay, Francia). El sensor Multiplex está orientado a la detección de enfermedades y a determinar la madurez y calidad de los frutos. El sensor Dualex (Figura 1.9C) está orientado a las carencias nutricionales, y aunque se clasifica como un sensor de fluorescencia, también es de transmitancia. Dualex mide los polifenoles (flavonoles y antocianos (opcional)) y la clorofila. Este sensor es una herramienta portátil con cinco fuentes luminosas LED: una UV-A, una verde, una roja y dos infrarrojas cercanas, y dispone de un GPS interno. Cuando la disponibilidad de N es baja, las plantas asignan el exceso de carbono a la síntesis de polifenoles. De hecho, Cartelat *et al.,* (2005) encontraron que la aplicación de N a los cultivos aumenta el contenido de clorofila y disminuye el de polifenoles de la hoja. El alto contenido de polifenoles es, por lo tanto, un indicador potencial del bajo estado nutricional nitrogenado de los cultivos (Cartelat *et al.,* 2005). Las mediciones con Dualex se toman pinzando la hoja con el sensor, no son destructivas y otorgan un valor instantáneo. Los valores que otorga el sensor son cuatro índices: índice de clorofila, índice de flavonoles, índice NBI®: estado de N y ratio clorofila/flavonoles, e índice de antocianos (ForceA, 2020). Al medir también la transmitancia, tiene la limitación de que son mediciones basadas en el contacto y, por tanto, no son adecuadas para las mediciones automatizadas (Padilla *et al.*, 2018).

1.4.2.3.3 Sensores de reflectancia

Los sensores de reflectancia proporcionan información sobre el estado nitrogenado del cultivo midiendo la luz reflejada por el follaje del cultivo (Knipling, 1970). Normalmente el tejido vegetal absorbe aproximadamente el 90% de la radiación visible (390 a 750 nm) y el 50% de la radiación en el infrarrojo cercano, NIR (750 a 1300 nm) (Knipling, 1970). El grado de absorbancia y reflectancia en las porciones visible y NIR del espectro varía con la concentración de N del cultivo, proporcionando información sobre el estado nutricional nitrogenado del cultivo. Los cultivos con deficiencia de N, generalmente, reflejan más visible y menos NIR que los cultivos con una correcta nutrición nitrogenada (Schepers *et al.,* 1996).

1.4.2.3.3.1 Sensores proximales (proximal sensing)

En las últimas décadas se ha determinado que los sensores proximales que miden la reflectancia del cultivo son herramientas valiosas para el ajuste de la dosis del fertilizante nitrogenado (Ali *et al.*, 2015; Mulla, 2013). Para la medida de la reflectancia de la cubierta proximal, los sensores se colocan relativamente cerca del cultivo (*e.g.*, a 0,4-3,0 m de la cubierta vegetal). Dentro de esta tipología, encontramos el sensor RapidScan CS-45 (Holland Scientific Inc., Lincoln, Nebraska, EUA), una de las herramientas utilizadas en este trabajo. RapidScan CS-45 (Figura 1.9D) es un sensor activo proximal que mide en tres longitudes de onda: 670 nm (rojo, R), 730 nm (rojo lejano, RE) y 780 nm (infrarrojo cercano, NIR). A partir de estos valores calcula dos índices de vegetación: NDVI (Índice de vegetación de diferencia normalizada) y NDRE (Índice de rojo lejano de diferencia normalizada). Esta herramienta mide la reflectancia de la cubierta vegetal, incorpora un GPS y almacena los datos. Los valores medios se obtienen haciendo pases por la zona a medir a una velocidad constante y a una altura aproximada de un metro con respecto a la superficie foliar.

Existen varios sensores más con características similares a las de RapidScan CS-45. Algunos se utilizan montados en un tractor, como los sensores GreenSeeker™ (NTech Industries, Inc., Ukiah, California, EUA), y Crop Circle (Holland Scientific, Lincoln, Nebraska, EUA).

1.4.2.3.3.2 Teledetección (remote sensing)

La teledetección es la adquisición de información sobre un objeto o fenómeno desde la distancia. Para esto se precisa un instrumento o sensor montado en una plataforma, como un satélite, una aeronave, un UAV (vehículo aéreo no tripulado), un UGV (vehículo terrestre no tripulado), o una sonda (Weiss *et al.,* 2020). El sensor normalmente mide la radiación electromagnética que es reflejada por el objetivo. Es necesario tener en cuenta que la iluminación y las condiciones atmosféricas influyen indirectamente, por lo que es importante detectar los diferentes artefactos que pueden interferir en la señal para que la información obtenida dependa sólo de las propiedades del objetivo (Roy *et al.,* 2002).

La teledetección desde cualquier plataforma muestra un gran potencial para la agricultura de precisión (Weiss *et al.,* 2020). A nivel de satélites, la disponibilidad de imágenes libres proporcionadas por Sentinel-2 o Landsat permite hacer un seguimiento del crecimiento de los cultivos. El satélite Sentinel-2, enviado por la Agencia Espacial Europea (ESA), aporta información en diversas longitudes de onda con una resolución espacial de 10 m y una resolución temporal de cinco días. Este progreso técnico hace que la agricultura de precisión sea asequible y rentable y, por lo tanto, operativa. Sin embargo, aun disponiendo de equipos de alta tecnología y tecnologías de última generación, es necesario saber cómo hacer uso de la información obtenida mediante ellos, y trasladar esta información a campo para el manejo óptimo de cada parcela, tipo de suelo, condiciones climáticas, cultivo y variedad. La utilización de esta información para dar recomendaciones de fertilización nitrogenada en cultivos extensivos requiere de un conocimiento exhaustivo, tanto en lo referente a la obtención de valores de referencia como a las actuaciones a llevar cabo para cada valor de referencia.

1.5 Justificación del trabajo

Tal y como se ha expuesto en la introducción de este trabajo, los sistemas de producción agroganadera deben ser capaces de satisfacer el progresivo aumento en la demanda de alimentos asequibles y de calidad que genera el continuo crecimiento de la población mundial, y debe hacerlo mediante la adopción de tecnologías y métodos de producción que no pongan en riesgo la sostenibilidad ambiental. El incremento productivo de las últimas décadas ha estado ligado, entre otros factores, a la utilización intensiva de fertilizantes minerales. Sin embargo, la baja eficiencia en su uso y el elevado consumo energético asociado a su producción, en especial de los fertilizantes nitrogenados, causa un impacto ambiental que debe ser afrontado. En este sentido, la utilización de subproductos orgánicos de origen ganadero como fertilizantes es una alternativa interesante, bien para reducir la dosis de fertilizante mineral, o bien como fertilizante único en la agricultura ecológica. Los distintos tipos de subproductos de origen ganadero tienen distintas propiedades y, por tanto, distintos patrones de mineralización en el suelo, lo que se traduce en potenciales agronómicos diferentes. Además, es necesario ajustar las cantidades de N a aportar en función de los requerimientos de los cultivos, en este caso del trigo, para lograr un ahorro en fertilizantes y un beneficio económico y ambiental. Las estimaciones del N que un suelo puede suministrar al cultivo a lo largo de su ciclo de crecimiento y el uso de sensores ópticos para determinar el estado nutricional del cultivo pueden ayudar a optimizar las cantidades de N que hay que aportar al cultivo. Los sensores ópticos también pueden utilizarse para hacer un seguimiento del estado nutricional nitrogenado del cultivo durante su crecimiento vegetativo para poder detectar si existen carencias, y así no comprometer los rendimientos o la calidad del grano. En resumen, este trabajo pretende, por un lado, mejorar el conocimiento sobre la capacidad de los suelos para suministrar N, tanto del propio suelo como del aportado por subproductos orgánicos de la ganadería, y por el otro, desarrollar las metodologías relacionadas con el uso de sensores ópticos para la correcta nutrición nitrogenada del cultivo de trigo.

1.6 Objetivos

Nota: Los estados fenológicos mencionados en el siguiente apartado están basados en la escala de Zadoks (Zadoks et al., 1974, ver apartado 1.2.2 Fenología del trigo y necesidades de nitrógeno)

El objetivo general de este trabajo es la mejora de la fertilización nitrogenada del cultivo de trigo (*Triticum aestivum* L.) en condiciones de clima mediterráneo húmedo, mediante la generación e integración de nuevo conocimiento acerca de: i) los factores que influyen en la capacidad de mineralización de varios suelos de Araba; ii) la disponibilidad de N tras el aporte de distintos subproductos orgánicos de origen ganadero; y iii) la utilización de sensores ópticos proximales para el ajuste de la dosis de N mineral y para el seguimiento del estado nitrogenado del cultivo en estados fenológicos clave.

Para ello, se establecieron los siguientes objetivos específicos:

1. Evaluación en un ensayo de invernadero y en distintos suelos de Araba de la dinámica del N_{min} a lo largo del ciclo de cultivo del trigo, tanto en los estados fenológicos clave para el rendimiento como para el contenido de proteína en grano.

2. Estudio en invernadero del efecto de la aplicación de distintos subproductos orgánicos de origen ganadero (estiércol de vacuno de carne, gallinaza y purín de vacuno de leche) a en distintas dosis de aporte (170 y 340 kg N ha^{-1}) sobre (i) la disponibilidad de N en el suelo y ii) el rendimiento productivo del cultivo de trigo, y el contenido de proteína en grano. Asimismo, se evalúa el efecto de la presencia/ausencia del cultivo de trigo sobre las actividades enzimáticas del suelo, y las dinámicas de carbono orgánico y N_{min} en la solución del suelo.

3. Estudio de la utilidad de los sensores de detección proximal Yara N-Tester^{TM} y RapidScan CS-45 en el ajuste de la dosis óptima de N a aportar en el inicio de encañado (GS30) del cultivo de trigo, tras la adición de subproductos orgánicos en fondo, en condiciones de clima mediterráneo húmedo.

4. Estudio de la utilidad de los sensores Yara N-Tester^{TM} y RapidScan CS-45 para la realización del seguimiento del índice nutricional nitrogenado (INN) durante el ciclo fenológico del cultivo de trigo mediante un modelo estandarizado o modelos específicos,

evaluando también la idoneidad del tipo de valores: normalizados *vs.* absolutos.

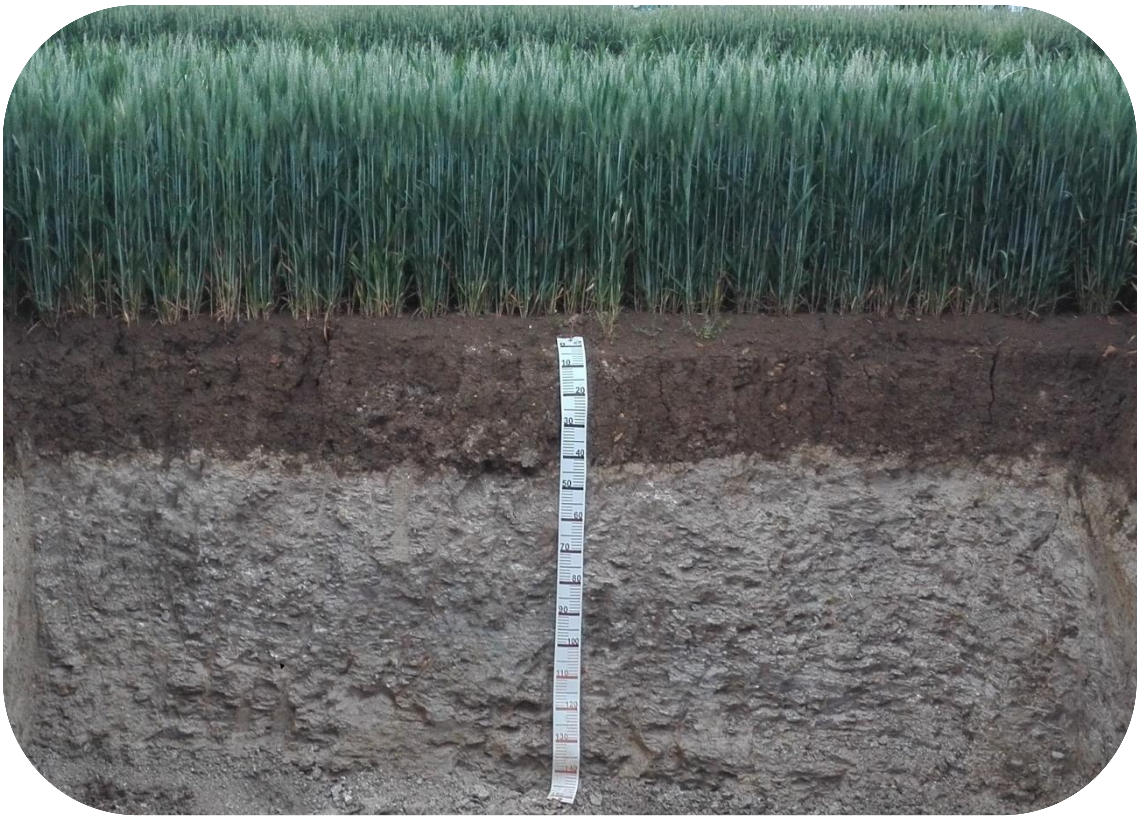
5. Determinación de los valores NDVI umbral obtenidos a partir del sensor proximal RapidScan CS-45 en distintas etapas de la fenología del cultivo (p.ej. GS30, GS32, GS37 y GS65) con el fin de maximizar el rendimiento productivo del trigo blando de invierno.

6. Determinación de la necesidad de aportar 40 kg N ha^{-1} en inicio de ahijado (GS21) tras la adición de subproductos orgánicos en fondo.

7. Evaluación de la relación entre las lecturas del medidor de clorofila en el estado GS65 y los valores de proteína en grano, con el fin de obtener un valor de lectura límite para garantizar valores de proteína en grano superiores al 12,5%.

Chapter 2

Soil properties for predicting soil mineral nitrogen dynamics throughout a wheat growing cycle in calcareous soils



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2 Soil properties for predicting soil mineral nitrogen dynamics throughout a wheat growing cycle in calcareous soils

2.1 Introduction

Few agroecosystems supply enough nitrogen (N) to sustain satisfactory crop production without fertilizers. Throughout agricultural history, agriculturists have attempted to maintain fertility levels in the soil, depending on biologically fixed N, through the application of organic amendments and the decomposition of soil organic matter (SOM) to provide N to crops. In cereal cropping systems, N is one of the most important elements controlling crop development (Samborski *et al.*, 2009). Thus, to assure that the potential yield is achieved each year, N is frequently applied in excessive amounts without determining the appropriate N fertilization rate, which usually leads to N losses. To comply with economic and ecological regulations in recent years, concerns about the need for improving nitrogen use efficiency (NUE) in cereal production have increased. The N fertilizer demand is dependent on the plant available N supplied by soils and the potential yield, which varies from year to year. Available soil resources should be taken into account for the determination of appropriate N fertilization rates to enhance the efficiency of agricultural systems and ecosystem health.

It is necessary to provide better insight into the capacity of soils to provide N to crops (soil N supply) to understand the factors that control N mineralization in soils and therefore improve N fertilization recommendations for cereal (Zebarth *et al.*, 2009). In the field, different climatic and agronomic parameters affect wheat yield production, and among them, the contribution of soil N dynamics is very relevant. Nitrogen cycling in the soil–plant system is very complex and involves interactions between soil and plant factors. Only a small portion of N is biologically active, serving as a substrate for N mineralization (Parton *et al.*, 1987). To determine the rates of N fertilizer application, it is necessary to take into account the inorganic N of the soil and the organic N mineralized during crop growth (Martínez *et al.*, 2018; Van Groenigen *et al.*, 2015).

The method used most in Western Europe for N fertilization application in cereals is the N_{min} method, which is based on the measured amount of soil mineral N in the main rooting depth before N fertilizer application at the beginning of the rapid period of crop growth. The calculation of the N fertilizer recommended rate is made using the predicted N demand for the target yield minus the measured soil N_{min} value. However, with this method, the N that has been mineralized during the remainder of the growing cycle is not taken into account. In addition, taking soil samples in a narrow period of time and analysing the mineral N in each individual field and each season is not practical. Therefore, with the aim of measuring the N supply capacity of the soil during the entire growing season, the potentially mineralizable N (N_o) or bioavailable N is measured (Ros *et al.*, 2011). The standard method for measuring N_o was defined by Stanford and Smith (Standford and Smith, 1972), who developed a method based on long-term aerobic incubations, in which soil was maintained under optimum conditions (35 °C and field capacity). However, this method is impractical for routine laboratory analysis due to the long incubation periods required (32 weeks). With the purpose of avoiding the long period required, several chemical methods have been developed to estimate N_o, such as extractions with different saline solutions. Different methodologies have been used to build a global strategy with the aim of estimating crop N availability (Serna and Pomares, 1992; Dessureault-Rompré *et al.*, 2010; Velthof and Oenema, 2010; Dessureault-Rompré *et al.*, 2015; Martínez *et al.*, 2018). However, no one method has yet obtained general approval (Luce *et al.*, 2011). In a previous article, in the area where this study was carried out (Araba, Basque Country, northern Spain), with calcareous soils and under humid Mediterranean conditions, Villar *et al.* (2014) determined that the most appropriate laboratory technique to estimate the amount of available N that soils are able to provide to wheat was hot KCl extraction.

In Araba (Basque Country, northern Spain), traditionally, two applications of N fertilizer are supplied at following growing stages (GS): GS21 (beginning of tillering) and GS30 (stem elongation) for wheat, according to the Zadoks scale (Zadoks *et al.*, 1974). As soils differ in their composition and mineralization patterns, the N rate applied in these two main periods should be specific with respect to the available N (Aranguren *et al.*, 2018). Furthermore, as in many other wheat-producing countries, the demand for high grain protein content (GPC) has also increased. Therefore, it is essential to estimate the amount of N mineralized from SOM to adjust the rate of N fertilizer required to optimize crop yield and quality, reducing the negative impacts of excessive N on the environment.

The main objective of this study was to assess the soil N_{min} dynamics throughout the wheat growing season at crucial stages for plant yield and GPC. To this aim, we analyzed the utility of different characteristics of the soil before sowing: (i) the commonly used routine soil physicochemical analyses (SOM, Ntot, pH, $CaCO_3$ and texture), (ii) potentially mineralizable N (N_o), analyzed using aerobic incubation, and (iii) different extraction methods to estimate N_o

2.2 Materials and Methods

2.2.1 Experimental Setup

A greenhouse experiment was established in Derio (Bizkaia, Basque Country, Spain) at NEIKER-tecnalia experimental facilities using 16 field soils collected from Villanañe, Soportilla, Lantaron, Arangiz, Betolaza, Gauna, and Tuesta (Araba, Basque Country, Spain) from the 0–30 cm layers (Table 2.1). No organic amendments had been applied to the studied soils for several years before sample collection or N fertilization in the previous months. Moreover, no leguminous crops were grown in the fields preceding soil collection. Soils were air-dried and sieved though a 2 mm mesh and poured into pots (height of 30 cm, and diameter of 22 cm). Before sowing, 300 mL of a nutrient solution without N (Velthof and Oenema, 2010) was added to the soil to ensure that there were no nutrient limitations to the plants. Twenty seeds of soft red winter wheat (*Triticum aestivum* L., var Soissons) per pot were sown (30 May 2011), and after germination, the number of plants was reduced to 14 seedlings to simulate the common sowing dose of 240 kg ha^{-1}. There were three replicates per soil that were distributed in a completely randomized experimental design. Pots were kept at field capacity during the whole experiment. The experiment was extensively described in a previous article (Villar *et al.*, 2014).

2.2.2 Plant Sampling

Wheat aboveground biomass was sampled at GS30 (stem elongation), GS37 (leaf flag emergence), and harvest (19 December 2011). Fresh biomass samples were weighed and oven dried, and the dried biomass samples were again weighed for dry matter content determination. Biomass was estimated, and the N concentration was determined using dry combustion with LECO equipment (TrueSpec® CHN-S, LECO Corporation, Michigan, USA). At harvest, grain and straw were separated and dried at 70 °C for two days to obtain the dry matter content. Grain yield was measured, and grain and straw N concentration were determined using dry combustion with LECO equipment (TrueSpec® CHN-S). Nitrogen uptake was calculated, and GPC was determined by multiplying the total grain N concentration by 5.7 (Teller, 1932).

Table 2.1. Location, and physical and chemical characteristics, of the soils from Araba (Basque Country, northern Spain) used in the greenhouse experiment.

Soil	Location	Soil texture	Sand[a] %	Silt[a] %	Clay[a] %	SOM[b] %	Ntot[c] %	pH[d] %	$CaCO_3$[e] %
1	Villanañe	Clay-loam	30.5	37.1	32.4	1.8	0.12	8.3	29.0
2	Soportilla	Loam	35.9	39.3	24.8	1.0	0.07	8.5	29.4
3	Lantaron	Loam	31.7	43.0	25.4	1.3	0.09	8.4	56.5
4	Arangiz	Silty-loam	19.4	55.9	24.6	1.6	0.12	8.3	56.3
5	Arangiz	Clay-loam	26.1	45.6	28.3	2.0	0.16	8.3	21.7
6	Arangiz	Silty-clay-loam	16.2	56.9	27.0	2.0	0.13	8.3	53.0
7	Betolaza	Silty-clay-loam	12.1	58.6	29.3	3.1	0.24	8.3	29.3
8	Gauna	Sandy-clay-loam	47.4	24.9	27.6	2.0	0.15	8.1	8.0
9	Tuesta	Silty-loam	18.8	54.8	26.4	1.4	0.11	8.3	55.8
10	Arangiz	Silty-clay-loam	17.5	55.3	27.2	2.0	0.12	8.3	56.8
11	Arangiz	Silty-clay-loam	18.0	52.8	28.5	1.8	0.11	8.4	40.9
12	Gauna	Clay-loam	38.5	32.6	29.0	2.0	0.12	8.1	9.2
13	Arangiz	Silty-loam	19.8	54.8	25.4	1.5	0.10	8.4	53.8
14	Tuesta	Clay-loam	36.8	28.4	34.8	1.9	0.15	8.2	16.4
15	Gauna	Sandy-clay-loam	47.6	24.5	27.9	2.2	0.17	8.0	12.1
16	Gauna	Clay loam	44	25.9	30.1	2.1	0.16	8.0	7.2

[a]Texture using a pipette method (Gee and Bauder, 1886); [b]Soil organic matter (Walkey and Black, 1934); [c]Soil total N (dry combustion using a LECO TruSpec CHN); [d]pH (1:2.5 soil:water); [e]CaCO3 (NH4AcO; (MAPA, 1994)).

2.2.3 Soil Samples

Soil was sampled with a soil sampling rod (full depth from each pot) at sowing, GS30 (stem elongation), GS37 (leaf flag emergence), GS60 (beginning of flowering), harvest, post-harvest, and pre-sowing to determine the ammonium and nitrate levels. NH_4^+ and NO_3^- were spectrophotometrically determined (Cawse, 1967; Nelson, 1983). N_{min} was calculated as the sum of NH_4^+ plus NO_3^-.

2.2.4 Aerobic Incubation

Aerobic incubation was performed following the method described by Stanford and Smith (1972) and modified by Campbell *et al.* (1993). Fifteen grams of each soil sample was air-dried and sieved (2 mm mesh) and then mixed with an equal amount of quartz sand. Soils were incubated aerobically at field capacity for 32 weeks in a culture chamber at 35 °C. Samples were leached every 2 weeks during the first 8 weeks and every 4 weeks thereafter with a 0.01 M $CaCl_2$ solution. Mineral N was determined in each sample spectrophotometrically, and N_o was estimated by fitting the accumulated N_{min} against time to a first-order kinetic exponential model. N mineralized in the first two weeks (N2wk) and accumulated after 30 weeks of incubation (N30wk), and potentially mineralizable N (N_o) were calculated. The procedure of the experiment was extensively described in a previous article (Villar *et al.*, 2014).

2.2.5 Chemical Extractions

2.2.5.1 The 0.01 M $CaCl_2$ Extraction

Calcium chloride extraction was performed using the method described by Houba *et al.* (1986) and modified by Velthof and Oenema (2010). Soil samples were divided into three parts: the first part was air-dried (30 °C), the second part was dried at 40 °C, and the last part was dried at 105 °C. After drying, 6 g of soil was extracted with 60 mL of 0.01 M $CaCl_2$ via shaking for 2 h. After extraction, the samples were centrifuged for 5 min at 2328 × *g*. Nitrate and ammonium were determined spectrophotometrically. Two mineralization indices were calculated: MI-$CaCl_2$ I and MI-$CaCl_2$ II. MI-$CaCl_2$ I was calculated as the difference between the ammonium extracted at 105 °C and the ammonium extracted from the air-dried samples. MI-$CaCl_2$ II was calculated as the difference between the ammonium extracted at 105 °C and the ammonium extracted at 40 °C.

2.2.5.2 The KCl and HotKCl Extraction

Ten grams of soil were extracted with 20 mL of 2 M KCl at room temperature (Gianello and Bremmer, 1986). After extraction, the samples were centrifuged at 2328× *g* for 5 min and analyzed spectrophotometrically to determine the concentrations of NO_3^- and NH_4^+.

The value of N_{min} obtained using hotKCl was determined by heating 1.5 g of soil with 10 mL of 2 M KCl solution on a digestion block set at 100 °C for 4 h. After extraction, the NO_3^- and NH_4^+ concentrations were determined as described for the room temperature procedure.

The N mineralization index (MI-hotKCl) was calculated as the difference between the amount of NH_4^+ extracted using hotKCl and that extracted at room temperature.

2.2.5.3 The 0.01 M $NaHCO_3$ Extraction

$NaHCO_3$ extraction was carried out using the method described by MacLean (1964) and modified by Serna and Pomares (1992). Two grams of soil was mixed with 40 mL of 0.01 M $NaHCO_3$; the samples were then centrifuged at 2328× *g* for 5 min and filtered through Whatman No. 42 filters (Whatman International Ltd., Maidstone, England) and the absorbance of the filtrate was then measured at 205 and 260 nm (205ABS and 260ABS, respectively). The procedure of the experiment was extensively described in a previous article (Villar *et al.,* 2014).

2.2.6 Statistical Analysis

For analyzing N_{min} differences among soils, a one-way ANOVA was performed for each growing stage: sowing, GS30, GS37, GS60, harvest, post-harvest, and pre-sowing (R Core Team, 2013). For analyzing differences among the soils' final N uptake, yield, and GPC, one-way ANOVA was performed (R Core Team, 2013). To separate the means, Duncan's test was used ($p \leq 0.05$) using the R package *agricolae V. 1.2-4* (De Mendiburu, 2009).

Pearson's correlation analysis of the soil properties, aerobic incubation and chemical extractions with 1) N_{min} values at sowing, GS30, GS37, GS60, harvest, post-harvest, and pre-sowing and with the 2) nitrogen uptake by the plant between sowing and GS30, GS30–GS37, GS37-harvest, and yield and GPC was performed. In addition, Pearson's correlation analysis was performed between N_{min} and N uptake by the plant, sowing and GS30, GS30–GS37, GS37-harvest, and yield and GPC.

2.3 Results

2.3.1 Range of Soil Physical and Chemical Characteristics

The soils varied widely in their physical and chemical characteristics. Sixteen soils were classified into five texture classes: clay-loam (S-1, S-5, S-12, S-14, and S-16), loam (S-2 and S-3), silty-loam (S-4, S-9, and S-13), silty-clay-loam (S-6, S-7, S-10, and S-11), and sandy-clay-loam (S-8 and S-15). The pH values were high (8.0–8.5). Twelve soils were calcareous (>15% $CaCO_3$), and the remaining four soil values varied between 7.0 and 12.1%. Soil organic matter values were low (SOM ≤ 1.9%; S-1, S-2, S-3, S-4, S-9, S-11, S-13, and S-14), moderate (SOM 1.9–2.2%; S-5, S-6, S-8, S-10, S-12, S-15, S-16, and S-17), or high (SOM 3.1%; S-7).

2.3.2 Availability of Mineral N in Soil

Soil mineral N availability values were different among soils and phenological stages (Table 2.2). Remarkably, N_{min} did not follow the same dynamic pattern throughout the crop cycle in different soils. Soil N_{min} values decreased from sowing to GS37 in the vast majority of the soils, except for S-9, S-3, and S-5, which increased their N_{min} values from sowing to GS30. N_{min} values increased from GS37 to GS60 in each soil and then remained similar. Differences were especially evident at GS30, where S-9 had the highest value and S-2, S-8, S-10, S-12, S-14, and S-16 had the lowest values. Soils with the highest N availability in GS37 were S-1, S-6, S-7, S-8, S-12, and S-14, whereas at GS60, S-7, S-8, and S-14 had the highest N availability.

2.3.3 Wheat N Uptake, Yield, and GPC

There were significant differences in wheat N uptake at harvest, yield, and GPC depending on the soil (Figure 2.1). The soil with the highest N uptake (mg pot^{-1}) was S-1. The lowest N uptake was in S-2 and S-8. In the case of wheat yield (g pot^{-1}), the highest values were achieved in S-3, S-9, and S-15 and the lowest in S-2, S-8, and S-16. Regarding GPC (%), the highest values were achieved in S-14, and the lowest were in S-2.

Table 2.2. Soil mineral nitrogen (N_{min}; mg kg^{-1}) evolution in 16 soils from Araba throughout the wheat growing season in a greenhouse experiment. Different letters represent significant differences ($p \leq 0.05$) among soils for each growing stage: sowing, stem elongation (GS30), leaf flag emergence (GS37), flowering (GS60), harvest, post-harvest, and pre-sowing.

Soil	N_{min} (mg kg^{-1})													
	Sowing		GS30		GS37		GS60		Harvest		Post-harvest		Pre-sowing	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
1	16.6 AB	4.0	10.2 ABC	3.2	2.6 A	0.5	7.5 ABC	0.7	4.7 BC	0.9	7.2 AB	1.2	3.3 ABC	1.0
2	8.5 EFG	2.1	3.7 C	0.9	0.4 B	0.1	5.2 C	1.0	3.8 CD	0.6	3.0 C	0.7	2.5 C	0.9
3	10.3 ABC	1.8	12.3 AB	1.7	1.7 AB	0.5	6.2 BC	2.2	3.7 CD	0.8	5.6 BC	0.3	3.2 ABC	0.6
4	12.6 ABC	6.1	6.1 BC	1.9	2.2 AB	0.7	7.1 ABC	2.0	4.9 B	0.9	8.0 AB	1.2	3.2 ABC	0.6
5	11.3 ABC	2.3	9.0 ABC	1.9	1.7 AB	0.9	8.2 ABC	2.2	4.6 BC	0.8	6.5 BC	0.9	3.3 ABC	0.6
6	11.4 ABC	5.9	5.3 BC	1.7	2.9 A	0.3	8.0 ABC	0.8	5.1 B	0.5	6.8 BC	0.8	3.6 ABC	0.5
7	6.5 FG	0.4	6.6 BC	0.9	3.0 A	0.7	8.1 A	0.9	6.6 A	0.9	8.9 A	0.6	3.8 ABC	1.0
8	7.3 EFG	2.6	3.3 C	0.9	2.9 A	0.6	7.5 A	1.8	3.1 D	0.7	4.3 BC	1.0	2.7 C	0.2
9	9.7 CDE	3.5	16.4 A	1.2	0.9 AB	0.1	5.9 AB	1.0	4.4 BC	0.6	4.4 BC	0.8	3.2 ABC	0.2
10	12.7 ABC	2.9	4.2 C	0.7	2.2 AB	0.4	8.3 ABC	0.7	4.9 BC	0.9	5.5 ABC	1.0	3.9 A	0.5
11	11.6 ABC	3.2	4.9 BC	1.1	1.6 AB	0.7	7.4 ABC	1.4	5.3 BC	1.7	7.0 AB	1.3	3.6 ABC	0.7
12	5.7 G	2.0	4.4 C	0.7	2.7 A	0.7	8.2 ABC	1.9	5.1 BC	1.2	5.2 ABC	0.7	3.3 ABC	0.8
13	9.4 CDE	1.8	5.0 BC	0.9	1.3 BC	0.6	9.6 AB	1.4	4.7 BC	1.2	5.7 ABC	0.8	3.5 ABC	1.7
14	10.3 CDE	6.0	3.7 C	0.8	2.8 A	0.3	10.2 A	1.2	5.2 BC	1.3	7.1 AB	0.7	4.0 AB	0.9
15	13.7 A	3.5	5.0 BC	1.3	1.5 AB	0.4	8.1 ABC	2.0	4.3 BC	1.0	5.8 ABC	1.7	3.5 ABC	0.2
16	7.9 EFG	1.3	2.8 C	0.8	1.2 AB	0.5	4.9 C	2.3	4.5 BC	1.4	5.4 ABC	0.7	3.0 ABC	0.8

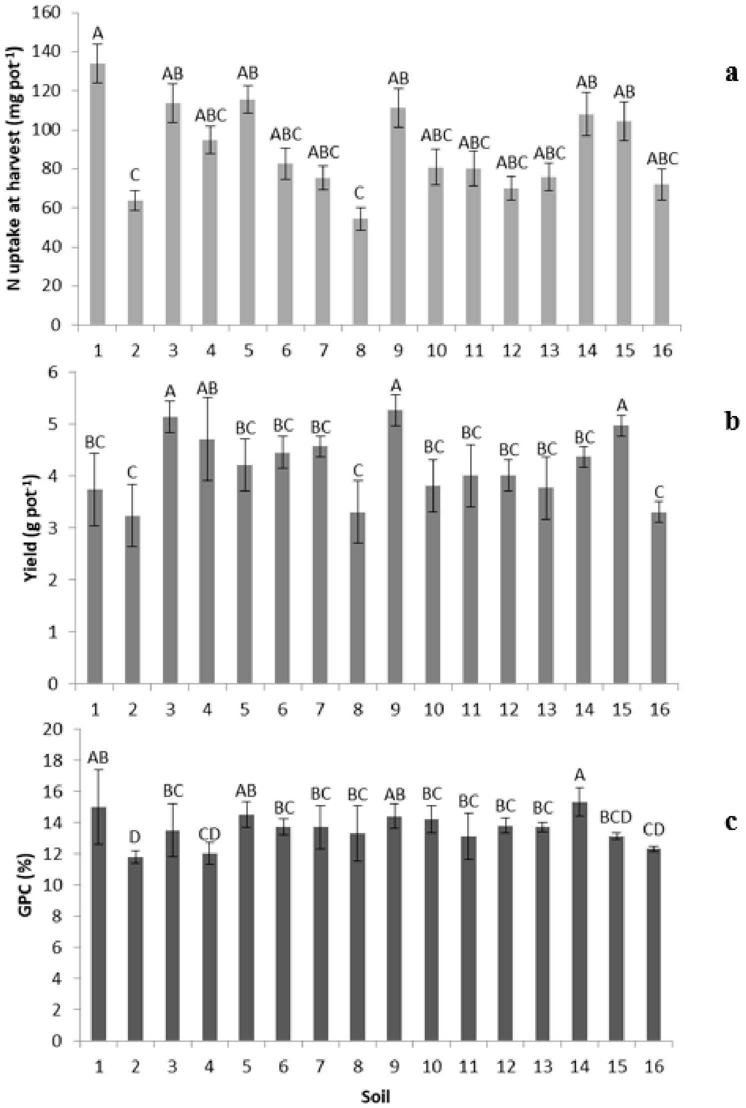


Figure 2.1. (**a**) Wheat N uptake at harvest (mg pot^{-1}), (**b**) yield (g pot^{-1}), and (**c**) GPC (%) in 16 soils from Araba. Different letters represent significant differences ($p \leq 0.05$) among soils. Values are the mean of three replicates ± SD.

2.3.4 Relationships between Initial Soil Characteristics with Nmin throughout the Growing Cycle

Regarding the soil physicochemical properties (Table 2.3), sand had a negative correlation with N_{min} values at harvest, silt had a positive correlation with N_{min} values at harvest, and clay had a positive correlation with N_{min} values at GS37 and GS60. Ntot was positively correlated with N_{min} at GS37, harvest, and post-harvest. SOM had a positive correlation with N_{min} values at GS37, GS60, harvest, and post-harvest. In the case of aerobic incubations, N2wk had a positive correlation with the sowing values, and N30wk had a positive and significant correlation with the GS60, harvest, post-harvest, and pre-sowing N_{min}. N_o was positively correlated with the GS60 and pre-sowing N_{min} values. Regarding the chemical extractants used to estimate N_o, MI $CaCl_2$ I was correlated with the N_{min} values at GS60. MI-hotKCl was positively correlated with N_{min} values from GS37 to pre-sowing. For $NaHCO_3$, only 205 ABS was correlated with the sowing and pre-sowing N_{min} values. Remarkably, SOM, N30wk, and hotKCl were correlated with the N_{min} values after harvest.

2.3.5 Relationships between Soil Characteristics and Plant N Uptake, Yield, and GPC

Regarding the soil initial properties (Table 2.4), clay had a positive correlation with GPC. Ntot had a positive correlation with the N uptake between sowing and GS30 and with the N uptake between GS30 and GS37. In the aerobic incubations, N30wk and N_0 showed a positive and significant correlation with the N uptake between GS37 and harvest and GPC. Concerning the chemical extractants, only 205ABS had a positive correlation with yield.

The soil N_{min} values (Table 2.5) at sowing and GS30 were correlated positively with N uptake between GS37 and harvest. The N_{min} values at GS30 and GS60 had positive correlations with yield and GPC, respectively.

Table 2.3. Pearson correlation coefficients (r) between soil properties, N mineralization indices calculated from the aerobic incubations and chemical extractions with N_{min} values at sowing, stem elongation (GS30), leaf flag emergence (GS37), flowering (GS60), harvest, post-harvest, and pre-sowing.

Soil characteristics		N_{min}													
		Sowing		GS30		GS37		GS60		Harvest		Post-harvest		Pre-sowing	
		r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Soil properties	Sand	-0.11	*ns*	-0.25	*ns*	0.00	*ns*	-0.01	*ns*	-0.63	**	-0.48	*ns*	-0.47	*ns*
	Silt	0.09	*ns*	0.27	*ns*	-0.12	*ns*	-0.11	*ns*	0.50	*	0.36	*ns*	0.33	*ns*
	Clay	0.05	*ns*	-0.11	*ns*	0.55	*	0.56	*	0.38	*ns*	0.40	*ns*	0.47	*ns*
	SOM	-0.16	*ns*	-0.24	*ns*	0.70	**	0.50	*	0.60	**	0.58	*	0.49	*ns*
	Ntot	-0.16	*ns*	-0.12	*ns*	0.57	*	0.43	*ns*	0.54	*	0.59	**	0.44	*ns*
	pH	0.04	*ns*	0.22	*ns*	-0.47	*ns*	-0.38	*ns*	0.06	*ns*	-0.03	*ns*	-0.07	*ns*
	CaCO3	0.33	*ns*	0.40	*ns*	-0.29	*ns*	-0.25	*ns*	0.09	*ns*	0.10	*ns*	0.22	*ns*
Aerobic incubations	N2wk	0.54	*	0.02	*ns*	0.25	*ns*	0.30	*ns*	0.05	*ns*	0.14	*ns*	0.46	*ns*
	N30wk	0.37	*ns*	0.15	*ns*	0.43	*ns*	0.57	**	0.51	*	0.61	**	0.83	***
	No	0.29	*ns*	0.16	*ns*	0.43	*ns*	0.65	**	0.38	*ns*	0.49	*ns*	0.73	**
Chemical extractions	MI $CaCl_2$ I	0.19	*ns*	-0.44	*ns*	0.35	*ns*	0.55	*	0.16	*ns*	0.37	*ns*	0.43	*ns*
	MI $CaCl_2$ II	0.34	*ns*	-0.41	*ns*	0.31	*ns*	0.52	*	-0.03	*ns*	0.29	*ns*	0.38	*ns*
	MI-Hot KCl	0.36	*ns*	-0.29	*ns*	0.62	**	0.70	*	0.50	*	0.69	**	0.64	**
	205ABS	0.59	**	0.07	*ns*	-0.16	*ns*	0.23	*ns*	0.18	*ns*	0.30	*ns*	0.59	**
	260ABS	0.31	*ns*	0.08	*ns*	-0.15	*ns*	0.15	*ns*	0.26	*ns*	0.32	*ns*	0.33	*ns*

ns, not significant ($p > 0.05$). *, **, *** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.

Table 2.4. Pearson correlation coefficients (r) among soil properties, N mineralization indices calculated from the aerobic incubations and chemical extractions with N uptake values between sowing and stem elongation (GS30), GS30 and leaf flag emergence (GS37), GS37 and flowering (GS60), and yield and GPC (grain protein content).

Soil characteristics		N uptake						Yield		GPC	
		Sowing-GS30		GS30-GS37		GS37-harvest					
		r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Soil properties	Sand	-0.06	*ns*	-0.04	*ns*	0.01	*ns*	-0.22	*ns*	-0.06	*ns*
	Silt	-0.02	*ns*	0.09	*ns*	-0.10	*ns*	0.22	*ns*	-0.10	*ns*
	Clay	0.39	*ns*	-0.26	*ns*	0.45	*ns*	-0.07	*ns*	0.73	***
	SOM	0.46	*ns*	-0.47	*ns*	-0.10	*ns*	0.09	*ns*	0.31	*ns*
	Ntot	0.52	*	0.49	*	0.06	*ns*	0.24	*ns*	0.26	*ns*
	pH	-0.43	*ns*	0.33	*ns*	-0.06	*ns*	-0.16	*ns*	-0.22	*ns*
	$CaCO_3$	-0.24	*ns*	0.33	*ns*	0.08	*ns*	0.32	*ns*	-0.13	*ns*
Aerobic incubations	N2wk	0.30	*ns*	-0.24	*ns*	0.39	*ns*	0.26	*ns*	0.40	*ns*
	N30wk	0.39	*ns*	-0.25	*ns*	0.64	**	0.34	*ns*	0.75	**
	No	0.47	*ns*	-0.38	*ns*	0.64	**	0.43	*ns*	0.74	**
Chemical Extractions	MI $CaCl_2$ I	-0.01	*ns*	-0.21	*ns*	-0.11	*ns*	-0.07	*ns*	0.04	*ns*
	MI $CaCl_2$ II	-0.06	*ns*	-0.14	*ns*	-0.05	*ns*	-0.07	*ns*	0.03	*ns*
	MI-Hot KCl	0.43	*ns*	-0.35	*ns*	0.21	*ns*	-0.07	*ns*	0.05	*ns*
	205ABS	0.29	*ns*	-0.24	*ns*	0.24	*ns*	0.57	*	0.00	*ns*
	260ABS	-0.11	*ns*	0.10	*ns*	-0.04	*ns*	0.10	ns	-0.08	*ns*

ns, not significant ($p > 0.05$). *, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels, respectively.

Table 2.5. Pearson correlation coefficients (r) among the soil N_{min} values, N uptake values between sowing and stem elongation (GS30), GS30 and leaf flag emergence (GS37), GS37 and flowering (GS60), and yield and GPC (grain protein content).

Soil N_{min}	N uptake						Yield		GPC	
	Sowing-GS30		GS30-GS37		GS37-Harvest					
	r	*p*	r	*p*	r	*p*	r	*p*	r	*p*
Sowing	0.01	*ns*	0.18	*ns*	0.53	*	0.22	*ns*	0.09	*ns*
GS30	-0.02	*ns*	0.31	*ns*	0.65	**	0.59	**	0.31	*ns*
GS37	0.27	*ns*	-0.25	*ns*	0.00	*ns*	-0.04	*ns*	0.38	*ns*
GS60	0.22	*ns*	-0.31	*ns*	0.09	*ns*	-0.07	*ns*	0.53	**
Harvest	0.38	*ns*	-0.25	*ns*	0.00	*ns*	0.22	*ns*	0.24	*ns*

ns, not significant ($p > 0.05$). *, ** Significant at the 0.05 and 0.01 probability levels, respectively.

2.4 Discussion

The tested soils differed in their initial N_{min} values and their physical and chemical properties (Villar *et al.*, 2014), which significantly influenced the N mineralization patterns. Depending on their characteristics, soils mineralize in different ways, making available different N_{min} values (Table 2.2). The first step in mineralization is ammonification, which is the conversion of organic N into ammonium by soil microbes. This process is carried out exclusively by heterotrophic microorganisms that utilize C as an energy source. Nitrate production is mediated via two groups of autotrophic bacteria (*Nitrosomonas* and *Nitrobacter*) that convert ammonium into nitrate by the process called nitrification. Nitrogen availability relies on both the initial availability of N_{min} and the rate of mineralization or immobilization (Mohanty *et al.*, 2013), as well as the previous N uptake by the crop, influencing the yield and GPC.

There were no correlations between the soil properties and N_{min} values at sowing or GS30. It should be mentioned that the soil preparation (drying, sieving, and rewetting) prior to the experiment could have affected soil structure and functioning. This could explain the lack of correlation in the early stages. It should be mentioned that soil rewetting often causes abundant mineralization because microorganisms recover their activity (Griffin, 2008). However, Mikha *et al.* (2005) suggested that N immobilization occurred in response to the easily accessible C due to the rapid increase in microbial activity. Later, in the growing cycle, from GS37 onwards, SOM, Ntot, sand, silt, and clay were relatively effective predictors of soil N_{min} dynamics.

Soil organic matter (SOM) is a heterogeneous mixture of organic compounds that vary in their nutrient composition, molecular characteristics, age, and biological stability. Increasing SOM by adding carbon is a beneficial agronomic practice that stimulates microbial communities and enhances soil N and C pools (Urra *et al.*, 2018). The youngest compounds are the most biologically active compounds, and the materials with intermediate ages contribute to soil physical characteristics (Mohanty *et al.*, 2013). We found positive correlations from GS37 to post-harvest with the N_{min} values (Table 2.3). Ros *et al.* (2011) found that SOM explained 78% of the variation in mineralizable N, whereas other soil properties only explained 8%. In some studies, SOM fractions have been preferred to total SOM for predicting N_o due to the easy release of labile compounds during the extractions (Wander, 2004). However, other studies suggested that none of those SOM fractions is an a priori preferable indicator of N_o (Haynes, 2005; Ros *et al.*, 2011).

Debosz and Kristensen (1995) found that Ntot content had a positive relationship with N mineralization. Similarly, Dessureault-Rompré *et al.* (2010) showed that Ntot was one of the best predictors of soil mineralizable N pools. However, in our case, Ntot only had positive correlations with the N_{min} values at harvest and post-harvest (Table 2.3). It is remarkable that only approximately 1–4% of the Ntot is mineralized as plant-available N (NH_4^+ and NO_3^-) each year (Debosz and Kristensen, 1995). Many authors have found that soil Ntot and SOM were the best predictors of N_o (Dessureault-Rompré *et al.*, 2010; Dessureault-Rompré *et al.*, 2015).

The mineralization of N is often affected by the clay content, likely due to SOM binding to mineral particles. Clay was correlated with N_{min} at the end of the growth cycle and with the GPC. In this experiment, soils with the highest GPC were S-1, S-5, S-9, and S-14 (15–16%), where the clay values were 32.4, 28.3, 26.4, and 34.8%, respectively. Clay has indirect affects through the formation of aggregates that protect SOM, and therefore microbial biomass, and direct effects with the stabilization of organic N (Tisdale *et al.*, 1985). Hassink (1997) found that the mineralization of organic N was negatively affected by a high clay content due to SOM binding to mineral particles. Similarly, Ros *et al.* (2011) found that clay had a negative influence on mineralizable N because mineralization in clayey soils was lower than that in sandy soils. In contrast, in this experiment, clay was positively correlated with N_{min} at GS37 and GS60, and sand was negatively correlated with N_{min} at GS60 (Table 2.3). Some clayey soils are able to fix and release ammonium, but the regulation of N availability is not fully understood (Nieder *et* *al.,* 2011). Chantigny *et al.* (2004) showed that in clay soils, the fixation of the recently added ammonium was higher than that in sandy soil (34% and 11%, respectively). The recently fixed ammonium that can be derived from added fertilizer or from soil organic matter (Nieder and Benbi, 2008) is quickly fixed by clay minerals and later released slowly during the crop growth season due to the increased crop demand. In a greenhouse experiment, Dou and Steffens (1995) found that 90–95% of the recently fixed ammonium was released during a 14-week period. Under field conditions, 66% of the recently fixed ammonium was released 86 days after fixation (Kowalenko, 1978). Provision of root exudates by plants improved the activity of heterotrophic microorganisms, which foster the release of fixed ammonium (Nieder *et al.,* 2011), retarding nitrification. The silt fraction has also been reported to bind NH_4^+ in a non-exchangeable form (Nieder *et al.,* 2011); in our study, it was correlated with N_{min} at harvest. In S-9, where high values of GPC were achieved (15%), the silt content was 54.8.

The pH and $CaCO_3$ did not present any effect on N mineralization. Dessureault-Rompré *et al.* (2015) found that the effect of pH on soil mineralization was very low. In other studies, soil pH, moisture and temperature were often non-linearly related to the dynamics of N (Rodrigo *et al.,* 1997; Paul, 2016). The pH range among our soils was very low.

With respect to wheat N uptake (Table 2.4), among the soil properties, only soil Ntot was positively correlated with the wheat N uptake at two times: from sowing to GS30 and from GS30 to GS37. In the case of aerobic incubation, N30wk and N_0 were correlated with the N uptake from GS37 to harvest and with GPC. However, only soil N_{min} at sowing and GS30 was correlated with the N uptake from GS37 to harvest (Table 2.5). Historically, soil N availability has been seen as an inaccurate indicator of plant N availability because plant roots are considered poor competitors for inorganic N against soil microorganisms (Schimel and Bennet, 2004). This idea could explain the lack of correlation between the soil N availability and the N uptake from the crop. Conversely, it has been determined that a cereal crop was able to accumulate a greater amount of added inorganic N than microorganisms (Inselsbacher *et al.,* 2010). The results showed that different soils followed different mineralization patterns affecting yield and GPC. The soil N_{min} at GS30 and at GS60 was positively correlated with the yield and GPC, respectively (Table 2.5). This suggests that the N status at those times is essential for determining the yield (Aranguren *et al.*, 2018) or GPC (Fuertes-Mendizabal *et al.*, 2010;

Ortuzar-Iragorri *et al.,* 2017). Soils with the lowest N availability at GS30 and GS60 as S-2 and S-16 presented the lowest yields and GPC, respectively. However, when the N availability was high in these growth stages, the yields and GPC values were high. Remarkably, none of the soil properties was correlated with yield, but clay was positively correlated with GPC (Table 2.4). However, one of the methods of chemical extraction (205 ABS) was correlated with the yield.

The key to optimizing high yields, wheat quality (GPC), and environmental protection is to achieve synchronicity between the N supply and crop demand, while accounting for spatial and temporal variability in soil N. As previously observed, many factors affect soil N mineralization, and therefore wheat N uptake. In Western Europe, the soil N_{min} at the end of winter is used to correct the values of N fertilizer rates calculated from the potential yield. Nevertheless, it implies laborious and expensive sampling and analysis. In Araba (Basque Country, northern Spain), the usual last and greater N dressing application occurred at GS30, but there were no correlations between soil initial properties and N_{min} values at GS30 (Table 2.3). This is remarkable because a high N availability at GS30 is key for achieving high yields (Aranguren *et al.*, 2018). As the last N dressing is at GS30, it is common to have low N in wheat plants at the end of the growing cycle (GS60–harvest) in Araba (Ortuzar-Iragorri *et al.,* 2017). Moreover, the climatic conditions in this area, humid Mediterranean, can lead to very high yields and therefore low protein concentrations in grain. Fuertes-Mendizabal *et al.* (2010) showed that late N availability (GS37 onwards) in wheat under humid Mediterranean conditions increased GPC, especially when no N was applied in the late stages, as in the area of study. As stated above, clay apparently allowed higher N availability at GS37 and GS60. This could be explained by the positive correlation between N_{min} at GS60 and GPC or the correlation between clay and GPC. However, soils presented a narrow clay range (24.6–34.8%) to confirm that finding. Inside the aerobic incubation, N30wk and N_o were able to estimate the N available for the wheat crop at the end of the growing cycle and thus the GPC. Identifying soils in Araba where it would be possible to have late N availability with the aim of improving GPC would be interesting. In the humid Mediterranean climatic conditions of Araba, a third application at GS37 is possible since rain water usually allows the utilization by wheat of this N applied late (Fuertes-Mendizabal *et al.,* 2010; Ortuzar-Iragorri *et al.,* 2017). However, the chemical methods that avoid the long periods required for aerobic incubation did not correlate properly with the N uptake values in the late growth stages.

In order to make N recommendations that guarantee adequate levels of GPC, methods for the diagnosis of soil available N must be improved, especially in the later stages of the growing cycle. In this sense, it is necessary to explore quick and simple methods because the most effective ones require periods of incubations that are too long. This is even more important in certain circumstances, such as organic farming, where it is difficult to make late applications of N with authorized fertilizers (organic fertilizers).

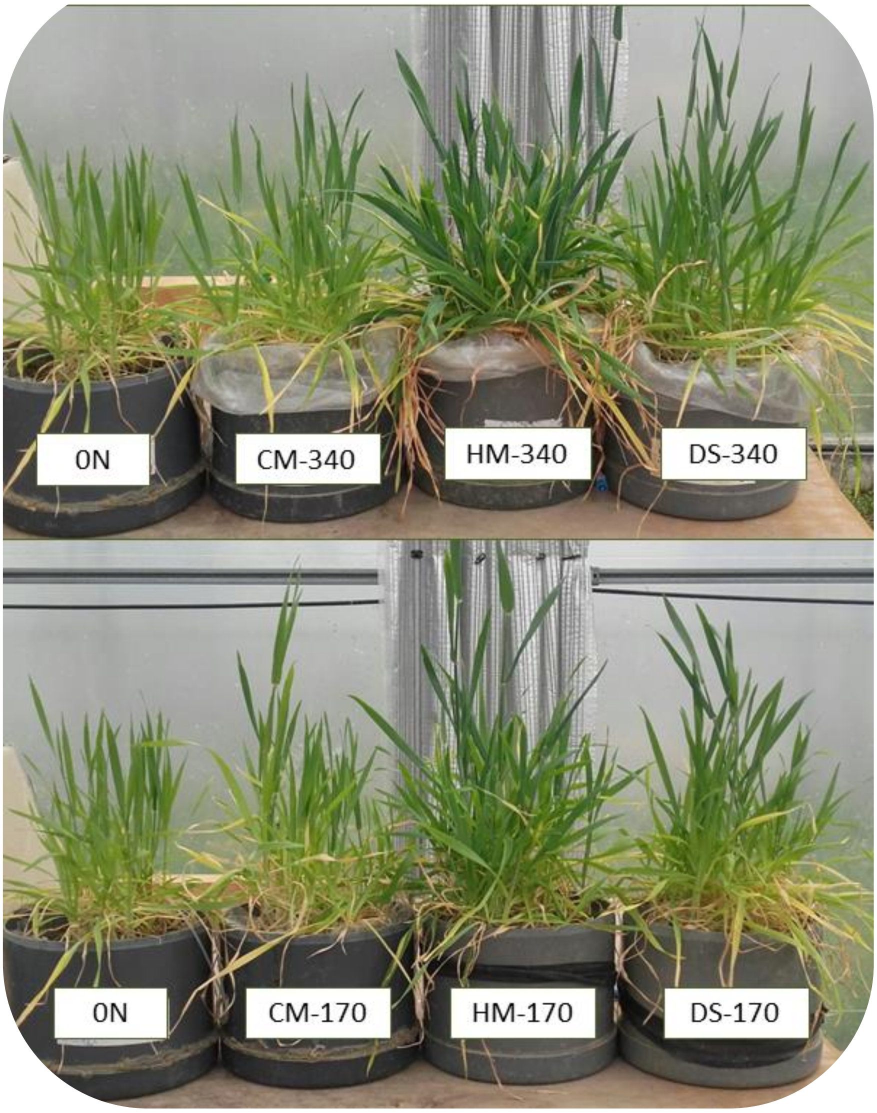
2.5 Conclusions

Even in a relatively small cropping area where the variability of soil properties is narrow, the dynamics of soil nitrate and ammonium throughout the cropping season were variable, and therefore, so was the crop N uptake. The soil N_{min} values at early wheat growth stages were well correlated with yield, and at late stages, they were well correlated with GPC.

N_0 was correlated with late N uptake and GPC. However, the chemical methods that avoid the long periods required for N_0 determinations were not correlated properly with the N uptake in the late wheat growth stages or GPC. Conversely, clay was positively correlated with the late N_{min} values and GPC, although the clay range was not very wide. Chemical methods were unable to estimate the available soil N in the later stages of the growing cycle. Consequently, as incubation methods are too laborious for their widespread use, further research must be conducted.

Chapter 3

Influence of wheat crop on carbon and nitrogen mineralization dynamics after the application of livestock manures



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3 Influence of wheat crop on carbon and nitrogen mineralization dynamics after the application of livestock manures

3.1 Introduction

The rising global demand for livestock products has increased the scale of livestock farming. Additionally, the disposal and management of large quantities of manures has become a serious environmental challenge. Animal manures constitute an important nutrient source in crop production (Whalen *et al.*, 2019). The suitable application of these products to soils improves soil quality and crop productivity, maintains soil fertility, recycles locally available nutrients (Mohanty *et al.*, 2013), and decreases the need for inorganic fertilizers (Nevens and Reheul, 2003). The synthesis of inorganic nitrogen fertilizers by the Haber-Bosh process is associated with major environmental concerns because it is very energy-intense and requires nonrenewable feedstock (Cherkasov *et al.*, 2015). Bouwman *et al.* (2013) estimated that by 2050, agricultural land will be supplied with approximately 130-153 Tg of N from animal manure, whereas the N input from mineral fertilizers is estimated to be 83-109 Tg. It is important to apply manures judiciously because they might cause negative effects on the environment, such as water eutrophication and greenhouse gas emissions (Delgado, 2002), or introduce diverse pollutants (Urra *et al.*, 2019). The mineralization of organic N in manures is influenced by soil temperature, soil moisture, soil properties, environmental conditions, microbial activity, and manure physical and chemical characteristics (Whalen *et al.*, 2019). Most of these factors cannot be precisely predicted; thus, only an approximation of manure N mineralization following the application is possible (Eghball *et al.*, 2002). In this sense, it is necessary to carry out mineralization studies with manure-amended agricultural soils for different regions and considering the crop N uptake.

The chemical composition of manures depends on several factors: animal species and diet, additives, bedding, water, etc. Manures contain both organic and inorganic forms of N. Organic N is a mixture of proteinaceous compounds, purines, nucleic acids, and uric acid, among other compounds (Whalen *et al.*, 2019). Inorganic N is considered to be the preferred N form taken up by higher plants. To release plant-available N, organic N compounds undergo a biochemical transformation called mineralization, which is mediated by enzymes (St. Luce *et al.*, 2011). The mineralization process is carried out exclusively by heterotrophic microorganisms that utilize C substances as an energy source and release NH_4^+ (Mohanty *et al.*, 2013). The mineralization of organic N in the soil depends on the biochemical quality of the substrates. The most labile substrates mineralize faster than the most recalcitrant substrates: urea > amino acids > proteins > nucleic acid > amino sugars > humic nitrogen (Coyne, 1999). Sequential reactions of aminization and ammonification occur during this process (St. Luce *et al.*, 2011). Aminization requires enzymes, such as proteases and proteinases, which break down complex proteins into simpler amino acids and amino sugars (Whalen and Sampedro, 2010). Amino acid compounds are further broken by enzymes, such as arylamidase or amidase, during ammonification to produce NH_4^+. Amino sugars are phosphorylated by kinases and then released as NH_4^+ (Whalen and Sampedro, 2010). In the nitrification process, NH_4^+ is transformed to NO_3^-, which is mediated primarily through two groups of autotrophic bacteria: *Nitrosomonas* and *Nitrobacter* (Mohanty *et al.*, 2013).

Usually, livestock manures are voluminous, wet, and odorous, and transporting them far from livestock farms is expensive and requires large amounts of energy. For that reason, manures are generally applied to agricultural soils within 20–30 km of livestock production facilities (Whalen *et al.*, 2019). In the area where this study was carried out (Basque Country, Northern Spain), animal manures that are generated in greater quantity are cattle manure (CM), hen manure (HM) and dairy slurry (DS) (Gobierno Vasco, 2008). Usually, CM has a very low mineral N content and a high organic N content, thus providing a very slow N availability rate for the crop (Whalen *et al.*, 2019). The content of uric acid in HM is high and rapidly converted to urea and NH_4^+ by the activity of various enzymes, such as uricase or urease (Murakami *et al.*, 2011), and DS contains high amounts of readily available N for the crop (Rochette *et al.*, 2001). Therefore, it is very important to improve the use efficiency of these manures by understanding the processes involved in N transformations because using different types and rates of manure fertilizer can result in different N dynamics in the soil. Thus, the rate and timing of application of organic fertilizers should be estimated through the N release characteristics of the amendments (Eghball *et al.*, 2002); otherwise, large amounts of ammonia and nitrate might be lost to the atmosphere or groundwater, respectively. In addition, N monitoring in the soil solution is necessary to better understand the relations of fertilization practices with crop responses (Peralta-Antonio, 2019) because plants obtain most of their nutrients from the soil solution (Nacry *et al.*, 2013).

To our knowledge, few studies have performed exhaustive monitoring of N mineralization of three different organic by-products applied to soil throughout the wheat growing cycle (and without wheat to assess the influence of the crop) by investigating the soil solution and enzymatic activities related to the N cycle.

A better knowledge of N availability when livestock manures are applied to the soil will help to improve the efficient use of these materials as fertilizers and avoid risky applications. The aims of this work are to evaluate the effect of three livestock manures (CM, HM, and DS) at two different N application rates (170 and 340 kg N ha^{-1}) on wheat yield and grain protein content as well as N uptake and to compare the effect of growing wheat (*Triticum aestivum* L.) on i) the protease, amidase, and urease soil enzymatic activities and ii) the dissolved organic carbon (DOC), ammonium and nitrate dynamics in the soil solution from an agronomic and environmental point of view.

3.2 Materials and Methods

3.2.1 Experimental Setup and Treatments

A pot experiment was carried out in a greenhouse in Derio (43° 17’ N, 2° 52’ W; Bizkaia, Basque Country, Spain) at the NEIKER facilities, and the upper layer (0-30 cm) was a Typic Calcixeroll soil (USDA, 2015). The soil was collected from the experimental field station of Arkaute (42° 85´ N, 2° 62´ W; Araba, Basque Country, Spain) and had rapeseed (*Brassica napus* L.) as the preceding crop, and it was collected on November 5, 2015. The soil had a high pH, was calcareous, had a moderate organic matter content in the upper layer, and had not received any organic amendments for several years before collection. The soil texture, soil organic matter, carbonate, nutrient contents, and mineralogy were analysed by X-ray diffraction (XRD) for the fraction < 2 µm, and the results showed that smectite was the most abundant clay (58%), followed by illite (30%) and kaolinite (12%) (Table 3.1). XRD analysis was carried out with a Philips PANalytical X'Pert PRO diffractometer (Philips Analytical, The Netherlands) with CuKα radiation (λ = 0.15406 nm), operating at 40 kV and 40 mA between 1-30º (2θ).

Table 3.1. Physical, chemical and mineralogical properties of the soil used for the greenhouse experiment.

Coarse sand (250 to 2000 μm) (g 100 g^{-1})[a]	7.5
Fine sand (50 to 250 μm)(g 100 g^{-1})[a]	35.1
Silt (2 to 50 μm) (g 100 g^{-1})[a]	24.4
Clay (<2 μm)(g 100 g^{-1})[a]	33.0
Textural classification[b]	Clay loam
pH (1:2.5)[c]	8.1
Organic matter (g 100 g^{-1})[d]	2.3
$CaCO_3^{2-}$ (g 100 g^{-1})[e]	30.1
Ca (cmol+ kg^{-1})[f]	33.1
Mg (cmol+ kg^{-1})[f]	0.93
K (mg kg^{-1})[f]	207
P (mg kg^{-1})[g]	65
NO_3^- (mg kg^{-1})[h]	6.8
NH_4^+ (mg kg^{-1})[h]	1.8
Dry Matter (g 100 g^{-1})	98
N_{min} (kg ha^{-1})[i]	37
Quartz (g 100 g^{-1})[j]	49
Calcite (g 100 g^{-1})[j]	4
Plagioclase (g 100 g^{-1})[j]	3
Potassium feldspar (g 100 g^{-1})[j]	4
Clays (g 100 g^{-1})[j]	40
Illite (g 100 g^{-1})[j]	12
Kaolinite (g 100 g^{-1})[j]	4.8
Smectite (g 100 g^{-1})[j]	23.2

[a]Texture by pippete method (Gee and Bauder, 1986); [b]Classification (USDA, 1999); [c]pH (1:2.5 soil:water using a pH-Meter CG840); [d]Organic matter (Walkey and Black, 1934); [e]CO_3^{2-}(NH4AcO, MAPA, 1994); [f]Ca, Mg & K (MAPA, 1994); [g]P (Watanabe and Olsen, 1965); [h]NO_3^-& NH_4^+ (Cawse, 1967; Nelson, 1983; Wei *et al.*, 2008); [i]Nmin= NH_4^+ + NO_3^-; [j]Analysis by X-ray diffraction

The CM was collected from an ecological extensive farm; the HM was collected from an intensive hen-layer facility; and the DS was collected from an intensive dairy farm. The manures were chemically characterised (Table 3.2), showing that they varied widely in their chemical composition.

Table 3.2. Chemical properties of the cattle manure, hen manure and dairy slurry applied as fertilizers in wheat in the greenhouse experiment.

	Cattle manure (CM)	Hen manure (HM)	Dairy slurry (DS)
D.M. (g 100 g^{-1})[a]	22.6	29.2	7.2
Organic matter (g 100 g^{-1})[b]	15	20	6.4
Total C (%)[c]	9.5	10.9	3.6
Total N (%)[c]	0.7	1.9	0.3
NH_4^+-N (%)[d]	0.03	0.8	0.2
C:N (%)	14.5	5.5	10.8
N-NH_4^+/Ntot (%)	4.5	40.9	60.6
Uric acid[e]/Ntot (%)	ND	60	ND
K (g kg^{-1})[e]	25.1	12.7	479.7
P (g kg^{-1})[e]	38.6	1.6	85.7
Ca (g kg^{-1})[e]	38.8	53.7	296
Mg (g kg^{-1})[e]	113.5	319.2	75.6
S (g kg^{-1})[e]	124.2	117.5	65
Cu (mg kg^{-1})[e]	28773	14438	510
Zn (mg kg^{-1})[e]	153.7	121	2748.7
Fe (mg kg^{-1})[d]	1045.4	1135.9	12575
Mn (mg kg^{-1})[e]	43812.2	1942.4	2748.7
Cd (mg kg^{-1})[e]	2946.3	1055	4
Pb (mg kg^{-1})[e]	4.6	1.63	17.3
Cr (mg kg^{-1})[e]	187.7	69.1	136.3
Ni (mg kg^{-1})[d]	113.8	45.6	71.4
Hemicellulose (g 100 g^{-1})[f]	14	14	16.7
Cellulose (g 100 g^{-1})[f]	22.9	12.7	14.2
Lignin + Cutin (g 100 g^{-1})[f]	19.2	1.4	7.5

[a]Organic matter on a fresh weight basis (gravimetrically following AFNOR, 1979); [b]Ctot and Ntot on a fresh weight basis (dry combustion using LECO, TrueSpec®CHNs); [c]N-NH_4^+ on a fresh weight basis (Kjeldahl digestion, MAFF, 1986); [d]Uric acid on a fresh weight basis (High-Performance Liquid Chromatography); [e]P, K, Ca, Mg, S, Cu, Zn, Fe, Mn, Cd, Pb, Cr, Ni on a fresh weight basis (acid digestion and ICP UV spectrometry, Cary 100, Varian Inc.); [f]Ankom 220 Fiber Analyzer on a dry weight basis (gravimetrically using Ankom Technology); ND (no data).

Manures were tested at two total N rates (Table 3.3): 170 and 340 kg N ha^{-1}. The 340 kg N ha^{-1} rate was used with the aim of analysing trends from an environmental perspective, although it is considered too high for field application. Pots of 0.0283 m^2 area were lined with a plastic bag to prevent leaching, and manures were applied to 3.9 kg soil; after mixing, they were poured into the pots. The soil had been previously air-dried and sieved to pass through a 20 mm mesh. Wheat (*Triticum aestivum* var. Cezanne) was sown (18/01/2016) in half of the pots (treatments with plants, P). Eighteen seeds were sown per pot, and 14 seedlings were left after germination to simulate the common sowing dose of 220 kg ha^{-1}. In the other half of the pots, the soil was left bare (treatments with no plants, NP). Pots were placed in the greenhouse along a north-south orientation. In each pot, two Rhizons Flex MOMs (RhizonFlex, Rhizosphere) with a diameter of 2 mm were placed at 5 and 10 cm from the pot bottom for soil solution extraction. Pots were irrigated and maintained at field capacity (32% w/w) during the whole experiment by weighing the pots and refilling them with water. Irrigation water NO_3^- and NH_4^+ concentrations were measured (2.7 mg L^{-1} and <0.05 mg L^{-1}, respectively). There were four replicates per treatment, and the experimental design was a three-factor (manure type, N rate, and presence of wheat plants or not) randomized complete block design. Apart from the treatments, two controls were established: 0N-P with plants and 0N-NP without plants (Table 3.3).

Table 3.3. Description of the treatments applied according to the three factors evaluated: type of manure, N dose and presence or absence of wheat plants in the greenhouse experiment.

Treatment	Farmyard manures	Dose (kg N ha^{-1})	Plant / No Plant
CM-170-P	Cattle manure (CM)	170	Plant
CM-170-NP	Cattle manure (CM)	170	No Plant
CM-340-P	Cattle manure (CM)	340	Plant
CM-340-NP	Cattle manure (CM)	340	No Plant
HM-170-P	Hen manure (HM)	170	Plant
HM-170-NP	Hen manure (HM)	170	No Plant
HM-340-P	Hen manure (HM)	340	Plant
HM-340-NP	Hen manure (HM)	340	No Plant
DS-170-P	Dairy slurry (DS)	170	Plant
DS-170-NP	Dairy slurry (DS)	170	No Plant
DS-340-P	Dairy slurry (DS)	340	Plant
DS-340-NP	Dairy slurry (DS)	340	No Plant
0N-P	No organic compound	0	Plant
0N-NP	No organic compound	0	No Plant

When applying manures at the N rates tested (170 and 340 kg N ha^{-1}), the amounts of organic fertilizer applied varied: CM, 25.75 and 51.5 t ha^{-1}; HM, 8.6 and 17.2 t ha^{-1}; and DS, 51.5 and 103 t ha^{-1}, respectively. To attribute the observations directly to N addition rates, unfertilized control pots (0N-P and 0N-NP) were included. Basal fertilization with P, K or other nutrients was not necessary because the soil K and P contents (Table 3.1) were adequate for the expected nutrient extractions by wheat in the pots. In addition, the soil was taken from an experimental field station where micronutrient deficiencies had never been detected for this crop. Therefore, the differences in the results are mainly attributed to the manure N input. Because the N content is the main component of manure for fertilization, the manure application rate is generally based on its N content.

Wheat seeds started germinating at day 10 after the beginning of the experiment, three leaves emerged (GS13, Zadoks *et al.,* 1974) 17 days after sowing, the beginning of tillering (GS21) took place at day 30, stem elongation (GS30) occurred at day 60, leaf flag emergence (GS37) occurred at day 95, flowering (GS65) occurred at day 117, and the wheat grain was harvested at crop maturity at day 156. Grain yields as well as straw production were recorded at crop maturity on 21/06/2016. For comparisons among pots, grain yields were converted to a 12% dry matter basis. The grain and straw samples were oven-dried at 70°C for at least 48 hours and then ground through a 1 mm mesh before determination of the total N concentration by the Kjeldahl procedure (AOAC, 1999). The grain protein concentration (GPC) was determined as the product of the grain N concentration by 5.7 (Teller, 1932). The wheat crop N uptake at harvest was calculated as follows:

$$\textit{Crop Total N (kg N ha}^{-1}\textit{) = Grain Total N (kg N ha}^{-1}\textit{) + Straw Total N (kg N ha}^{-1}\textit{),} \qquad (3.1)$$

where Grain Total N content was calculated as;

$$\textit{Grain Total N (kg N ha}^{-1}\textit{) = Grain Yield (kg ha}^{-1}\textit{) x Grain N concentration (\%)} \qquad (3.2)$$

where Straw Total N content was calculated as;

$$\textit{Straw Total N (kg N ha}^{-1}\textit{) = Straw Yield (kg ha}^{-1}\textit{) x Straw N concentration (\%)} \qquad (3.3)$$

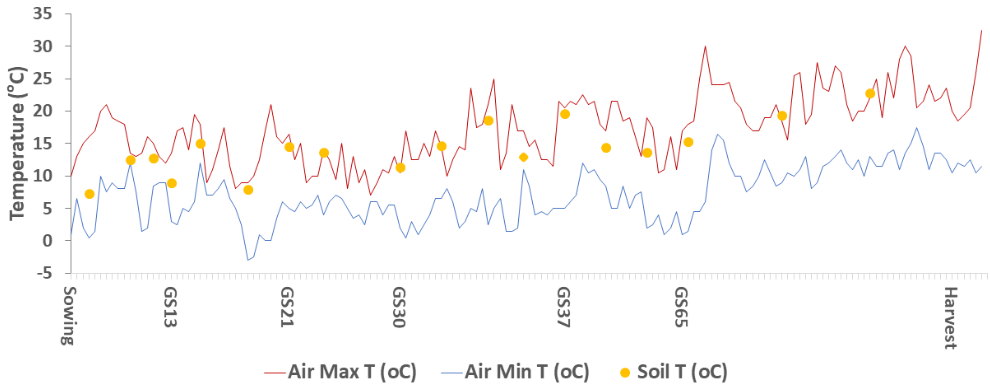

Figure 3.1. Air maximum and minimum temperatures (°C) and soil temperature (°C) in the wheat greenhouse experiment from sowing until harvest. GS13, three leaves emerged; GS21, tillering; GS30, stem elongation; GS37, leaf flag emergence; GS65, flowering (Zadoks *et al.*, 1974).

3.2.2 Enzymatic Activities

Three enzymatic activities related to the N cycle were measured: protease (mg tyrosine $kg^{-1}h^{-1}$) following Geisseler and Horwath (2008), amidase (mg β-naphthylamine $kg^{-1}h^{-1}$) following Acosta-Martinez and Tabatabai (2000) and urease (mg NH_4^+ $kg^{-1}h^{-1}$) following Kandeler and Gerber (1988). Soil cores were taken with a soil sampler at full depth in the pot at four sampling times: at sowing (3 days after sowing), at seed germination (10 days after sowing), at GS13 (17 days after sowing), and at the preharvest period (two weeks before harvest). Proteases catalyse the hydrolysis of large proteins to oligopeptides and from oligopeptides to amino acids. Amidase catalyses the hydrolysis of C-N other than peptide bonds in linear amides to monocarboxylate and NH_3. Urease catalyses the hydrolysis of urea to CO_2 and NH_3, with the formation of carbamate as an intermediate product. The released ammonia is hydrolysed to ammonium in the soil solution, which releases a hydroxide ion ($NH_3 + H_2O \rightarrow NH_4^+ + OH^-$).

3.2.3 Soil Solution Samples

Soil solution samples were collected (RhizonFlex, Rhizosphere) 22 times from sowing to harvest to analyse dissolved organic carbon (DOC), ammonium (NH_4^+), and nitrate (NO_3^-). Soil solution samples were collected twice per week during the first month and once per week thereafter. The first soil solution sample was discarded, and then 40 mL of soil solution was taken from each pot, 20 mL from each RhizonFlex. The contents of the two syringes were mixed to obtain one sample per pot. Soil solution DOC, ammonium and nitrate were determined spectrophotometrically (Wei *et al.*, 2008; Cawse, 1967; Nelson, 1983, respectively). Soil solution ammonium and nitrate concentrations were used as proxy variables to estimate N mineralization dynamics.

3.2.4 Statistical Analysis

In the case of yield, GPC, and wheat N uptake at harvest, the factors taken into account in the statistical analysis were manure type and N rate. A two-way analysis of variance (ANOVA; R core Team, 2013) was performed. Positive interactions were detected; therefore, it was necessary to analyse each factor depending on the other factor. An ANOVA was performed to analyse differences between applied manures depending on the N rate. Another ANOVA was performed to analyse differences between applied N rates depending on the manure. To separate the means, Duncan's test was used ($p \leq 0.05$) using the R package *agricolae* (De Mendiburu, 2009). Control (0N) was not included in the ANOVA. Normality was checked using the Shapiro–Wilk test before the ANOVAs. The Levene test was carried out with the aim of determining whether the variance in the populations to be tested was equal or not. When variances were unequal, Welsh's ANOVA was implemented.

In the case of enzymatic activities (protease, amidase, and urease) and for the variables related to soil solution (DOC, ammonium, and nitrate), the factors analysed in the statistical analysis were manure type, N rate and presence (or not) of plants by a three-way analysis of variance for each sampling time (ANOVA; R core Team, 2013). Because triple positive interactions (manure type, N rate, and presence (or not) of plants) occurred, two-way ANOVAs were performed between the mentioned factors. Positive interactions were also detected; therefore, each factor was analysed depending on the other factors. An ANOVA was performed to analyse differences between applied manures depending on the N rate and the effect of the plant for each sampling time. Another ANOVA was performed to analyse differences between applied N rates depending on the manure and the effect of plants for each sampling day. Finally, an ANOVA was performed to analyse the effect of the presence of plants depending on the manure applied and the N rate applied for each sampling time. To separate the means, Duncan's test was used ($p < 0.05$) in all cases using the R package *agricolae* (De Mendiburu, 2009). Controls (0N) were not included in the ANOVAs. Normality and homogeneity of the variances were checked using the Shapiro–Wilk and Levene tests, respectively, before ANOVAs. When the variances were unequal, Welsh's ANOVA was implemented. In the case of enzymatic activities, when significant differences were not found, the results were not plotted. Letters from Duncan's tests to indicate the differences among significantly different means are not shown in Figure 3.2 because it would make their visualization difficult. Thus, letters are presented in Table A3.1. To simplify the results, only the significant results were mentioned in the text.

3.3 Results

3.3.1 Wheat Yield

The highest wheat grain yields (kg ha^{-1}) were achieved with the HM treatments, followed by DS and CM (Table 3.4). In the DS treatment, higher yields were achieved at the highest N rate. Similar to the yield, the highest GPC (%) and the highest wheat N uptake (straw + grain) were achieved with the HM treatments, followed by DS and CM (Table 3.4). In the HM and DS treatments, higher GPC (%) values and higher N uptake (kg N ha^{-1}) were achieved at the highest N rate.

Table 3.4. Wheat grain yield, grain protein content (GPC) and wheat crop N uptake at harvest (grain + straw) in the different treatments applied in the greenhouse experiment.

Manure type	N rate (kg N ha^{-1})	Treatment	Grain Yield (kg ha^{-1})		GPC (%)		Wheat N uptake (kg N ha^{-1})	
			mean	*sd*	mean	*sd*	mean	*sd*
CM	170	CM-170-P	2196 B	220	9.2 B	0.5	68 C	7
HM	170	HM-170-P	3236 A	625	11.5 A	1.3	123 A	9
DS	170	DS-170-P	2637 B	339	9.2 B	0.1	81 B	9
CM	340	CM-340-P	2601 B	231	9.0 B	0.3	80 C	8
HM	340	HM-340-P	3571 A	707	15.4A#	2.2	162A#	10
DS	340	DS-340-P	3236 B *	260	10.6 B*	0.3	108 B *	7
Control	0N	0N-P	1649	316	52.0	3.4	52.0	3

CM, cattle manure; HM, hen manure; DS, dairy slurry; different capital letters represent differences among fertilizers (CM, HM, DS) for each N rate (170 and 340 kg N ha^{-1}) in grain yield (kg ha^{-1}), GPC (%), and wheat N uptake at harvest (kg N ha^{-1}). #, significantly higher mean between N rates (170 and 340 kg N ha^{-1}) in HM; *, significantly higher mean between N rates (170 and 340 kg N ha^{-1}) in DS.

3.3.2 Enzymatic Activities

In protease enzymatic activity (Figure 3.2a), at the highest N rate at sowing, CM had higher activity than DS and HM (Table A3.1). In the CM treatment, the highest N rate had higher protease values. Regarding amidase enzymatic activity (Figure 3.2b), at the highest N rate at sowing, DS had higher enzymatic activity than CM and HM. In the DS treatment, the highest N rate had higher protease values. In the case of urease enzymatic activity (Figure 3.2c), the treatments with plants and the lowest N rate had higher values than those with the highest N rate (Table A3.1). In addition, all the manures applied at the lowest rate had higher values than treatments without plants. The control with plants (0N) presented higher ammonium concentrations than the control without plants (0N).

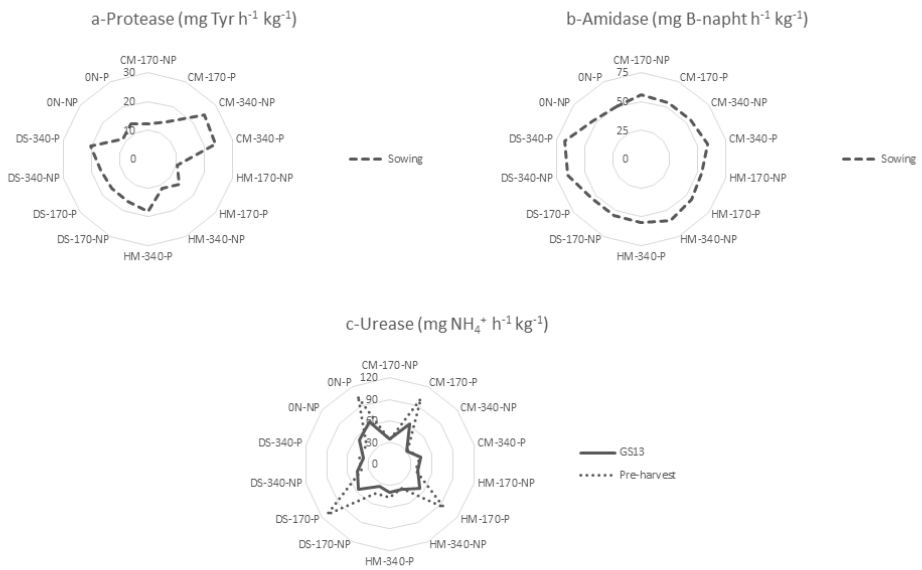

Figure 3.2. Protease (a), amidase (b) and urease (c) enzymatic activities in soil. CM, cattle manure; HM, hen manure; DS, dairy slurry; 0N, control. 170, 170 kg N ha^{-1} application rate; 340, 340 kg N ha^{-1} application rate. P, treatments with plants; NP, treatments without plants (bare soil). **- - -,** sowing; ——, GS13; ••••••••, pre-harvest. Tyr, Tyrosine; Napht, naphthylamine, NH_4^+, urease

3.3.3 Dissolved Organic Carbon, Ammonium and Nitrate in Soil Solution

Dissolved Organic Carbon, DOC

In treatments with the lowest N rate (Figure 3.3a and Figure 3.3b), the day just after sowing DS had higher DOC values (150-200 mg L^{-1}) than HM and CM (50-75 mg L^{-1}).

Four days after sowing, DOC values decreased and remained similar in all treatments until harvest (0-50 mg L^{-1}). In treatments with the highest N rate (Figure 3.3c and Figure 3.3d) and at the first day after sowing, the DS treatment had higher DOC values (400-450 mg L^{-1}) than HM and CM (75 mg L^{-1}). Four days after sowing, DOC values decreased for the three livestock manures (50-100 mg L^{-1}). Subsequently, DOC values remained similar and constant in all treatments until harvest (0-50 mg L^{-1}). DS was the only manure type with higher nitrate values in the highest N rate on the day after sowing.

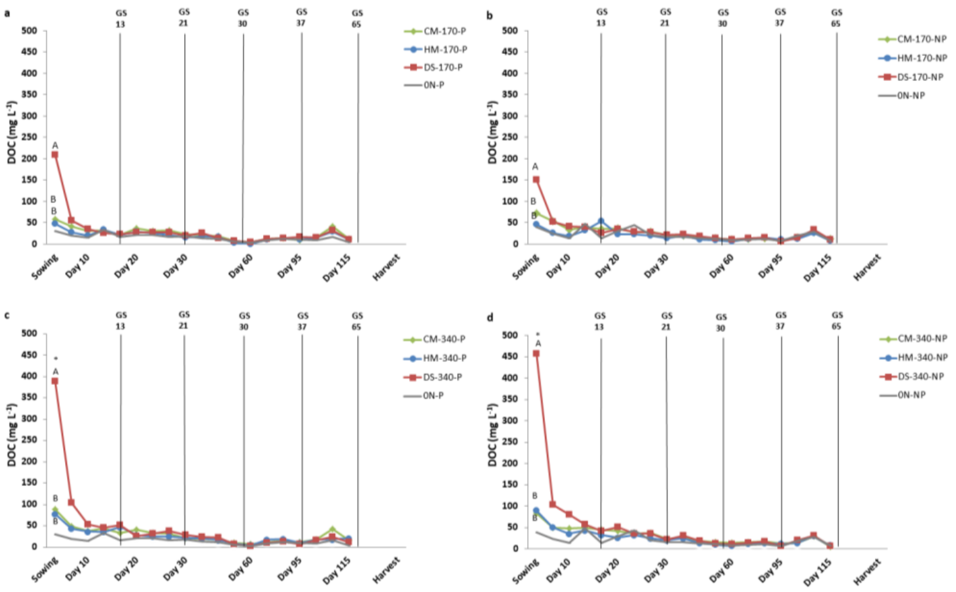

Figure 3.3. Dissolved organic carbon (DOC) concentration dynamics in the soil solution. a) Manures applied at 170 kg N ha^{-1} and presence of plants; b) Manures applied at 170 kg N ha^{-1} and absence of plants (bare soil); c) Manures applied at 340 kg N ha^{-1} and presence of plants; d) Manures applied at 340 kg N ha^{-1} and absence of plants (bare soil). ◆, cattle manure (CM); ●, hen manure (HM); ■, dairy slurry (DS); —, control (0N). *, significantly higher mean between N rates in DS and the presence (A vs C) or absence (B vs D) of plants. Capital letters represent differences among fertilizers for each N rate and the presence or absence of plants. Growing stages for wheat: GS13, three leaves emerged; GS21, tillering; GS30, stem elongation; GS37, leaf flag emergence; GS65, flowering (Zadoks *et al.* 1974). Figures for treatments without plants also show growing stages for comparisons among figures.

Ammonium

Regarding the lowest N rate (Figure 3.4a and Figure 3.4b), on the day after sowing, the DS treatments achieved higher ammonium values than CM and HM. In the treatments with DS, ammonium concentrations in the soil solution decreased from the day after sowing (4.5-6 mg L^{-1}) to the fourth day after sowing (1.2 mg L^{-1}). In the treatments with HM, the ammonium concentration in the soil solution increased slightly between the day after sowing and the fourth day after sowing (0 to 1-3 mg L^{-1}). Treatments with CM had very low ammonium concentrations during the whole experiment (not higher than 1.5 mg L^{-1}).

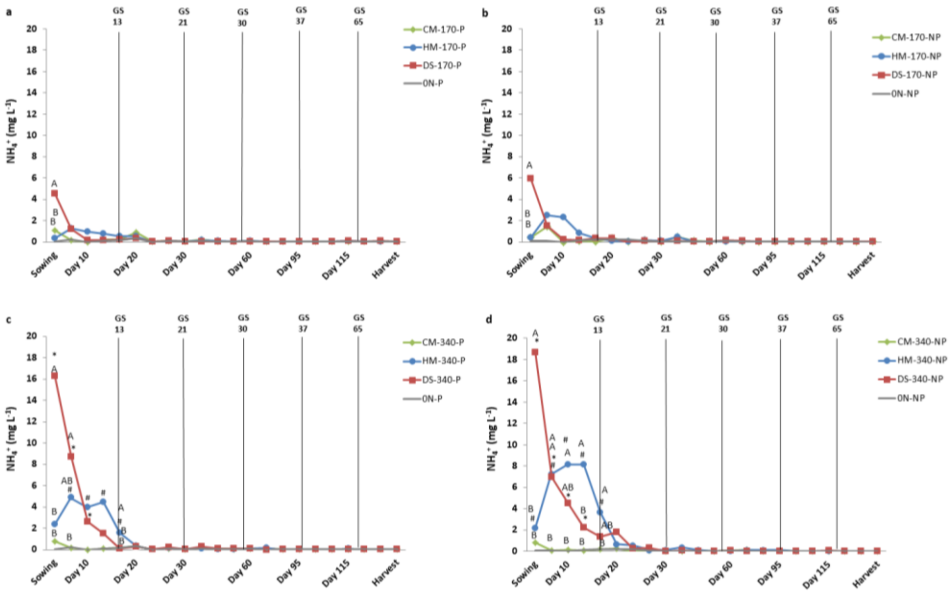

Figure 3.4. Ammonium (NH_4^+) concentration dynamics in the soil solution. a) Manures applied at 170 kg N ha^{-1} and presence of plants; b) manures applied at 170 kg N ha^{-1} and absence of plants (bare soil); and c) manures applied at 340 kg N ha^{-1} and presence of plants; d) Manures applied at 340 kg N ha^{-1} and absence of plants (bare soil). ◆, cattle manure (CM); ●, hen manure (HM); ■, dairy slurry (DS); —, control (0N). #, significantly higher mean between both N rates in HM and the presence (A vs C) or absence (B vs D) of plants; *, significantly higher mean between both N rates in DS presence (A vs C) or absence (B vs D) of plants. Capital letters represent differences among fertilizers for each N rate and the presence or absence of plants. Growing stages for wheat: GS13, three leaves emerged; GS21, tillering; GS30, stem elongation; GS37, leaf flag emergence; GS65, flowering (Zadoks *et al.* 1974). Figures for treatments without plants also show growing stages with the aim of comparison among figures.

Regarding the highest N rate (Figure 3.4c and Figure 3.4d), on the day after sowing, DS had the highest ammonium concentration in the soil solution (16.5-19 mg L^{-1}). By the fourth day after sowing, the DS ammonium concentration values drastically decreased while HM ammonium concentration values increased, with similar values observed between them (5-8 mg L^{-1}) and higher values than CM (0 mg L^{-1}). In the second week after sowing, the DS values decreased steadily until reaching very low values (0-2 mg L^{-1}) while HM increased, reaching 5-8 mg L^{-1}. From the second week after sowing onwards, HM reached values close to 0 mg L^{-1}. The CM treatment had a very low ammonium concentration during the entire growing cycle.

In the case of DS, the ammonium concentration in the soil solution was higher at the highest N rate than at the lowest N rate in the first two weeks of the experiment. In the HM treatments, the highest N rate had higher ammonium values than the lowest N rate from the fourth day until two weeks after sowing.

Nitrate

From one week before GS21 to harvest, the nitrate concentrations in the treatments without plants (Figure 3.5b and Figure 3.5d) were higher than those in the treatments with plants (Figure 3.5a and Figure 3.5c) for every fertilizer and N rate.

Focusing on the treatments with the lowest N rate (Figure 3.5a and Figure 3.5b), on the day after sowing, the nitrate concentrations were approximately 350-400 mg L^{-1} and increased by the second week in the DS and HM treatments until 400-600 mg L^{-1}. However, in the CM treatment, the nitrate concentrations remained constant. From GS13 onwards, HM had higher nitrate concentrations in the soil solution (900-1000 mg L^{-1}) than DS and CM (500 and 350 mg L^{-1}, respectively). In the treatments with plants (Figure 3.5a), the nitrate concentrations for the three manures decreased for the week before GS21 (0-150 mg L^{-1}) and then remained negligible until harvest. However, in the treatments without plants (Figure 3.5b), the nitrate concentrations in the HM treatment (250 mg L^{-1}) were higher than those in the treatments with DS and CM (50-100 mg L^{-1}) and remained different until two weeks before GS37. After that time, the nitrate values remained constant (50-100 mg L^{-1}).

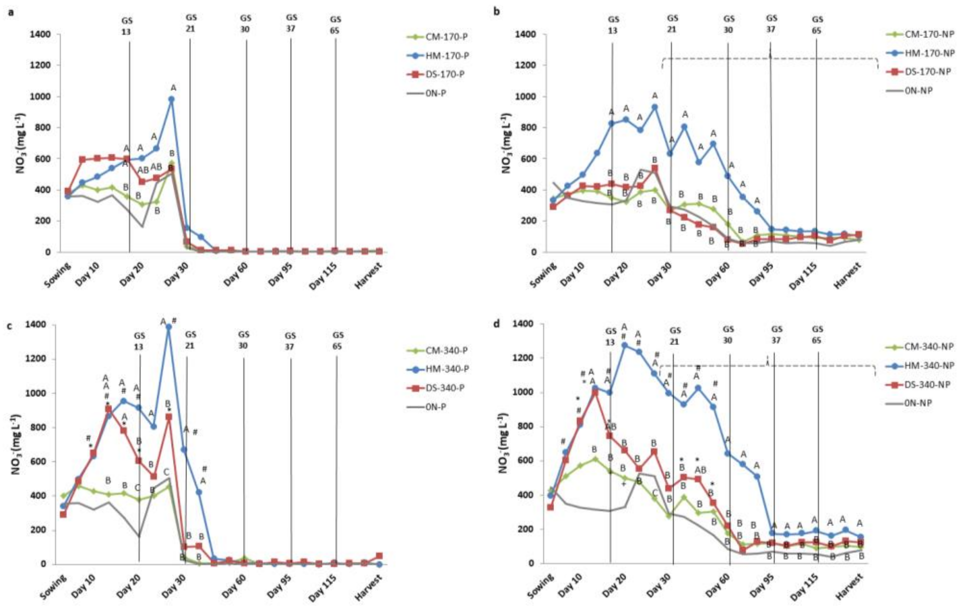

Figure 3.5. Nitrate (NO_3^-) concentration dynamics in the soil solution. a) Manures applied at 170 kg N ha^{-1} and presence of plants; b) manures applied at 170 kg N ha^{-1} and absence of plants (bare soil); c) manures applied at 340 kg N ha^{-1} and presence of plants; and d) manures applied at 340 kg N ha^{-1} and absence of plants (bare soil). ◆, cattle manure (CM); ●, hen manure (HM); ■, dairy slurry (DS); —, control (0N). +, significantly higher mean between both N rates in CM and the presence (A vs C) or absence (B vs D) of plants; #, significantly higher mean between both N rates in HM and the presence (A vs C) or absence (B vs D) of plants; *, significantly higher mean between both N rates in DS and the presence (A vs C) or absence (B vs D) of plants. Capital letters represent differences among fertilizers for each N rate and the presence or absence of plants. Brackets determine the period where differences between the presence and absence of plants were significant for all fertilizers for the 170 kg N ha^{-1} rate (A vs B) and 340 kg N ha^{-1} rate (C vs D) and were disposed when the highest nitrate concentrations were found. Growing stages for wheat: GS13, three leaves emerged; GS21, tillering; GS30, stem elongation; GS37, leaf flag emergence; GS65, flowering (Zadoks *et al.* 1974). Figures for treatments without plants also show growing stages with the aim of comparison among figures.

In the treatments with the highest N rate (Figure 3.3c and Figure 3.5d), on the first day after sowing, the nitrate concentrations were approximately 400 mg L^{-1} and increased by 10 days after sowing in DS and HM treatments to values of approximately 750 mg L^{-1}, while in CM, the nitrate concentrations remained constant (400 mg L^{-1}). The nitrate concentration continued to rise in the HM treatment until one week before GS21 (reaching values of approximately 1300 mg L^{-1}) but decreased in the DS treatment. By one week before GS21, in the treatments with plants (Figure 3.5c), the nitrate concentrations in soil solution began to decrease drastically for all the manures applied. By GS21, the nitrate concentrations were low (approximately 50 mg L^{-1}) in DS and CM, while in HM, the nitrate concentrations remained at approximately 700 mg L^{-1}. The nitrate values in HM decreased to 20 mg L^{-1} two weeks after GS21. In treatments without plants (Figure 3.5d), by one week before GS21, the nitrate concentrations started decreasing and did not vary from two weeks before GS37 until harvest (50-170 mg L^{-1}). One week before GS21 until harvest, the nitrate concentration in the treatments with HM (approximately 160 mg L^{-1}) was higher than that in the treatments with DS and CM (approximately 100 mg L^{-1}).

Regarding the differences in the N rates applied, HM had higher nitrate values at the highest N rate from the fourth day after sowing to GS30 in the treatments with plants and without plants. The DS had higher values in the highest N rate from 10 days after sowing to GS13 (with and without plants) and in the period from GS21 to GS30 (without plants). The CM had higher nitrate values at the highest N rate only at GS13 and four days after GS13 in the treatments without plants (Figure 3.5b and Figure 3.5d).

3.4 Discussion

The livestock manures used in the present experiment differed widely in their physical and biochemical composition (Table 3.2), which would influence the rate of organic N mineralization and the dynamics of the enzymatic activities (Mohanty *et al.*, 2013). The manures had C:N ratios below 15 (Table 3.2), and Tisdale *et al.* (1985) mentioned that when the C:N ratio was lower than 20, N was released into the soil solution. However, some authors reported that immobilization can still occur with low C:N because differences in the manures' biochemical composition are not reflected in the C:N ratio (Mohanty *et al.*, 2013).

Regarding the C-based compounds, two fractions can be distinguished: a) nonfibre C, such as starch, sugars and pectines, and b) fibre C, such as cellulose, hemicellulose and lignin (Sauvant *et al.*, 2004). Nonfibre C is often a small part of manure, and although it is unlikely to make a large difference in estimating the total nutrient supply of manure, it may change the dynamics of nitrogen release (Daud *et al.*, 2010). The lignin fraction has been identified as relevant for manure mineralization (Mohanty *et al.*, 2013) because N mineralization decreases with the presence of lignin due to its resistance to degradation (Pansu *et al.*, 2003). In our case, CM had more lignin-cutin compounds (Table 3.2) than

DS and HM. On the other hand, in CM, the quantitatively most important part of N is proteins (Whalen *et al.*, 2019), while the ammonium content was very low (Table 3.2). In treatments with CM, protease enzyme activity was higher than in the treatments with DS and HM three days after sowing when applying the highest N rate. Additionally, the CM treatment had higher protease activity when applying the highest N rate than when applying the lowest rate. Protease enzymes hydrolyse large proteins to oligopeptides and can usually degrade most nonstructural proteins (Chadwick *et al.*, 2000; Miller and Cramer, 2005). Its higher activity related to CM might be explained by its protein-rich composition. In addition, it has been shown that easily metabolizable C sources, amino acids or NH_4^+, may repress protease enzyme production (Allison and Macfarlane, 1992), and HM and DS have higher contents of these compounds/molecules than CM (low initial NH_4^+/Ntot values (Table 3.2)). In the case of HM, low amounts of lignin + cutin compounds were measured (Table 3.2). Uric acid represents 60% of the total N and ammonium 40% (Table 3.2), although protein molecules have also been described by other authors (Pan *et al.*, 2009). It should be mentioned that uric acid is not always reported as a component of organic N due to its rapid hydrolysis to urea (Whalen *et al.*, 2019). Due to the high amount of uric acid, it was hypothesized that urease enzymatic activity might be higher in the treatments with HM, and Cook *et al.* (2008) identified HM as an ideal environment for the growth of ureolytic microorganisms. However, there were no differences with the other manures or with the control for urease activity during the wheat growing period. DS had the lowest amounts of lignin + cutin (Table 3.2). Slurries contain organic compounds that are chemically recalcitrant to microbial decomposition (van Kessel *et al.*, 2000). Milkhouse wastewater mixed with dairy manure and urine forms a semisolid mixture (slurry) with soluble organic substrates (Whalen *et al.*, 2019). However, urease activity did not increase with the application of DS throughout the growing season, even though urea is known to be released from mammalian urine (Whalen *et al.*, 2019). Contrary to our results, Bol *et al.* (2003) showed that urease activity increased with DS application. Regarding amidase enzymatic activity, the highest values were measured at sowing when applying DS at the highest N rate. The amidase enzyme breaks the C-N bonds of peptides and amides, which releases ammonia. When applying slurry, a pool of liquid soluble organic substrates is added where amino acids (Bol *et al.*, 2003), among other compounds, are present.

The differences related to different manures were only detected at sowing in the case of protease and amidase, which might be explained by the diverse pool of microorganisms found in manures contributing to the enzyme pools (Durso *et al.*, 2011). However, those differences did not persist during the wheat growing period, likely due to the dilution effect with the soil (He and Zhang, 2014) or due to short-term survival of the organic amendment-borne microorganisms on soils (Saison *et al.*, 2006). In the case of urease, differences related to the added livestock manures were not detected during the wheat growing period, although urea is released with mammalian urine (Whalen *et al.*, 2019), and it is the product of uric acid, which is present in high proportions with HM (Pan *et al.*, 2009). According to some authors, a large proportion of the urease activity in soil is extracellular and associated with soil particles, especially soil organic matter and clay minerals (Geisseler *et al.*, 2010), which may explain the lack of differences among manures and with the control. Burns *et al.* (1972) showed that urease exists in soil as an enzyme-organic matter complex. As in the present study, Zantua and Bremner (1976) found that the addition of urea did not increase urease activity, which may be due to the high proportion of soil matrix-protected urease.

With the application of livestock manures in the soil, different dynamics were observed in the soil solution. When DS was applied to the soil, DOC and ammonium concentrations presented similar patterns, with both concentrations being higher than in the other two fertilizers (Figure 3.3 and Figure 3.4), which was likely due to their higher initial availability. Ammonium was initially present in DS (Table 3.2). Norris *et al.* (2018) reported that much of the N supplied from dairy slurries during the first year after application was likely from the inorganic N initially present at the time of application. Whalen *et al.* (2019) mentioned that slurries are rich in soluble organic substrates that are susceptible to enzymatic hydrolysis, which might explain the higher DOC concentrations (Figure 3.3). When applying DS, ammonium and DOC concentrations in the soil solution decreased during the first week after sowing. Sørensen (1998) showed that part of the slurry ammonium is quickly immobilized by microbial biomass that decomposes slurry organic matter, particularly compounds such as volatile fatty acids. Part of this N remains immobilized for several years (Sørensen, 2004). Kirchman and Lundvall (1993) mentioned that the concentration of volatile fatty acids decreased to nondetectable levels six days after slurry application, and ammonium and nitrate were immobilized. In CM and HM, the DOC concentrations in the soil solution were also higher during the first week after sowing and then remained constant (25-50 mg L^{-1}) until harvest (Figure 3.3). Kidd *et al.* (2007) and Villar (2014) reported similar DOC values (approximately 50 mg L^{-1}) when constant concentration values were reached after sludge application. The DOC fraction has been considered an immediate energy source for microorganisms, and once consumed, microbial activity will depend on more recalcitrant organic fractions, such as cellulose (Martín-Olmedo and Rees, 1999). In the HM treatments, ammonium concentrations increased from sowing to 10 days after sowing at the highest N rate, and this change was likely due to the high amount of uric acid present, which is considered a labile compound (Pan *et al.*, 2009). Sharifi *et al.* (2009) also reported that uric acid applied with HM was hydrolysed to urea and ammonium in the first 3-14 days after its application to soil. Regarding CM, the previously mentioned recalcitrant composition and the low initial ammonium availability explained the low ammonium concentrations from sowing until harvest (Figure 3.4). Griffiths *et al.* (2012) mentioned that the C-rich compounds in the bedding materials stimulated microbial growth while the low ammonium availability would cause the rapid immobilization of ammonium by soil microorganisms, leaving very low ammonium concentrations in soil solution. Grignani and Zavattaro (2000) reported that ammonium concentrations in the soil solution were less than 2 mg L^{-1} in 99% of the samples after applying 369-509 kg N ha^{-1} $year^{-1}$ in crop-livestock integration systems with corn and barley production. Kirschke *et al.* (2019) and Yan *et al.* (2018) reported that the maximum increase in ammonium content in soil solution occurred three days (8 mg L^{-1}) and 10 days after urea application (2-2.5 mg L^{-1}), respectively. Villar (2014) performed a pot experiment and reported that after sludge application, ammonium concentrations in soil solution decreased after two weeks to values close to 0 mg L^{-1}. Decreases in ammonium in the soil solution might be due to different reasons, such as immobilization by soil microorganisms, ammonium fixation in soil clays, volatilization, nitrification, and/or uptake by wheat plants (Fuertes-Mendizabal *et al.*, 2013). Rochette *et al.* (2001) and Kelleher *et al.* (2002) pointed out that volatilization is generally the major N loss mechanism of slurries and HM, respectively. However, in this case, manures were incorporated into the soil, which has been reported as a good practice to minimize the rates of volatilization (Rochette *et al.*, 2001). In the present study, ammonium in the soil solution was transformed to nitrate in the first 15-18 days (Figure 3.4 and Figure 3.5). During this period, as ammonium decreased, nitrate started increasing in the soil solution. The nitrate concentrations (Figure 3.5) were much higher than the ammonium concentrations in soil solution (Figure 3.4), which was likely due to the transitory form of ammonium in the mineralization process (Azeez and Van Averbeke, 2010). Yan *et al.* (2018) and Kirschke *et al.* (2019) also showed that the soil solution contained significantly higher nitrate values than ammonium because nitrate is the primary form of mineral N in the solution. During the first month after livestock manure application, the nitrate values increased in the soil solution in comparison with the controls (Figure 3.5). There was a nitrate spike just before GS21 in most treatments, even in the controls, and it coincided with an increase in the greenhouse air maximum temperatures (21°C, Figure 3.1). Chantigny *et al.* (2004) mentioned that in clayey soils, the nitrate concentration was significantly lower in the first 14 days after slurry application and attributed to the clay fixation of the added ammonium. In DS, the nitrate values exponentially increased with the highest N rate, whereas the curve was much flatter with the lowest N rate, suggesting that the ammonium applied with the highest N rate exceeded the retention capacity of the soil. In HM, the nitrate increase dynamics were similar at both N application rates, reaching higher values with the highest N application rate. Delin (2011) attributed high nitrate increases in poultry manure to the sum of uric acid and ammonium N. Saka *et al.* (2017) performed a field experiment and reported maximum nitrate peak values in soil two and a half months after poultry manure incorporation under treatments with 140 kg N ha^{-1}, 114 mg NO_3^- kg^{-1}; 540 kg N ha^{-1}, 230 mg NO_3^- kg^{-1}, and 1668 kg N ha^{-1}, 250 mg NO_3^- kg^{-1}. Expressing the data from the present study in mg NO_3^- kg^{-1} soil, the maximum nitrate peaks in HM would be 448 mg NO_3^- kg^{-1} at the highest N rate and 320 mg NO_3^- kg^{-1} at the lowest N rate, which were higher than those reported by Saka *et al.* (2017). In the present study, in the CM treatment, the nitrate values remained constant in the first month, which was probably due to its low ammonium availability and its recalcitrant composition (Table 3.2). Qian and Schoenau (2002) showed that when the CM C:N ratio was 13 to 15, there was little impact on short-term N release, which is consistent with this study, and tended to decrease with C:N ratios over 15. In other studies carried out in pot experiments, the nitrate concentrations were similar to those reported in the present experiment and reached 1300 mg L^{-1} after applying 200 kg N ha^{-1} as $(NH_4)_2SO_4$ (Yanai *et al.* 1998) or 1500-1000 mg L^{-1} after applying limed sludge and nonlimed sludge, respectively (Villar 2014). Under hydroponic conditions, Urrestarazu (2004) reported that optimum nitrate concentrations were 700 mg L^{-1} for tomato and approximately 1000 mg L^{-1} for cucumber. Given that horticultural crops are more demanding on N than wheat, we may infer that nitrate values in soil solution are higher than needed, especially at the beginning of the growing cycle. The nitrate concentrations under field conditions reported in other studies were lower (1 to 150 mg L^{-1} in a crop-livestock integration system (Grignani and Zavattaro, 2000) and 180 mg L^{-1} after green manure application (Peralta-Antonio *et al.*, 2019)) than those reported in this experiment. Significant differences were found between treatments with plants and without plants in nitrate concentrations from GS21 to the end of the experiment (Figure 3.5). Yanai *et al.* (1998) found that changes in nitrate concentration in soil solutions with and without wheat plants were significant from day 20 until the end of the experiment. In treatments without plants (Figure 3.5b and Figure 3.5d), the nitrate values decreased two months after sowing in CM and DS and by three months in HM, and then the concentrations remained constant. Kirschke *et al.* (2019) reported that maximum nitrate values in soil solution without plant cultivation (200 mg L^{-1}) were achieved one month after urea application (270 kg N ha^{-1}). The reduction in nitrate concentrations in the treatments without plants might be explained by different hypotheses, such as microbial immobilization (Burger and Jackson, 2003), leaching and/or denitrification (St. Luce *et al.,* 2011). Regarding microbial immobilization, Burger and Jackson (2003) reported high nitrate immobilization rates and suggested that when high OM inputs are added into soil, greater C availability supports a more active microbial biomass with a greater N demand, thus promoting immobilization and recycling nitrate. Morvan *et al.* (1997) accounted for 10% more N immobilized in bare soil than in the treatments with ryegrass when applying slurry. Denitrification is a microbial process where nitrate and nitrite are reduced to gaseous forms of N, such as oxides of N or molecular N (N_2). Denitrification has been traditionally considered to be carried out in anaerobic conditions, although it is also carried out in the presence of oxygen (Chen and Strous, 2013). Although lower cumulative nitrous oxide losses have been detected at field capacity than when soil is water saturated, gaseous emissions cannot be ignored (Clough *et al.,* 2005). Although the pots were lined with a plastic bag to prevent leaching, the rhizons were placed at 5 and 10 cm above the pot bottom; therefore, nitrate might also be concentrated at the deepest part of the pot when watering. Haynes and Williams (1992) reported that leaching nitrate resulted in equivalent losses of Ca^{2+} and Mg^{2+}, which are the major counter ions for nitrate in soil solution. In the present experiment, Ca^{2+} and Mg^{2+} decreased following a trend very similar to nitrate (data not shown). In our assay, this can be explained by the fact that as nitrate disappears from the soil solution, equivalent amounts of Ca and Mg must no longer be in solution.

In treatments with plants, nitrate drastically decreased around GS21 (Figure 3.5a and Figure 3.5c), occurring five more days later in HM than in CM and DS likely due to its composition (Table 3.2). The GS21 stage, when lateral tillers start emerging in wheat, is considered a key time concerning N fertilization as it is the beginning of plant biomass accumulation (Satorre and Slafer, 1999). From GS21 until harvest, the nitrate concentration in the soil solution was nearly negligible because wheat plants absorbed nitrate as it was released. Villar (2014) also found low nitrate concentrations (<20 mg L^{-1}) in the soil solution after GS30 in a pot experiment where wheat was grown, and different rates of sewage sludge were applied. The high diffusion coefficient of nitrate and its much higher concentration in comparison with other N forms makes nitrate readily available for plant roots (Miller and Cramer, 2005). Because wheat plants rapidly absorbed nitrate from the soil solution, the treatments without plants allowed us to observe that mineralization was higher when HM was applied, followed by DS and CM. It should be mentioned that mineralization can be enhanced by plant roots (Herman *et al.*, 2006). The highest wheat grain yields, wheat N uptake values at harvest, and GPC (%) values were achieved with the HM treatments, where the highest nitrate values were measured at both N rates, followed by DS and CM. The lack of differences in HM yields between both N rates might be attributed to the pot size, which might have limited wheat growth because the maximum nitrate concentrations were much higher at the highest N rate. In HM, wheat crop N uptake at harvest (kg N ha^{-1}) and GPC (%) were significantly higher at the highest N rate applied (Table 3.4), which might suggest that in some periods, although nitrate values in soil solution drastically decreased around GS21 (Figure 3.5a and Figure 3.5c), the N uptake by the plants at the highest N rate was higher than that at the lowest rate. In DS, the highest N treatment achieved higher yields. Four days before GS21, the highest N rate in the DS treatments had significantly higher nitrate concentrations than the lowest N rate, which might have promoted wheat tillering. Tillering has been reported to be important for reaching adequate wheat yields because good tiller establishment allows a greater potential for biomass accumulation (Magney *et al.*, 2016). In CM, differences in nitrate concentrations at the different N rates were not as high as in DS or HM due to the mentioned recalcitrant composition and low ammonium availability (Table 3.2), thus explaining the lack of differences in yield, wheat crop N uptake at harvest and the GPC values. As in the present experiment, Shah *et al.* (2013) and Delin and Engström (2010) reported that crop N uptake was higher from poultry manure than from dairy manure and dairy slurry. Following the effect of the wheat plant on the nitrogen dynamics, when the N availability was lower for the plants, urease enzymatic activity increased at GS13 and at preharvest for the three types of manure without differences among them (Figure 3.2c). Follmer (2008) mentioned that the urease enzyme can be produced by plants, while Jonnasson *et al.* (2006) reported that plants can enhance the rate of N turnover in the rhizosphere because root excretions stimulate soil microbial biomass and activity (Bais *et al.*, 2006). Herman *et al.* (2006) also mentioned that microbial-root interactions are likely involved in accelerating the flux of N from organic sources to the plant available ammonium pool. These data suggested that when wheat plants had low N availability, urease activity increased to enhance N transformation from organic nitrogen to ammonium. Furthermore, that effect would be time-persistent because at preharvest, the nitrate concentrations in soil solution in the treatments with plants were close to 0 mg mL^{-1} at both N rates while the activity of urease remained higher at the lowest N rate. The time persistence of urease activity in the soil might be explained by its stabilization in the soil matrix as Lorenz and Dick (2011) reported. However, differences in ammonium and nitrate between rates were only significant in HM and DS but not in the case of CM. Although nitrate is considered the preferred N form taken up by wheat (Miller and Cramer, 2005), it has been reported that organic N forms, such as amino acids (Gioseffi *et al.,* 2012; Owen and Jones, 2001) or even peptides (Hill *et al.*, 2011), are present in the soil solution and may constitute a significant source of N for wheat when mineral N concentrations are low (Gioseffi *et al.*, 2012). When CM is applied, most of the N enters soil as proteins (Chadwick *et al.,* 2000), which are hydrolysed to peptides and to amino acids by protease enzymes. As mentioned, the protease enzyme presented higher activity values after sowing in CM than in DS and HM at the highest N rate (Figure 3.2a). Because higher concentrations of a N source (such as peptides or amino acids) other than nitrate or ammonium in the soil solution are possible, the highest N rate of the CM treatment might justify the higher urease activity in the lowest N treatment of CM. Even if there was a higher presence of amino acids in the CM treatments with the highest N rate, increased urease enzyme activity was observed and the wheat yield or GPC did not differ at harvest between the nitrogen rates. Nacry *et al.* (2013) mentioned that in studies supplying organic and inorganic forms of N, the uptake rates of amino acids by plants are only slightly affected by N treatments in comparison to nitrate and ammonium uptake. Similar to our results, Reddy *et al.* (1987) found that the application of sludge reduced urease activity in soils without plants but increased urease activity in soils with plants, and they pointed out that if sludge application was higher (120 t ha^{-1} vs. 40 t ha^{-1}), reduced urease activity might be due to the application of higher levels of available heavy metals. In the present study, heavy metals were always lower than the maximum value established in the Royal Decree RD 849 (1986) for the Regulation on Public Water Domain, although more heavy metals was applied at the higher N dose.

As shown in our results, the three manures had different mineralization dynamics, which implies that the time of application should be different. When applying livestock manure containing a highly readily available N pool such as HM, plant N requirements could be met by applying manure adjusted to the crop demand (Whalen *et al.,* 2019). However, manures with these characteristics are applied when the crop N demand is low or if the applied rate is too high, which might cause N losses to the atmosphere or groundwater (Delgado, 2002). In Araba (Basque County, Northern Spain), manures are typically applied as basal dressing before winter cereal sowing (usual rate, 40 t ha^{-1}) due to the humid climate in winter and spring, which usually hinders the entrance of heavy machinery to fields. Consequently, it is not recommended to apply poultry manures at sowing. Delin (2011) mentioned that barley yields were higher when poultry manure was applied in early spring than when it was applied at sowing. In the case of slurry, due to its high ammonium content, it should also be applied as close to crop demand as circumstances allow (Delin and Engström, 2010; Whalen *et al.*, 2019). Finally, the N released at both N rates (even under the highest) is limited to the early stages of the crop cycle. From a very early stage (at stem elongation), when the crop N demand starts to be high, the amount of N released from the organic fertilizer is low, suggesting that a topdressing application with mineral N would be necessary, especially when high grain protein contents want to be achieved. The level of nitrate provided by CM manure is lower than that in HM and DS and therefore the risk of N losses is reduced, which makes it a more suitable fertilizer for winter crops, such as wheat.

The initial N availability and the dynamics of mineral N release from livestock manures are also determined by the properties of the soils to which manures are applied (Shah *et al.,* 2013; Whalen *et al.,* 2019). Consequently, the findings from this study provide recommendations on the livestock manures and soil type used in this study, which is a Calcixeroll and represents approximately 5% of European soils, mainly in the southern areas (JRC, 2005).

3.5 Conclusion

The HM treatment produced the highest yields and GPC (%) values, followed by DS and CM due to the higher availability of the N applied. Protease and amidase enzyme activities increased just after the application of CM and DS at the highest N rate, and urease activity increased with the presence of wheat plants at the lowest N rate (170 kg N ha^{-1}). When applying HM, ammonium in the soil solution increased initially and the nitrate concentrations were also highest, followed by DS and CM. However, the nitrate values decreased in the treatments with plants at the beginning of tillering and remained at the same level as the control until harvest. Thus, when plants had a high N demand (beginning of stem elongation), the nitrate and ammonium concentrations were very low. Therefore, even when a high N rate is applied with organic fertilizers, the crop N demand is not satisfied. However, the necessary amount of mineral N at topdressing to fulfil the crop N demand after the application of organic fertilizers would be lower in the case of HM, followed by DS and CM.

As determined in this study, the three livestock manures have different mineralization dynamics, which implies that the time of application should be different. HM and DS should be applied in spring crops or near the time of maximum uptake in winter crops because they have high contents of uric acid and ammonium, respectively. Both compounds can be rapidly transformed to nitrate, which can be easily lost through leaching unless absorbed by the crop. In contrast, CM is more suitable for basal application in winter crops due to the low nitrate level released, which reduces the risk of losses to the environment but also satisfies crop N needs.

3.6 Appendices

Table A3.1. Ducan's test ($p < 0.05$) results of the differences in protease, amidase, urease enzymatic activities when ANOVAs were significant.

Protease Sowing	CM-170-P	ns	CM-340-P	A +
	HM-170-P	ns	HM-340-P	B
	DS-170-P	ns	DS-340-P	B
	CM-170-NP	ns	CM-340-NP	A +
	HM-170-NP	ns	HM-340-NP	B
	DS-170-NP	ns	DS-340-NP	B
Amidase Sowing	CM-170-P	ns	CM-340-P	B
	HM-170-P	ns	HM-340-P	B
	DS-170-P	ns	DS-340-P	A *
	CM-170-NP	ns	CM-340-NP	B
	HM-170-NP	ns	HM-340-NP	B
	DS-170-NP	ns	DS-340-NP	A *
Urease GS13	CM-170-P	ns + ●	CM-340-P	ns
	HM-170-P	ns # ●	HM-340-P	ns
	DS-170-P	ns * ●	DS-340-P	ns
	CM-170-NP	ns	CM-340-NP	ns
	HM-170-NP	ns	HM-340-NP	ns
	DS-170-NP	ns	DS-340-NP	ns
Urease Preharvest	CM-170-P	ns + ●	CM-340-P	ns
	HM-170-P	ns # ●	HM-340-P	ns
	DS-170-P	ns * ●	DS-340-P	ns
	CM-170-NP	ns	CM-340-NP	ns
	HM-170-NP	ns	HM-340-NP	ns
	DS-170-NP	ns	DS-340-NP	ns

CM, cattle manure; HM, hen manure; DS, dairy slurry. 170, 170 kg N ha^{-1} application rate; 340, 340 kg N ha^{-1} application rate. P, treatments with plants; NP, treatments without plants (bare soil). Capital letters represent differences among three manures for each N rate and presence of plants or absence plants. $^{+}$, significant higher mean between both N rates in CM and presence of plants or absence of plants; $^{\#}$, significant higher mean between both N rates in HM and presence of plants or absence of plants; *, significant higher mean between both N rates in DS and presence of plants or absence of plants. ●, significant higher mean between presence of plants and absence of plants for three fertilizers (CM, HM, DS) and for two rates (170 and 340 kg N ha^{-1} rate).

Chapter 4

Crop sensor-based in-season nitrogen management of wheat with manure application

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4 Crop sensor-based in-Season nitrogen management of wheat with manure application

4.1 Introduction

Few cereal agroecosystems supply enough nitrogen (N) to sustain satisfactory crop production without fertilizers. To ensure that the potential yield is reached each year, fertilizers are often applied in excessive quantities, causing N loss to the atmosphere and water. In agricultural systems, mineral N is mainly lost through ammonia volatilization, denitrification and leaching (Cameron *et al.*, 2013). Correct dose and application timing of N fertilizer is important so that crops make best use of the N applied with minimum risk of losses and adverse environmental impacts.

Agronomic decisions in cereals are implemented by using a growth-stage key which provides a common reference for describing crop development. Management by growth stage is critical to optimize N fertilization strategies. The Zadoks Cereal Growth-Stage Key (Zadoks *et al.*, 1974) is the most commonly used growth-stage key for cereals, in which the development of the cereal plant is divided into 10 distinct development phases covering 100 individual growth stages. Individual growth stages are denoted by the prefix GS (growth stage). The principle Zadoks growth stages used in relation to N management are the beginning of tillering (GS21) and beginning of stem elongation (GS30). GS21 is the stage when tillers (lateral shoots emerging at the base of the main stem of the plant) start emerging. Each tiller has the potential to produce a spike. GS30 is the stage in which the final spikelet can be observed within the stem of the main tiller.

The application of fertilizers consists of two methods, basal application and topdressing application. In the former, fertilizers are distributed over the field and mixed with soil before sowing. In the latter, fertilizers are applied in the soil surface. In cereal crops, local farmers commonly apply organic manures as basal fertilizers rotationally every two or three years. Organic manures are typically applied as a basal dressing before sowing due to the humid climate in winter and spring that usually hinders the entrance of machinery to fields. The application of organic fertilizers is generally combined with the application of mineral fertilizers. Conversely, when organics are not applied as initial fertilizers, mineral N is applied in two topdressing applications: 40 kg N ha^{-1} at GS21 to encourage tillering and a second and greater application at GS30.

Organic manure is a heterogeneous material collected from livestock raising facilities. The manure's physical status depends on farm practice and storage conditions. Farmyard manures are solids that are commonly mixed with lignocellulosic bedding materials. Slurries are liquids generated by mixing the solid manures with wastewater, washing water or urine. In recent decades, with the rise in livestock industry, the disposal of these farmyard manures and slurries has increased (FAO, 2005). There is an excess of manure in many regions around the world, and the amount of manure available for land application is increasing (Whalen *et al.*, 2019). The application of these manures into agricultural soil allows the recycling of their nutrient value for fertilizing crops and increasing soil fertility, making better use of resources and economic sense (Defra, 2017). However, organic manures can pose a considerable environmental risk if they are not carefully treated and applied. It is important to point out that the N applied with the organic manures is generally less available to crops than N in mineral fertilizers (Defra, 2017). In addition, it is difficult to predict the amount of available N from organic manures for crops since nutrient mineralization from manures is influenced by manure characteristics, soil and environmental temperature, soil moisture, soil properties and microbial activity (Cameron *et al.*, 2013). The capacity of the soil itself must be taken into account in order to provide N to crops and to improve cereal N fertilizer recommendations. In this sense, soil organic matter (SOM) is one of the most relevant soil compounds, as it has been reported that SOM accounted for 78% of the variation in mineralizable N, whereas other soil properties only accounted for 8% (Ros *et al.*, 2011). N mineralization in SOM is enhanced by increasing temperature (Eghball, 2000) and when soil moisture is near field capacity (Eghball *et al.*, 2002). It must not be forgotten that the weather varies considerably from year to year, causing large differences in yield potential in the same site (Arregui *et al.*, 2006; Basso *et al.*, 2012); consequently, crop N fertilizer demand widely varies.

In Western Europe, a soil test for estimating available mineral N (ammonium plus nitrate) in the soil profile has been widely used to measure the quantity of N fertilizer which needs to be applied. The technique is called N_{min} method. Generally, for winter wheat (as for many other crops), crop N need is calculated at the end of winter or early spring based on a target yield. The recommended rate of N fertilizer is calculated by the predicted N demand for the target yield minus the measured soil N_{min} value at the end of winter, where the rapid period of crop growth starts. Even if soil analysis should give reliable information, it is often perceived as imprecise for several reasons (Ravier *et al.*, 2016). In fact, translating a few values of the soil samplings to a heterogeneous field makes the method imprecise. Apart from that, both the sampling and the determination of N_{min} require time-consuming procedures.

The plant itself is considered a relevant indicator of N availability from any origin (organic manure, soil N supply or mineral fertilizer) within the growing season. Strategies based on plant indicators have been developed for the adjustment of N fertilizer application during the growing season. Ravier *et al.* (2016) showed some decision rules for determining N fertilizer application on the basis of the crop Nitrogen Nutrition Index (NNI). The NNI is calculated relative to the critical N concentration of the aerial parts of the crop defined as the minimal concentration required for the maximum production of aerial dry matter (Ravier, 2017c). However, NNI determination requires destructive and time-consuming procedures of the plant N content and crop biomass which make it impractical for farmers.

Various types of optical sensing tools have been developed for assessing the N status of plants within the growing season (Antille *et al.*, 2018; Diacono *et al.*, 2013). However, these tools are unable to measure N content of the crop directly (Raun *et al.*, 2002), therefore they are based on the measurement of compounds, such as chlorophyll (Ravier *et al.*, 2017c). Optical crop sensing is relatively easy to perform, and a range of sensing techniques and sensors are commercially available (Antille *et al.*, 2018). Regarding transmission or absorption sensors, hand-held chlorophyll meters such as Yara N-Tester^{TM} can provide rapid results for diagnostic purposes (Diacono *et al.*, 2013; Samborski *et al.*, 2009). Chlorophyll meter readings have been widely proven to be well correlated with leaf chlorophyll and N concentrations in wheat; therefore, chlorophyll content can be used to diagnose the N status of plants (Lemaire *et al.*, 2008; Ortuzar-Iragorri *et al.*, 2005; Piekelek and Fox, 1992), making them interesting tools for modulating the N rate. Furthermore, they can be used to decide whether a supplementary dose is to be applied in order to increase grain N content (Eghball *et al.*, 2002; Ortuzar-Iragorri *et al.*, 2017. The use of chlorophyll meter measurements as an alternative to the NNI has been tested (Prost and Jeuffroy, 2007; Debaeke *et al.*, 2006; Ravier *et al.*, 2017a).

When considering reflectance sensors, ground-based active crop canopy reflectance sensors have been identified as potentially valuable tools for site-specific N management in cereals (Shanahan *et al.*, 2008; Ali *et al.*, 2015; Mulla, 2013), because these sensors are not affected by clouds, unlike aerial or satellite sensing. Ground-based sensors have been developed to assess the plant nutritional status and guide variable-rate N application for different grain crops (Raun *et al.*, 2002; Ravier *et al.*, 2017; Cao *et al.*, 2015). Spectral data collected by RapidScan CS-45 is converted into canopy green area measurements by calculating vegetation indices such as the Normalized Difference Vegetation Index (NDVI) or Normalized Difference Red Edge (NDRE). Marti *et al.* (2007) found significant correlations between the NDVI, yield and biomass in wheat. Lu *et al.* (2017) determined that RapidScan CS-45 was useful for non-destructively estimating the NNI of rice and Li *et al.*, 2018 (2018) showed a great potential for monitoring rice leaf N status. Similarly, Bonfil *et al.*, (2017) showed that the use of RapidScan CS-45 allows rapid and accurate crop monitoring and yield estimation. Zhang *et al.* (2019) predicted grain yield based on RapidScan CS-45 measurements. In a previous study, we demonstrated that the normalized readings of the proximal sensing tools, Yara N-Tester^{TM} (chlorophyll meter) and RapidScan CS-45 (ground-based active-light proximal sensor), were good indicators of the N nutritional status of the plant, as the NNI or N_{min}. The mineral fertilizer rate applied at stem elongation (GS30, following Zadoks *et al.*, 1974) could be modulated with the use of those tools (Aranguren *et al.*, 2018).

In this study, we annex a third year when the weather conditions in spring after the second and greater N application at GS30 were very dry (it did not rain in the following 20 days after application) in comparison with the first two years. The climate of the area is Temperate–Mediterranean according to the temperature regime of the Papadakis classification and humid Mediterranean according to the water regime (Papadakis, 1966), with an average rainfall of 779 mm year^{-1} and an average annual temperature of 11.5 °C. This climate covers a great portion of the territory of Araba (The Basque Country, norther Spain) and, although it is classified as Mediterranean, maritime and temperate characteristics are very evident (Euskalmet, 2018). In this area, three of the ten years have dry periods around the second and greater application at GS30 (media 1978–2017; period 15 March–1 May), as in the third year of this study. Weather conditions are variable from year to year (Aranguren *et al.*, 2018) and the mineralization of nutrients from SOM and farmyard manures is influenced by many factors such as manure characteristics, soil and air temperature, soil moisture, soil properties and microbial activity (Eghball, 2000). Therefore, making the prediction of the N needed by the crop in different situations is necessary but complicated. Yara N-Tester^{TM} and RapidScan CS-45 have shown promise for making N recommendations (Lemaire *et al.*, 2008; Piekelek and Fox, 1992; Ortuzar-Iragorri *et al.*, 2017; Lu *et al.*, 2017; Li *et al.*, 2018; Zhang *et al.*, 2019) and provide a practical and affordable option for on-farm implementation. The aim of this study was to assess the usefulness of the proximal sensing tools, Yara N-Tester^{TM} and RapidScan CS-45, for adjusting the optimum N rate at GS30 when farmyard manures are applied before sowing in the variable humid Mediterranean climate conditions. We also aimed to decide whether the 40 kg N ha^{-1} rate at GS21 is necessary when organic manures are applied as basal fertilizers. In addition, the utility of a reference area on the field with non-limited N supply is questioned (Diacono *et al.*, 2013), making the diagnosis more complicated for farmers.

4.2 Materials and Methods

Three field trials were established in Arkaute (Araba, Basque Country, Spain) at NEIKER-Tecnalia facilities (42°85’N, 2°62’W; elevation 515 m above sea level) in three consecutive wheat-growing seasons: 2014–2015, 2015–2016 and 2016–2017 (defined as 2015, 2016 and 2017) in different fields under rainfed conditions. There was a 130 meter (m) distance between the three field trials. In the field trial carried out in 2015, a soil pit was made and after describing and analyzing its horizons, the soil was classified as Hypercalcic Kastanozem (IUSS, 2014). The mineralogical properties of the soil were analyzed by X-ray diffraction. The soil contained 40% clay (clay < 2 µm: Illite (%) = 30; Kaolinite (%) = 12; Smectite (%) = 58). In the field trials carried out in 2016 and 2017, several prospective holes were observed, verifying that the three soils had similar characteristics. The three fields were flat.

Representative soil samples were taken from each field trial to analyze the physical and chemical properties before wheat sowing from depths of 0–30 and 30–60 centimeter (cm). Soil texture was analyzed by the Pippete method (Gee and Bauder, 1986) and classified (0–30 cm, sandy clay loam and 30–60cm, clay loam) (SSS, 1999). Soils that had high pH values (1:2.5 soil:water using a pH-Meter CG840; 8.0–8.5), were calcareous (3.6–58% according to soil depth) (MAPA, 1994) and had moderate organic matter content (Walkey and Black, 1934) in the upper layer (2–2.5%).

4.2.1 Experimental Setup and Treatments

In the area (Figure 4.1) where this study was carried out (Araba, Basque Country, norther Spain), the application of organic fertilizers is generally combined with the application of mineral fertilizers. Farmyard manures are typically applied as a basal dressing before sowing. For mineral fertilizers, an application is made at GS21 (Zadoks *et al.*, 1974) and the last and greater mineral N dressing application is made at GS30 (Zadoks *et al.*, 1974).

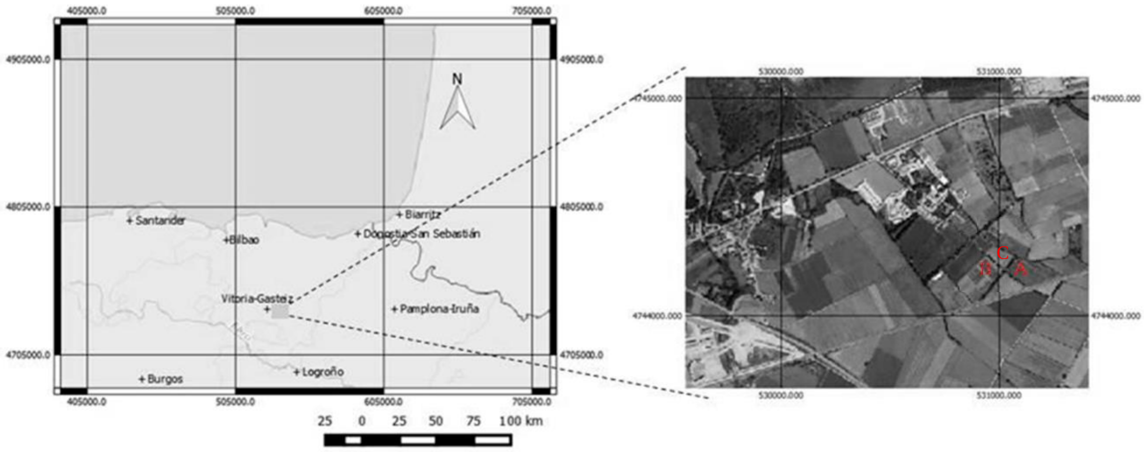


Figure 4.1. Location of the study (Arkaute, Araba) in the Basque Country, northern Spain. Letters “A”, “B” and “C” represent the field trials in the 2015, 2016 and 2017 wheat-growing seasons, respectively.

Three kinds of initial fertilization were applied: dairy slurry (40 tons per hectare t ha^{-1}), sheep farmyard manure (40 t ha^{-1}) and conventional treatment (no organic fertilizer basal dressing and 40 kg N ha^{-1} at tillering). These three types of fertilization were combined with five N rates (calcium ammonium nitrate, NAC 27%) in the topdressings applied at GS30 (0, 40, 80, 120 and 160 kg N ha^{-1}). Apart from the treatments, two controls were established: a control without N fertilization (0N) and an overfertilized control plot (80 kg N ha^{-1}; 80 kg N ha^{-1} applied at tillering and 200 kg N ha^{-1} applied at GS30; Table 4.1). The experiment was a factorial randomized complete block design with three factors (year, initial fertilization and N rate at GS30) and four replicates. The area of each plot was 4 m in width and 8 m in length.

Organic amendments were applied on 13 November 2014, 4 November 2015 and 17 November 2016. Slurry and manure were sampled and analyzed for total N and NH_4^+ (Table 4.1). We decided to apply manure at 40 t ha^{-1} and slurry at 40 t ha^{-1} because this is the usual rate at which organic amendments are applied as initial fertilizers in Araba, Basque Country, Spain.

Wheat (*Triticum aestivum* var. Cezanne) was sown on 24 November 2014, 06 November 2015 and 18 November 2016, and was harvested on 21 July 2015, 2 August 2016 and 2 August 2017. The sowing rate was 220 kg seed ha^{-1}. The preceding crops were flax (*Linum usitatissimum*), rapeseed (*Brassica napus*) and wheat (*Triticum aestivum*), respectively.

4.2.2 Mineral N Samples (Nmin) and Biomass Samples for the Nitrogen Nutrition Index (NNI)

Three soil samples from replicates I, II and III (there were four replicates per treatment) and three rows of aboveground biomass of wheat of one meter in length (four replications) were taken for each kind of initial fertilization treatment (dairy slurry, sheep farmyard manure and conventional treatment), in 40N + 0N, DS + 0N and SM + 0N treatments (Table 4.1) at 0–30 at two times: (1) At GS21 (09 March 2015, 19 January 2016 and 02 March 2017) just before mineral N fertilization in the conventional treatment; (2) at GS30 (04 April 2015, 17 March 2016 and 06 April 2017) just before mineral N fertilization. The soil samples were analyzed for soil mineral nitrogen (NH_4^+ plus NO_3^-) by spectrophotometry (Cawse, 1967; Nelson, 1983).

Table 4.1. N application rates and timing for three initial fertilization treatments for field trial (2015, 2016 and 2017) and control (0N) and overfertilized (280N) plots. GS21 is the beginning of tillering (end of winter (Zadoks *et al.*, 1974)); GS30 is the beginning of stem elongation (Zadoks *et al.*, 1974).

	2015		2016		2017		2015-2016-2017		
Initial fertilization	**Total N[a] (kg ha^{-1})**	**N-NH_4^{+}[b] (kg ha^{-1})**	**Total N[a] (kg ha^{-1})**	**N-NH_4^{+}[b] (kg ha^{-1})**	**Total N[a] (kg ha^{-1})**	**N-NH_4^{+}[b] (kg ha^{-1})**	**Topdressing at GS21 (kg N ha^{-1})**	**Topdressing at GS30 (kg N ha^{-1})**	**Treatment identification**
Conventional [--]	--	--	--	--	--	--	40	0	40N+0N
								40	40N+40N
								80	40N+80N
								120	40N+120N
								160	40N+160N
Dairy Slurry (DS) [40 t ha^{-1}]	192	104	144	80	120	68	--	0	DS+0N
								40	DS+40N
								80	DS+80N
								120	DS+120N
								160	DS+160N
Sheep manure (SM) [40 t ha^{-1}]	336	0	592	200	448	--	--	0	SM+0N
								40	SM+40N
								80	SM+80N
								120	SM+120N
								160	SM+120N
Control	--	--	--	--			--	--	0N
Overfertilized	--	--	--	--			80	200	280N

[a]Total N (dry combustion using a LECO TruSpec®CHNs, [b]Kjeldahl digestion

Fresh biomass samples were weighed and oven dried, and the dried biomass samples were again weighted for dry matter content determination. Biomass was estimated and N concentration was determined by Kjeldahl's method (AOAC, 1999 to calculate the NNI (4.1) following Lemaire *et al.* (2008):

$$NNI = Na/Nc \tag{4.1}$$

The NNI was calculated as a ratio of plant N concentration (N_a) and the critical N concentration (N_c) in aerial biomass (shoots). N_c (4.2) is defined as the minimum concentration of N necessary to achieve maximum aerial biomass at any stage of vegetative growth (Lemaire *et al.*, 1984). For the wheat critical N concentration, dilution curves were developed for winter wheat in France (AOAC, 1999) as a universal relationship. In 2012, another was built in China (Yue *et al.*, 2012) with the aim of adjusting the original to the conditions in China. Since we did not have sufficient data to develop a specific function for our region, we used the original dilution curve developed by Justes *et al.* (1994) due to the proximity to France.

$$Nc = 5.35 * DM^{-0.442} \tag{4.2}$$

where DM is the amount of dry matter accumulated in the aerial biomass expressed in t ha^{-1}. Nc is expressed in % DM.

4.2.3 Proximal Sensing Tools for Adjusting the Optimum N Rate at GS30

The proximal sensing tools for the diagnosis of the N nutritional status tested were Yara N-Tester™ (Yara International ASA, Oslo, Norway) and RapidScan CS-45 (Holland Scientific, Lincoln, NE, USA). Yara N-Tester™ is a clip-on hand-held chlorophyll meter which measures light transmitted by the plant leaf at two different wavelengths, 650 (red light) and 940 nm (near infrared light, NIR). The ratio of the light transmitted at these wavelengths, in addition to the ratio determined with no sample, is processed by the instrument to produce a digital reading. The measurement point should be in the middle of the blade of the youngest, fully developed leaf. The values obtained are unitless and they express relative chlorophyll content. Thirty random measurements are recorded to get the representative value in each sampling point. RapidScan CS-45 is a portable entirely self-contained ground-based active crop canopy sensor that integrates a data logger, graphical display, GPS, active crop sensor and power source into a small and compact instrument. It measures crop reflectance at 670, 730 and 780 nm and provides the NDVI and NDRE. The measurements with RapidScan CS-45 were taken as the sensor was passed over the crop surface at approximately 1 m at constant walking speed. The sensor´s unit was handheld and two rows per elemental plot were scanned. NDVI and NDRE values were averaged to generate a value for that plot.

Measurements with the proximal sensing tools were taken in four replications in each kind of initial fertilization treatment (dairy slurry, sheep farmyard manure and conventional treatment) just before applying the topdressing at GS30. Measurements were taken in 40N + 0N, DS + 0N and SM + 0N treatments (Table 4.1). In addition, samples were taken in the control (0N) and overfertilized treatments (280N). We will refer to the Yara N-Tester^{TM} absolute values as abs_NTester and the RapidScan CS-45 absolute values as abs_NDVI and abs_NDRE. The measurements with Yara N-Tester^{TM} and RapidScan CS-45 were taken as described in Aranguren *et al.* (2018).

Normalized values for the Yara N-Tester^{TM} and RapidScan CS-45 measures were calculated to avoid the noise encountered by variables other than N fertilizer. These values were calculated as a percentage by assigning the 100% value to the overfertilized treatment (280N) described previously, similar to the technique suggested by Follett and Follett (1992). We will refer to the Yara N-Tester^{TM} normalized values as nor_NTester and the RapidScan CS-45 normalized values as nor_NDVI and nor_NDRE.

4.2.4 Grain Yield

Yields were recorded at crop maturity using a plot harvester (1.5 × 8 m; Wintersteiger AG, Ried, Austria). For comparisons between fields, yields were converted to a 12% dry matter basis.

To show grain yield response to N fertilization at GS30, the grain yield was determined by fitting a Quadratic Plateau Function. It has been shown to best describe the yield response to N fertilization in humid Mediterranean climate conditions of Araba (Basque Country, northern Spain) after comparison with other models (quadratic and square root) as was shown by Ortuzar-Iragorri *et al.* (2010), who selected those models because other authors reported that they were frequently used for studying the relationship between yield and N fertilization. Ortuzar-Iragorri *et al.* (2010) used the NLIN procedure (SAS Institute 1998) to adjust the data to the proposed models to obtain statistical parameters. The most important attribute of the function is where yield becomes relatively insensitive to increases in N fertilizer addition at GS30. The optimum N rate was determined following Ortuzar-Iragorri *et al.* (2010). In the neighborhood region of Navarra (northern Spain), with similar climatic conditions, Arregui *et al.* (2006) also used a Quadratic Plateau Function for wheat yield response to N fertilization. In the case of corn, a Quadratic Plateau Function was also shown to be the best model for yield response to N fertilization (SAS, 1998; Roberts *et al.,* 2012; Scharf *et al.,* 2006). A Quadratic Plateau Function (4.3) was used to indicate the optimum N rate at GS30 to achieve the maximum yield (yield vs. N fertilizer rate at GS30):

$$y = a + bN + cN^2 \qquad (4.3)$$

where *y* is the dependent variable (yield, kg ha^{-1}), N is the N rate applied at GS30 (kg N ha^{-1}) and a, b and c are coefficients.

The equations obtained with the Quadratic Plateau Function were used to determine the economically optimal dose of N (4.4), following the technique suggested by Aizpurua *et al.* (2010). According to their findings, the revenues obtained can be calculated as:

$$Revenue\ yield = w\,Y - fN \qquad (4.4)$$

where *w* is the wheat price (€ kg^{-1}), *f* is the fertilizer price (€ kg^{-1}), *N* is the nitrogen rate (kg ha^{-1}) and Y is the quadratic plateau function.

When the revenue yield is derived with respect to the N rate and equals to zero, maximum revenues would be obtained, and that would be the economical optimum rate based on yield. The wheat grain price used was 0.18 € kg^{-1}, and the fertilizer price was 1.19 € kg N (MAPAMA, 2017).

4.2.5 Statistical Analysis

The Quadratic Plateau Function was used for the wheat yield response. A nonlinear regression procedure was carried out using *R 3.2.5'* software (2013) to plot curves that best described the yield response to N fertilizer application.

Both soil mineral nitrogen (N_{min}) and NNI measurements were conducted before applying the N fertilization rates at GS21 and at GS30. The proximal sensing tool (Yara

N-Tester^{TM} and RapidScan CS-45) measurements (abs_NTester, abs_NDVI and abs_NDRE) were conducted before applying the N fertilization rate at GS30. Overfertilized plots were used to normalize absolute values (nor_NTester, nor_NDVI and nor_NDRE). The factors analyzed for statistical analysis in all cases were growing season and initial fertilization by analyses of variance (ANOVA) using *'R 3.2.5'* software (2013). There was a significant interaction between growing season and initial fertilization for all these measurements. Therefore, an ANOVA was performed to analyze differences among initial fertilization treatments in each growing season. Another ANOVA was performed to analyze the differences among the wheat-growing seasons in each initial treatment. To separate the means, the Tukey test was used ($p \leq 0.05$), utilizing the *R package agricolae* (De Mendiburu, 2009).

4.3 Results

4.3.1 Grain Yield

Optimum N Rate at GS30

The optimum N rate at GS30 was different for each kind of initial fertilizer (conventional treatment, slurry or sheep manure) in each wheat-growing season. In 2015 (Figure 4.2a), the optimum N rate at GS30 was 98 kg N ha^{-1} in the conventional treatment (plus 40 kg N ha^{-1} at tillering). In the organic treatments, the optimum N rate at GS30 was approximately 118 kg N ha^{-1}. Maximum wheat grain yields were 8456, 8240 and 8356 kg ha^{-1} for conventional treatments, slurry and manure, respectively.

In 2016 (Figure 4.2b), the optimum N rate at GS30 in the conventional treatment was 109 kg N ha^{-1} (plus 40 kg N ha^{-1} at tillering). In the organic treatments, the optimum N rate in the slurry treatment was 98 kg N ha^{-1} and in the manure treatment was 147 kg N ha^{-1}. Maximum wheat grain yields were 10,227, 10,271 and 10,723 kg ha^{-1} for the conventional, slurry and manure treatments, respectively.

In 2017 (Figure 4.2c), the optimum N rate at GS30 in the slurry treatment was 128 kg N ha^{-1} and in the manure treatment, it was 156 kg N ha^{-1}. In the conventional treatment, the maximum yield was not achieved. Therefore, the maximum rate applied at GS30 (160 kg N ha^{-1}) was taken as the optimum N rate at GS30. Maximum wheat grain yields were 5841 and 6205 kg ha^{-1} for slurry and manure treatments, respectively.

Economically Optimal Dose

In 2015 and 2016, the economically optimal dose at GS30 was almost the same as the optimum N rate at GS30. In 2015, the economically optimal dose was 98 kg N ha^{-1} for conventional treatment and 117 kg N ha^{-1} for organic amendments. In 2016, the economically optimal dose was 110, 100 and 141 kg N ha^{-1} for conventional, slurry and manure initial treatments, respectively. However, in 2017 the economically optimal dose at GS30 was lower than the optimum N rate at GS30 (131, 111 and 130 kg N ha^{-1} for conventional, slurry and manure initial treatments, respectively).

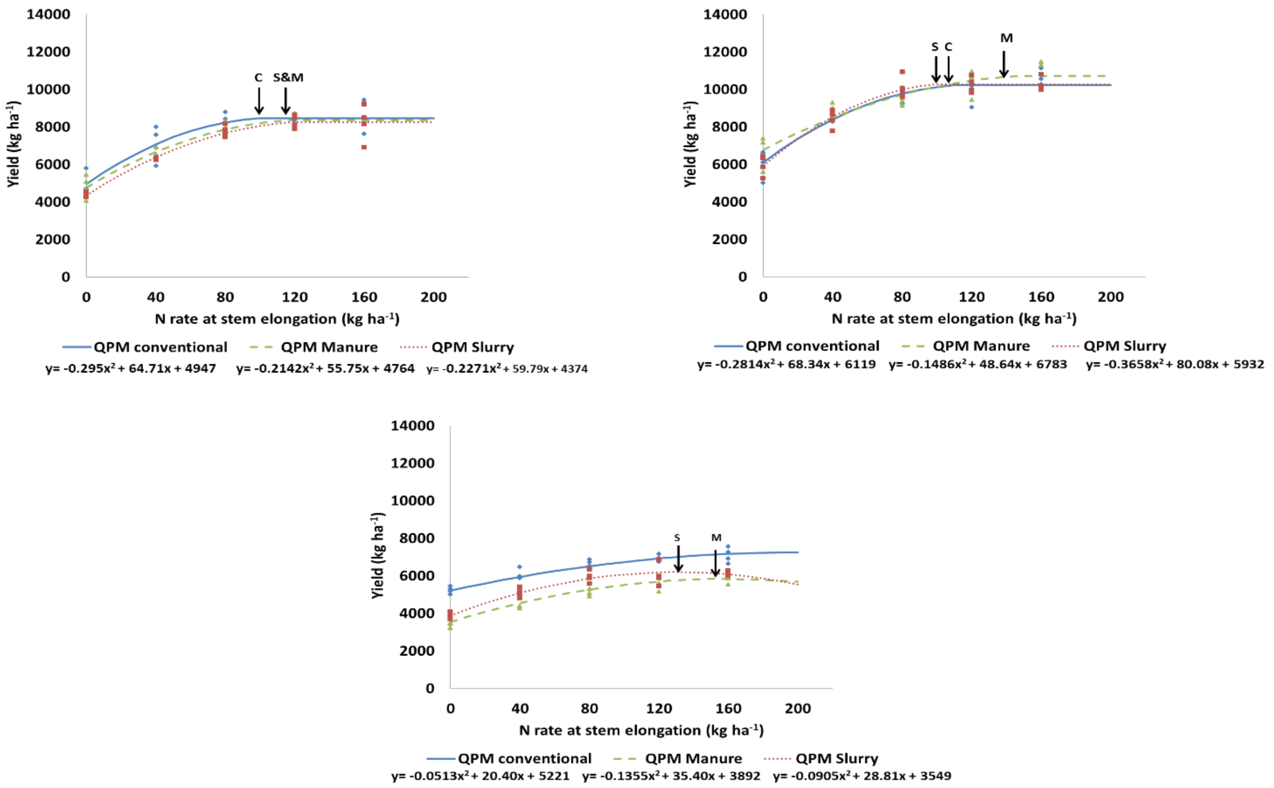


Figure 4.2. Effect of the N fertilization rate at stem elongation (GS30) on yield (kg ha^{-1}) in 2015 (**a**), 2016 (**b**) and 2017 (**c**); wheat-growing seasons with respect to initial fertilization: conventional treatment, dairy slurry, sheep manure. A quadratic plateau model was used to study the yield response. The arrow marked with C represents the N fertilization rate when the maximum yield was achieved by conventional treatment (blue line). S represents the N fertilization rate when the maximum yield was achieved by dairy slurry treatment (red line), and M represents the N fertilization rate when the maximum yield was achieved by sheep manure treatment (green line).

4.3.2 Soil Mineral Nitrogen (Nmin) and Total Rainfall

Before sowing, soil N_{min} in 2015 was 50 kg N ha^{-1}; in 2016, it was 42 kg N ha^{-1} and in 2017, it was 34 kg N ha^{-1} (Table 4.2). At GS21, N_{min} values (Table 4.2) were lower in 2015 than in 2016 and 2017 in organic fertilization treatments. During the 2016 and 2017 wheat-growing seasons, the lower rainfall (Figure 4.3) allowed higher N_{min} values (around 30 kg N ha^{-1}) at GS21 (Table 4.2). In 2015 at GS21, conventional treatment had significantly higher N_{min} values than dairy slurry and sheep manure (Table 4.2). At GS30, 0N and conventional treatment presented higher values in 2017 than in 2015 and in 2016. N_{min} values in 2016 were extremely low in all cases (Table 4.2). No differences among treatments were detected at GS30 in any growing cycle.

Table 4.2. Soil N_{min} content (kg N ha^{-1}; 0–30 cm) at the beginning of the three wheat-growing seasons (2015, 2016 and 2017), beginning of tillering (GS21) and beginning of stem elongation (GS30) in Arkaute.

Growing season	Treatments	Nmin (0-30cm; kg N ha^{-1})				
		Initial	**GS21**		**GS30**	
		Mean values	Mean values	sd	Mean values	sd
2015	0N	50	22 A	5	12 b	5
	40+0N		22A	5	13 b	4
	DS+0N		4 B b	1	13	9
	SM+0N		9 B b	3	12	5
2016	0N	42	30	9	1 c	1
	40+0N		30	9	3 b	1
	DS+0N		32 a	4	4	0
	SM+0N		30 a	2	1	4
2017	0N	34	33	12	26 a	3
	40+0N		33	12	32 a	13
	DS+0N		36 a	11	16	3
	SM+0N		16 ab	12	14	11

Means followed by a different capital letter indicate significant differences among initial treatments for each year (Tukey, $p \leq 0.05$). Means followed by a different lower-case letter indicate differences among each initial treatment for different years (Tukey, $p \leq 0.05$). Both 0N and 40 + 0N have the same value at GS21 because the measurement was taken before topdressing application in 40 + 0N at GS21.

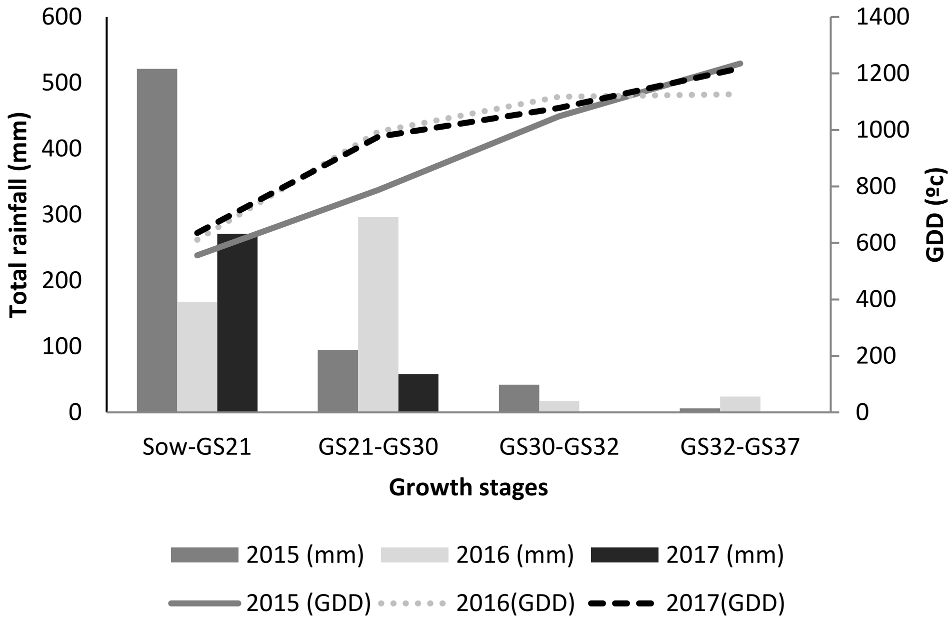


Figure 4.3. Total rainfall (mm) and growing degree days (GDD, ˚C) between wheat growth stages (Zadoks *et al.*, 1974) in three wheat-growing seasons (2015, 2016 and 2017) in Arkaute. Sow, sowing.

4.3.3 NNI

NNI values at GS21 (Table 4.3) in 2016 were significantly higher than in 2015 and 2017, and dairy slurry and sheep manure treatments had significantly higher values than the treatments without organics as initial fertilizer. However, there were no differences in 2015 and 2017 among initial fertilization treatments.

At GS30 (Table 4.3), in treatments with organics as initial fertilizer and in 0N, NNI values were significantly higher in 2016 than in 2015 and 2017. However, conventional treatment presented higher values in 2016 and 2017 than in 2015. There were significant differences in 2016 and 2017 among initial fertilization treatments. In 2017, the overfertilized plot presented the highest NNI followed by the conventional treatment and 0N and organic treatments. There were no differences between 0N and organic treatments. In 2016, the overfertilized plot presented the highest NNI and there no differences among the rest of the treatments. In 2015, there were no differences among initial fertilization treatments.

Means followed by a different capital letter indicate significant differences among initial treatments for each year (Tukey, $p < 0.05$). Means followed by a different lower-case letter indicate differences among each initial treatment for different years (Tukey, $p < 0.05$). ND means no data. sd means standard deviation. 0N, 40 + 0N and OverFert treatments have the same value at GS21 because the measurement was taken before topdressing application in 40 + 0N and OverFert at GS21.

Table 4.3. Nitrogen Nutrition Index (NNI) at the beginning of tillering (GS21) and at stem elongation (GS30) for three initial fertilization treatments, as well as control (0N) and overfertilized (280), in three field trials (2015, 2016 and 2017) in Arkaute.

Growing season	Treatments	NNI			
		GS21		GS30	
		Mean values	sd	Mean values	sd
2015	OverFert	0.37 b	0.02	ND	ND
	0N	0.37 b	0.02	0.26 b	0.03
	Conventional	0.37 b	0.02	0.35 b	0.09
	Dairy Slurry	0.34 b	0.05	0.37	0.08
	Sheep manure	0.38 b	0.06	0.29 b	0.02
2016	OverFert	0.60 B a	0.08	0.77 A a	0.10
	0N	0.60 B a	0.08	0.42 B a	0.04
	Conventional	0.60 B a	0.08	0.55 B a	0.01
	Dairy Slurry	0.67 A a	0.03	0.51 B	0.03
	Sheep manure	0.68 A a	0.05	0.51 B a	0.07
2017	OverFert	0.23 c	0.02	0.80 A	0.05
	0N	0.23 c	0.02	0.33 C b	0.03
	Conventional	0.23 c	0.02	0.53 B a	0.05
	Dairy Slurry	0.26 c	0.02	0.38 C	0.04
	Sheep manure	0.23 c	0.04	0.37 C b	0.05

4.3.4 Proximal Sensing Tools

4.3.4.1 Absolute Values

The absolute values of RapidScan CS-45 (abs_NDVI and abs_NDRE) at GS30 were significantly higher in 2016 than in 2015 and 2017 in all treatments (Table 4.4). The absolute values of Yara N-Tester^{TM} (abs_NTester) were significantly higher in 2016 than in 2015 (Table 4.4) in dairy slurry and sheep manure treatments. In the conventional treatment, there were no significant differences among years (Table 4.4). Finding values to adjust the optimum N rate with absolute values was complicated because variability among years was high.

Means with different lower-case letters (a, b) represent significant differences among initial treatments in Yara N-Tester™ (abs_NTester) measurements for each year (Tukey, $p \leq 0.05$); means with different capital letters in italics (*A*, *B*) represent significant differences among initial treatments in NDVI RapidScan CS-45 measurements (abs_NDVI) for each year (Tukey, $p \leq 0.05$); means with different underlined capital letters (A, B) represent significant differences among initial treatments in NDRE RapidScan CS-45 measurements (abs_NDRE) for each year (Tukey, $p \leq 0.05$); means with different lower-case letters (y, z) represent significant differences among the three years in Yara N-Tester™ measurements (abs_NTester) for each treatment (Tukey, $p \leq 0.05$); means with different capital letters in italics (*Y*, *Z*) represent significant differences among the three years for NDVI RapidScan CS-45 measurements (abs_NDVI) in each treatment (Tukey, $p \leq 0.05$) and means with different underlined capital letters (Y, Z) represent significant differences among the three years for NDRE RapidScan CS-45 measurements (abs_NDRE) in each treatment (Tukey, $p \leq 0.05$).

Table 4.4. Absolute values obtained with tools for the diagnosis of the N nutritional status (Yara N-Tester™ and RapidScan CS-45) at stem elongation (GS30) for three initial fertilization treatments and control (0 N) in three wheat-growing seasons (2015, 2016 and 2017) in Arkaute.

Growing season	**Treatments**	**abs_Ntester**		**RapidScan CS-45**			
				abs_NDVI		**abs_NDRE**	
		Mean values	**sd**	**Mean values**	**sd**	**Mean values**	**sd**
2015	0N	304b z	34	0.35 *B Z*	0.04	0.12 B Z	0.01
	40N	460 a	22	0.54 *A Z*	0.04	0.20 A Z	0.02
	DS	334 b z	16	0.39 *B Z*	0.04	0.14 B Z	0.02
	SM	332 b z	10	0.39 *B Z*	0.05	0.13 B Z	0.02
2016	0N	403 c y	8	0.55 *B Y*	0.05	0.18 B Y	0.02
	40N	477 a	14	0.67 *AY*	0.02	0.24 A Y	0.15
	DS	438 b y	15	0.65 *AY*	0.04	0.23 A Y	0.02
	SM	442 b y	28	0.66 *AY*	0.03	0.23 A Y	0.02
2017	0N	377 b z	13	0.39 *C Y*	0.06	0.12 B	0.01
	40N	507 a	54	0.59 *A Z*	0.03	0.19 A	0.01
	DS	382 b z	53	0.43 *B Z*	0.04	0.13 B	0.01
	SM	389 b z	12	0.41 *C Z*	0.04	0.13 B	0.01

4.3.4.2 Normalized Values

The normalized values of RapidScan CS-45 and Yara N-Tester™ (as a percentage compared to the overfertilized (280 N) treatment) were significantly higher in 2016 (Figure 4.4b) than in 2015 and 2017 (Figure 4.4a,c) in all treatments with the exception of nor_NTester values in the conventional treatment. In 2015 (Figure 4.4a), the normalized measurements of both tools agreed with each other for all treatments, showing differences between the conventional treatment (88%), initial organic fertilization treatments (65%) and 0N (57%). In 2016 (Figure 4.4b), Yara N-Tester™ showed differences between the conventional treatment (92%), control (78%) and both organic treatments (85%). RapidScan CS-45 showed differences between the conventional treatment (88%) and 0N (72%), but the tool did not detect differences between the conventional treatment and organic treatment (84–87%). In 2017 (Figure 4.4c), the measurements of both proximal tools detected differences between the conventional treatment (83–87%) and the remaining treatments (control and organics). In 2017, only nor_NDRE detected differences between control and organic treatments.

It was possible to find a relationship between normalized values of RapidScan CS-45 and Yara N-Tester^{TM} and optimum N rate at GS30 to achieve the maximum yield in the conventional treatment and in the slurry treatment (Table 4.5). When both proximal sensing tool values were 60–65%, the optimum N rate at GS30 to achieve the maximum yield was 118–128 kg N ha^{-1} in dairy slurry treatment (Table 4.5). When the readings were 85–90%, the optimum N rate was 100–110 kg N ha^{-1} in dairy slurry and conventional treatment (Table 4.5). In the sheep manure treatment, there was no clear relationship between sensor values and the optimum N rate. When values were 60–65%, the N recommendation was 117 or 155 kg N ha^{-1} and 147 kg N ha^{-1} for readings around 89%.

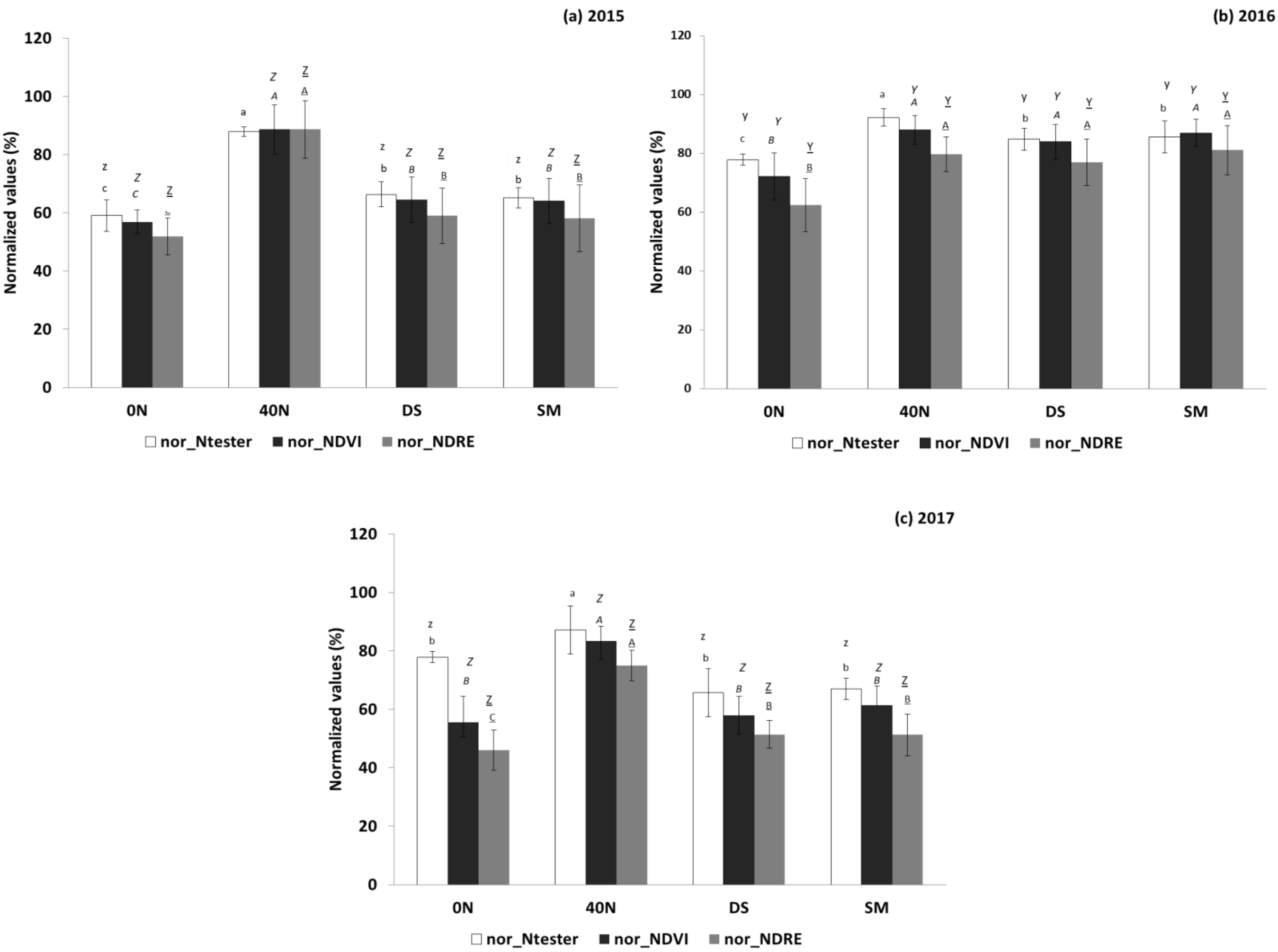


Figure 4.4. Relation between initial fertilization (conventional treatment (40N), dairy slurry (DS) and sheep manure (SM)) and control (0N) and values obtained with tools for the diagnosis of the N nutritional status (RapidScan CS-45, Yara N-Tester^{TM}) at stem elongation (GS30) in the 2015 (**a**), 2016 (**b**) and 2017 (**c**) wheat-growing seasons. Values were normalized assigning the 100% value to the overfertilized (280N) plot. Means with different lower-case letters (a, b) represent significant differences among initial treatments in Yara N-Tester^{TM} (nor_NTester) measurements for each year (Tukey, $p \leq 0.05$); means with different capital letters in italics (*A, B*) represent significant differences among initial treatments in NDVI RapidScan CS-45 measurements (nor_NDVI) for each year (Tukey, $p \leq 0.05$); means with different underlined capital letters (A, B) represent significant differences among initial treatments in NDRE RapidScan CS-45 measurements (nor_NDRE) for each year (Tukey, $p \leq 0.05$); means with different lower-case letters (y, z) represent significant differences among the three years in Yara N-Tester^{TM} measurements (nor_NTester) for each treatment (Tukey, $p \leq 0.05$); means with different capital letters in italics (*Y, Z*) represent significant differences among the three years for NDVI RapidScan CS-45 measurements (nor_NDVI) in each treatment (Tukey, $p \leq 0.05$) and

means with different underlined capital letters (Y, Z) represent significant differences among the three years for NDRE RapidScan CS-45 measurements (nor_NDRE) in each treatment (Tukey, $p \leq 0.05$).

Table 4.5. Normalized values (nor_Ntester, nor_NDVI, nor_NDRE) obtained with tools for the diagnosis of the N nutritional status (Yara N-Tester and RapidScan CS-45) at stem elongation (GS30) and their corresponding optimal N application at GS30 for dairy slurry and conventional treatments.

Initial fertilization	Proximal tool readings at GS30 (%)	Optimal N application at GS30 (kg N ha^{-1})
Dairy Slurry	60 - 65	118 - 128
Dairy Slurry/Conventional	85 - 90	100 - 110

4.4 Discussion

Studies focused on the use of proximal sensing tools for adjusting topdressing N in wheat have been conducted in other climates. In this region with variable rainfall, it was necessary to study the usefulness of proximal sensing tools for an optimum N mineral rate application when organic manures had been applied as basal fertilizers.

4.4.1 Mineral N Fertilizer Reduction When Organic Fertilizer Was Applied before Sowing

In this study, maximum wheat grain yields were different in each growing season with 2016 being the year with the highest yields. Within each year for 2015 and 2016, maximum yields between the conventional treatment and organics as initial fertilizers treatment were comparable (Figure 4.2a,b). However, in 2017, there were differences among initial fertilization treatments, with the conventional treatment being 1300–1700 kg ha^{-1} more productive than the organic treatments depending on each individual N rate at GS30 (Figure 4.2c). In this regard, the optimum N rate at GS30 was different for each growing season and for each initial fertilization treatment. In fact, the application of organic manures as initial fertilizers reduced the mineral N rate used at GS30 in 2015 and 2016. In 2015, when organic manures were used as initial fertilizers, approximately 20 kg N mineral ha^{-1}less than in the conventional treatment was necessary to achieve the maximum wheat grain yield. In 2016, when applying slurry as an initial fertilizer, 51 kg N mineral ha^{-1} less than in the conventional treatment was necessary to achieve the maximum wheat grain yield. In 2016, when using manure as initial fertilizer, the same mineral N dose as in the conventional treatment was necessary to achieve the maximum wheat grain yield. However, in the manure treatment, the maximum yield was 500 kg ha^{-1} higher than in the conventional treatment. According to results shown in 2015 and 2016, the 40 kg N ha^{-1} application at the GS21 could be avoided when organic manures were applied even when N_{min} at GS21 was very low (4–9 kg N ha^{-1}) as in 2015 (Table 4.2). In 2017, following mineral N application at GS30, it did not rain until leaf flag emergence (GS37 (Zadoks *et al.,* 1974); Figure 4.3). In 2017, yields were higher in the conventional treatment where 40 kg N ha^{-1} had been applied at GS21 even if N_{min} values at GS21 were high (Table 4.2).

Both economic and environmental costs are key considerations when N fertilization management strategies are being developed (Aizpurua *et al.,* 2010). The economically optimum N rate at GS30 was similar to the optimum N rate in 2015 and 2016. However, in 2017, when mineral fertilizer price and wheat prices are considered, the N rate is lower than the optimum N rate. The low precipitation after the mineral N application at GS30 did not allow N absorption by the wheat crop until one month later, negatively affecting N use efficiency, hence reducing the economic N rate and the economic benefit of the fertilizer.

4.4.2 Soil N Availability

As the N_{min} values showed, the soil N supply in soils amended with animal manure may be very variable (Table 4.2). The recommended rate of N fertilizer is calculated by the predicted N demand for the target yield minus the measured N_{min} at the end of winter. However, some factors make this technique imprecise. On the one hand, using a target yield is not feasible because, each growing season, yields vary depending on the weather (Figures 4.2 and 4.3). On the other hand, soil N_{min} at GS21 depends not only on the mineralization (SOM, applied organic compounds and weather conditions) but also on crop uptake. Although N_{min} values at GS21 in 2016 and 2017 were similar, yields achieved in 2016 (10,200–10,700) were higher than yields in 2017 (5800–6200; Figure 4.2). As the results showed (Tables 4.2 and 4.3), it is possible to have high N availability in soil and plants with low N nutritional status (as in 2017), as well as low N availability in soil and acceptable N nutritional statuses in plants (as in 2016).

The weather has a significant effect on yield. Since the weather can be difficult to predict in the long term, predicting the mineralization of organic forms of N and other nutrients into plant available mineral forms and the wheat demand remains a challenge (Mohanty *et al.,* 2013). Many factors control the decomposition of SOM and the mineralization of nutrients, rendering the prediction difficult (Walley *et al.,* 2002). Arregui and Quemada (2006), in similar climate conditions (humid Mediterranean), showed that due to the high rainfall, low evapotranspiration and low crop demand, from sowing to mid-tillering (GS25), most of the mineral N present in the soil before sowing was lost by nitrate leaching, as could have happened in 2015. The lower N_{min} values in the treatments where organics were applied in 2015 could be explained by immobilization (Mohanty *et al.,* 2013). Basso *et al.* (2012) showed that wheat yield production in the Mediterranean environment is highly affected by spring rainfall and the amount of soil water stored in soil before and during the growing season. In the area where the study was carried out (humid Mediterranean) (Papadakis, 1966), three of the ten years have dry periods (media 1978–2017; period 15 March–1 May) when N fertilizer topdressing is applied at GS30 at the stage of highest N uptake by the wheat crop. It should be mentioned that applying organics as initial fertilizers will probably not synchronize with the highest N demand by the crop because a long period of time passed between application and plant N uptake. However, the wet conditions in winter in Araba hinder the entrance of machinery to fields in spring. The unique alternative being their application in autumn, likely causing N loss by leaching.

Soil characteristics also have an effect on N mineralization. In our study, the soil presented 40% clay (58% Smectite). Chantigny *et al.* (2004) suggested that clay fixation may have a negative effect on N availability during the crop growing season. Other studies also demonstrated that there is a significant interaction between manure and soil regarding the net mineralization and that the net N mineralization of cattle manures, cattle slurry and plant recovery is lower in clay soils is associated with the clay fixation of NH_4^+ (Shah *et al.,* 2003).

It is also remarkable that, in 2016, the preceding crop was rapeseed (*Brassica napus*). It has been shown that in the humid Mediterranean region of Spain, including rapeseed in the crop rotation increases wheat yields by about 10% (INTIA, 2017). Gallejones *et al.* (2012) concluded that rapeseed as the preceding crop for wheat probably caused higher N mineralization.

Many factors affect soil N mineralization and soil N availability, making it difficult to estimate the amount of N mineralized from SOM to adjust the rate of N fertilizer required to optimize crop yield and also quality, even if soil N_{min} is known at the end of winter. Otherwise, fields are heterogeneous, and it implies laborious and expensive sampling and analysis (Sylvester-Bradley *et al.,* 2009). Ravier *et al.* (2016) highlighted many sources of uncertainty in soil N analysis (lack of standardized procedure for soil sampling or the choice of the sampling zone and extrapolation) that led to the exclusion of decision rules based on the monitoring of soil mineral content. Recommendations for the start of the crop cycle are usually based on soil parameters, and the recommendations for later stages in the crop cycle are based on plant indicators. Plants integrate soil variability, climate, crop management and other environmental influences, which are good indicators of the nutrient needs.

4.4.3 Nitrogen Nutrition Index (NNI)

The topdressing N fertilizer recommendation rate can be adjusted according to the NNI, which can indicate the magnitude of the N surplus or deficiency (Lemaire *et al.*, 2008). In our experiment, all treatments were under N deficiency (NNI < 0.9), even the overfertilized ones. However, the NNI was able to detect differences among treatments in the same growing season and differences among the wheat-growing seasons for the same treatment. Different thresholds of the NNI have been proposed in on-farm applications to diagnose the N status of plants (Ravier, 2017c; Cilia *et al.,* 2014; Xia *et al.,* 2016). Ravier *et al.* (2017a) determined the NNI threshold that the wheat crop can tolerate in different growing stages without reducing the maximum yields. Thus, they consider NNI values <0.4 as situations that should be avoided, and when the NNI is between 0.4 and 0.7 before ear has reached 1 cm in length (before GS30, following Zadoks *et al.,* 1974), they recommend the application of 40 kg N ha^{-1} at that stage. In our case, in 2016 at GS21, NNI values were 0.60–0.68. In 2016, yields were comparable between the conventional treatment (where 40 kg N ha^{-1} was applied at GS21) and treatments where organics were applied as initial fertilizers without N application at GS21. This fact suggests that increasing the N rate at GS30 can reverse the N deficiency at GS21 and organics could match the conventional treatment (where 40 kg N ha^{-1} was applied at GS21). In 2015 at GS21, NNI values were lower than 0.4 (0.34–0.38), which means that the crop had a severe deficiency and that the yields will be reduced. In 2015, yields were lower than in 2016 but as in 2016, yields between the conventional treatment (where 40 kg N ha^{-1} was applied at GS21) and organics treatment were comparable. In 2017, NNI values were 0.23–0.26 at GS21 and it did not rain after N application at GS30 until GS37, causing low yields. In 2017, the application of 40 kg N ha^{-1} at GS21 in the conventional treatment had a significant effect on the yield, allowing higher yields in the conventional treatment than in the organic treatment. Those events in 2015 and 2016 suggested that the tolerable N deficiency in GS21 could be lower (0.3) than the one identified by Ravier *et al.* (2017a) and that crop yield can be restored with the topdressing N application at GS30 when the N deficiency is not very severe, as in 2017. However, they also concluded that the extent of tolerable N deficiencies in the early stages is less clear than in more advanced stages. In fact, a low NNI occurring during tillering can lead to a low number of stems, but a higher NNI during GS30 can lead to an increase in the number of grains per ear (Ravier *et al.,* 2017a). It is important to highlight that tolerating N deficiency gives the crop the opportunity to absorb the available soil mineral N before applying fertilizer (Ravier *et al.,* 2016). The determination of the NNI requires representative samples to be taken, the determination of dry matter content and the determination of N concentration by laboratory analytics, making the determination of the NNI difficult and time-consuming. Moreover, as with soil sampling, farmers cannot instantly know the N status of plants.

4.4.4 Proximal Sensing Tools and Vegetation Indices

Abs_NTester readings increased as NNI values increased. For NNI = 1 values, Ziadi *et al.* (2008) determined an absolute chlorophyll meter reading with Minolta SPAD-502 of 42.5 at GS30 in wheat. Similar absolute chlorophyll meter readings (ranging from 39 to 45, depending on the site-year) for NNI = 1 in wheat were also reported by Peltonen *et al.* (1995) and Bundy and Andraski (2004). The crop sensor performance for N status diagnosis is influenced by many other variables apart from N: seasonal variation, plant water status, diseases and pests, plant growth stage, genotype, etc. (Bonfil, 2017). Normalized readings have been recommended to reduce the year effect (Prost and Jeuffroy, 2007; Ravier *et al.,* 2017a) and the noise promoted by other variables other than N fertilization (Ortuzar-Iragorri *et al.,* 2017).

In normalized values, chlorophyll meter readings have been correlated with the NNI (Prost and Jeuffroy, 2007; Debaeke *et al.,* 2006). Ziadi *et al.* (2008) established the critical relativized chlorophyll meter readings ranging from 0.89 to 0.95 for NNI = 1. However, the normalization of the chlorophyll meter readings does not entirely remove the year effect (Ravier *et al.*, 2017b), but these readings better predict the NNI (Ziadi *et al.*, 2008). Prost and Jeuffroy (2007) suggested that the overfertilized plot should have an NNI higher than 1, indicating that N is not limiting. Ravier *et al.* (2017b) concluded that the use of normalized values is problematic because it is essential to ensure that a well-fertilized strip is not N deficient. They determined that using relativized values from an N-deficient overfertilized plot (NNI < 0.9) may lead to an error of 0.34%. The overfertilized treatments of our dataset did not obtain NNI ≥ 0.9 (Table 4.3) even after the application of large amounts of N. Yao *et al.* (2014) showed that normalized values were more correlated with the NNI at later stages of development than at early stages. In our results, both absolute values and normalized values were able to similarly detect differences among treatments. However, finding values to adjust the optimum N rate was complicated with absolute values because variability among years was higher than with relativized values. Regarding normalized values, in dairy slurry treatment, when both proximal sensing tool values were 60–65%, the optimum N rate at GS30 to achieve the maximum yield was 118–128 kg N ha^{-1} (Table 4.5). In dairy slurry and conventional treatments, when the readings were 85–90%, the optimum N rate was 100–110 kg N ha^{-1} (Table 4.5). In the sheep manure treatment, there was no clear relationship between sensor values and the optimum N rate. When values were 60–65%, the N recommendation was 117 or 155 kg N ha^{-1} and 147 kg N ha^{-1} for readings around 89%. That may be due to the heterogeneous nature of manures that makes it difficult to predict how quickly and how much N will be transformed in plant-available N during the wheat-growing season. The composition of manures depends on animal diet, the amount of bedding, water and nutrient loss during storage and land application (Whalen *et al.*, 2019). However, in this study, the main factor that brings variability is the maturation (curing) phase. Thus, sheep manure characteristics were different depending on the year: in 2016, the sheep manure was “fresh”, containing NH_4^+ (0.33% of total N; Table 4.1), whereas in 2015 and 2017, it was “old” and the manures did not present any NH_4^+. In dairy slurry treatments, the NH_4^+ content in the three wheat-growing seasons was around 0.55% of total N, having lower variability compared to sheep manure. In 2016, the N availability in sheep manure treatment was high when the crop N demand was high, allowing greater tool values than in 2015 and 2017 (Table 4.4and Figure 4.4). However, the N recommendation at GS30 was much higher in sheep manure treatments than in treatments with dairy slurry as initial fertilizer, even if tools' values were similar at GS30 (Table 4.4 and Figure 4.4). Manures also contain organic N that becomes available to crops after enzymatic hydrolysis. The amount of N mineralized from organic N depends on its physical–chemical characteristics, soil properties and climatic conditions (Sharifi *et al.*, 2011). Typically, slurries are more susceptible to enzymatic hydrolysis than solid manures that have bedding materials made of lignified compounds (Whalen *et al.*, 2019). This can also justify the higher and variable N required by the crop when sheep manure was applied than when slurry was applied, especially when crop N requirements were high, as in 2016. Moreno-García *et al.* (2018) and Zhao *et al.* (2015) found that the higher availability of micronutrients provided by the organic fertilizers allowed higher yield than in conventional treatment and suggested that it is necessary to establish overfertilized plots for each type of fertilization.

These results look promising in order to adjust the N application rate at GS30 with the Yara N-Tester^{TM} and RapidScan CS-45 normalized readings when applying dairy slurry before sowing. However, in the sheep manure treatment, it is more difficult to find a relationship between sensor readings and yield. Both handheld N diagnostic tools were able to detect differences among N nutritional status in plants. Siband *et al.* (2001) suggested that the NNI responded faster to N applications than chlorophyll meters, which is probably related to the time between N uptake and chlorophyll synthesis (Debaeke *et al.*, 2006). Ziadi *et al.* (2008) saw that the whole plant can be less N deficient than the uppermost collared leaf. In this sense, evaluating the whole plant would be more precise than just a leaf. At early growth stages, evaluating the NDVI would be more accurate, although some authors such as Broge and Mortensen (Broge *et al.*, 2002) highlighted that, when the wheat canopy is not closed, soil background exposure reduces the reliability of using reflectance for the estimation of crop N status. However, at the end of the growing cycle, the use of a chlorophyll meter is suitable (Ortuzar-Iragorri *et al.*, 2005), as 35% of N in the grain comes from the last developed leaf (Fuertes-Mendizabal *et al.*, 2002). The utility of proximal tools to detect the N nutritional status of plants was supported by Arregui *et al.* (2006) and Ortuzar-Iragorri *et al.* (2005) who observed that chlorophyll meters enabled the prediction of the N status of plants. Furthermore, Mullen *et al.* (2003) showed that in-season N demand for added N fertilizer in winter wheat could be detected using NDVI readings collected at GS30. Moreover, algorithms using crop canopy reflectance sensing to make N recommendation for wheat have been identified, and it has been shown that active canopy sensors could be used in determining variable N rate applications in wheat from the mid-growing season (Raun *et al.*, 2005; Calvo *et al.*, 2015). Marti *et al.* (2007) found significant correlations between the NDVI, yield and biomass in wheat. On the other hand, Sylvester-Bradley *et al.* (2009) developed an alternative strategy for signaling soil N status and observed that the NDVI of young canopies can signal soil N status where N_{min} is lower than 120–140 kg N ha^{-1}. In our conditions, generally N_{min} values were lower than 120–140 kg N ha^{-1}. In our case, both proximal tools showed a greater sensitivity than N_{min} when differentiating the initial fertilization treatments applied in the field trial. All of these findings were very promising because the type of fertilization could be changed to achieve a more precise N rate adjusted to the wheat crop demand. It should be noted that measurements taken with RapidScan CS-45 were less time consuming than with Yara N-Tester™ and were taken on the plant canopy and not just in the uppermost fully expanded leaf, as with chlorophyll meters. Thus, samples taken with RapidScan CS-45 can better represent the spatial variation of the crop N status.

These hand-held tools are used during the wheat growth period (GS30), but many environmental variables (rainfall, temperature or relative humidity) affect crop growth and development after this stage until harvest (Aranguren *et al.*, 2018b). However, any method of diagnosing N nutritional status at a particular stage has the same limitation. Furthermore, it has been shown that it is always possible to correct N deficiency until the end of the cereal growth season (GS65) (Ravier *et al.*, 2017a) if soil is wet (Soenen *et al.*, 2017). Since remote sensing measurements are not invasive and can be repeated several times during the growth period, information obtained on N status dynamics of plants can be used for decision making in N fertilizer management. What is more, ground-based remote sensing tools can be applied to satellite or airborne remote sensing.

4.5 Conclusions

Experimental findings to date have shown that Yara N-Tester™ or Rapidscan CS-45 normalized readings look promising in order to adjust the N application rate at GS30 under rainfed conditions in humid Mediterranean climate conditions. For dairy slurry, when either proximal sensor readings were 60–65% of the reference plants with non-limiting N, the optimum N rate for maximizing yield was 118–128 kg N ha^{-1}. When the readings were 85–90%, the optimum N rate dropped to 100–110 kg N ha^{-1} for both dairy slurry and conventional treatments. However, in the sheep manure treatment, it is more difficult to find a relationship between sensor readings and yield due to the variable composition of the manure and subsequently, the available N.

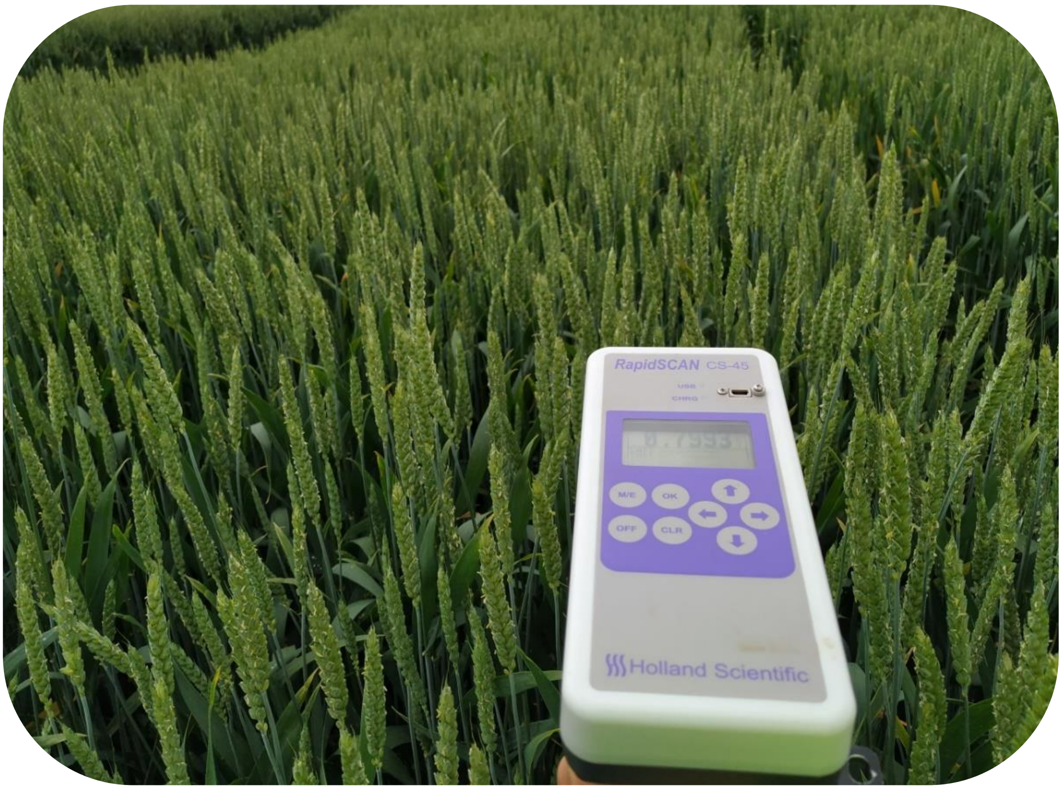
When rainfall conditions between mid-March and April are as usual (seven out of ten years), a low N status (60–65%) can be recovered with no adverse effect on yield when mineral N is applied at GS30. However, during those years in which dry periods occur (three of ten), when mineral N is applied at GS30, the N deficiency is not recovered because the lack of soil moisture prevents proper N uptake by the crop. In these cases, the application of 40 kg N ha^{-1} at GS21 leads to higher yields. As the N rate at GS30 can be modulated with the proximal tools, the application of 40 kg N ha^{-1} at GS21 is necessary to ensure an optimal N status from the beginning of wheat crop development.

These hand-held tools are used during the wheat-growing period (GS30), but many environmental variables may affect crop development until harvest. In order to address this, given that remote sensing measurements are not invasive, these measurements should be taken periodically to monitor crop N status in an effective way. Routine measurements throughout the growing season are particularly needed in climates with variable rainfall. Measurements taken with RapidScan C-45 were less time consuming and better represent the spatial variation as they are taken on the plant canopy.

While Yara N-Tester^{TM} or Rapidscan CS-45 look promising for adjusting N application rates, further research is needed to improve the use of these sensors. These research findings could be used in applicator-mounted sensors to make variable-rate N applications.

Chapter 5

Crop sensor based non-destructive estimation of nitrogen nutritional status, yield, and grain protein content in wheat



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5 Crop Sensor Based Non-destructive Estimation of Nitrogen Nutritional Status, Yield, and Grain Protein Content in Wheat

5.1 Introduction

To meet the globally increasing food demand, achieving high grain yields and high-quality grains has become fundamental. For those purposes, N fertilizer is a crucial factor because its application results in an increase in grain quality and grain yield. However, in cereal production, the excessive application of nitrogen fertilisers is common. The optimum management of N fertilization requirements needs a steady monitoring of crop N status throughout the vegetative period (Ravier *et al.*, 2017a).

Determining the cereal N status is very important for adjusting the necessary N dose, evaluating crop growth, and estimating yield and grain protein content (GPC) (Raun *et al.*, 2001; Hansen *et al.*, 2003; Zhao *et al.*, 2018). In this sense, the nitrogen nutrition index (NNI) has been commonly utilized to determine the N status of plants during the growing season (Lemaire *et al.*, 2009). The NNI, determines if the N concentration needed to achieve the greatest biomass production is optimum based on a crop’s current biomass (Ravier *et al.*, 2017a). The NNI could be helpful to follow N dynamics in a crop canopy and, in this way, identify the deficiencies that suggest a yield decrease. NNI dynamics may be useful under circumstances where cereals are destined to lose their yield. Ravier *et al.* (2017a) identified a threshold NNI path for the wheat growing cycle to determine N fertilizer application timing and suggested the minimum NNI values needed for each key growing stage to achieve high yields together with a lower risk of nitrate pollution. Reliable information on crop nutritional status throughout the vegetative period could reveal the need for additional N fertilizer (Ravier *et al.*, 2018; Denuit *et al.*, 2002) and may help develop an innovative method to manage N fertilization. However, to determine NNI, laboratory analytical procedures are needed, thereby making the calculations complicated and time-consuming. To achieve precise N fertilizer management, crop N status should be analysed in-season and at specific sites.

Optical sensing techniques estimate the N content in a plant indirectly, as such techniques cannot measure N content directly. Measurements are rapid, low cost, and can be done intensively over space and time, thereby providing the necessary resolutions required for N fertilizer management (Zhao *et al.,* 2018). In this sense, non-destructive and instantaneous measurements can be taken for crop blades with chlorophyll meters to use them as estimators of the crop N nutritional status. A previous study showed a good relationship between leaf N concentration and measurements taken with chlorophyll meters (Denuit *et al.,* 2002; López-Bellido *et al.,* 2004; Arregui *et al.,* 2006). Chlorophyll meters and wheat grain yield were related in different studies and used to identify responses to additional fertilizers (Arregui *et al.,* 2006; Aranguren *et al.,* 2018) and for recommendations on fertilizer management [Aranguren *et al.,* 2019; Ortuzar-Iragorri *et al.,* 2017; Debaeke *et al.,* 2006). Moreover, the possibility of using chlorophyll meters to decide if an extra fertilizer dose is required to increase GPC has been studied [Denuit *et al.,* 2002; Arregui *et al.,* 2006]. The readings provided by chlorophyll meters have also been well correlated with the NNI [Ravier *et al.,* 2017b; Ortuzar-Iragorri *et al.,* 2005) and with wheat leaf N concentrations and leaf chlorophyll (Denuit *et al.,* 2002; López-Bellido *et al.,* 2004).

Active crop canopy sensors have their own light sources, so they are not limited by changeable light conditions, thus making them practical for on-farm management. Plant tissue normally reflects nearly 50% of the near infra-red (NIR) and absorbs nearly 90% of the visible radiation (Knipling, 1970). Information related to crop N status is provided because the ratio of reflectance and absorbance changes with crop N and biomass (Padilla *et al.*, 2019). Then, vegetation indexes can be calculated with the spectral data collected by the crop sensors. Mistele and Schmidhalter (2008) concluded that the NNI can be determined using spectra-based measurements, and Marti *et al.* (2007) positively correlated Normalized Difference Vegetation Index (NDVI) values with wheat yield and biomass. RapidScan CS-45 (Holland Scientific, Lincoln, NE, USA) is a portable ground-based active canopy sensor with a built-in GPS that measures crop reflectance at red (R; 670 nm), red-edge (RE; 730 nm), and near infra-red (NIR; 780 nm) spectra and provides the NDVI and the Normalized Difference Red Edge (NDRE). Previous studies showed that RapidScan CS-45 estimates NNI in rice (Lu *et al.,* 2017) and allows the fast and precise crop tracking of N status and yield estimations in wheat (Bonfil *et al.,* 2017).

Proximal sensor measurements can be repeated several times throughout the wheat growing season, and the information obtained and related to crop N status may be utilized to follow crop N dynamics in real time (López-Bellido *et al.,* 2004; Cao *et al.,* 2013; Magney *et al.,* 2016; Zhang *et al.,* 2019). However, remote sensing measurements are usually taken in the middle wheat-growing period to adjust the N fertilizer rate

(Aranguren *et al.,* 2019; Bijay-Singh *et al.,* 2011). In our area, the highest amount of N is applied at stem elongation (GS30), but the time until harvest is long, and many factors may affect the subsequent N uptake by the crop. However, if the soil is wet (Arregui *et al.,* 2006), it is possible to amend N deficiency until late in the wheat growing season (GS65 (Ravier *et al.,* 2017a); mid-flowering; (Zadoks *et al.,* 1974)). In our area, it is possible to use a third application of N fertilizer at leaf-flag emergence (GS37) because there will likely be sufficient rain (Arregui *et al.,* 2006; Ortuzar-Iragorri *et al.,* 2017) to permit N uptake by the crop. Therefore, it is desirable to follow the crop N status during the vegetative growing season to make decisions related to the optimization of N fertilizer applications (Ravier *et al.*, 2018; Ortuzar-Iragorri *et al.*, 2017) or to predict the yields and GPC values. In this sense, optical sensing tools could help us understand easily how climate and N rates affect N uptake via crops and, therefore, affect yield and GPC. Ravier *et al.* (2018) developed decision rules for determining N fertilizer application through the wheat growing season as a function of the crop N status or NNI reference values in the key growing stages. However, there are no optical sensing reference values for evaluating wheat crop N status during the vegetative growing period.

The usefulness of the proximal sensing tools for predicting NNI (Ortuzar-Iragorri *et al.,* 2005; Lu *et al.,* 2017) and for predicting yield (Raun *et al.,* 2017; Marti *et al.,* 2007) and GPC (Hansen *et al.,* 2003; Magney *et al.,* 2016) has been studied with mixed results. Predictions depend on the agroecosystem environment, and specific correlations for each climate should be developed (Magney *et al.*, 2016). The present study was developed under humid Mediterranean conditions to (i) determine if the NNI can be predicted using the proximal sensing tools RapidScan CS-45 and Yara N-Tester^{TM} and if a single or unique model for all growing stages (from GS30 to mid-flowering (GS65)) could be used to predict NNI (or if growing stage-specific models would be necessary); (ii) to determine if grain yield and GPC can be predicted using the RapidScan CS-45 and Yara N-Tester^{TM} and (iii) to determine if the predictions are improved using normalized values rather than absolute values.

5.2 Materials and Methods

5.2.1 Study Site

Three field trials were established during three consecutive wheat growing seasons (2014–2015, 2015–2016, and 2016–2017) in Arkaute (Araba, Basque Country, northern

Spain) at NEIKER installations (Figure 5.1) under unirrigated conditions. We refer to the growing seasons as 2015, 2016, and 2017. The climate of the area where the study was carried out was Temperate–Mediterranean (Papadakis *et al.*, 1966). The soil texture was analyzed by the pipette method (Gee *et al.*, 1986) and classified (0–30 cm, sandy clay loam and 30–60 cm, clay loam) (SSS, 1999). pH values (8.0–8.5) were high in the soil, which was calcareous (MAPA, 1994) and had moderate organic matter content (Walkey *et al.*, 1934) in the upper layer (2%–2.5%). The soil was classified as Typic Calcexeroll (SSS, 2015). Further experimental details were described by Aranguren *et al.* (Aranguren *et al.*, 2019).

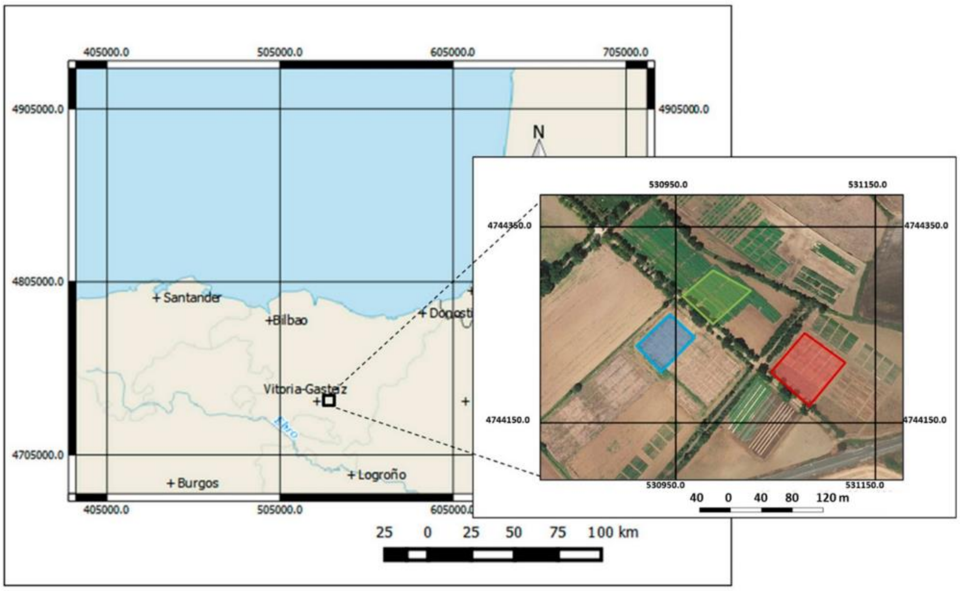

Figure 5.1. Location of the three field experiments in Arkaute, Araba, Basque Country, northern Spain. The field experiment in 2015 was carried out in the red field. The field experiment in 2016 was carried out in the blue field. The field experiment in 2017 was carried out in the green field.

5.2.2 Treatments

Three different initial fertilizers were used: dairy slurry (40 t ha^{-1}), sheep farmyard manure (40 t ha^{-1}), and conventional treatment (no basal dressing but 40 kg N ha^{-1}, 18 kg S ha^{-1}, and 45 kg K ha^{-1} at tillering (GS21, (Zadoks *et al.*, 1974)). The dairy slurry N content was 192, 144 and 120 kg N ha^{-1} in 2015, 2016, and 2017, respectively. The sheep farmyard manure N content was 336, 592 and 448 kg N ha^{-1} in 2015, 2016, and 2017, respectively. Five N rates, applied at GS30 (0, 40, 80, 120, and 160 kg N ha^{-1}), were combined with the three types of initial fertilization. Regarding mineral fertilization, N was applied as calcium-ammonium-nitrate 27% (NAC) and S and K were applied as potassium sulphate 50%. A control without N (0 N) and an over-fertilized control plot (280 kg N ha^{-1}) were also established. The treatments are shown in Table 5.1. Organic fertilizers were applied in mid-November each growing season. In the area where this study was carried out, organics are usually applied in combination with mineral fertilizers. The application rate in the experiment (40 t ha^{-1}) is the usual rate applied in the area. Wheat (*Triticum aestivum* var. Cezanne) was sown just after application of the organics. The experiment used a factorial randomized complete block design. The area of each plot was 4 m wide and 8 m long.

Table 5.1. Fertilization treatments for the field trials (2015, 2016, and 2017) and the N dose applied in each of them. Beginning of tillering (GS21; end of winter (Zadoks *et al.*, 1974)) and stem elongation (GS30; (Zadoks *et al.*, 1974).

Initial Fertilization	**2015-2016-2017**		**Treatment identification**
	Topdressing at GS21 (kg N ha^{-1})	**Topdressing at GS30 (kg N ha^{-1})**	
Conventional (--)	40	0	40N + 0N
		40	40N + 40N
		80	40N + 80N
		120	40N + 120N
		160	40N + 160N
Dairy Slurry (DS) (40 t ha^{-1})	--	0	DS + 0N
		40	DS + 40N
		80	DS + 80N
		120	DS + 120N
		160	DS + 160N
Sheep manure (SM) [40 t ha^{-1})	--	0	SM + 0N
		40	SM + 40N
		80	SM + 80N
		120	SM + 120N
		160	SM + 160N
Control (--)	--	--	0N
Overfertilized (--)	80	200	280N

(–), no initial fertilization.

Yields were harvested at crop maturity. Total N concentration was determined following the Kjeldhal procedure (AOAC, 1999). GPC was determined by multiplying the total N concentration of the product by 5.7 (Teller *et al.,* 1932).

5.2.3 Plant Biomass and Nitrogen Nutrition Index (NNI)

Plant biomass samples were taken at GS30, GS37, and GS65 in all conventional treatments DS + 0N, and SM + 0N and in two control treatments (0N and 280N). Plant biomass sampling was done according to Aranguren *et al.* (Aranguren *et al.*, 2019). The biomass was measured and N concentration was determined following Kjeldahl's method (AOAC, 1999) to calculate the Nitrogen nutrition index (NNI) (Ravier *et al.,* 2017a):

$$\mathrm{NNI} = \frac{Na}{Nc} \qquad (5.1)$$

where *Na* represents the present wheat N uptake, and *Nc* represents the critical N uptake that corresponds to the present shoot wheat biomass W (t ha^{-1}) (Justes *et al.*, 1994):

$$Nc = 5.35\ x\ W^{-0.442} \qquad (5.2)$$

when the NNI values are close to one, wheat has an optimum N status; values lower than 0.8 indicate N deficiency, and values higher than one indicate non-limiting N.

5.2.4 Crop Sensors for Following Crop N Status

Crop sensor readings (CSR) were taken with a Yara N-Tester^{TM} (Yara International ASA, Oslo, Norway) and RapidScan CS-45 (Holland Scientific, Lincoln, NE, USA) at GS30, GS37, and GS65 (Zadoks *et al.,* 1974) in four replications of all treatments (Table 5.1). The Yara N-Tester^{TM} is a chlorophyll meter that measures and processes the ratio of the light transmitted at 650 and 940 nm wavelengths, in addition to the ratio determined with no sample, to produce a digital reading. It is a clip-on hand-held tool whose measurement point is placed in the middle of the blade of the youngest fully developed leaf. To acquire a representative value for each measured treatment, thirty random measurements were recorded. The RapidScan CS-45 is a ground-based active crop canopy sensor that measures crop reflectance at 670, 730, and 780 nm and provides the NDVI and NDRE (Equations (5.3) and (5.4)). Plot measurements were taken when the sensor was passed over the crop at approximately 1 m at a constant walking speed. Two rows per plot were scanned, and the NDVI and NDRE values were averaged to generate a value for the plot.

We refer to the Yara N-Tester^{TM} measurements as abs_N-Tester. We refer to the RapidScan CS-45 measurements as abs_NDVI and abs_NDRE. Measurements with both tools were taken as described by Aranguren *et al.* (2019):

$$\text{NDVI} = \frac{R_{NIR780} - R_{RED670}}{R_{NIR780} + R_{RED670}} \tag{5.3}$$

$$\text{NDRE} = \frac{R_{NIR780} - R_{RED-EDGE730}}{R_{NIR780} + R_{RED-EDGE730}} \tag{5.4}$$

The normalized values for crop sensor readings (nor_CSR: nor_N-Tester, nor_NDVI, and nor_NDRE) were calculated according to Aranguren *et al.* (2019):

$$\text{nor}_{\text{CSR}} = \frac{\text{CSR}}{\text{CSRoverfertilized}}\ 100 \tag{5.5}$$

Thus, each absolute crop sensor reading (CSR; abs_N-Tester, abs_NDVI, and abs_NDRE) was divided by the CSR values of the overfertilized plot (280N; CSR_overfertilized) at the same growing stage and in the same growing season (Wang *et al.*, 2014).

5.2.5 Models to be Fitted

Based on a literature review, the models for predicting NNI (Ravier *et al.*, 2017b; Lu *et al.*, 2017; Cao *et al.*, 2013), yield (Marti *et al.*, 2007; Zhang *et al.*, 2019), and GPC (Hansen *et al.*, 2003; Bijay-Singh *et al.*, 2011) in cereals from crop sensors readings were selected.

5.2.5.1 The Linear Model

This model assumes that the NNI, yield, and GPC increase steadily with the CSR (or nor_CSR).

The NNI is defined as follows:

$$Y = a + b \times CSR \qquad (5.6)$$

where *Y* is the NNI (or yield or GPC), CSR (or nor_CSR) is the measured value with the crop sensor, and a and b are the parameters of the linear trend that are estimated when the model is fitted to the experimental data.

5.2.5.2 The Exponential Model

This model does not feature a constant increase in *NNI*, yield, or GPC with *CSR* (or nor_*CSR*) (unlike the linear model) and is defined as follows:

$$Y = a + e^{b \times CSR} \qquad (5.7)$$

where *Y* is *NNI* (or yield or GPC), *CSR* (or nor_*CSR*) is the measured value with the crop sensor, and a and b are the parameters of the exponential trend that is estimated when the model is fitted to the experimental data.

The three-year dataset was divided into two subsets: 75% for fitting the coefficients of determination and 25% for the validation dataset. The 25% dataset was always taken from the same block in the field experiment. The coefficients of determination (R^2) were calculated using the R 3.2.5 software (R Core Team, 2013). R^2 was calculated for the relationship between the *CSR* (abs_N-Tester, abs_*NDVI*, and abs_*NDRE*) and *NNI* and between nor_*CSR* (nor_N-Tester, nor_*NDVI*, and nor_*NDRE*) and *NNI* in each growing stage (GS30, GS37, and GS65). R^2 was calculated for the relationship between the *CSR* and *NNI* and between nor_*CSR* and the NNI for all growing stage readings together (general model). R^2 was calculated for the relationship between the CSR and the yield and between nor_CSR and the yield for each growing stage (GS30, GS37, and GS65). R^2 was calculated for the relationship between the CSR and GPC and between nor_CSR and GPC for each growing stage (GS30, GS37, and GS65).

Only when the above-mentioned relationships were statistically significant were the relationships plotted. Moreover, when these relationships were significant, the NNI values predicted from the different indexes and models were plotted against the NNI values measured from the remaining samples (25%) using the R 3.2.5 software (R Core

Team, 2013).

The output of the models was assessed by comparing the $R^{2,}$ RMSE (root mean square error), and AIC (Akaike Information Criterion (Akaike, 1973)). The RMSE defines the best-fit function that captures the relationship between NNI, yield, or GPC and CSR (or nor_CSR), which is defined as follows:

$$\text{RMSE} = \sqrt{\frac{1}{N}\sum^{N}(Y - Y')^2} \quad (5.8)$$

where *Y* is the measured NNI, and *Y'* is the estimated NNI.

The AIC describes to what degree the model is explained by the data. The models were compared, and that with the least amount of information loss was used (Burnham *et al.*, 2002). The models close to reality had lower AIC values (Ravier *et al.*, 2017b).

The highest precision and accuracy of the model for predicting crop N status (NNI), yield, or GPC was chosen based on (i) the highest R^2, and (ii) the lowest RMSE and AIC. In the results of the models, the highest value was the R^2 and the lowest was the RMSE and AIC in all cases. Therefore, only the R^2 will be mentioned in the results.

5.3 Results

5.3.1 Relationship between the NNI and Crop Sensor Readings

The correlations were fitted between the absolute and normalized CSR and NNI for each different growing stage (GS30, GS37, and GS65; Figures 5.2 and 5.3) as well as a general correlation across growing stages (Figure 5.4). For growing stage-specific models, the RapidScan CS-45 indexes predicted a better NNI (Figures 5.2 and 5.3) than the Yara N-Tester^{TM} for both models (linear and exponential). The Yara N-Tester^{TM} did not present a significant relationship to NNI, thus, relationships were not plotted. With RapidScan, the exponential models predicted the NNI more successfully than the linear models in all cases (a higher R^2 and lower AIC and RMSE). For absolute values, the abs_NDVI values better explained the NNI variability (Figure 5.2a–c) than the abs_NDRE values (Figure 5.3a,b) in every growing stage (R^2 values higher than 52 in all cases). The relationship between abs_NDRE at GS65 and the NNI was not significant, thus, it was no plotted. For the normalized values, the nor_NDRE (Figure 5.3d,e) values fit the NNI slightly better at GS37 and GS65 (R^2 = 0.7) than the nor_NDVI values (Figure 5.2e,f). However, at GS30, nor_NDVI predicted the NNI better than nor_NDRE (Figures 5.2d and 5.3c). The general model (Figure 5.4), especially the abs_NDVI values (Figure 5.4a), had good accuracy when estimating the NNI (R^2 = 0.7, similar to the models for different growing stages). In the general model, there were no significant relationships between the Yara N-Tester™ and NNI, like that found in the growing stage-specific models (data not shown).

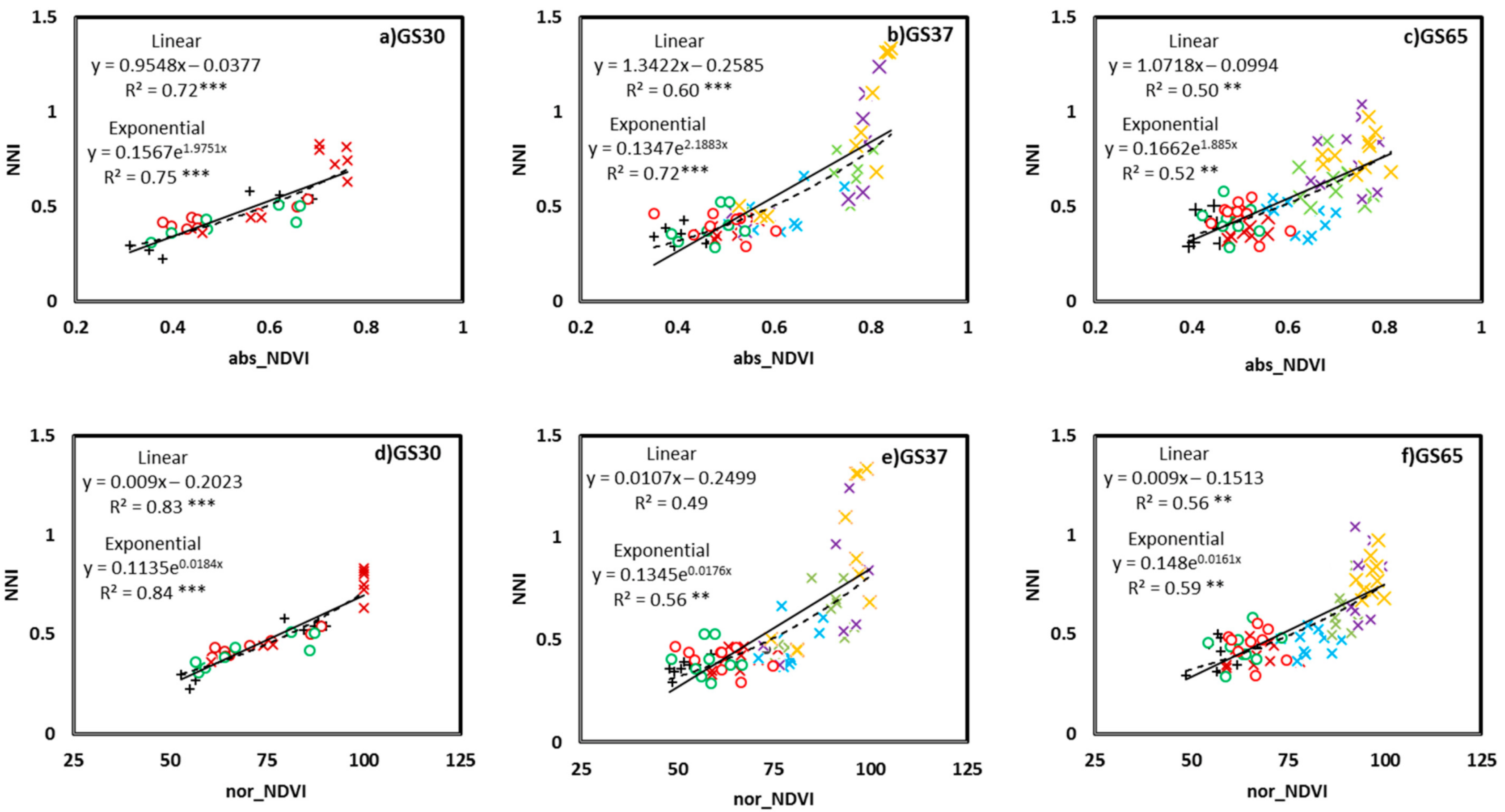

Figure 5.2. Relationship between the NNI (Nitrogen Nutritional Index) and abs_NDVI values at GS30 (**a**), GS37 (**b**), and GS65 (**c**) and between the NNI and nor_NDVI values at GS30 (**d**), GS37 (**e**), and GS65 (**f**). Two models were fitted: linear, solid line; exponential, dashed line. **, *** Significant at 0.01 and 0.001 probability levels, respectively. +, 0N; ×, 40N + 0N; ×, 40N + 40N; ×, 40N + 80N; ×, 40N + 120N; ×, 40N + 160N; o, DS + 0N; o, SM + 0N. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index.

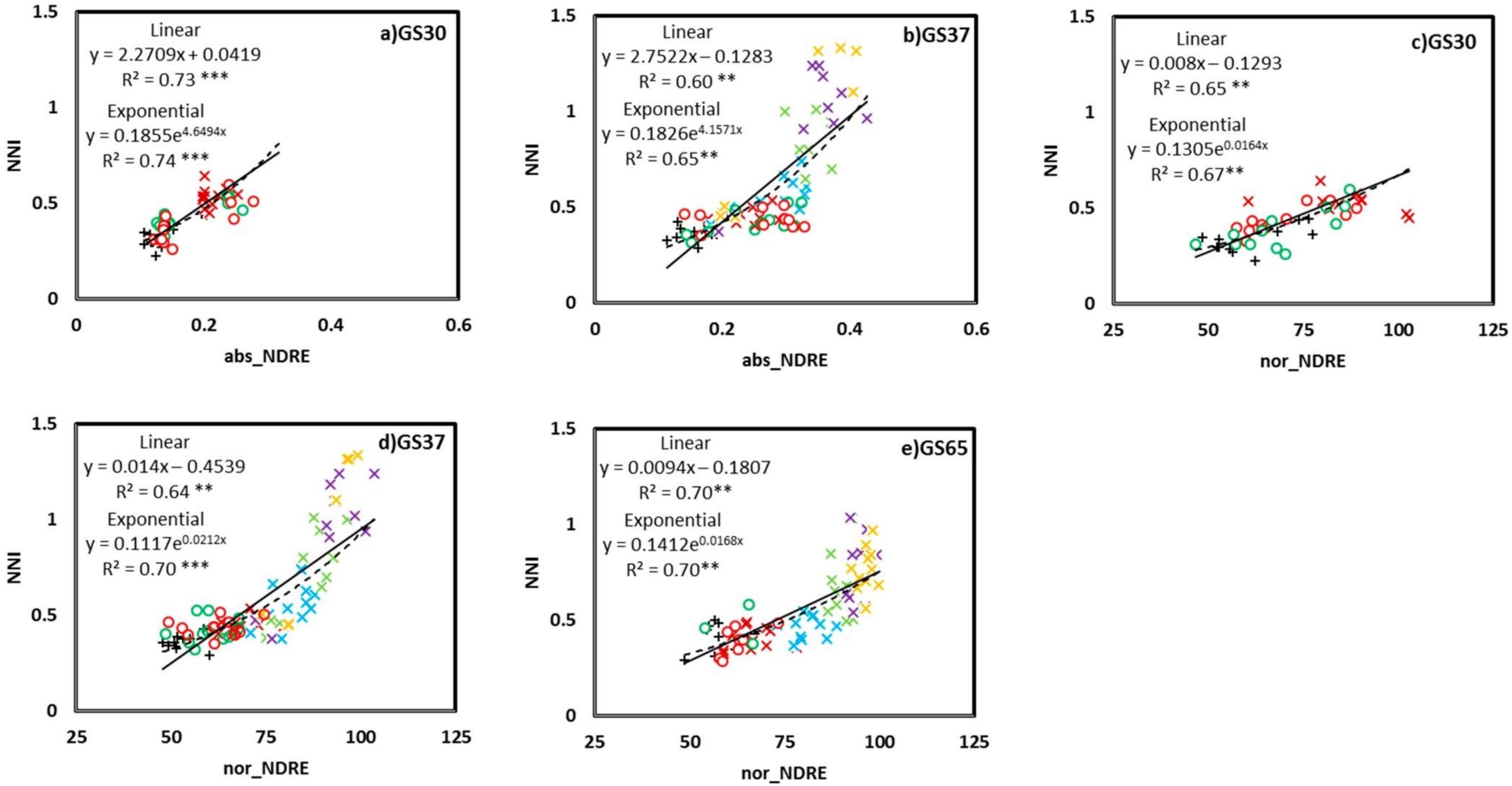

Figure 5.3. Relationship between the NNI (Nitrogen Nutritional Index) and abs_NDRE values at GS30 (**a**) and GS37 (**b**) and between the NNI and nor_NDRE values at GS30 (**c**), GS37 (**d**), and GS65 (**e**). Two models were fitted: linear, solid line; exponential, dashed line. **, *** Significant at 0.01 and 0.001 probability levels, respectively. +, 0N; ×, 40N + 0N; ×, 40N + 40N; ×, 40N + 80N; ×, 40N + 120N; ×, 40N + 160N; o, DS + 0N; o, SM + 0N. abs, absolute values; nor, normalized values; NDRE, Normalized Difference Red Edge Index.

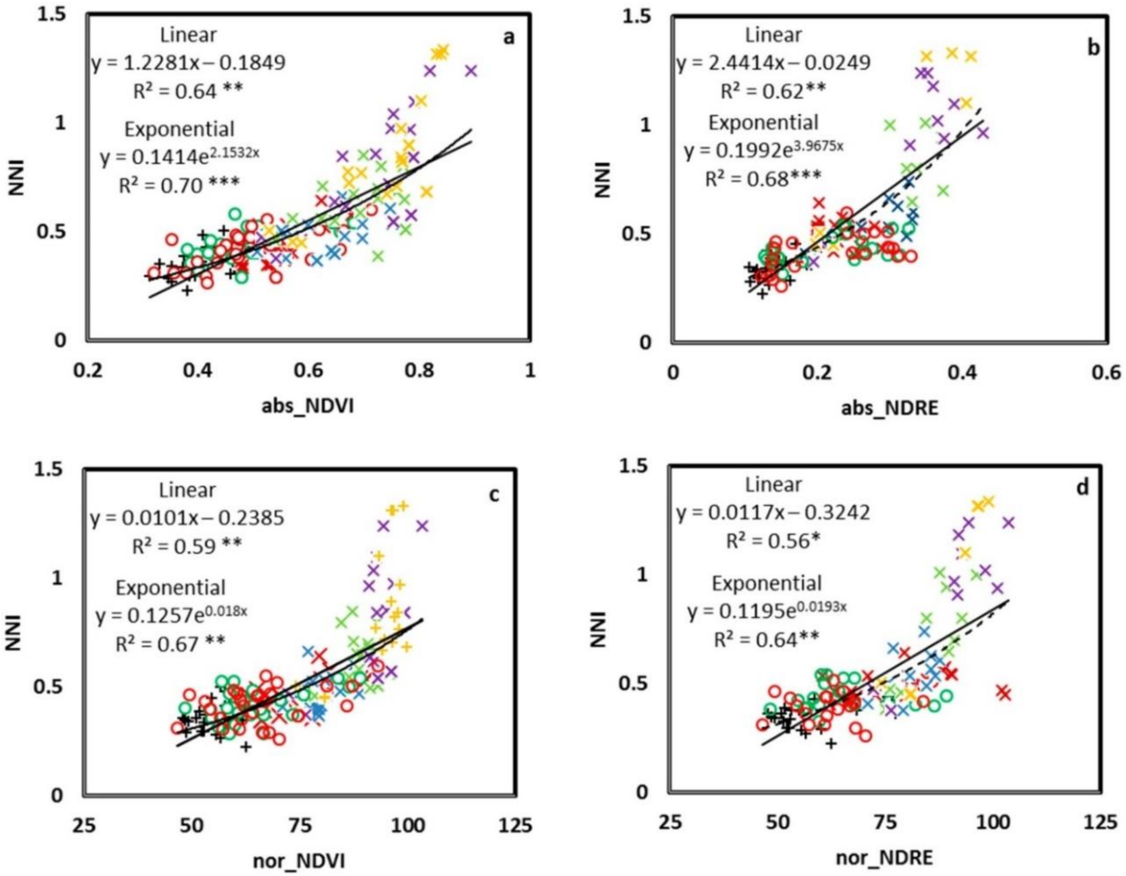

Figure 5.4. Relationship between the abs_NDVI (**a**) and abs_NDRE (**b**) values for all growing stages measurements together and the NNI (Nitrogen Nutritional Index) and the nor_NDVI (**c**) and nor_NDRE (**d**) values for all growing stage measurements together and the NNI. Two models were fitted: linear, solid line; exponential, dashed line. *, **, *** Significant at 0.05, 0.01 and 0.001 probability levels, respectively. +, 0N; ×, 40N + 0N; ×, 40N + 40N; ×, 40N + 80N; ×, 40N + 120N; ×, 40N + 160N; o, DS + 0N; o, SM + 0N. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index; NDRE, Normalized Difference Red Edge Index.

Saturation effects were detected for both the NDVI and NDRE indexes when NNI > 0.8 (Figures 5.2-4). The RapidScan CS-45 indexes did not increase more than 0.8 for the NDVI (Figures 5.2and 5.4) or more than 0.4 for the NDRE (Figures 5.3 and 5.4), even though the NNI values continued increasing above 0.8. Although NDVI and NDRE reached saturation at a similar point, the NDRE value range was slightly wider for NDVI values around 0.8 (0.35–0.40; Figure5.5). The values located from NNI = 0.8 to NNI = 1.4 were not quantified by RapidScan CS-45 (Figures 5.2-5.4).

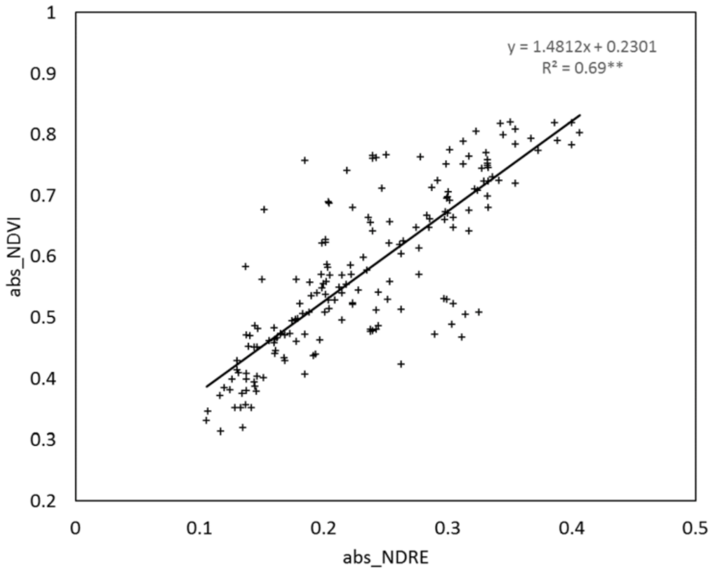

Figure 5.5. Relationship between the abs_NDVI and abs_NDRE values from the three growing seasons. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index; NDRE, Normalized Difference Red Edge Index. **, significant at 0.01 probability level.

5.3.2 Relationship between the Yield and GPC and Crop Sensor Readings

Correlation coefficients of the relationship between the absolute and normalized CSR at each different growing stage (GS30, GS37, and GS65) and grain yield (Figure 5.6) and GPC were fitted. The lineal models and exponential models predicted similar yields from CSR. The yield prediction capacity was high with abs_NDVI at GS65 (Figure 5.6a; R^2 = 0.72), nor_NDVI at GS37 (Figure 5.6b; R^2 = 0.76), and nor_NDRE at GS37 (Figure 5.6c; R^2= 0.70), whereas the yield prediction capacity with abs_N-Tester at GS37 (Figure 5.6d; R^2= 0.53) and that with nor_ N-Tester at GS65 (Figure 5.6e; R^2 = 0.57) was low. The remaining relationships between yield and CSR (abs_NDVI at GS30 and GS37; abs_NDRE at GS30, GS37 and GS65; abs_N-Tester at GS30 and GS65) and nor_CSR (nor_NDVI at GS30 and GS65; nor_NDRE at GS30 and GS65; nor_N-Tester at GS30 and GS37) were not significant (data not shown). The GPC prediction capacity with CSR and that with nor_CSR was not significant in any of the cases (the best relationship was observed between abs_N-Tester at GS65 (R^2 =0.35)).

5.3.3 *NNI Estimation Performances from Proximal Sensing Tools*

The NNI values predicted from the different indexes and models were plotted against the measured NNI values from the remaining samples (25%) when the correlations were significant (Figures 5.7–5.9). In the models specific to the growing stage, the order of the accuracy of the correlations for validation was similar to the accuracy of the prediction correlations (Figures 5.7 and 5.8). There was a significant agreement between the estimated NNI and the measured NNI for the vast majority of the correlations, with the exception of nor_NDRE at GS30 (Figure 5.8b). NDVI had a greater potential for predicting NNI than NDRE, as represented by its higher R^2 and lower RMSE and AIC. In the general model (Figure 5.9), there was a significant agreement between the estimated NNI and the measured NNI with abs_NDVI and abs_NDRE (Figure 5.9a,c), especially with abs_NDVI in the exponential model (Figure 5.9a). For nor_NDRE and nor_NDVI, the predicted NNI and the measured NNI did not agree (Figure 5.9b,d). NDVI had a high potential for predicting NNI, especially with the exponential model (R^2 = 0.74; RMSE = 0.12; AIC = −89), even in the general model.

5.3.4 *Grain Yield and GPC Estimation Performance from Proximal Sensing Tools*

The predicted yield values from the different indexes and models were plotted against the measured yield values from the remaining samples (25%) when the correlations were significant (Figure 5.10). Figure 5.10 shows that there was a significant agreement between the estimated yields and the measured yields in all cases. However, for abs_N-Tester at GS37 and nor_N-Tester at GS65, the agreement was lower than that for nor_NDVI and nor_NDRE at GS37 and abs_NDVI at GS65, as shown by the higher R^2 values and lower RMSE and AIC values. Since no index or model could predict the GPC values, GPC estimation performance was studied.

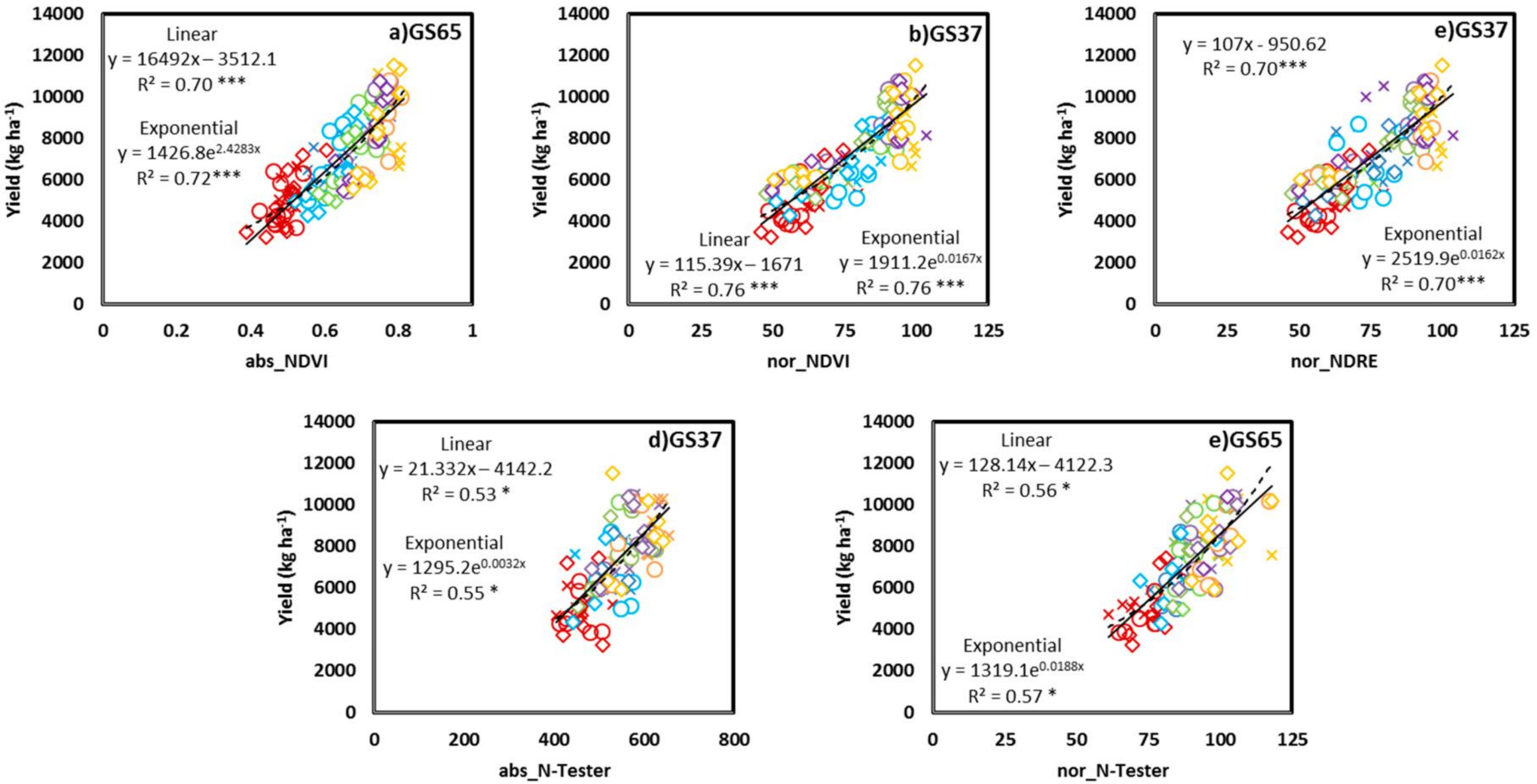

Figure 5.6. Relationship between the yield (kg ha^{-1}) and abs_NDVI values at GS65 (**a**), nor_NDVI at GS37 (**b**), nor_NDRE at GS37 (**c**), abs_N-Tester at GS37 (**d**), and nor_N-Tester at GS65 (**e**). Two models were fitted: linear, solid line; exponential, dashed line. *, *** Significant at 0.05 and 0.001 probability levels, respectively. Initial fertilizers: ×, conventional; o, dairy slurry; ♦, sheep manure. N rate at GS30: red, +0N; blue, +40N; green, +80N; purple, +120N; orange, +160N. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index; NDRE, Normalized Difference Red Edge Index.

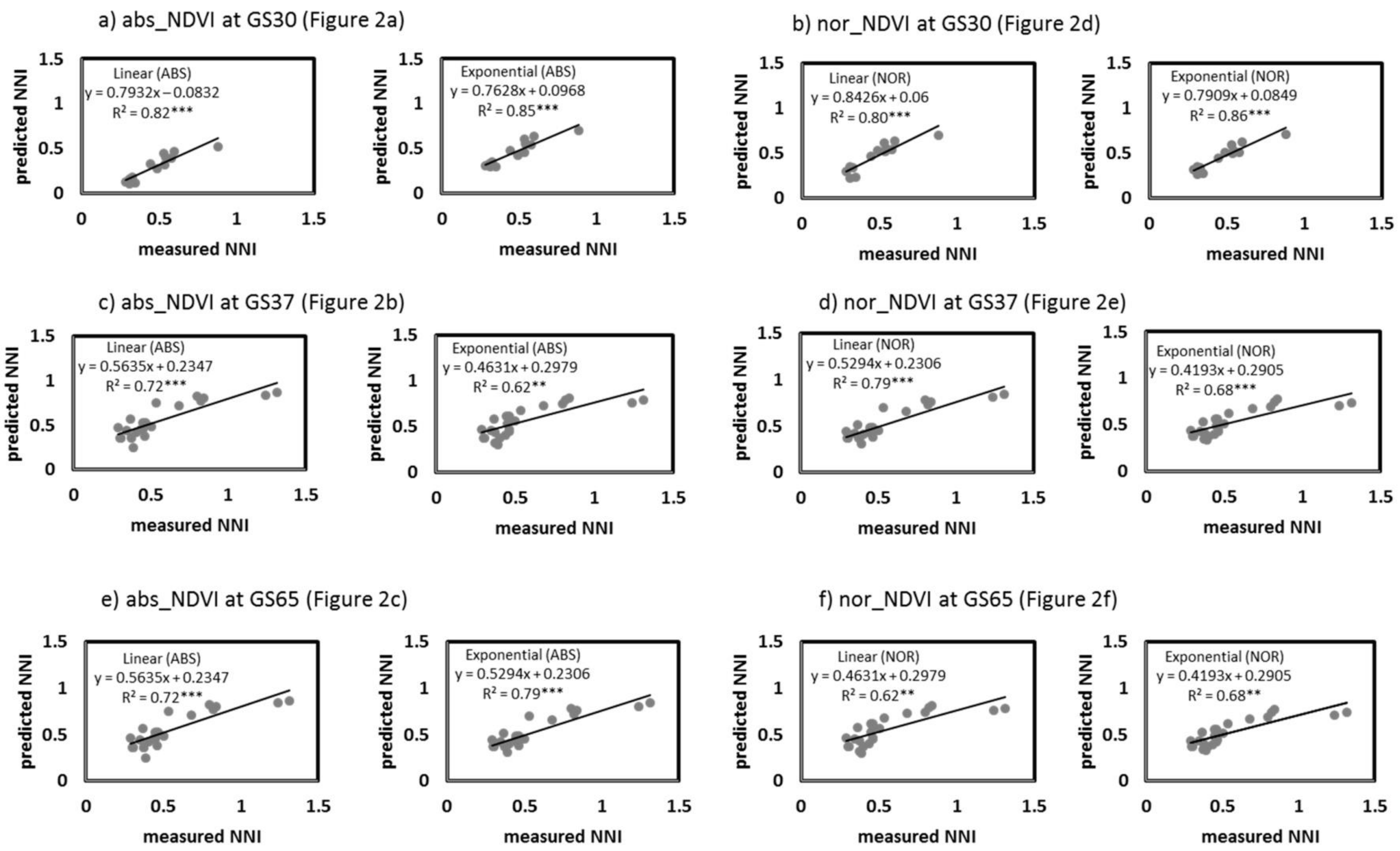

Figure 5.7. Relationships between the predicted NNI (Nitrogen Nutritional Index) values (from abs_NDVI at GS30 (**a**), nor_NDVI at GS30 (**b**), abs_NDVI at GS37(**c**), nor_NDVI at GS37 (**d**), abs_NDVI at GS65 (**e**) and nor_NDVI at GS65 (**f**) values in the growing stage-specific models) and the measured NNI values from 25% of the samples. **, *** Significant at 0.01 and 0.001 probability levels, respectively. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index.

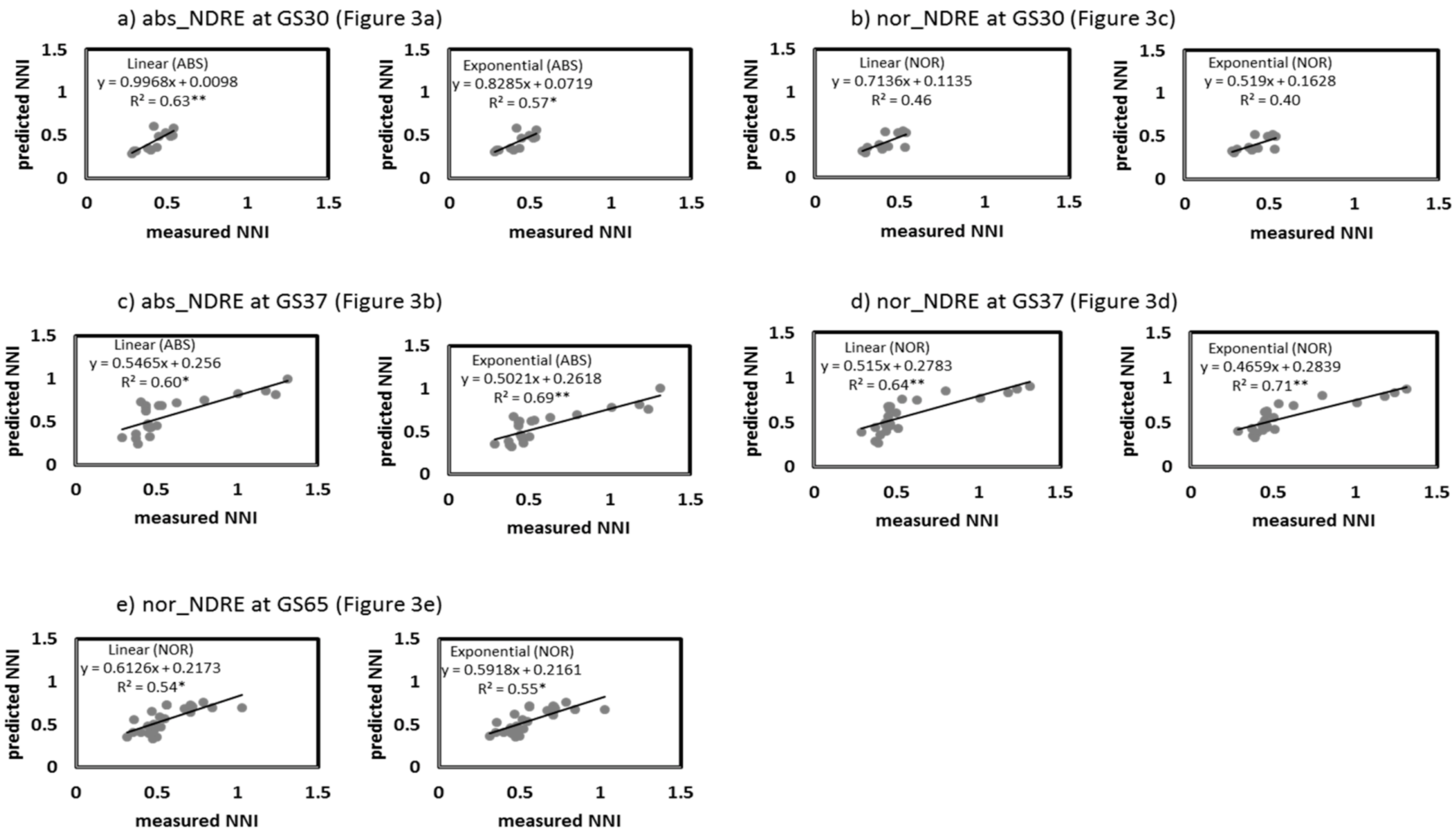

Figure 5.8. Relationships between the predicted NNI (Nitrogen Nutritional Index) values (from abs_NDRE at GS30 (**a**), nor_NDRE at GS30 (**b**), abs_NDRE at GS37(**c**), nor_NDRE at GS37 (**d**), and nor_NDRE at GS65 (**e**) values in the growing stage-specific models) and the measured NNI values from 25% of the samples. *, ** Significant at 0.05 and 0.01 probability levels, respectively. abs, absolute values; nor, normalized values; NDRE, Normalized Difference Red Edge Index

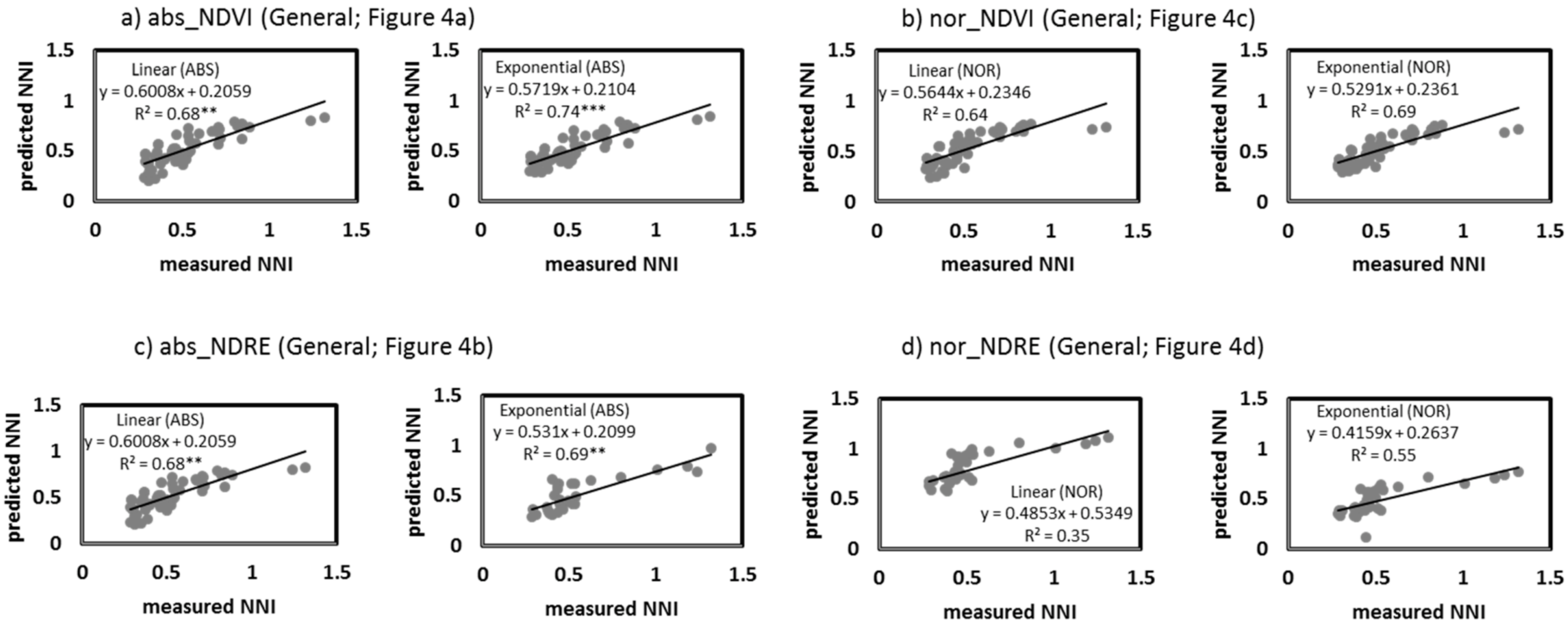

Figure 5.9. Relationships between the predicted NNI (Nitrogen Nutritional Index) values (from abs_NDVI (**a**) and nor_NDVI (**b**) and abs_NDRE (**c**) and nor_NDRE (**d**) values in the general models) and the measured NNI values from 25% of the samples. **,*** Significant at 0.01 and 0.001 probability levels, respectively. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index; NDRE, Normalized Difference Red Edge Index.

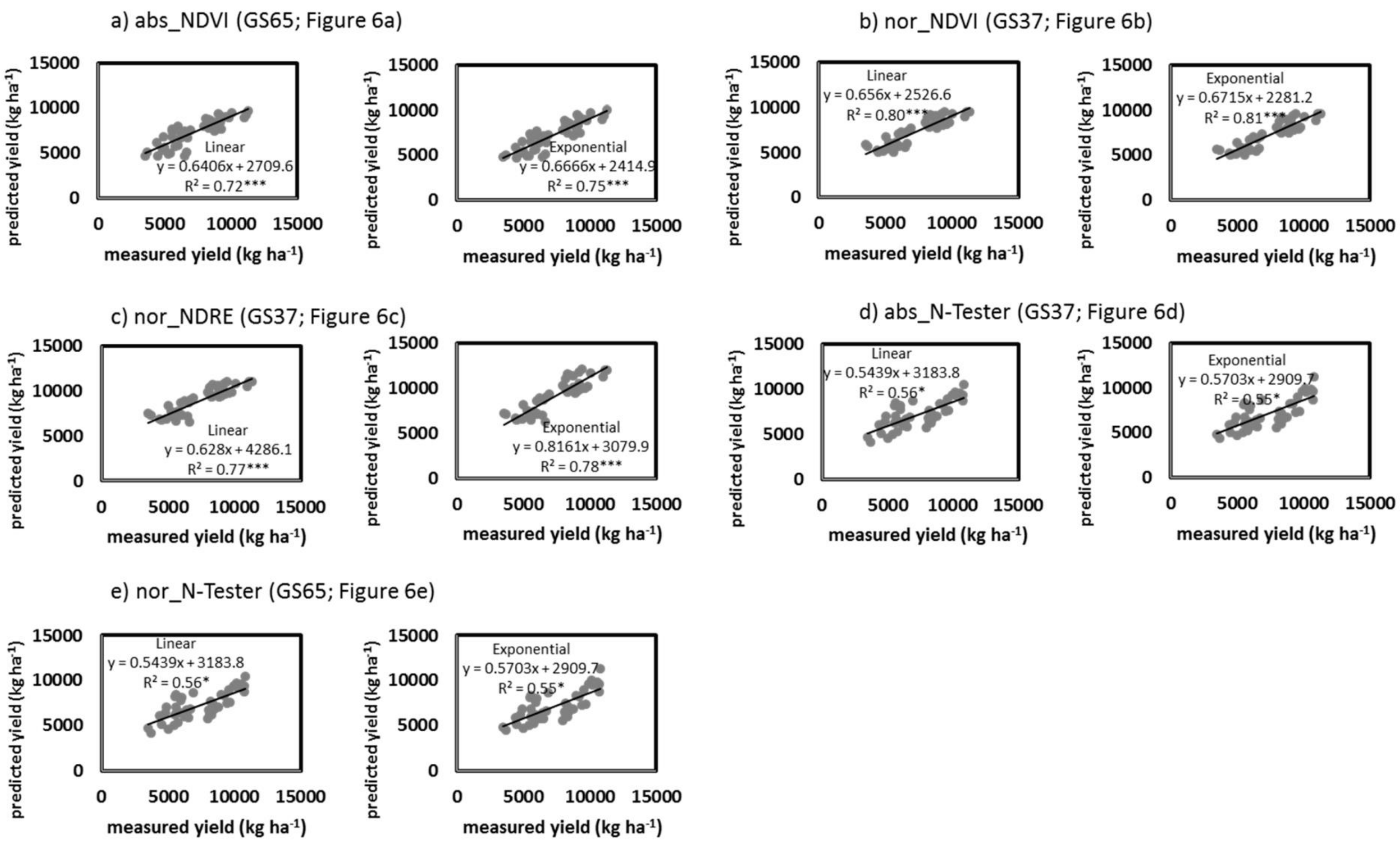

Figure 5.10. Relationships between the predicted yield values (from abs_NDVI at GS65 (**a**), nor_NDVI at GS37 (**b**), nor_NDRE at GS37 (**c**) abs_N-Tester at GS37 (**d**) and nor_N-Tester (**e**)) and the measured yield values from 25% of the samples. *,*** Significant at 0.05 and 0.001 probability levels, respectively. abs, absolute values; nor, normalized values; NDVI, Normalized Difference Vegetation Index; NDRE, Normalized Difference Red Edge Index.

5.4 Discussion

The NDVI has been the most commonly used vegetation index in agriculture over the last four decades (Tucker, 1979) and is a common measure for determining crop N status. The vast majority of the models for predicting the in-season N rate use NDVI (Cao *et al.*, 2005). Similar to the results of the present study, Xue *et al.* (2014) described a good relationship between N status and the vegetative period of rice (R^2 = 0.70–0.90), and Cao *et al.* (2015) found that the NDVI explained 47% of NNI changeability across growing stages and growing seasons in wheat.

Usually, in scientific studies, ground-based values are normalized with an overfertilized reference strip where non-limiting N has been applied. However, this approach has limitations because it is not easy for a control fringe to be representative of the field. Moreover, using normalized values would make the use of these tools more difficult for the farmer. Furthermore, Ravier *et al.* (2017b) showed that it is not easy to ensure that an overfertilized fringe is not N deficient, thereby problematizing the use of normalized data. In our case, the absolute values were normalized using a strip fertilized with 280 kg N ha^{-1}. However, these overfertilized treatments did not obtain NNI ≥ 1 in some situations. Moreover, when extending the method to large scale tools as the satellite, the utilization of absolute values is convenient, as having an overfertilized strip at each field for data normalization would be a challenging issue. However, if the measurements are not normalized it would be necessary to adjust the crop sensor measurements to different conditions, locations, and varieties (Craigie *et al.*, 2013).

Many authors have noted that the correlations between the NNI and proximal sensors fit better with growing stage-specific models. Sembiring *et al.* (2000) and Mistele and Schmidhaltel (2008) (using spectral reflectance) and Cao *et al.* (2012), Ravier *et al.* (2017b) (using chlorophyll meters), and Lu *et al.* (2017) (using RapidScan CS-45 sensors) have showed that the highest diagnostic accuracy obtained for cereals differs depending on the growing stage. In our study, the general model, especially abs_NDVI values, showed good accuracy in its NNI estimations, similar to the models for different growing stages (Figure 5.4). The accuracy of the correlations for validation was also high for abs_NDVI (Figures 5.7and 5.9a), especially in the exponential model. Remarkably, it would thus be easier to use a unique model for on-farm implementations than growing stage-specific models. As in the present study, other authors have also supported using general models with active canopy sensors for winter wheat (Cao *et al.*, 2018) and with chlorophyll meters for durum wheat (Debaeke *et al.,* 2006).

Saturation effects were detected for both the NDVI (around 0.8) and NDRE (around 0.4) indexes when NNI > 0.8. Although NDVI and NDRE reached saturation at a similar point, the NDRE value range is slightly wider for NDVI values around 0.8 (0.35–0.40; Figure 5.5). However, the NNI threshold values needed to achieve the maximum yields in wheat proposed by Ravier *et al.* (2017a) were always NNI < 0.8 or lower. In that case, the saturation effect of the RapidScan CS-45 indexes would not be a problem, as the values related to NNI = 0.8 were close to NDVI = 0.8 and NDRE = 0.37 (Figures 5.2–5.5). Therefore, the NDVI values obtained in our conditions would be useful for us making a good nitrogen nutritional diagnosis. The saturation effects on NDVI have been also shown by other authors for winter wheat (Cao *et al.,* 2015; Erdle *et al.,* 2011) and for rice (Gnyp *et al.,* 2014). It has been reported that when crops achieve a critical canopy or critical chlorophyll content, the NDVI saturation effect is relevant (Cao *et al.,* 2015; Moriondo *et al.,* 2007; Sharma *et al.,* 2015). This saturation effect is relevant when the canopy is very close and the NIR and visible light break into the crop canopy differently, thereby diminishing the normalization effect of the calculations (Knipling, 1970). When the canopy is close, the NIR reflectance increases while the red reflectance hardly changes (Knipling, 1970). The transmittance of the visible light through the canopy is low, so it is dominated by the top leaves of the plants (Knipling, 1970). However, the NIR detects the biomass bellow as it has a higher transmittance through the crop canopy (Knipling, 1970). The saturation effect can be reduced by using red-edge-based vegetation indices, such as NDRE (Cao *et al.,* 2018). Indices including red-edge channels could have higher sensitivity to chlorophyll content in crops, as Cao *et al.* (2018) and Zhang *et al.* (2019) detected that the red-edge bands were more suitable for determining crop N status than the NDVI, and Sharma *et al.* (2015) concluded that NDRE would be better for developing late-season N application algorithms. Conversely, Bonfil (2017) showed that the NDVI reaches the same accuracy as the NDRE with RapidScan CS-45 in wheat, similar to the present study.

The yield potential may vary between growing seasons because of the temporal variability in rainfall, temperature, or relative humidity (Ravier *et al.*, 2017a). In this study, the rainfall patterns were very different between years (data not shown), giving more variability to the data. Royo *et al.* (2003) and Martí *et al.* (2007) concluded that the prediction of wheat yield is better when the absolute NDVI readings from later in the season (flowering; GS60–GS65) are used (when yield estimates stabilize). In late growing stages, crop development is advanced in its phenology, and fewer abiotic effects can affect the grain yield. However, similar to our results, Magney *et al.* (2016) showed that the strongest predictions of wheat yield are made prior to heading (before GS50). Otherwise, the yield predictive capacity of the NDVI decreases during grain filling (Magney *et al.,* 2016).

The GPC prediction capacity with CSR and nor_CSR was not possible in the present study. The best relationship was observed between abs_N-Tester at GS65, where the GPC values could be partially explained (R^2 = 0.36). GPC is a product of the N assimilated by the crop prior to grain filling and of the environmental conditions that the crop undergoes in that period (Masclaux-Daubresse *et al.*, 2010). Contrary to our results, the literature showed the potential of using chlorophyll meters to estimate the GPC in wheat (Denuit *et al.,* 2002; Arregui *et al.,* 2006). As also verified by other authors (Hansen *et al.,* 2003), in this study, the NDRE and NDVI could not explain the GPC variability in any of the growing stages. Magney *et al.* (2016) showed that the NDVI's predictive capacity for GPC never exceeds 0.2. As much as 75%–90% of the total N in plants at harvest may come from the preanthesis (growing stage prior to GS60) N uptake in cereals (Dupont and Altenbach, 2003), significantly influencing grain quality (Montemurro *et al.,* 2003). However, it is not possible for crop sensors to estimate N translocation efficiency from the vegetative portion to the grain.

As the results showed, both tools have different behaviors in the prediction of NNI, yield, and GPC. These differences can be explained by the intrinsic differences between the proximal sensing tools, which sense different physical variables and different targets. The RapidScan CS-45 measurements are related to the photosynthetically active crop canopy biomass, while the Yara N-Tester measurements are related to leaf chlorophyll content. On the other hand, RapidScan CS-45 measures the crop reflectance, and Yara N-Tester measures the transmittance of the light on a particular leaf. The operations for the measurements are also different; the measurements using RapidScan CS-45 are simpler, faster, and cover a much larger area as they measure the whole canopy of the crop and thus better represent spatial variability. However, the Yara N-Tester only measures the central zone of the last fully developed leaf, even if this leaf has a relevant role in plant N nutrition.

N fertilization recommendations must be made based on remote sensing indexes that were studied and developed for NNI (Ravier *et al.*, 2018). NNI measurements require great time and labour and are not instantaneous as subsequent laboratory analyses are needed. Active crop sensor measurements, on the other hand, are not invasive, their information obtained for crop N status is instantaneous and well correlated with the NNI, and measurements can be taken at many time-points during the growing season. Following crop N status is not only important in conventional fertilization but also when organics are applied as initial fertilizers. Organic manures differ in their physical and chemical characteristics; they possess different N mineralization patterns and, therefore, leave uncertain quantities of N available for the crop (Aranguren *et al.*, 2018). Moreover, the same organic fertilizer may have different N release dynamics depending on the year, especially considering the long period between the application and beginning of N uptake by the plant (3–4 months).

Some authors have noted that having only two or three wavebands are a limitation to developing optimum vegetation indices (Colaço and Bramley, 2018). These authors have suggested the use of tools with more wavebands to calculate more complex indexes or indexes that also include green. Satellite remote sensing, where the waveband spectrum is more complete, may have the potential to improve ground-based sensor performance. However, for the accurate interpretation of satellite-based data necessary for making large-scale N fertilization recommendations, we must first understand the information obtained in ground-based areas through field-trials, where different fertilization strategies are tested and indexes are measured at various growing stages, as done in this study.

5.5 Conclusions

This study demonstrated that RapidScan CS-45 indexes are able to follow the NNI throughout the entire wheat growing season. A single model (for all growing stages) with absolute NDVI data can, therefore, be used for NNI prediction. The RapidScan CS-45 indexes were able to predict yield with normalized values at GS37 better than at GS65. RapidScan CS-45 and Yara N-Tester™ were not able to predict GPC. Data normalization improved the model for yield but not for NNI prediction. Therefore, following crop N status throughout the growing season using proximal sensing may allow for better adjustment of the N fertilizer to the crop requirements.

Chapter 6

Wheat Yield Estimation with NDVI Values Using a Proximal Sensing Tool

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6 Wheat Yield Estimation with NDVI Values Using a Proximal Sensing Tool

6.1 Introduction

One aim of agriculture has focused on the increase in cereal grain yields to feed an increasing population. Nitrogen (N) fertilization is an essential aspect for achieving this, as its application enhances grain yields. The optimization of the balance between yields and the environment needs fertilization strategies based on the regulation between N supply and crop needs (Diacono *et al.,* 2013). N fertilizer should be applied to satisfy crop demand, taking into account the soil mineral N, the N mineralized from applied organic amendments (as farmyard manures, slurries, etc.), and the N mineralized from previous crop residues (Padilla *et al.,* 2018). Besides, weather conditions vary considerably from growing season to growing season, releasing different amounts of N from organic matter, representing varying crop N needs within a single year throughout the season or among many years.

A key factor to obtain high yield is the optimal timing of N application (Fuertes-Mendizábal *et al.,* 2010). The main Zadoks wheat growth stages (Zadoks *et al.,* 1974) where topdressing N fertilizer is applied in the area where this experiment was performed (Araba, Basque Country, northern Spain) are the beginning of tillering (GS21) and the beginning of stem elongation (GS30). GS21 is the stage when tillers start emerging, where each one has the potential of producing a spike. Adequate N availability during the stem elongation stages determines the number of panicles and promotes crop growth (Thu *et al.,* 2014). In Araba, Basque Country, Spain, the mentioned growing stages are clues for establishing the required N fertilization, thus, usually, 40–60 kg N ha^{-1} is applied at GS21 and the greater and variable application is carried out at GS30. However, crop response to applied N is highly dependent upon the weather that occurs throughout the growing season (Aranguren *et al.,* 2019). The time elapsed between GS30 and grain harvest is long, and several factors might influence the N uptake by crops (Basso *et al.,* 2012).

For achieving the maximum benefit with the minimum environmental impact, it is necessary to balance the maximum yield and the minimum cost for production. Regarding grain yield, some periods of N deficiency in cereals are critical and others have no impact (Jeffroy and Bouchard, 1999). Ravier *et al.* (2017a) showed that even intense N deficiencies in wheat crops may have no impact on grain yield as long as they occur around the early growing stages (GS30). However, N deficiencies should decrease in intensity later in the growing season to maintain satisfactory crop production. Jeuffroy and Bouchard (1999) observed that the greatest losses in grain number were when the N deficiency was maintained until flowering (GS60 to GS69) (Zadoks *et al.,* 1974). Ravier *et al.* (2017a) detected threshold nitrogen nutrition index (NNI) values throughout key growing stages (GS30, GS32, GS39, and GS60), above which the yield did not decrease. However, NNI determination is time-consuming and needs destructive measurements of plant biomass and N content. Several authors have found correlations between NNI and optical sensing tool measurements (Lu *et al.,* 2017; Ravier *et al.,* 2017b; Li *et al.,* 2018). In a previous paper, Aranguren *et al.* (2020) studied if NNI could be predicted using RapidScan CS-45 or the chlorophyll meter Yara N-Tester™, and they concluded that the NNI and the normalized difference vegetation index (NDVI) calculated from RapidScan CS-45) were well correlated in the humid Mediterranean conditions of Araba ($R^2 = 0.70$), opening the possibility of establishing threshold NDVI values throughout key wheat growing stages for achieving maximum yields. Using crop diagnostic tools is more practical than using NNI and offers real-time decision support tools for farmers. Besides, proximal sensing study might help in satellite-based remote sensing implantation. However, for accurate interpretation of satellite-based data necessary for making large-scale N fertilization recommendations, first, it is necessary to understand the information obtained in field-trials through proximal sensing, where different fertilization strategies are tested and indexes are measured at various growing stages, as done in the present study.

To achieve high yields without negative effects on the environment, it is necessary to synchronize N fertilization to wheat N needs. Thus, tracking the crop N status throughout the wheat growing season is key to finding the balance between crop N requirements and fertilizer needs. The present study aims to establish NDVI values for the soft winter wheat yield in humid Mediterranean conditions at the GS30, GS32, GS37, and GS65 growth stages with the RapidScan CS-45 instrument for the purpose of setting fertilizer requirements to maximize yields while avoiding N losses.

6.2 Materials and Methods

6.2.1 Study Site

Three field trials were established in Arkaute (Araba, Basque Country, Spain) at NEIKER facilities in three consecutive wheat growing seasons, defined as 2015, 2016, and 2017 in different fields under rainfed conditions (Figure 6.1). The three field trials were flat, presented similar characteristics, and the distances among them were 130 m. Soils had high pH values, were calcareous, presented a sandy clay loam texture, and had a moderate organic matter content (2–2.5%) in the first 0–30 cm. The soil was classified as Typic Calcexeroll (SSS, 2015). Further details were described in a previous research study (Aranguren *et al.*, 2019).

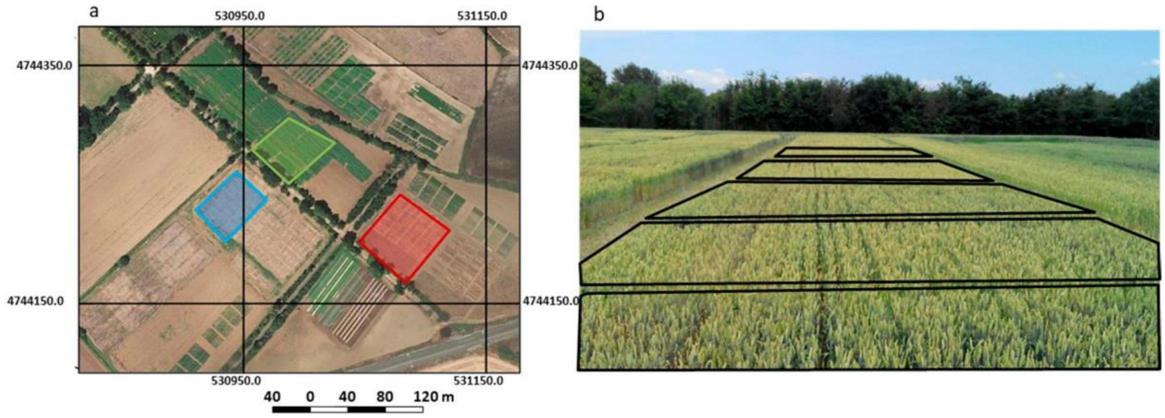


Figure 6.1. Location (**a**) and view (**b**) of the field experiments in Arkaute, Araba, Basque Country, northern Spain. (**a**) The 2015 field experiment was located in the red field, the 2016 field experiment was located in blue field, and the 2017 field experiment was located in the green field. (**b**) Marked plots in the field where the experiments were carried out.

6.2.2 Climate

The climate of the area was humid Mediterranean according to the water regime of Papadakis' (1966) classification. The meteorological conditions in the three growing seasons are described in Table 6.1.

6.2.3 Experimental Setup and Treatments

The experiment was of a factorial randomized complete block design with three factors (year, initial fertilization, and N rate at stem elongation) and featured four replicates. Three kinds of initial fertilization were applied (conventional, no organic fertilizer at basal dressing but 40 kg N ha^{-1} at GS21, dairy slurry, 40 t ha^{-1}, and sheep manure, 40 t ha^{-1}) which were combined with five topdressing N rates (0, 40, 80, 120, and 160 kg N ha^{-1} at GS30) (Table 6.2). The fertilizer used was calcium–ammonium–nitrate (NAC, 27%). Both a control without N fertilization (0N) and an overfertilized control plot (280N) were also established (Table 6.2). Information about the organic manure application and characteristics was described in (Aranguren *et al.*, 2019), where their effect in yield was deeply discussed.

Table 6.1. Total rainfall (mm) and days elapsed between wheat growing stages (Zadoks *et al.*, 1974) in three growing seasons (2015, 2016, and 2017) in the field experiment located in Arkaute.

Growing season	Growth stage	Total rainfall (mm)	Days elapsed
2015	Sowing (24/11) to GS21 (09/03)	521.2	106
	GS21 (09/03) to GS30 (04/04)	94.6	30
	GS30 (04/04) to GS32 (29/04)	43.2	21
	GS32 (29/04) to GS37 (11/05)	6	12
	GS37 (11/05) to GS65 (28/05)	13.8	17
	GS65 (28/05) to harvest (21/07)	55.5	54
2016	Sowing (06/11) to GS21 (19/01)	168.1	74
	GS21 (19/01) to GS30 (17/03)	296.1	56
	GS30 (17/03) to GS32 (30/03)	16.5	13
	GS32 (30/03) to GS37 (06/04)	24.3	7
	GS37 (06/04) to GS65 (25/05)	71.2	49
	GS65 (25/05) to harvest (02/08)	70.5	69
2017	Sowing (18/11) to GS21 (02/03)	271.3	105
	GS21 (02/03) to GS30 (06/04)	57.6	35
	GS30 (06/04) to GS32 (12/04)	0	6
	GS32 (12/04) to GS37 (25/04)	0	13
	GS37 (25/04) to GS65 (30/05)	82.4	35
	GS65 (30/05) to harvest (02/08)	114.3	63

Wheat (*Triticum aestivum* var. Cezanne) was sown at a 220 kg seed ha^{-1} rate on 24-11-2014, 06-11-2015, and 18-11-2016, and was harvested on 21-07-2015, 02-08-2016, and 02-08-2017 (Table 6.1). Yields were harvested at crop maturity using a plot harvester (1.5 m × 8 m) and were converted to 12% dry matter basis.

Table 6.2. Nitrogen (N) application rates and timing in three initial fertilization

treatments and in three growing seasons (2015, 2016, and 2017) in the field experiment located in Arkaute. Control (0N) and overfertilized (280N). GS21, beginning of tillering; GS30, stem elongation (Zadoks *et al.,* 1974).

Treatment name	**Initial fertilization**	**Topdressing at GS21 (kg N ha^{-1})**	**Topdressing at GS30 (kg N ha^{-1})**
40N + 0N	Conventional [-]	40	0
40N + 40N			40
40N + 80N			80
40N + 120N			120
40N + 160N			160
DS + 0N	Dairy slurry (DS) [40 t ha^{-1}]	--	0
DS + 40N			40
DS + 80N			80
DS + 120N			120
DS + 160N			160
SM + 0N	Sheep manure (SM) [40 t ha^{-1}]	--	0
SM + 40N			40
SM + 80N			80
SM + 120N			120
SM + 120N			160
0N	Control [-]	--	--
280N	Overfertilized [-]	80	200

6.2.4 Measurements with RapidScan CS-45

In previous research, Aranguren *et al.* (2020) found that NNI could be predicted using NDVI absolute values from the portable active crop canopy sensor RapidScan CS-45 (Holland Scientific, Lincoln, NE, USA). Following that finding, RapidScan CS-45 NDVI measurements from the key growing stages of the crop phenology (GS30, GS32, GS37, and GS65) were used with the aim of identifying and defining the NDVI threshold values that did not lead to a decrease in yield. Measurements were taken in all treatments in four replications and at control (0N) and overfertilized treatments (280N; Table 6.2).

The RapidScan CS-45 sensor features three optical photodetector channels that measure the reflectance from the crop at 670, 730, and 780 nm wavelengths. The RapidScan CS-45 sensors makes height not dependent on reflectance measurements, presenting a wide measurement range (from 0.3 m to 3 m). The field of view is approximately 45 degrees by 10 degrees. Measurements are sunlight independent, as RapidScan CS-45 is equipped with a modulated polychromatic lamp as an active light source. Besides, it is equipped with an internal GPS for geolocation with an accuracy <1 m. It presents a low noise performance. The measurements with RapidScan CS-45 were taken as the sensor was passed over the crop surface at approximately 1 m, always maintaining a 90° inclination with the crop at a constant walking speed. The sensor was handheld and two rows per plot were scanned. With both measurements, the coefficient of variation was calculated to measure the dispersion between readings. The coefficient of variation was always less than 5%. The NDVI values were averaged to generate a value from each plot.

The NDVI is the most widely known vegetation index (6.1) and is determined as follows:

$$NDVI = \frac{R_{NIR} - R_{RED}}{R_{NIR} + R_{RED}} \quad (6.1)$$

The accumulated NDVI (∑NDVI) was calculated (6.2) for each treatment throughout the wheat growing season with the measurements taken at GS30, GS32, GS37, and GS65 as follows:

$$\sum NDVI = NDVI_{GS30} + NDVI_{GS32} + NDVI_{GS37} + NDVI_{GS65} \quad (6.2)$$

6.2.5 Statistical analysis

Two different statistical analyses were carried out: different analyses of variance (ANOVAs) were performed for the factors affecting wheat grain yield and NDVI, and a coefficient of determination (R^2) was performed for the relationship between ∑NDVI and wheat grain yield. The yield-influencing three factors analyzed were the growing season, initial fertilization treatment, and N rate at GS30, determined via analyses of variance (ANOVA) using the R 3.2.5 software package. There was an interaction among the growing season, initial fertilization treatment, and N rate at GS30. Therefore, it was necessary to analyze each factor depending on the other factors. An ANOVA was performed to analyze differences among the N rate at GS30 in each initial fertilization treatment and each growing season (2015, 2016, and 2017). Another ANOVA was performed to analyze the differences among the initial fertilization treatment in each N rate at GS30 and growing season (2015, 2016, and 2017). To separate the means, Duncan's test was used ($p < 0.05$), using the R package "agricolae" (De Mendiburu, 2009). 0N and 280N were not included in the ANOVA. The statistical significance of the difference in yield between each treatment and 0N, as well as each treatment and 280N, were determined according to Welsh's *t*-test (for unequal variances) or via a pooled variance *t*-test (for equal variances). Levene's test was carried out with the aim of determining if the variance in the two populations to be tested was equal or not. The results of the *t*-tests are presented in Table A6.1 (differences with 0N) and Table A6.2 (differences with 180N).

As in the case of yield, it was necessary to analyze each factor depending on the others. The same was done for analyzing differences in the NDVI, since the aim was to relate NDVI values with yield. An ANOVA was performed to analyze the differences in the NDVI values among the N rate applied at GS30 in each initial fertilization treatment and growing season (2015, 2016, and 2017) using R 3.2.5. Another ANOVA was performed to analyze the differences among initial fertilization treatment in each N rate at GS30 and growing season (2015, 2016, and 2017). To separate the means, Duncan's test was used ($p < 0.05$) using the R package "agricolae" (De Mendiburu, 2009). The letters from Duncan's tests were used to see the differences among means and are not presented in the tables (Tables 6.3–6.5) because it would make their visualization difficult and heavy. Here, letters are presented in Tables A6.3 and A6.4. Nevertheless, when differences among NDVI values of N fertilization treatments were significant, each growing stage was marked with *** (i.e., significant at a 0.001 probability level) in Tables 6.3–6.5. When there was no difference, each growing stage was marked with "ns." Here, 0N and 280N were not included in the ANOVA. As with the yield, the statistical significance of the differences in NDVI between each treatment and 0N, as well as each treatment and 280N, were determined according to Welsh's *t*-test (unequal variances) or a pooled variance *t*-test (equal variances). Levene's test was carried out with the aim of determining if the variance in the two populations to be tested was equal or not. The results obtained from the *t*-tests are presented in Table A6.1 (differences with 0N) and Table A6.2 (differences with 180N).

A coefficient of determination (R^2) for the relationship between ∑NDVI and wheat grain yield was performed using R 3.2.5.

6.3 Results

6.3.1 NDVI throughout Wheat-Growing Season

6.3.1.1 Period Comprehended from GS21 to GS30

The NDVI values at GS30 in 2016 (Table 6.4) were higher than the ones in 2015 and 2017 (Tables 6.3 and 6.5), especially for treatments with organics as initial fertilizers. In 2015 and 2017, the values at GS30 were different for three initial fertilization treatments, where the values at the conventional treatment were higher (0.55–0.60) than those of the organic treatments (0.40–0.45). However, in 2016, the values for three initial fertilization treatments at GS30 were similar (0.65). In 2016, even the control (0N) reached a value higher than 0.5. More time elapsed in 2016 from GS21 to GS30 than in 2015 and 2017 (Table 6.2). Besides, in 2016 it rained far less before GS21 (Table 6.2) than in 2015 and 2017. Regarding the overfertilized plots, the NDVI value in 2015 was 0.6, in 2016 was 0.75, and in 2017 was 0.7.

6.3.1.2 Period Comprehended from GS30 to GS32

In 2016, for the treatments where 0 kg N ha^{-1} was added at GS30, the values descended from GS30 to GS32 (Table 6.4). In 2015, following the nitrogen application at GS30, the NDVI values rapidly increased, showing an increase in the photosynthetically active biomass. Besides, the 0N values also increased but in a smaller degree. In 2015 (Table 6.3) at GS32 in conventional treatment, there were no differences in the NDVI values when N was applied at GS30 (Table A6.3). In treatments where organics were applied, there were no differences among the highest N rates (80, 120, 160 kg N ha^{-1}). In 2016 (Table 6.4), at GS32, more differences among treatments were appreciable according to the N rate applied at GS30 (the higher the N rate, the higher the NDVI value). In 2017, the NDVI values did not increase from GS30 to GS32 in any of the treatments and there were no differences among the N rates applied at GS30 (Table 6.5). Regarding the NDVI values in the overfertilized plot in 2017, they decreased from GS30 (0.7) to GS32 (0.65).

6.3.1.3 Period Comprehended from GS32 to GS37

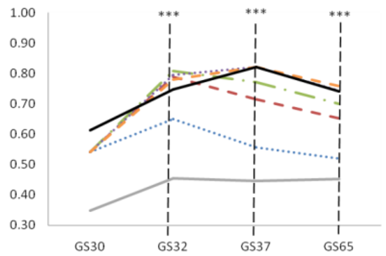
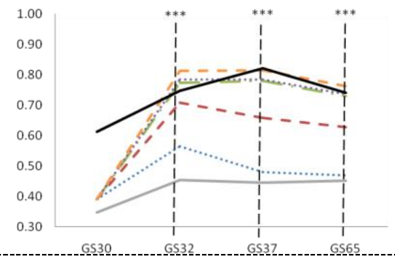
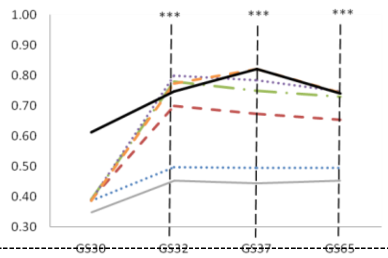
In 2015, the NDVI values showed that from GS32 onwards the organic treatments and conventional treatment with 80, 120, 160 kg N ha^{-1} rates applied at GS30 were similar (Table 6.3), reaching values above 0.75. In 2016, the NDVI values differed according to the different rates applied at GS30 in three initial fertilization treatments from GS32 on (Table 6.4). In 2017, the different N rates applied at GS30 were not reflected by different

NDVI values in any of the initial fertilizer treatments. However, differences in the NDVI values between conventional treatment and organic treatments persisted until GS37 (Table 6.5). In 2017, in the overfertilized plot, the NDVI values remained much higher than the rest of treatments (0.7).

6.3.1.4 Period Comprehended from GS37 to GS65

In 2015, the NDVI values in the majority of treatments decreased from GS37 to GS65 (Table 3). In 2016, the NDVI values in all treatments remained the same from GS37 to GS65 (Table 6.4). In 2017, the differences among NDVI depending on the N rates applied at GS30 were not measurable until GS65 in three initial fertilization treatments (Table 6.5). Thus, the higher the N rate applied at GS30, the higher the NDVI values at GS65. The NDVI values at the conventional treatments remained higher than the organics for the lower N rates applied at GS30 (0 and 40 kg N ha^{-1}).

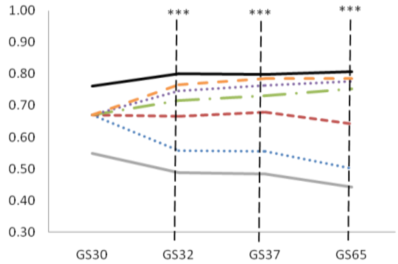
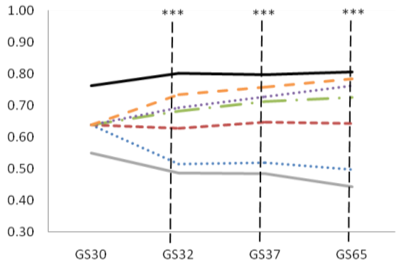
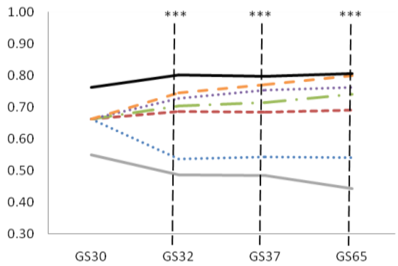
Table 6.3. Wheat grain yield (kg ha^{-1}) and normalized difference vegetation index (NDVI) readings collected with RapidScan CS-45 in 2015 at stem elongation, second node, leaf-flag emergence, and mid-flowering (GS30, GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) in the field experiment located in Arkaute.

Growing season	Initial fertilization	Treatment	Yield	sd	NDVI readings
2015	Conventional	40N + 0N	4942 C	555	
		40N + 40N	7078 B	912	
		40N + 80N	8215 A	548	
		40N + 120N	8230 A	144	
		40 N+ 160N	8688 A	812	
	Dairy slurry	DS + 0N	4378 C	145	
		DS + 40N	6271 B	56	
		DS + 80N	7762 A	316	
		DS + 120N	8275 A	345	
		DS + 160N	8181 A	961	
	Sheep manure	SM + 0N	4807 C	588	
		SM + 40N	6525 B	261	
		SM + 80N	7966 A	244	
		SM + 120N	8154 A	368	
		SM + 160N	8525 A	452	
	Control	0N	4119	277	
	Overfert.	280N	nd	nd	

Control 0N 40N 80N 120N 160N Overfert

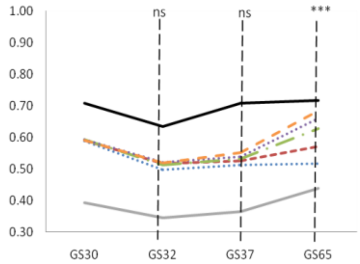
ANOVAs (analyses of variance) were performed to analyze differences in the yield and NDVI values (at GS32, GS37, and GS65) among N rates applied at GS30 (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) in each initial fertilization treatment (conventional, dairy slurry, and sheep manure). Different capital letters represent differences among yields at different N application treatments at GS30 for each initial fertilization treatment. Differences from Duncan's test in NDVI values are shown in Tables A6.3 and A6.4. ***, significant at a 0.001 probability level. 0N was not included in the ANOVAs. The statistical significance of the differences in yield and NDVI between each treatment (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) and 0N are shown in Tables A6.1 and A6.2. sd, standard deviation. nd, no data.

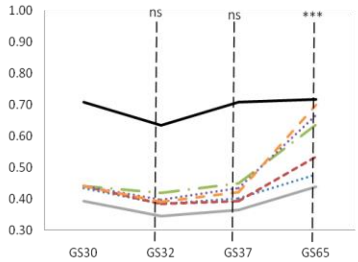
Table 6.4. Wheat grain yield (kg ha^{-1}) and NDVI readings collected with RapidScan CS-45 in 2016 at stem elongation, second node, leaf-flag emergence, and mid-flowering (GS30, GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) in the field experiment located in Arkaute.

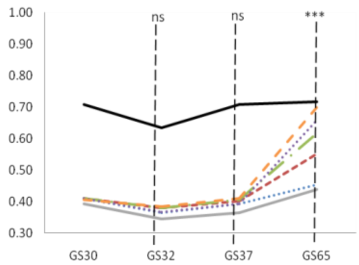
Growing season	Initial fertilization	Treatment	Yield	sd	NDVI readings
2016	Conventional	40N + 0N	6083 C	755	
		40N + 40N	8507 B	203	
		40N + 80N	9682 A	357	
		40N + 120N	9933 A	630	
		40N + 160N	10,554 A	401	
	Dairy slurry	DS + 0N	5969 C	525	
		DS + 40N	8431 B	480	
		DS + 80N	10,136 A	560	
		DS + 120N	10,221 A	426	
		DS + 160N	10,262 A	373	
	Sheep manure	SM + 0N	6659 D	801	
		SM + 40N	8803 C	424	
		SM + 80N	9518 BC	336	
		SM + 120N	10,446 AB	681	
		SM + 160N	10,772 A	726	
	Control	0N	5243	182	
	Overfert.	280N	10,375	911	

ANOVAs (analyses of variance) were performed to analyze differences in yield and NDVI values (at GS32, GS37 and GS65) among N rates applied at GS30 (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) in each initial fertilization treatment (conventional, dairy slurry and sheep manure). Different capital letters represent differences among yields at different N application treatments at GS30 for each initial fertilization treatment. Differences from Duncan's test in NDVI values are shown in Tables A6.3 and A6.4. ***, significant at a 0.001 probability level. Here, 0N and 280N were not included in the ANOVAs. The statistical significance of the differences in yield and NDVI between each treatment (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) and 0N, and each treatment (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) and 280N are shown in Tables A6.1 and A6.2. sd, standard deviation.

Table 6.5. Wheat grain yield (kg ha^{-1}) and NDVI readings collected with RapidScan CS-45 in 2017 at stem elongation, second node, leaf-flag emergence and mid-flowering (GS30, GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) in the field experiment located in Arkaute.

<table>
<tr><th>Growing season</th><th>Initial fertilization</th><th>Treatment</th><th>Yield</th><th>sd</th><th>NDVI readings</th></tr>
<tr><td rowspan="17">2017</td><td rowspan="5">Conventional</td><td>40N + 0N</td><td>5239 C a</td><td>192</td><td rowspan="5">


</td></tr>
<tr><td>40N + 40N</td><td>5905 B a</td><td>488</td></tr>
<tr><td>40N + 80N</td><td>6492 AB a</td><td>450</td></tr>
<tr><td>40N + 120N</td><td>6941 A a</td><td>202</td></tr>
<tr><td>40N + 160N</td><td>7095 A a</td><td>407</td></tr>
<tr><td rowspan="5">Dairy slurry</td><td>DS + 0N</td><td>3879 C b</td><td>168</td><td rowspan="5">


</td></tr>
<tr><td>DS + 40N</td><td>5081 B b</td><td>248</td></tr>
<tr><td>DS + 80N</td><td>5965 A b</td><td>322</td></tr>
<tr><td>DS + 120N</td><td>6056 A b</td><td>589</td></tr>
<tr><td>DS + 160N</td><td>6137 A b</td><td>104</td></tr>
<tr><td rowspan="5">Sheep manure</td><td>SM + 0N</td><td>3472 D b</td><td>196</td><td rowspan="5">


</td></tr>
<tr><td>SM + 40N</td><td>4704 C b</td><td>445</td></tr>
<tr><td>SM + 80N</td><td>5287 B b</td><td>413</td></tr>
<tr><td>SM + 120N</td><td>5537 AB b</td><td>290</td></tr>
<tr><td>SM + 160N</td><td>5923 A b</td><td>314</td></tr>
<tr><td>Control</td><td>0N</td><td>3348</td><td>320</td><td></td></tr>
<tr><td>Overfert.</td><td>280N</td><td>8020</td><td>268</td><td></td></tr>
</table>

Control ······ 0N — 40N — 80N ······ 120N — 160N — Overfert

ANOVAs (analyses of variance) were performed to analyze differences in yield and NDVI values (at GS32, GS37 and GS65) among N rates applied at GS30 (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) in each initial fertilization treatment (conventional, dairy slurry and sheep manure). Different capital letters represent differences among yields at different N application treatments at GS30 for each initial fertilization treatment. Different lowercase letters represent differences among yields for different initial fertilization treatment at the same N application treatment at GS30. Differences from Duncan's test in NDVI values are shown in Tables A6.3 and A6.4. ns, not significant. ***, significant at a 0.001 probability level. Here, 0N and 280N were not included in the ANOVAs. The statistical significance of the differences in yield and NDVI between each treatment (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) and 0N, and each treatment (X + 0N, X + 40N, X + 80N, X + 120N, X + 160N) and 280N are shown in Tables A6.1 and A6.2. sd, standard deviation.

6.3.2 NDVI Values for Maximum Grain Yield

Grain yield was significantly influenced by the amount of mineral N fertilizer at GS30 in the three growing seasons. Different NDVI combinations for similar yields were detected throughout the three growing seasons (Tables 6.3–6.5). It is remarkable that yields in 2016 were higher than in the other two growing seasons, and in 2017 yields were especially low (with the exception of the overfertilized plot (8000 kg ha^{-1})). Overall, and taking into account the controls, wheat grain yield varied between 4100 and 8700 kg ha^{-1} in 2015 (Table 6.3), 5200–10,700 kg ha^{-1} in 2016 (Table 6.4) and 3350–8000 kg ha^{-1} in 2017 (Table 6.5). In 2015, the maximum wheat yield was achieved in all the initial fertilization treatments (conventional, dairy slurry, and sheep manure) with 80 kg N ha^{-1} at GS30. Regarding the NDVI values, the treatments with 80, 120, and 160 kg N ha^{-1} rates applied at GS30 were comparable (Tables 6.3 and A6.3) in three initial fertilization treatments reaching values above 0.75, 0.76, and 0.69 at GS32, GS37, and GS65, respectively. The absence of significant differences in the NDVI values among treatments with the 80, 120, and 160 kg N ha^{-1} rates matched with the N rate with which the maximum yield was achieved (80 kg N ha^{-1} at GS30). In fact, for treatments with lower N application rates at GS30 (0 y 40 kg N ha^{-1}), the NDVI values (NDVI = 0.50–0.70) did not reach the 2015 NDVI values mentioned for the high yield producing treatments. In 2016 and 2017 (Tables 6.4 and 6.5), with conventional and dairy slurry treatments, the maximum wheat yield was achieved with a 80 kg N ha^{-1} rate and in sheep manure treatment with a 120 kg N ha^{-1} rate at GS30. In 2016, the NDVI values at the treatments that achieved the highest yield were 0.70 at GS32 and 0.75 at GS37 and GS65 in three initial fertilization treatments (Table 6.4). In the treatments where the highest yields were not achieved, the NDVI values did not reach the mentioned 2016 values (0.70 at GS32 and 0.75 at GS37 and GS65) corresponding to the most productive treatments. In 2017, the organics applied as initial fertilizers produced less than the conventional treatments for each N rate applied at GS30. In conventional treatment, the NDVI values at GS32 and GS37 were 0.5 for all different N rates applied at GS30. In the case of organics as initial fertilizers, the NDVI values at GS32 and GS37 were around 0.4 for all different N rates applied at GS30. The higher NDVI values achieved in conventional treatments than in organic treatments at those growing stages (Table A6.4) meant a difference of 500–1500 kg ha^{-1} in yield, depending on the N rate applied at GS30. At GS65, the NDVI values at the treatments that achieved the highest yield in three types of initial fertilization were 0.65 (Table 6.5), while in the rest of treatments the NDVI remained lower (0.4–0.6). That increase at GS65 meant a difference of up to 2000 kg ha^{-1} in yield between the treatments with the maximum yields and the lowest yields (the ones that received 0 kg N ha^{-1} at GS30). In 2017, in the overfertilized plot, the NDVI values remained higher than the rest of treatments from GS30 to GS65 (0.7 at GS30, 0.65 at GS32, 0.7 at GS37, and 0.7 at GS65; Table A6.2), which meant a difference in yield of 1500 kg ha^{-1} in comparison with maximum yields in conventional treatment, and 2000–2500 kg ha^{-1} in comparison with the maximum yields in the organic treatments.

Different NDVI combinations for similar yields were detected throughout the three growing seasons (Tables 6.3–6.5). For example, for achieving yields around 8500 kg ha^{-1}, different NDVI values at GS30, GS32, GS37, and GS65 were possible, specifically, 0.65, 0.69, 0.69, and 0.65, respectively (in treatment 40 + 40N in 2016), or 0.38, 0.79, 0.81, and 0.75, respectively (in treatments DS + 160N and SM + 160N in 2015). The ∑NDVI throughout the growing season could explain in a proper way the yields achieved (R^2 = 0.79, Figure 6.2). The higher the ∑NDVI, the higher the achieved yields, as in 2016, where yields were higher than in 2015 and 2017. In 2016, the ∑NDVI ranged between 2.1 and 3.1, in 2015 between 1.8 and 2.8, and in 2017 between 1.3 and 2.4. For example, for achieving yields around 8000 kg ha^{-1}, it is necessary to exceed ΣNDVI = 2.6.

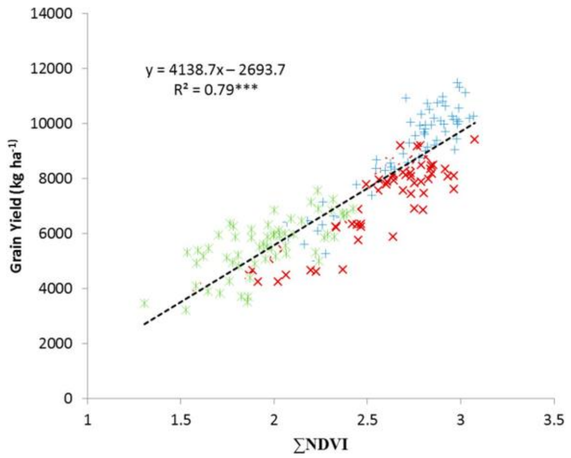


Figure 6.2. Relationship between wheat grain yield (kg ha^{-1}) and accumulated NDVI (∑NDVI) in three wheat growing seasons (2015 (**x**) 2016 (+) and 2017 (*)) at Arkaute. Linear model was fitted. ***, significant at a 0.001 probability level.

6.4 Discussion

These results show the high potential of the RapidScan CS-45 instrument to track the N status of wheat plants with NDVI during the crop growth, as Li *et al.* (2018) have also shown for rice. In fact, the NDVI has been considered a good indicator of green biomass or N content, focusing on the plant canopy (Colaço and Bramley, 2018), and has been a widely used vegetation index in agriculture for the last 40 years (Tucker, 1979). For following the physiological processes that control plant N uptake and yield, real-time information about crop nutritional status is necessary (Magney *et al.*, 2016). Each phenological period has an influence on the wheat harvest metrics, and thus the N deficiencies present a different effect depending on the intensity and duration in each moment of the crop growth (Jeuffroy and Bouchard, 1999). Some periods of N deficiency in wheat are critical and others have no impact or are tolerable (Ravier *et al.*, 2017a), making yields variable among years. In the present study, different NDVI evolution patterns were detected, causing different effects on grain yield (Tables 6.3–6.5). Ravier *et al.* (2017a) detected threshold NNI values throughout key growing stages that were tolerable for wheat without decreasing yields. NNI values should always exceed 0.4, 0.7, 0.7, and 0.8 at growth stages GS30, GS32, GS39, and GS60, respectively.

6.4.1 NDVI Values at Key Growing Stages

In the present study the minimum NDVI values for achieving the highest yields were 0.7–0.75 at GS32, GS37, and GS65. However, the NDVI threshold values at GS30 were not clear (0.4–0.65). In a previous paper (Aranguren *et al.*, 2020), good correlations between the NNI and NDVI were found; therefore, those correlations were used to compare the results of this study with the ones published by Ravier *et al.* (2017a). For achieving the NNI values proposed by Ravier *et al.* (2017a), the NDVI values should be 0.5 at GS30, 0.75 at GS32, 0.75 at GS37, and 0.8 at GS65. Similarities and differences were found when comparing the results of the present study with the ones proposed by Ravier *et al.* (2017a). At GS30, the results obtained in the present study and the ones proposed by Ravier *et al.* (2017a) diverged. However, at GS32, GS37, and GS65, the values obtained from this study were similar to the ones obtained by Ravier *et al.* (2017a).

Focusing on the GS30 growing stage, in all treatments in 2016 and only for the conventional treatments in 2017 and 2015 were the NDVI values higher than the ones proposed by Ravier *et al.* (2017a), 0.4 for the NNI and 0.5 for the NDVI according to the relationship established by Aranguren *et al.* (2020). The same happened for all overfertilized treatments, where the NDVI values were 0.75, 0.70, and 0.6 in 2016, 2017, and 2015, respectively. Tillering (GS21 to GS29) has been reported to be important for reaching an adequate wheat yield (Magney *et al.*, 2016; Asseng and Herwaarden, 2003), as a good tiller establishment allows a greater potential for biomass accumulation (Magney *et al.*, 2016). At this early growing stage (before GS30), when the soil water content is high, the most limiting contributor to slow tillering could be the soil temperature and anaerobic conditions (Magney *et al.*, 2016). The tillering period is related with wheat yield as it is involved in the determination of the spike number (Borràs-Gelonch *et al.*, 2012). Prolonging the tillering period results in a higher number of tillers formed (Magney *et al.*, 2016), which is probably what happened in 2016. The time elapsed from GS21 to GS30 was longer in 2016 (56 days) than in 2017 (35 days) and in 2015 (30 days) (Table 6.1), promoting more efficient N incorporation (higher N uptake), leading to a higher photosynthetically active biomass. The longer the time elapsed from GS21 to GS30, the higher the achieved NDVI values (0.65 in 2016, 0.45–0.6 in 2017, and 0.38–0.55 in 2015). Otherwise, in 2015 and 2017, it rained much more than in 2016, with a difference of 353 mm and 103 mm, respectively, from the same period in 2016 (Table 6.2).These heavy rains caused soil waterlogging, visible puddles, and anaerobic conditions, explaining the lower NDVI values. Soil waterlogging in winter in this area is not uncommon in very wet years. In fact, the experimental area is located in the Quaternary aquifer of Vitoria-Gasteiz, where the existence of several historically built trenches that 0.5 m deep with the purpose of avoiding flooding in agricultural areas have been described (Arrate *et al.,* 1997). Besides, those environmental conditions have provoked less N availability from the organics applied as initial fertilizers, explaining the lower NDVI values in the treatments with organics in 2015 and 2017 (0.4–0.45) than the conventional treatments (Tables 6.3 and 6.5). Otherwise, those values were lower than the threshold values proposed by Ravier *et al.* (2017a), 0.4 for NNI and 0.5 for NDVI according to the relationship established by Aranguren *et al.* (2020). In 2015, the yields were comparable between organic treatments and conventional treatments for each N rate applied at GS30. That fact suggests that a N deficiency at GS30 can be partially corrected if the plant is able to absorb enough N in the following growing stages, as in the year 2015 (0.4, 0.7, 0.7 (organics) and 0.55, 0.7, 0.7 (conventional) for GS30, GS32, and GS37, respectively). Similarly, Morris *et al.* (2006) reported that a N deficiency in early growing stages results in maximum or near maximum yields in winter wheat. Conversely, in 2017, the yields in organic treatments were significantly lower than the yields in conventional treatments for the same N rate applied at GS30, as the NDVI values in conventional treatments remained higher than in organics, at least until GS37 (Table A6.4; discussed in the following paragraphs). Yields in 2016 were generally higher than in 2015 and 2017, suggesting that the period before GS30 always has an important effect on crop yield if it maintained in the following key growing stages (from GS32 to GS65). No specific value was found for the GS30 growing stage, as the data volume was small for all possible options presented in the following key wheat growing stages (GS32, GS37, and GS65). That high variability made the establishment of the GS30 value difficult, thus, new data obtained from more experiments might help in its adjustment. Ravier *et al.* (2017a) also remarked that the N threshold at GS30 was not so clear in terms of the comparison with the other stages.

Focusing on stem extension (GS32 to GS37), the values detected in the present study for achieving maximum yields and the ones proposed by Ravier *et al.* (2017a) were similar (a NDVI value around 0.75). Adequate N availability at stem extension promotes crop growth through the development of viable tillers, increasing the future sink capacity of the plant, being related to the spike numbers and the grain number per unit area (Jeuffroy and Bouchard, 1999). A N deficiency in this period leads to the greatest losses in grain number (Jeuffroy and Bouchard, 1999). It is relevant that in 2017, during this period, the NDVI values were very low (Table 6.5) in most of the treatments (0.4–0.55) because of the lack of rain after N application at GS30 (Table 6.1), justifying the lower yields achieved in 2017 in comparison with 2015 and 2016. In fact, the treatments with organics presented lower NDVI values (NDVI = 0.40–0.45) than the conventional treatments (NDVI = 0.52–0.55), explaining the significantly higher yields in conventional treatments, as mentioned in the previous section. The NDVI values were significantly higher in the 280N treatment (Table A6.2, NDVI = 0.65–0.70) because of the high N rate applied at GS21 (80 kg N ha^{-1}), explaining the higher yields achieved (8020 kg ha^{-1}). Anyway, these values were lower than the threshold values proposed by Ravier *et al.* (2017a) and the ones detected in the present study (NDVI around 0.75). Villegas *et al.* (2001) showed that drought stress at stem extension resulted in lower rates of biomass accumulation. Adequate water availability for crops after the application of N fertilizer can be beneficial for a good N status and for achieving high yields (Tremblay and Bélec, 2016), as in 2015 and 2016 (Table 6.1), where after the mineral fertilizer application at GS30 the soil was sufficiently moist, making the N uptake possible. The rainfall quantity necessary for the absorption of a N application by the crops is at least 15 mm in the fortnight after the application (Arvalis, 2017).

At the GS65 growing stage, the NDVI values achieved at the treatments with the highest yields were similar to the ones proposed by Ravier *et al.* (2017a), with NDVI values around 0.75. In the 2017 growing season, this was the first growing stage where differences in the NDVI values among different N rates were appreciable (Table 6.5). The previous dry period and the rainfall that occurred after GS37 made the uptake of the N applied at GS30 viable. The N absorption by the crop from GS37 to GS65 was evident when comparing the yields obtained at the lower and higher doses applied at GS30, although it has been reported by other authors that this is late for increasing yields (Altman *et al.,* 1983). Similar to the present study, Asseng *et al.* (2016) reported that it is a viable method to improve yields via nitrogen application at GS59 (before flowering) if the soil is wet. Anyway, in 2017, there was a smaller increase in yield per kg of N applied at GS30 in comparison with the 2015 and 2016 growing seasons, which is appreciable in the lower yields achieved and in the narrow difference of up to 2000 kg ha^{-1} in yield between the treatments with the maximum yields and the lowest yields.

6.4.2 NDVI Dynamics and Wheat Grain Yield

In the present study, it was shown that when values at GS30 were 0.55–0.6 (higher than the ones proposed by Ravier *et al.* (2017a)) and around 0.75 at GS32, GS37, and GS65, maximum yields were achieved. It is remarkable that different dynamics in wheat NDVI values throughout the growing cycle were possible for achieving a specific yield, as mentioned in the results. There were treatments with a low N status at GS30 that raised their NDVI values by GS32 or GS37, while there were other treatments that, due to the low N rate applied at GS30, reduced their NDVI values by GS32 or GS37. During the wheat growing cycle, the circumstances in the early, middle, and late growth stages are strongly associated with the number of tillers, grain per spike, and 1000 grain weight, respectively (Wang *et al.*, 2014). A low N status during a given growing period, thus causing a deficiency in one of these parameters, may not have an effect on yield if the N status is higher in another key period. Thus, a nitrogen deficiency that may affect the number of tillers can be supplied by a higher N status when the grains per spike are established (Jeuffroy and Bouchard, 1999). The use of ∑NDVI is a way of testing that every key growing stage (GS30, GS32, GS37, and GS65) has an important effect on crop yield. The NDVI from early growing stages to anthesis holds a large amount of crop growth data, thus representing the impact of plant growth status on yield formation in wheat, as Wang *et al.* (2014) have shown. Besides, the ∑NDVI from GS30 to GS65 better explained the grain yield variability (80% of the variability, Figure 6.2) than just using the measurement of one growing stage alone. In a previous paper, Aranguren *et al.* (2020) found that NDVI absolute values could explain yields at GS65 (70% of the variability). In the case that a period of dryness occurs after the N application, as in 2017, where the N absorption did not occur until late growing stages, by using the ∑NDVI it is possible to predict how this will affect the wheat grain yield. Saturation effects have been reported for ∑NDVI values around 0.8 when NNI > 0.8 (Aranguren *et al.*, 2020). Anyway, the NNI threshold values for achieving maximum yields in wheat proposed by Ravier *et al.* (2017a) were always a NNI value of 0.8 or lower. Therefore, in this case, the saturation effect of the NDVI would not be problematic. Cao *et al.* (2018) reported that saturation effects can be decreased by using NDRE (a red-edge-based vegetation index), whereas in studies with the RapidScan CS-45 instrument it was shown that the NDRE reaches the same precision as the NDVI and a comparable saturation level (Aranguren *et al.*, 2020; Bonfil *et al.*, 2016). Besides, thinking of a possible future application of the images from the Sentinel satellite, it should be considered that the NDRE has a lower resolution than NDVI, as the red edge band (used for NDRE calculation) and red band (used for NDVI calculation) have radiometric resolutions of 20 m and 10 m, respectively (ESA, 2020)].

6.4.3 Proximal Sensing for Correcting Wheat Yield

For designing N fertilization schedules related to specific agronomic regions, it is necessary to obtain better knowledge of the critical moments and critical values for adjusting the requirements of the N fertilizer by the crop during the key vegetative growing stages. Remote sensing measurements are not laborious and can be taken periodically to monitor crop N status in an effective way until the end of the cereal-growing season. The present results show that following the NDVI dynamics throughout crop growth would be helpful for determining if there is a N deficiency, if it is possible to correct it by applying N, and therefore optimize crop N management for achieving high yields. In fact, it has been reported that it is always possible to correct a nitrogen deficiency until flowering (Ravier *et al.*, 2017a) if soil is humid (Ortuzar-Iragorri *et al.*, 2017). These NDVI threshold values have been defined for indicated edaphoclimatic conditions here and for the Cezanne variety. The fact that the results reported in the present study are similar to those obtained by Ravier *et al.* (2017a) in France with 215 treatments, different wheat varieties, and different locations, suggest that these values might be used in a wider range of conditions. Caution is needed regarding the universality of minimum NDVI values across wheat varieties and geographical locations. As Diacono *et al.* (2013) have reported, wheat variety should be considered, as each variety might have its own calibration requirements. They recommended the use of data normalization, but several concerns related to the use of normalized data have been found. Aranguren *et al.* (2020) found that the relationship between the NNI and normalized NDVI values is worse than using absolute NDVI values. Ravier *et al.* (2017b) reported that it is not easy to find a control strip to be representative of the entire field and ensure that an overfertilized fringe is not N deficient. Besides, data normalization makes the use of proxy tools more complicated for farmers. Bonfil *et al.* (2016) reported that using the RapidScan CS-45 instrument for cultivar classification, combining the data from a few monitoring days, was possible.

Understanding ground-based remote sensing could help with satellite remote sensing implementation, and this is why accurate interpretations of satellite-based data require robust ground-based reference data (Nguy-Robertson *et al.*, 2013). However, satellite remote sensing requires special training to process data, while active canopy sensors are simple to operate but are not adequate for extended surfaces.

6.5 Conclusions

The threshold NDVI values measured with a RapidScan CS-45 instrument for achieving the highest yields in the wheat Cezanne variety under humid Mediterranean conditions were 0.7–0.75 at the GS32, GS37, and GS65 growing stages. It was not clearly established here for the GS30 growing stage, as N deficiencies (NDVI = 0.4–0.45) may not affect wheat yields as long as the N status increases by GS32 and it is maintained thereafter.

Following the NDVI dynamics throughout the growing season could help to predict the yields at harvest time. Here, the ΣNDVI from GS30 to GS65 explained 80% of wheat yield variability. Therefore, a given yield could be achieved with different dynamics in wheat NDVI values throughout the growing cycle. The established ranges of NVDI values might be used for adjusting N fertilization to the wheat crop needs.

6.6 Appendix

Table A6.1. Statistical significance of the differences between each treatment (X + 0N, X + 40N, X + 80N, X + 120N, and X + 160N) and 0N for wheat grain yield and for NDVI readings measured with RapidScan CS-45 at stem elongation, second node, leaf-flag emergence, and mid-flowering (GS30, GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) at the 2015, 2016, and 2017 growing seasons according to Welsh's *t*-test (unequal variances) or the pooled variance *t*-test (equal variances).

Treatment	2015					2016					2017				
	Yield	NDVI readings				Yield	NDVI readings				Yield	NDVI readings			
		GS 30	GS 32	GS 37	GS 65		GS 30	GS 32	GS 37	GS 65		GS 30	GS 32	GS 37	GS 65
40N + 0N	*	***	**	*	*	*	***	**	*	*	***	***	**	***	**
40N + 40N	***	--	***	***	***	***	--	***	***	***	***	--	***	***	***
40N + 80N	***	--	***	***	***	***	--	***	***	***	***	--	***	***	***
40N + 120N	***	--	***	***	***	***	--	***	***	***	***	--	***	***	***
40N + 160N	***	--	***	***	***	***	--	***	***	***	***	--	***	***	***
DS + 0N	Ns	***	***	ns	ns	ns	**	ns	ns	ns	*	ns	ns	ns	ns
DS + 40N	***	--	***	***	***	***	--	***	***	***	***	--	ns	ns	***
DS + 80N	w***	--	***	***	***	***	--	***	***	***	w***	--	ns	ns	***
DS + 120N	***	--	***	***	***	***	--	***	***	***	***	--	ns	ns	***
DS + 160N	***	--	***	***	***	***	--	***	***	***	***	--	ns	ns	***
SM + 0N	Ns	ns	ns	ns	ns	ns	**	ns	ns	**	ns	ns	ns	ns	ns
SM + 40N	***	--	***	***	***	***	--	***	***	***	***	--	ns	ns	***
SM + 80N	**	--	***	***	***	***	--	**	**	***	w***	--	ns	ns	***
SM + 120N	w***	--	***	***	***	***	--	**	***	***	***	--	ns	ns	***
SM + 160N	***	--	***	***	***	***	--	**	***	***	***	--	ns	ns	***

*, **, and *** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ns, not significant. w, Welsh's *t*-test (unequal variances).

Table A6.2. Statistical significance of the differences between each treatment (X + 0N, X + 40N, X + 80N, X + 120N, and X + 160N) and 280N for wheat grain yield and for NDVI readings measured with RapidScan CS-45 at stem elongation, second node, leaf-flag emergence, and mid-flowering (GS30, GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) at the 2015, 2016, and 2017 growing seasons according to Welsh's *t*-test (unequal variances) or the pooled variance *t*-test (equal variances).

Treatment	2015					2016					2017				
	Yield	NDVI readings				Yield	NDVI readings				Yield	NDVI readings			
		GS30	GS32	GS37	GS65		GS30	GS32	GS37	GS65		GS30	GS32	GS37	GS65
40N + 0N	Nd	***	***	***	***	***	**	***	*	*	***	***	**	***	**
40N + 40N	Nd	--	***	***	***	***	--	***	***	***	***	--	***	***	***
40N + 80N	Nd	--	***	***	***	***	--	***	***	***	***	--	***	***	***
40N + 120N	Nd	--	***	ns	***	***	--	***	***	ns	***	--	***	***	***
40N + 160N	Nd	--	***	ns	ns	ns	--	***	ns	ns	***	--	***	***	***
DS + 0N	Nd	***	*	***	***	***	***	***	***	***	***	***	***	***	***
DS + 40N	Nd	--	ns	***	***	***	--	***	***	***	***	--	***	***	***
DS + 80N	Nd	--	ns	ns	ns	ns	--	***	***	***	***	--	***	***	***
DS + 120N	Nd	--	ns	ns	ns	ns	--	***	***	***	***	--	***	***	***
DS + 160N	Nd	--	***	ns	ns	ns	--	***	ns	ns	***	--	***	***	**
SM + 0N	Nd	***	**	***	***	***	***	***	***	***	***	***	***	***	***
SM + 40N	Nd	--	***	***	***	***	--	***	***	***	***	--	***	***	***
SM + 80N	Nd	--	***	***	ns	**	--	***	***	***	***	--	***	***	***
SM + 120N	Nd	--	**	ns	ns	ns	--	***	***	***	***	--	***	***	***
SM + 160N	Nd	--	***	ns	ns	ns	--	**	ns	ns	***	--	***	***	ns

*, **, and *** significant at 0.05, 0.01, and 0.001 probability levels, respectively. ns, not significant.

Table A6.3. Duncan's test ($p < 0.05$) results of the differences for NDVI readings measured with RapidScan CS-45 at the second node, leaf-flag emergence, and mid-flowering (GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) among different N application rates at GS30 (0, 40, 80, 120, 160 kg N ha^{-1}) for each initial fertilization treatment (conventional, dairy slurry, and sheep manure) and each growing season (2015, 2016, 2017).

Initial fertilization	Treatment	2015			2016			2017		
		NDVI readings			NDVI readings			NDVI readings		
		GS32	GS37	GS65	GS32	GS37	GS65	GS32	GS37	GS65
Conventional	40N + 0N	B	C	C	C	C	C	ns	ns	C
	40N + 40N	A	B	B	B	B	B			B
	40N + 80N	A	AB	AB	AB	AB	A			AB
	40N + 120N	A	A	A	A	A	A			A
	40N + 160N	A	A	A	A	A	A			A
Dairy Slurry	DS + 0N	B	C	C	C	C	C	ns	ns	C
	DS + 40N	B	B	B	B	B	B			B
	DS + 80N	A	A	A	AB	A	AB			A
	DS + 120N	A	A	A	AB	A	A			A
	DS + 160N	A	A	A	A	A	A			A
Sheep manure	SM + 0N	B	C	C	C	C	C	ns	ns	C
	SM + 40N	B	B	B	B	BC	B			B
	SM + 80N	A	A	A	AB	B	AB			A
	SM + 120N	A	A	A	A	A	AB			A
	SM + 160N	A	A	A	A	A	A			A

Different capital letters represent differences in NDVI readings among different N application rate at GS30 (0, 40, 80, 120, 160 kg N ha^{-1}) for each initial fertilization treatment (conventional, dairy slurry, and sheep manure) and each growing season (2015, 2016, 2017). ns, not significant.

Table A6.4. Duncan's test ($p < 0.05$) results of the differences in NDVI readings measured with RapidScan CS-45 at stem elongation, second node, leaf-flag emergence, and mid-flowering (GS30, GS32, GS37, and GS65, respectively (Zadoks *et al.* 1974)) among different initial fertilization treatments (conventional, dairy slurry, and sheep manure) for each N application rate at GS30 (0, 40, 80, 120, 160 kg N ha^{-1}) and each growing season (2015, 2016, 2017).

N rate at GS30	Initial fertilizer	2015				2016				2017			
		NDVI readings				NDVI readings				NDVI readings			
		GS30	GS32	GS37	GS65	GS30	GS32	GS37	GS65	GS30	GS32	GS37	GS65
0N	Conventional	A								A	A	A	
	Dairy Surry	B	ns	ns	ns	ns	ns	ns	ns	B	B	B	ns
	Sheep manure	B								B	B	B	
40N	Conventional										A	A	
	Dairy Surry	--	ns	ns	ns	--	ns	ns	ns	--	B	B	ns
	Sheep manure										B	B	
80N	Conventional										A	A	
	Dairy Surry	--	ns	ns	ns	--	ns	ns	ns	--	B	B	ns
	Sheep manure										B	B	
120N	Conventional										A	A	
	Dairy Surry	--	ns	ns	ns	--	ns	ns	ns	--	B	B	ns
	Sheep manure										B	B	
160N	Conventional										A	A	
	Dairy Surry	--	ns	ns	ns	--	ns	ns	ns	--	B	B	ns
	Sheep manure										B	B	

Different capital letters represent differences in NDVI readings among each initial fertilization treatment (conventional, dairy slurry, and sheep manure) for each different N application rate at GS30 (0, 40, 80, 120, 160 kg N ha^{-1}) and each growing season (2015, 2016, 2017). ns, no significant.

Chapter 7

Estimation of the wheat grain protein content using a chlorophyll meter under humid Mediterranean conditions

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7 Estimation of the wheat grain protein content using a chlorophyll meter under humid Mediterranean conditions

7.1 Introduction

One aim of agriculture has focused on the increase in wheat grain yields to feed the increasing population and also to achieve high-quality grain. Grain quality is characterized in different ways, such as hardness, specific weight, Chopin Alveograph, Zeleny volume, and Hagberg number (Lopez-Bellido *et al.,* 2004), but the main indicator is the grain protein content (GPC, Fuertes-Mendizábal *et al.,* 2010). GPC prediction is complex because it depends on several aspects related to crop nitrogen (N) utilization, such as genetics (variety), environmental factors, and agronomic management practices such as N fertiliser application (Gaju *et al.,* 2014). N fertilisation is a crucial factor for increasing yields and GPC. The amount of N applied to wheat must be carefully managed to the balance yield, grain quality, and environment needs, adjusting the N supply and crop requirements (Diacono *et al.,* 2013). However, it is difficult to increase grain yield and quality concurrently due to its negative relationship (Simmonds, 1995).

In the area where this study was located (Araba, Basque Country, northern Spain), low GPC values have been reported due to the high yields achieved (Fuertes-Mendizábal *et al.,* 2010). Additionally, the varieties commonly used have been selected to obtain high yields rather than obtaining high GPC. In this area, the beginning of tillering (GS21, Zadoks *et al.,* 1974) and stem elongation (GS30, Zadoks *et al.,* 1974) growing stages are clues to establishing the required N fertilisation. The usual application rate is 40–60 kg N ha^{-1} at GS21 and a greater but variable application at GS30. In some cases, a third late N fertiliser application at leaf-flag emergence (GS37, Zadoks *et al.,* 1974) has also been considered to increase the grain N concentration because weather conditions are humid around the fertilisation moment (beginning of May in our conditions; Fuertes-Mendizábal *et al.,* 2010).

The N utilization of crops involves several processes, such as N uptake, assimilation, translocation, and remobilization (Fuertes-Mendizábal *et al.,* 2012). Grain N is derived from two different N sources: N that is absorbed in the post-anthesis period from the soil (GS60–GS90; Zadoks *et al.,* 1974) and N remobilized to the grain that was accumulated in vegetative organs in the pre-anthesis period (until GS60). After anthesis, vegetative organs behave as N sources, protein hydrolysis occurs and amino acids are transported to the grain (Fuertes-Mendizábal *et al.,* 2012). Thus, a large proportion of N in grain (60%–95%) might come from N remobilized rather than being taken from the soil (Lopez-Bellido *et al.,* 2004), but it might depend on the weather conditions (Fuertes-Mendizábal *et al.,* 2010). Thus, because the grain-filling process depends on several factors, the in-season assessment of GPC remains challenging in cereals. The recommended level of GPC for high-quality bread-making flour should be higher than 12.5% (RD 677/2016). Farmers who grow cereals to achieve high-quality grain cannot predict if the crop will have the required protein standard (Lopez-Bellido *et al.,* 2004). The main concern is to ensure the GPC values that the crop will achieve and determine in advance whether an extra N rate is required. Not applying it when there is no need would benefit the farmer economically and would be environmentally friendly, avoiding N leaching and N gaseous losses (Ortuzar-Iragorri *et al.,* 2017). Additionally, a better estimation of the GPC would help cooperatives improve the planning of their sales strategies because they could determine beforehand the percentage of grain that could be sold for bread-making flour or as animal feed.

Because a significant proportion of N in the grain has been remobilized from the leaves, stem, and roots of the plant in the post-anthesis period, it is reasonable to measure the N content from the shoot part at mid-anthesis (GS65, Zadoks *et al.,* 1974) to predict GPC and anticipate whether a late N supply is necessary. Leaves are the most important organs in terms of N reserves, accounting for up to 50–62% of the total N of the plant (Fuertes-Mendizabal *et al.,* 2012). The leaf flag was shown as a good indicator of the whole shoot N status at GS65 (Lopez-Bellido *et al.,* 2004) and has been used to predict GPC in winter wheat (Wang *et al.,* 2004). Measuring N in the plant leaves is not practical because it requires destructive and time-consuming procedures such as sampling and laboratory analysis, making it impractical for farmers. In this sense, it is necessary to have rapid results and easy measurements of the shoot N status to develop reference values to decide whether a late N supply is necessary and predict the GPC at harvest.

Chlorophyll meters such as Minolta SPAD (Minolta corporation, Ltd., Osaka, Japan) or Yara N-Tester™ (Yara International ASA, Oslo, Norway) can provide instantaneous results for diagnostic purposes. Arregui *et al.* (2006) and Ortuzar-Iragorri *et al.* (2005) found good relationships between chlorophyll meters and N content of plant leaves in the same climatic conditions of the present field experiment, making them interesting to obtain rapid results. Relationships between remote sensing tool measurements against GPC have been studied in cereals, but there were no consistent results across locations and years (Hansen *et al.,* 2003; Magney *et al.,* 2016; Wang *et al.,* 2004). In a previous study, Aranguren *et al.* (2020) showed no general relationship across years between RapidScan CS-45 (NDVI and NDRE) or Yara N-Tester™ and GPC values under humid Mediterranean conditions. However, they detected that the relationship between the absolute chlorophyll meter values at GS65 (mid-anthesis; Zadoks *et al.,* 1974) and GPC across years (R^2=0.35) was improved compared with NDVI and NDRE (Aranguren *et al.,* 2020). They attributed that low predictability to the mentioned lack of consistency across years, as other authors have shown (Lopez-Bellido *et al.,* 2004). As previously mentioned, several variables, such as yield, the moment of crop N absorption or remobilisation efficiency, might affect the grain filling process (Hansen *et al.,* 2003). Therefore, based on previous results (Aranguren *et al.,* 2020) and literature review (Miller *et al.,* 1999; Ortuzar-Iragorri *et al.,* 2017; Lopez-Bellido *et al.,* 2004), it was hypothesized that chlorophyll meter readings might be helpful in understanding GPC in wheat but is necessary to study and understand the effect of other variables affecting the grain-filling process.

This study performed under humid Mediterranean conditions was aimed to i) evaluate the effect of the application of a variable N rate at stem elongation (GS30) when farmyard manure is applied as initial fertilisers to chlorophyll meter values at mid-anthesis (GS65) and GPC values, ii) determine the possibility of establishing a relationship between the chlorophyll meter values at GS65 and GPC, and iii) establish the minimum chlorophyll meter value needed at GS65 to obtain GPC values above 12.5%.

7.2 Materials and Methods

7.2.1 Study site

Three field trials were established in Arkaute (Araba, Basque Country, Spain) at NEIKER facilities in three consecutive wheat growing seasons defined as 2015, 2016 and 2017 in different fields under rainfed conditions. The three field trials were flat, presented similar characteristics and the distance among them was 130 m. Other soil properties were described previously (Aranguren *et al.,* 2019).

7.2.2 Climate

The climate of the area was humid Mediterranean according to the water regime of Papadakis' (1966) classification. The total rainfall (mm) and days elapsed between relevant key wheat growing stages in the three growing seasons are shown in Table A7.1.

7.2.3 Experimental Setup and Treatments

The experiment was a factorial randomized complete block design with three factors (year, initial fertilisation, and N rate at stem elongation) and four replicates. Three kinds of initial fertilisation were applied that were combined with five N rates (0, 40, 80, 120, and 160 kg N ha^{-1}) as top dressings applied at GS30. The mineral fertiliser was calcium-ammonium-nitrate (NAC 27%). Apart from the treatments, two controls were established (Table 7.1): a control without N fertilisation (0 N) and an overfertilised control plot (280 N). Information regarding the organic manure characteristics and application was previously described (Aranguren *et al.*, 2019). Soft wheat (*Triticum aestivum* var. Cezanne) was sown at a 220 kg seed ha^{-1} rate on 24-11-2014, 06-11-2015, and 18-11-2016 and was harvested on 21-07-2015, 2-08-2016 and 2-08-2017.

7.2.4 Yield and GPC

Grain yields were harvested at crop maturity using a plot harvester (1.5 m × 8 m) and were converted to a 12% dry matter basis. The grain samples were oven-dried at 70ºC for at least 48 hours and then ground through a 1-mm screen before the determination of the total N concentration using the Kjeldhal procedure (AOAC, 1999). GPC was determined as the product of the grain N concentration multiplied by 5.7 (Teller, 1932).

Table 7.1. N application rates and timing in three initial fertilisation treatments and three growing seasons (2015, 2016 and 2017) in the field experiment located in Arkaute. Control (0 N) and overfertilised (280 N). GS21, beginning of tillering; GS30, stem elongation (Zadoks *et al.*, 1974).

Initial fertilisation	Topdressing at GS21 (kg N ha^{-1})	Topdressing at GS30 (kg N ha^{-1})	Treatment identification
Conventional [-]	40	0	40N+0N
		40	40N+40N
		80	40N+80N
		120	40N+120N
		160	40N+160N
Dairy Slurry (DS) [40 t ha^{-1}]	--	0	DS+0N
		40	DS+40N
		80	DS+80N
		120	DS+120N
		160	DS+160N
Sheep manure (SM) [40 t ha^{-1}]	--	0	SM+0N
		40	SM+40N
		80	SM+80N
		120	SM+120N
		160	SM+120N
Control [-]	--	--	0N
Overfertilised [-]	80	200	280N

7.2.5 Increase in the N content in the Aerial Part during the Post-anthesis Period (from GS65 to harvest)

N accumulation in the aerial part of the wheat crop during the post-anthesis period (from GS65 to harvest) was measured in all conventional treatments, DS+0N, SM+0N and 0N (Table 7.1) and was calculated as follows:

$$\textit{Post-anthesis N increase (kg N ha}^{-1}\textit{) = Crop Total N – GS65 Total N} \qquad (7.1)$$

Crop Total N content was calculated as:

$$\textit{Crop Total N (kg N ha}^{-1}\textit{) = Grain Total N (kg N ha}^{-1}\textit{) + Straw Total N (kg N ha}^{-1}\textit{)} \qquad (7.2)$$

where Grain Total N content was calculated as:

$$\textit{Grain Total N (kg N ha}^{-1}\textit{) = Grain Yield (kg ha}^{-1}\textit{) x Grain N concentration (\%)} \qquad (7.3)$$

where Straw Total N content was calculated as:

$$\textit{Straw Total N (kg N ha}^{-1}\textit{) = Straw Yield (kg ha}^{-1}\textit{) x Straw N concentration (\%)} \qquad (7.4)$$

Total N at GS65 content was calculated as:

$$\textit{GS65 Total N (kg N ha}^{-1}\textit{) = Biomass (kg ha}^{-1}\textit{) x N (\%)} \qquad (7.5)$$

Biomass was calculated and the N concentration was analysed using the Kjeldhal procedure under all conditions (AOAC, 1999).

7.2.6 Measurements using Yara N-Tester^{TM}

Yara N-Tester^{TM} (Yara International ASA, Oslo, Norway) is a chlorophyll meter that measures light transmitted by the plant leaf at two different wavelengths, 650 (red light) and 940 nm (near-infrared light). The instrument processes a digital reading calculated from the light transmitted by the plant leaf and light transmitted with no sample.

The chlorophyll meter calculates a value (*M*) that is determined as follows:

$$M = k\ log10\,\frac{Io(650)I(960)}{I(650)Io(960)} \qquad (7.6)$$

where *Io* is the intensity of the incident monochromatic light and *I* is the intensity of the transmitted light. The K value is instrument dependent.

Yara N-Tester^{TM} readings were taken for all treatments (Table 7.1) and GS65. The measurements were taken in the last fully developed leaf in the middle of the blade. Thirty main-stem flag leaves were measured at random along with the plots. A mean value was calculated for each plot. The acquired values were expressed as the relative chlorophyll content and were unitless.

7.2.7 Statistical Analysis

The three factors that influence the GPC, yield, Yara N-Tester^{TM} at GS65, the N content in the post-anthesis period, and the grain total N content were the growing season, initial fertilisation treatment and N rate at GS30 were analysed by analysis of variance (ANOVA) using 'R 3.2.5' software. To separate the means, Duncan's test was used ($p < 0.05$) using the R package *agricolae*.

Coefficients of determination (R^2) were calculated for the relationships between the Yara N-Tester^{TM} values at GS65 and GPC (%) for each growing season (2015, 2016 and 2017) using 'R 3.2.5' software. Otherwise, the Cate–Nelson procedure was performed to determine the accuracy of Yara N-Tester^{TM} values at GS65 to predict GPC (%). The Cate–Nelson procedure can determine the critical level that best divides the data into two populations.

7.3 Results

The interaction among the growing season, initial fertilisation treatment and N rate at GS30 was significant. Therefore, each factor was analysed depending on the other factors. The differences among the growing seasons are not presented in Tables 7.2 and 7.3 because of the high volume of data; thus, they are presented in supplementary Tables A7.2 and A7.3. However, if differences were mentioned in the main text, they were considered statistically significant.

7.3.1 Wheat grain protein content (GPC) and Yield

The GPC varied from 7.4% to 10.9% in 2015, from 7.5 to 10% in 2016, and from 8.4 to 13.3% in 2017 (Table 7.2). In 2017, the GPC values were generally higher and the range of variation was wider than those in 2016 and 2015 (Table A7.2). In 2016, no differences were detected in the GPC values among the treatments (Table 7.2). However, in 2015 and 2017, significant differences were detected in the GPC values derived from the N rate applied at GS30 but not from the initial fertilisation treatments. In 2015, the highest GPC values were achieved with the 120 kg N ha^{-1} N rate in conventional and dairy slurry treatments; however, in sheep manure, the highest GPC values were achieved with 160 kg N ha^{-1}. In 2017, the GPC values increased together with the N rates applied at GS30.

The amount of mineral N fertiliser at GS30 significantly influenced the yield values in the three growing seasons. The wheat grain yield varied between 4,300 and 8,700 kg ha^{-1} in 2015, 6,000 and 10,800 kg ha^{-1} in 2016, and 3,800 and 7,000 kg ha^{-1} in 2017 (Table 7.2). The maximum wheat grain yields were achieved with 80 kg N ha^{-1} applied at GS30 in 2015 under all the initial fertilisation treatments (conventional, dairy slurry and sheep manure) and in 2016 and 2017 under conventional and dairy slurry initial fertilisation treatments. The maximum wheat grain yields were achieved with 120 kg N ha^{-1} applied at GS30 in 2016 and 2017 with sheep manure treatment.

Table 7.2. Wheat grain protein content (GPC), grain yield (kg ha^{-1}) and Yara N-Tester^{TM} readings at GS65 (mid-anthesis; Zadoks *et al.*, 1974) in the field experiment located in Arkaute in 2015, 2016 and 2017

Initial fertilisation	Treatment	2015			2016			2017		
		GPC (%)	**Yield (kg ha^{-1})**	**N-Tester GS65**	**GPC (%)**	**Yield (kg N ha^{-1})**	**N-Tester GS65**	**GPC (%)**	**Yield (kg N ha^{-1})**	**N-Tester GS65**
Conventional	**40 + 0N**	8.2 ± 2.4 BC	4942 ± 555 C	469 ± 47 C	8.3 ± 1.3	6083 ± 755 C	456 ± 65	8.4 ± 0.3 D	5239 ± 192 C a	478 ± 15 C
	40 + 40N	7.7 ± 0.3 C	7078 ± 912 B	530 ± 23 B	7.9± 1.3	8507 ± 203 B	474 ± 9	9.3 ± 0.5 C	5905 ± 488 B a	578 ± 18 B
	40 + 80N	8.6 ± 0.3 BC	8215 ± 548 A	585 ± 29A	8.0 ± 1.0	9682 ± 357 A	539 ± 42	10.8 ± 0.3 B	6492 ± 450 AB a	604 ± 48 B
	40 + 120N	9.7 ± 0.8 AB	8230 ± 144 A	614 ± 12A	9.5 ± 1.0	9933 ±630 A	551 ± 51	11.5 ± 0.9 B	6941 ± 202 A a	687 ± 3 A
	40 + 160N	10.4 ± 0.8 A	8688 ± 812 A	625 ± 9 A	9.5 ± 1.6	10554 ± 401 A	570 ± 57	12.9 ± 0.5 A	7095 ± 404 A a	710 ± 18 A
Dairy slurry	**DS + 0N**	8.1 ± 0.9 BC	4378 ± 145 C	489 ± 18D	9.6 ± 2.1	5969 ± 525 C	453 ± 28 B	8.6 ± 0.3 E	3879 ± 168 C b	460 ± 11 C
	DS + 40N	7.7 ± 0.4 C	6271 ± 56 B	547 ± 31 C	7.5 ± 0.9	8431 ± 480 B	467 ± 26 B	9.6 ± 0.4 D	5081 ± 248 B b	570 ± 22 B
	DS + 80N	8.7 ± 0.2 BC	7762 ± 316 A	570 ±19 B	8.5 ± 1.8	10136 ± 560 A	538 ± 35 A	10.9 ± 0.4 C	5965 ± 322 A b	605 ± 46 B
	DS + 120N	10.3 ± 0.8 A	8275 ± 345 A	601 ± 17 B	9.2 ± 2.0	10221 ± 426 A	562 ± 17 A	12.2 ± 0.4 B	6056 ± 589 A b	661 ± 26 A
	DS + 160N	10.6 ± 0.9 A	8181 ± 961 A	644 ± 18A	10.0 ± 3.3	10262 ± 373 A	610 ± 45 A	13.2 ± 0.6 A	6137 ± 104 A b	677 ± 8 A
Sheep manure	**SM + 0N**	7.4 ± 0.4C	4807 ± 588 C	503 ± 12 C	8.9 ± 1.6	6659 ± 801 D	452 ± 21 C	9.2 ± 0.4 E	3472 ± 196 D b	484 ± 5 C
	SM + 40N	7.7 ± 0.3 BC	6525 ± 261 B	521 ±28 C	10.0 ± 1.4	8803 ± 424 C	516 ± 33 B	10 ± 0.2 D	4704 ± 445 C b	562 ± 7 B
	SM + 80N	8.8 ± 0.3 BC	7966 ± 244 A	579 ± 20 B	7.7 ± 1.0	9518 ± 336BC	535 ± 39 B	10.8 ± 0.4 C	5287 ± 413 B b	604 ± 10 B
	SM + 120N	9.3 ± 1.7 B	8154 ± 368 A	623 ± 39A	8.4 ± 0.8	10446 ± 681 AB	575 ± 6 A	12 ± 0.4 B	5537 ± 290 AB b	653 ± 44 A
	SM + 160N	10.9 ± 1.0 A	8525 ± 452 A	639 ± 17A	8.6 ± 0.8	10772 ± 726 A	601 ± 48 A	13.3 ± 0.3 A	5923 ± 314 A b	683 ± 11 A
Control	**0N**	7.5 ± 0.1	4119 ± 277	652 ± 43	7.7 ± 1.1	5243 ± 182	409 ± 72	8.51 ± 0.3	3348 ± 320	478 ± 15
Overfert.	**280N**	nd	nd	657 ± 24	8.6 ± 1.1	9375 ± 911	550 ± 6	13.1 ± 0.2	8020 ± 268	683 ± 11

Uppercase letters represent differences ($p<0.05$) among different N rates applied at GS30 (X+0N, X+40N, X+80N, X+120N, X+160N) for each initial fertilisation treatment in each growing season. Lowercase letters represent differences ($p<0.05$) among different initial fertilisation treatments (conventional, dairy slurry or sheep manure) for each N rate applied at GS30 in each growing season. The absence of uppercase or lowercase letters indicates that no significant differences ($p>0.05$) were detected. *nd*, no data; ±, *sd* (standard deviation); 0N and 280N were not included in ANOVA

7.3.2 Yara N-Tester^{TM} readings and GPC Prediction

At the GS65 growing stage, the lowest Yara N-Tester^{TM} values were 470, 450 and 460 for 2015, 2016 and 2017, respectively, and the highest values were 660, 610 and 710 for the same years, respectively (Table 7.2). In 2015, with conventional treatment, the highest Yara N-Tester^{TM} values were achieved with the 80 kg N ha^{-1} rate applied at GS30, with dairy slurry treatment with the 160 kg N ha^{-1} rate and with sheep manure with the 120 kg N ha^{-1} rate. In 2016, with conventional treatment, no differences were found in the Yara N-Tester^{TM} readings. In 2016, with the dairy slurry treatment, the maximum Yara N-Tester^{TM} values were achieved with the 80 kg N ha^{-1} rate applied at GS30, and with sheep manure with the 120 kg N ha^{-1} rate. In 2017, (Table 7.2), under the three initial fertilisation treatments, the Yara N-Tester^{TM} maximum values were achieved with the 120-kg N ha^{-1} rate.

In 2016, the Yara N-Tester^{TM} values at GS65 could not explain the GPC variability (Figure 7.1b). However, in 2015 and 2017, the Yara N-Tester^{TM} values at GS65 could explain 67% and 77% of the GPC variability, respectively (Figure 7.1a and c). The Cate–Nelson procedure can identify the critical level that best divides the data into two populations. The points in quadrant II represent the population with the highest GPC values, and those in quadrant IV the population with the lowest GPC values. The critical level is that at which R^2 reaches a maximum. The Yara N-Tester^{TM} critical value dividing the two populations was 619 in 2015 and 591 in 2017. The GPC critical value obtained was 11.23 in 2015 and 10.29 in 2017. In 2015, the number of points in quadrant II was much lower than that in quadrant IV. In 2017, the dispersion of the points in the relationship was uniform in quadrants II and IV. The points located in quadrants I and III are considered outliers and correspond to the overestimation or underestimation of GPC, respectively. The error of the Yara N-Tester^{TM}, calculated as the percentage of outliers in relation to the total of points, was 28% in 2015 and 6% in 2017. In 2015, there were more error points mainly located in quadrant III (underestimation) and corresponding to yields higher than 8,000 kg ha^{-1}. Thus, the Yara N-Tester^{TM} and GPC values differ in their relationship when the yields are lower or higher than 8,000 kg ha^{-1}. When the yields are lower than 8,000 kg ha^{-1}, the relationship between the Yara N-Tester^{TM} values and GPC values increase linearly; when the yields are higher than 8,000 kg ha^{-1}, the relationship is unclear.

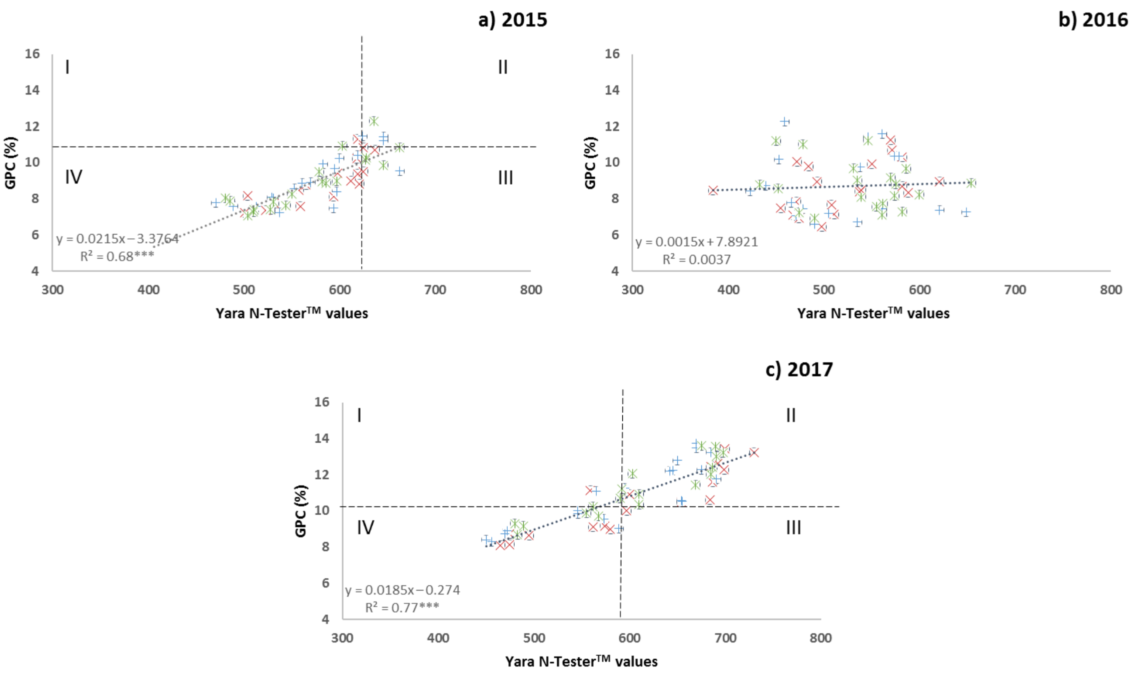

Figure 7.1. Relationship between the Yara N-Tester™ values at GS65 and % GPC in the 2015 (a), 2016 (b) and 2017 (c) growing seasons at Arkaute. Type of initial fertilisation: conventional **x**; DS: dairy slurry +; SM: sheep manure *. The linear model was fitted. ***, Significant at the 0.001 probability level. Strip lines indicate the critical *x* value and critical *y* value separating the data into four quadrants following the Cate–Nelson procedure. The Roman numerals indicate the quadrant of the plot. The data inside quadrants II and IV are concordant with the regression. The data inside quadrants I and III are not in concordance.

Therefore, the coefficients of determination (R^2) for the relationships between Yara N-Tester™ values at GS65 and GPC were calculated based on the 8,000 kg ha^{-1} yield. When the yields were lower than 8,000 kg ha^{-1}, the capacity to predict the GPC variability from Yara N-Tester™ readings was similar among the three growing seasons (R^2 = 0.75; Figure 7.2a). Yields lower than 8,000 kg ha^{-1} in 2015 occurred in the three lowest N rates (0, 40 and 80 at GS30) under DS and SM treatment and in the two lowest rates (0 and 40 at GS30) under conventional treatment. In 2016, yields lower than 8,000 kg ha^{-1} occurred with X+0N treatment, and in 2017 with all treatments. The error of Yara N-tester™ calculated as the percentage of outliers in relation to the total of points was 5%. When the yields were higher than 8,000 kg ha^{-1}, no significant relationship was found between GPC and Yara N-tester™ readings at GS65 (Figure 7.2b).

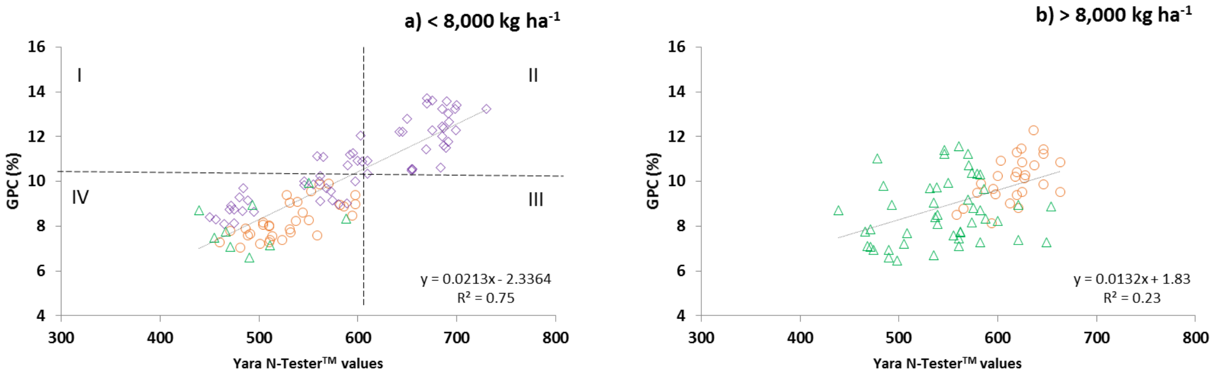

Figure 7.2. Relationship between the Yara N-Tester™ values at GS65 (mid-anthesis; Zadoks *et al.*, 1974) and GPC% when the wheat grain yields are lower than 8,000 kg N ha^{-1} (a) and when the wheat grain yields are higher than 8,000 kg ha^{-1} (b) in the field study at Arkaute. Wheat growing seasons: 2015○; 2016△; 2017◊. The linear model was fitted. ***, Significant at the 0.001 probability level. Strip lines indicate the critical *x* value and critical *y* value separating the data into four quadrants following the Cate–Nelson procedure. The Roman numerals indicate the quadrant of the plot. The data inside quadrants II and IV are concordant with the regression. The data inside quadrants I and III are not in concordance.

7.3.3 Factors that Might Affect the GPC Predictability

Regarding the grain total N at harvest (Table 7.3 and Table A7.3), in 2016, the values were generally higher (63–168 kg N ha^{-1}) than those in 2015 (51–149 kg N ha^{-1}) and 2017 (46–146 kg N ha^{-1}). The higher is the N rate at GS30, the higher are the grain total N values in the three growing seasons. In 2015, and 2016, the maximum values were achieved with the 120 kg N ha^{-1} rate applied at GS30; however, in 2017, the maximum value was achieved with the highest N rate (160 kg N ha^{-1}). In 2016, the DS+0N and SM+0N treatments presented higher values than the 40N+0N treatment.

No significant differences among the N treatments were detected in the post-anthesis N increase in the aerial part of the wheat crop from GS65 to harvest in any of the growing seasons (Table 7.3). However, treatments in the 2016 growing season presented a slightly higher N increase during the post-anthesis period (24–59 kg N ha^{-1}) than treatments in 2015 (6–32 kg N ha^{-1}) and 2017 (11–37 kg N ha^{-1}; Table A7.3). The differences between conventional treatment and treatments with organics as initial fertilisers without N application at GS30 (0 kg N ha^{-1}) were only significant in 2017. In that case, the conventional treatments achieved higher values (30 kg N ha^{-1}) than the treatments with organics as initial fertilisers (13–19 kg N ha^{-1}).

Table 7.3. Wheat crop total N content at harvest (kg N ha^{-1}) and increase in the N content in the aerial part of the crop (kg N ha^{-1}) during the post-anthesis period (from GS65 to harvest).

Treatment	2015				2016				2017			
	Grain Total N	***sd***	**Post-anthesis N increase**	***sd***	**Grain Total N**	***sd***	**Post-anthesis N increase**	***sd***	**Grain Total N**	***sd***	**Post-anthesis N increase**	***sd***
40N+0N	66 D	*17*	10	*1*	84 D	*18*	31	*11*	70 E	*3*	30 a	*6*
40N+40N	90 C	*14*	32	*9*	113 C	*17*	51	*18*	88 D	*11*	18	*5*
40N+80N	116 B	*12*	21	*6*	130 B	*19*	31	*2*	112 C	*6*	37	*11*
40N+120N	132AB	*14*	10	*5*	157AB	*17*	59	*22*	128 B	*13*	11	*6*
40N+160N	149 A	*20*	20	*4*	168 A	*33*	44	*18*	146 A	*6*	28	*9*
DS+0N	59 D	*8*	15	*5*	96 C	*24*	53	*26*	53 E	*4*	13 b	*5*
SM+0N	58 D	*5*	6	*2*	101 C	*29*	43	*14*	51 E	*3*	19 b	*3*
0N	51	*4*	17	*7*	63	*17*	24	*8*	46	*4*	11	*6*

Uppercase letters represent differences among different N rates applied at GS30 (40N+0N, 40N+40N, 40N+80N, 40N+120N, 40N+160N) for each initial fertilisation treatment. Lowercase letters represent differences ($p<0.05$) among different initial fertilisation treatments (conventional, dairy slurry or sheep manure) for the 0 kg N ha^{-1} rate applied at GS30 in each growing season. The absence of uppercase or lowercase letters indicates that no significant differences ($p>0.05$) were detected. *sd*, standard deviation. 0N was not included in ANOVA

7.4 Discussion

7.4.1 Periods Affecting Grain Protein Content (GPC) Prediction in Wheat

The N accumulated at pre-anthesis, mid-anthesis (GS65), and post-anthesis may affect N partitioning at the plant level (Monaghan *et al.*, 2001) and, thus, the GPC values in wheat crop.

In the present study, each kg of N applied at GS30 (pre-anthesis period) had a different effect depending on the growing season. Although grain total N was similar in 2015 and 2017 (Table 7.3), the GPC values were higher in 2017 than in 2015 (Table 7.2). In 2017, rain did not after the N application at GS30, and this dry period persisted until GS37 (Table A7.1), causing a late N uptake (from GS37 onwards), low yields (3,800–7000) and higher GPC values (8.4–13.3%). In the 2017 growing season, the NDVI values started increasing from GS37 and biomass accumulation (sink size) was low from GS30 to GS37 (data not shown). Lopez-Bellido *et al.* (2004) reported that high values of the grain N concentration and low yields were obtained in the years with dry conditions, such as 2017 (Table A7.1). Additionally, some authors have reported significant increases in the GPC in wheat in the same edaphoclimatic area when N is applied after GS37 (Fuertes-Mendizábal *et al.,* 2010; Ortuzar-Iragorri *et al.,* 2017). In relation to the 2016 growing season, the grain total N was higher than that in the other two growing seasons (Table 7.3). In 2016, the N applied at GS30 had a huge effect on the yield because, as the N rate applied at GS30 increased, the yield values increased more than that in the other two growing seasons. By contrast, the GPC values did not increase with the increases in the N rate applied at GS30 (Table 7.2). The grain total N values, high yields and achieved GPC values showed a clear dilution effect. In 2016, the NDVI values were high from GS30 to GS65 (data not shown). Thus, the crop sink capacity was high since early in the growing season. Bogard *et al.* (2010) showed that an increased crop sink capacity strongly affects the grain yield. The issue is that the grain yield and grain quality are difficult to improve simultaneously due to the negative relationship between them (Simmonds, 1995). Increasing the yield might have an important effect on reducing the GPC due to the dilution effect of carbon-based compounds (Lopez-Bellido *et al.*, 2004; Acreche and Slafer, 2009). Fuertes-Mendizábal *et al.* (2010) mentioned that climatic conditions in Araba could lead to very high yields, and, therefore, a low GPC due to the dilution effect.

Yara N-Tester™ readings taken at mid-anthesis (GS65) in the leaf flag could explain the GPC variability in the 2015 (Figure 7.1a) and 2017 (Figure 7.1c) growing seasons (R^2 = 67% and 77%, respectively). That correlation was reasonable because the flag-leaf provides N more directly to the spike and a strong depletion of more than 50% occurs in the flag-leaf N content during the grain-filling period (Fuertes-Mendizabal *et al.*, 2018). However, the relationship between the chlorophyll meter readings and GPC variability was growing season dependent. Thus, in 2016, it was not possible to explain the GPC variability from the Yara N-Tester™ readings (Figure 7.1b). The Cate–Nelson test showed that the critical values in 2015 divided the population into two different subpopulations (one with higher GPC values and the other with lower GPC values), whereas the Yara N-Tester™ and GPC values differed in their relationship. That division matched with yields approximately 8,000 kg ha^{-1}. When the yields exceeded 8,000 kg ha^{-1} (Figure 7.2b), no clear pattern in the relationship was observed between Yara N-Tester™ values and GPC values, similar to the findings for most of the treatments in 2016 (except for the treatments with 0 kg N ha^{-1} applied at GS30) and treatments with the highest N rates in 2015. However, when the yields were lower than 8,000 kg ha^{-1}, the GPC variability prediction capacity from Yara N-Tester™ readings was similar in the three growing seasons (R^2 = 0.75; Figure 7.2a). Other authors, such as Turley *et al.*, (2001) with yields ranging from 3,800 to 8,500 kg ha^{-1} and López-Bellido *et al.*, (2004) with yields ranging from 4,000 to 11,000 kg ha^{-1}, showed that taking chlorophyll meter readings at the leaf flag from GS60 to GS69 was adequate for GPC variability prediction. They remarked that, when the yields were high, chlorophyll meter readings presented higher variability than when the yields were lower. Le Bail *et al.* (2005) suggested that, together with the chlorophyll meter readings, the ear number per square metre should be measured to obtain a measurement related to the yield. Models to predict wheat yields have been developed (Zhang *et al.*, 2017) that allow the determination of the yields in advance and predict how the yield would affect the GPC values.

In 2016, the post-anthesis N increase in the aerial part was higher than that in the other two growing seasons (Table 7.3), likely because of the higher amount of biomass accumulated throughout the growing season, justifying the high yield values. Additionally, Mi *et al.* (2000) reported that a high sink size promotes the post-anthesis N uptake to meet the crop N requirements. The N uptake in the post-anthesis period can contribute from 5 to 50% of the grain N, but it depends on the environmental conditions (Fuertes-Mendizabal *et al.*, 2012). The mean rainfall of the area in the period between GS65 to harvest (1981–2010) was 81 mm (Euskalmet, 2019). In this study, wet conditions occurred in the three growing seasons after mid-anthesis (Table A7.1), with the total rainfall being 56 mm in 2015, 71 mm in 2016 and 114 mm in 2017. The number of days elapsed (Table A7.1) in 2016 from GS65 to harvest was more (69) than that in 2017 (63) and 2015 (54). In 2016, the soil moisture was higher and the crop had more time to absorb N than in the other two growing seasons. The higher uptake capacity and the more time elapsed in the post-anthesis period might explain the higher post-anthesis N increase. The effect of the post-anthesis N increase on the variability of the GPC values in winter wheat was also described by Monaghan *et al.* (2001). They concluded that the significance of the post-anthesis N absorbance in the GPC variability is related to the different N partitioning accumulated before and after anthesis. The N absorbed after anthesis is more efficiently destined to the grain because the N absorbed after anthesis does not include the crop growth response (Bogard *et al.* 2010). However, it is highly complex to predict how much N will be absorbed by the crop in the post-anthesis period (Magney *et al.*, 2016). The GPC value range was similar in 2015 and 2016, although the chlorophyll meter readings were lower in 2016 than in 2015. Another reason that could make the GPC variability prediction difficult is predicting how efficiently the plant will translocate N into the grain in the grain-filling period (GS60–GS90) (Magney *et al.,* 2016). In the present experiment, the amount of N translocated to grains from the crop total N was 80% or higher (data not shown). Fuertes-Mendizabal *et al.* (2018), using the same wheat variety and under the same edaphoclimatic conditions, found that the NHI values ranged between 75 and 82%.

In summary, the chlorophyll meter readings can predict the GPC values when the yields are lower than 8,000 kg N ha^{-1}, but not when the yields are higher. The established sink size and post-anthesis conditions may affect that prediction, as in 2016. Some authors have mentioned that the GPC predictability from chlorophyll meters is better when the readings are taken at the beginning of the milk stage (GS71, Zadoks *et al.,* 1974) than at the anthesis stage (Le Bail *et al.,* 2005). However, Lopez-Bellido *et al.* (2004) suggested that taking chlorophyll meter readings later in the growing season would improve the predictability, but leaf senescence (starting atmedium milk; GS75, Zadoks *et al.,* 1974) should be considered. Additionally, it would be late to perform N application.

7.4.2 Minimum Chlorophyll Meter Readings to Achieve GPC Values of 12.5%

The recommended level of GPC needed for the necessary bread-making quality should be higher than 12.5% (RD 677/2016). However, it is difficult to ensure that this value will be achieved because many factors influence the grain-filling process (GS60–GS90) (Monaghan *et al.,* 2001). As stated above, achieving the GPC needed for the necessary bread-making quality is complex under the humid Mediterranean conditions of Araba using the usual fertiliser practices (last N application at GS30). Additionally, the commonly used varieties, such as Cezanne variety, have been selected to obtain high yields rather than to obtain a high GPC. The 12.5% value was only achieved in 2017 with the highest N rate at GS30 (160 kg N ha^{-1}) in the three initial fertilisation treatments and with yields ranging from 6,000 to 7,000 kg ha^{-1}. To enhance the GPC, it would be useful to determine the minimum Yara N-Tester^{TM} reading value to achieve the required bread-making quality. As mentioned above, predicting the GPC variability using Yara N-Tester^{TM} in this area under the humid Mediterranean climate was only possible when the yields were lower than 8,000 kg ha^{-1}. Several authors have established minimum chlorophyll meter readings to achieve the GPC value of 12.5% (Lopez-Bellido *et al.,* 2004; Turley *et al.,* 2001; Miller *et al.,* 1999). Some of those studies used Minolta SPAD, which is a chlorophyll meter similar to Yara N-Tester^{TM}. Both tools measure the light transmitted at 650 and 940 nm and supply different units in readings, but they are highly correlated as observed in our previous calibration experiments (NTester reading = 14.1 × SPADreading − 61.54; R^2 = 0.90; data not shown). Arregui *et al.* (2006), under the same climatic conditions, also found that both tools are also highly correlated with a very similar equation. Turley *et al.* (2001) reported that, to achieve GPC values approximately 11% (with yields approximately 8,000 kg ha^{-1}), the Minolta SPAD reading values should be 48, which corresponds to a Yara N-Tester^{TM} value of 615 (calculated from the previously mentioned equation). In the present study, to achieve GPC values approximately 11%, the Yara N-Tester^{TM} reading values were similar (approximately 620; Figure 7.2a) to those proposed by Turley *et al.* (2001). Lopez-Bellido *et al.* (2004) showed that, to achieve GC values of 12.5% (with yields approximately 10,000 kg ha^{-1}), the necessary Minolta SPAD reading values at GS65 should be 51 or higher, corresponding to a Yara N-Tester^{TM} value of 658 (calculated from the previously mentioned equation). In the present study, the Yara N-Tester^{TM} values to achieve a GPC of 12.5% were higher (approximately 700, Figure 7.2a) than those proposed by Lopez-Bellido *et al.* (2004). Miller *et al.* (1999) reported that, tor achieving GPC values of 13% (with yields ranging from 1,100 to 8,000 kg ha^{-1}), the necessary Minolta SPAD readings should be higher than 40, which corresponds to Yara N-Tester^{TM} = 503 (calculated from the previously mentioned equation), with the values lower than those required in the present study (approximately 720; Figure 7.2a). The variation in the results demonstrates that it is necessary to be cautious regarding the universality of chlorophyll meter values across geographical locations, as Lopez-Bellido *et al.* (2004) reported. Additionally, the wheat variety should also be considered because each variety should have its own calibration (Diacono *et al.*, 2013). To generalize the relationship between the chlorophyll meter readings and GPC values, some authors have proposed the use of normalised values (Diacono *et al.*, 2013), which are calculated as a percentage by assessing 100% to a non-limiting area of the field. However, data normalisation has limitations because finding a control strip that is representative of the entire field is challenging. Ravier *et al.* (2017) showed that it is not easy to ensure that an overfertilised fringe is not N deficient, thereby complicating the use of normalised data. Additionally, using normalised values makes the use of chlorophyll meters more complicated for farmers. In the present study, the GPC predictability was the worst using Yara N-Tester^{TM} normalised values (data not shown). Hoel (2002) reported that correction factors (as +10 or -10, or +20 or -20) could be used to normalise the differences among varieties when absolute chlorophyll meter readings are used. Hoel (2002) reported that, with some varieties, it was not necessary to use a correction value because they presented similar leaf greenness.

7.5 Conclusions

The type of initial fertiliser did not affect the GPC values and chlorophyll meter readings at mid-anthesis. Generally, the higher is the mineral N applied at stem elongation, the higher are the chlorophyll meter readings and GPC values because the GPC values are yield dependent. The chlorophyll meter readings at mid-anthesis in wheat might be helpful in estimating the GPC values under humid Mediterranean conditions only when the yields are below 8,000 kg ha^{-1}. Yara N-Tester^{TM} readings at mid-anthesis should be higher than 700 in the wheat Cezanne variety to achieve the recommended level of GPC for high-quality bread-making flour (12.5%) at these yield levels. These results will allow farmers and cooperatives to make better decisions regarding late-nitrogen fertilisation and product sales, but it is necessary to adjust the values to the different varieties or cultivars. Future directions for wheat grain protein estimation should explore new fertilisation strategies including late N applications with granular mineral fertilisers or foliar applications but always avoiding the N rate increase.

7.6 Appendix

Table A7.1. Total rainfall (mm), cumulative growing degree days GDD (ºC) and days elapsed between wheat growing stages (Zadoks *et al.* 1974) in three growing seasons (2015, 2016 and 2017) in the field study at Arkaute

Growing season	Growing stage	Total rainfall (mm)	Cumulative GDD (°C)+	Days elapsed
2015	Sowing (24/11) - GS21 (09/03)	521.2	556	106
	GS21 (09/03) - GS30 (04/04)	94.6	788	30
	GS30 (04/04) - GS32 (29/04)	43.2	1048	21
	GS32 (29/04) - GS37 (11/05)	6	1235	12
	GS37 (11/05) - GS65 (28/05)	13.8	1432	17
	GS65 (28/05) - Harvest (21/07)	55.5	2462	54
2016	Sowing (06/11) - GS21 (19/01)	168.1	611	74
	GS21 (19/01) - GS30 (17/03)	296.1	994	56
	GS30 (17/03) - GS32 (30/03)	16.5	1118	13
	GS32 (30/03) - GS37 (06/04)	24.3	1126	7
	GS37 (06/04) - GS65 (25/05)	71.2	1721	49
	GS65 (25/05) - Harvest (02/08)	70.5	3005	69
2017	Sowing (18/11) - GS21 (02/03)	271.3	635	105
	GS21 (02/03) - GS30 (06/04)	57.6	976	35
	GS30 (06/04) - GS32 (12/04)	0	1078	6
	GS32 (12/04) - GS37 (25/04)	0	1218	13
	GS37 (25/04) - GS65 (30/05)	82.4	1718	35
	GS65 (30/05) - Harvest (02/08)	114.3	2980	63

+*The cumulative GDD was calculated with 5 °C as the baseline.*

Table A7.2. Duncan's test ($p<0.05$) results of the differences in the GPC values (%), yield (kg ha^{-1}), and Yara N-Tester values at mid-flowering (GS65, Zadoks *et al.*, 1974) among different wheat growing seasons (2015, 2016 and 2017) for each initial fertilisation treatment (conventional, dairy slurry and sheep manure) and each N dose (0, 40, 80, 120 and 160 kg N ha^{-1}) applied at stem elongation (GS30, Zadoks, *et al.*, 1974) in the field study at Arkaute

Initial fertilisation	Treatment	GPC (%)			Yield (kg N ha^{-1})			N-Tester GS65		
		2015	**2016**	**2017**	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**
Conventional	40 + 0N		*ns*		B	A	AB		*ns*	
	40 + 40N		*ns*		B	A	C	B	C	A
	40 + 80N	B	B	A	B	A	C	AB	B	A
	40 + 120N	B	B	A	B	A	C	B	C	A
	40 + 160N	B	B	A	B	A	C	B	C	A
Dairy slurry	DS + 0N		*ns*		B	A	B	A	B	B
	DS + 40N	B	B	A	B	A	B	A	B	A
	DS + 80N	B	B	A	B	A	B	AB	B	A
	DS + 120N	AB	B	A	B	A	B	B	C	A
	DS + 160N		*ns*		B	A	B	AB	B	A
Sheep manure	SM + 0N	B	A	A	B	A	B	A	B	A
	SM + 40N	B	B	A	B	A	B	B	B	A
	SM + 80N	B	C	A	B	A	B	A	B	A
	SM + 120N	B	B	A	B	A	B	A	B	A
	SM + 160N	B	C	A	B	A	B	AB	B	A

Different uppercase letters represent differences among wheat growing seasons (2015, 2016, 2017) for each initial fertilisation treatment (conventional, dairy slurry, sheep manure) and each N rate applied at GS30 (0, 40, 80, 120, 160 kg N ha^{-1}). ns, not significant

Table A7.3. Duncan's test ($p<0.05$) results of the differences in the grain total nitrogen (kg N ha^{-1}) and post-anthesis nitrogen increase (kg N ha^{-1}) among different wheat growing seasons (2015, 2016 and 2017) for each treatment in the field study at Arkaute .

Treatment	**Grain Total N**			**Post-anthesis N increase**		
	2015	**2016**	**2017**	**2015**	**2016**	**2017**
40N+0N		*ns*		B	A	A
40N+40N	B	A	B		*ns*	
40N+80N		*ns*			*ns*	
40N+120N		*ns*		B	A	B
40N+160N		*ns*		B	A	AB
DS+0N	B	A	B	B	A	B
SM+0N	B	A	C	C	A	B

Different uppercase letters represent differences among wheat growing seasons (2015, 2016, 2017) for each treatment. ns, not significant

Capítulo 8

Discusión general

8 Discusión general

La producción de la mayoría de los alimentos requiere fertilizantes minerales, que a su vez consumen mucha energía (Svanbäck *et al*., 2019). En el caso de los fertilizantes nitrogenados se utilizan combustibles fósiles para fijar el N atmosférico mediante el proceso de Haber-Bosch, y a nivel mundial un 1% de la energía se consume en la fabricación de estos fertilizantes (Dawson y Hilton, 2011). Los fertilizantes fosfóricos se obtienen a partir de depósitos fósiles de roca fosfórica, que debe ser procesada para aumentar su solubilidad (Sigurnjak *et al*., 2016), y se estima que los recursos de fósforo se agotarán a finales del siglo XXI (Chojnacka *et al*., 2020). En general, la explotación intensiva de materias primas agota los recursos disponibles a un ritmo bastante rápido, lo que hace aumentar su precio.

Por otro lado, la economía actual sigue un patrón lineal, lo que da lugar a la generación de grandes cantidades de productos de desecho, siendo muchos de ellos vertidos al medio ambiente (Sarsaiya *et al.*, 2019). Los subproductos orgánicos son una reserva valiosa de nutrientes, por lo que deben ser reciclados en la medida de lo posible. El concepto de Economía Circular fue introducido por la Comisión Europea como respuesta a problemas ambientales y sociales (Ritzén y Sandström, 2017). La idea de circularidad supone que los subproductos de un proceso de producción se usan como materias primas secundarias en otro (Hansen, 2018). En este sentido, la sustitución de los fertilizantes minerales por subproductos orgánicos representa una vía importante para su recuperación y la reducción del consumo de energía (Christel *et al.*, 2014). La Unión Europea (UE) espera que para 2030 los subproductos orgánicos reemplacen hasta el 30% de los fertilizantes minerales que se utilizan actualmente (Hansen, 2018). Sin embargo, para poder utilizarlos de forma idónea, en dosis correctas y en el momento adecuado, y para obtener un correcto estado nutricional del cultivo sin comprometer el medioambiente, es necesario llevar a cabo estudios donde se evalúe su dinámica de mineralización. Para llevar a cabo dichos estudios, tanto los ensayos de campo como los de invernadero constituyen herramientas muy útiles que nos permiten conocer mejor cuál es el aporte de nutrientes a la planta y en qué momento de su ciclo productivo ocurre.

8.1 Utilización de subproductos orgánicos de origen ganadero

En el Capítulo 3 de este trabajo, mediante un ensayo en invernadero, se ha determinado que tres de los subproductos orgánicos de origen ganadero más abundantes en la Comunidad Autónoma del País Vasco (CAPV) presentan distintos patrones de mineralización del N, sugiriendo que su momento de aplicación a los cultivos debe ser diferente. La gallinaza contiene altas cantidades de N fácilmente mineralizable, ya que tiene unas proporciones altas de ácido úrico y amonio respecto al N total, por lo que las necesidades de N del cultivo podrían verse satisfechas si se aplica de forma ajustada a la demanda de N por parte del cultivo. Lo mismo ocurre en el caso del purín de vacuno, ya que, debido a su alto contenido de amonio respecto al N total, es recomendable que su aporte se realice lo más cerca posible del momento de máxima necesidad de N por parte del cultivo, siempre que las circunstancias lo permitan. Sin embargo, la aplicación de este tipo de subproductos cuando la demanda de N del cultivo es baja, podría causar pérdidas de N a la atmósfera o a las aguas subterráneas (Delgado, 2002). En el caso del estiércol de vacuno, la liberación de nitrato es menor y más paulatina, por lo que el riesgo de pérdidas de N al medioambiente es más bajo. En Araba, la aplicación de subproductos orgánicos de origen ganadero se hace habitualmente en fondo, antes de la siembra del cereal de invierno. En este sentido, los estiércoles son los subproductos más adecuados para las aplicaciones en fondo de los cultivos de invierno. Las aplicaciones limitadas a la siembra del cereal en Araba se deben a que el clima húmedo de invierno y de primavera suele dificultar la entrada de maquinaria pesada en las parcelas. Bajo estas condiciones de humedad, el tráfico de maquinaria pesada por los campos de cultivo puede dañar la estructura del suelo, por lo que en el momento de salida de invierno suelen hacerse únicamente aplicaciones de N mineral, en las que no es necesario transportar una cantidad tan grande de abono. En el caso del purín se han descrito aplicaciones sin tanque utilizando mangueras remolcadas con un tractor conectadas a un camión o tanque fuera de la parcela, permitiendo la aplicación de purín durante el invierno e inicio de la primavera (Hansen, 2019), sin que ello suponga una fuerte compactación del suelo (Arvidsson, 1998). Este sistema se denomina "*drag-hose*" o inyección con manguera y permite hacer aplicaciones con purín cercanas al momento de máxima de demanda de nutrientes por parte del cultivo (Lovanh *et al.*, 2010).

En Europa existen distintos sistemas de procesamiento de los subproductos de origen ganadero. Pueden agruparse en separación de la fracción sólida y líquida, tratamientos anaeróbicos, tratamientos de la fracción sólida y tratamientos de la fracción líquida (Foged *et al.*, 2011), con el fin de reducir su volumen, facilitar su transporte, higienizar o concentrar su contenido de nutrientes (Bernal *et al.*, 2015). Mediante algunos de estos tratamientos, los subproductos de origen ganadero podrían utilizarse, al igual que los fertilizantes minerales, en los momentos en los que la demanda de N es más alta.

La separación del subproducto orgánico en dos fases con el objetivo de obtener una fracción sólida y otra líquida puede realizarse mediante diversas técnicas: centrifugación, sedimentación química, prensado o filtración más ósmosis inversa (Foged *et al.*, 2011). Estos tratamientos suelen hacerse como un paso previo a tratamientos posteriores más complejos (Bernal *et al.*, 2015).

La digestión anaeróbica es el proceso de degradación del material orgánico por microorganismos en ausencia de oxígeno, produciendo un biogás, compuesto principalmente por metano y dióxido de carbono, y un digestato (Nasir *et al.*, 2012). Los digestatos son ricos en nutrientes, lo que los convierte en posibles sustitutos de los fertilizantes químicos en la agricultura (Vaneeckhaute *et al.*, 2013; Tambone *et al.*, 2015). Por otro lado, el proceso de digestión puede realizarse en condiciones mesófilas o termófilas, y ambas condiciones resultan en la higienización efectiva del subproducto (Miller *et al.*, 2016). En la UE, a medida que aumenta el número de plantas de biogás, la producción de digestatos ricos en nutrientes también aumenta significativamente (Risberg *et al.*, 2017).

Dentro de los tratamientos de la fracción sólida existen distintas tecnologías como el compostaje de los subproductos de origen ganadero sólidos o de la fracción que contiene fibras en los subproductos líquidos, el secado térmico o la peletización (Foged *et al.*, 2011). El compostaje es un proceso de degradación biológica de la materia orgánica en condiciones aerobias que da lugar a un producto estable conocido como compost (St. Martin y Barthwaite, 2012). En este proceso, el estiércol solido puede reducir su volumen en un 50-60% (Alemu *et al.*, 2017). Sin embargo, durante el compostaje puede producirse una reducción considerable del contenido de nitrógeno del estiércol, debido a las pérdidas de nitrógeno como amoníaco (Van der Meer *et al.*, 2008; Hou *et al.*, 2017). En ello, influyen factores como la humedad, la temperatura, la aireación y volteo, y el tamaño de las partículas (Bernal *et al.*, 2009). El compost es rico en materia orgánica y mejora la fertilidad del suelo, la capacidad de retención de agua, la densidad aparente y sus propiedades biológicas (Whalen *et al.*, 2019). Además, las altas temperaturas alcanzadas durante el proceso de compostaje, debido al incremento de la actividad metabólica del inicio del proceso, permiten la reducción de la carga de organismos potencialmente patógenos, la disminución de elementos determinantes de resistencia antimicrobiana (*i.e.* compuestos antimicrobianos, genes de resistencia) y contaminantes orgánicos (Qian *et al.*, 2018), y reducen el número de semillas de plantas adventicias (Ershadi *et al.*, 2020). El secado térmico del estiércol y de los digestatos se hace habitualmente mediante una cinta dentro de una cámara cerrada y ventilada por un flujo de aire caliente (70-110°C). Esta solución requiere la captura y el tratamiento del aire de escape del secador por un depurador ácido antes de su emisión a la atmósfera. El propósito del proceso de secado es producir un fertilizante comercial, estable y fácil de transportar y aplicar, reduciendo el volumen y el peso del digestato o el estiércol y concentrando los nutrientes (N, P y K) (Bernal *et al.*, 2015). La peletización de los subproductos de origen animal, proporciona una reducción del volumen y peso de entre el 20 y el 50% del estiércol original y de entre el 50 y el 90% del compost, principalmente debido a la compresión (Hao y He, 2020). Mediante este proceso, también se reduce el olor de los subproductos de origen ganadero, creando un producto mejorado respecto al estiércol fresco por su menor costo de almacenamiento, transporte, manipulación y aplicación (Hao y He, 2020). En relación con este proceso se han diseñado diversas técnicas como, por ejemplo, la adición de determinados nutrientes para crear fertilizantes de diseño (Hara, 2001), más ajustados a las necesidades de determinados cultivos y áreas edafoclimáticas. De forma similar a la peletización, también se dan procesos de granulación, en los que se consigue un material similar a los pellets, pero no tan denso (Sakurada *et al.*, 2016). En algunas ocasiones, los términos de peletización y granulación se utilizan indistintamente (Rao *et al.*, 2007).

Finalmente, para los tratamientos de la fracción líquida, entre los que se encuentran los estiércoles diluidos o las fracciones líquidas obtenidas tras la separación, se encuentran técnicas para la eliminación de nutrientes y otras para su recuperación (Foged *et al.*, 2011). La nitrificación-desnitrificación biológica es la técnica más utilizada para eliminar el nitrógeno de la fracción líquida, y es considera una técnica de bajo coste. Esta técnica persigue la transferencia a la atmósfera del N inicialmente contenido en la fracción liquida en forma de N_2, un gas inerte. Obaja *et al.* (2005) observaron que la eliminación de N podía ser del 99% en la fracción líquida diluida de origen porcino. Este tratamiento parece interesante para las situaciones en las que la cantidad de subproducto es muy elevada en relación a la disponibilidad de parcelas para su aplicación y/o se han hecho aplicaciones durante largos periodos de tiempo (Riaño y García-González, 2014). La tecnología de recuperación de nutrientes puede seleccionarse en función de las características de los subproductos y de los productos finales a obtener (Vaneeckhaute *et* *al.*, 2017). Las técnicas utilizadas para la recuperación de nutrientes se basan en la fitodepuración con microalgas, lentejas de agua y macrófitas acuáticas (Zubair *et al.*, 2020). La recolección y gestión de la biomasa de las macrófitas en humedales artificiales puede ser el paso más complejo del proceso, especialmente si no existe una comercialización rentable del producto final (Bernal *et al.*, 2015). Zubair *et al.* (2020) mencionan que esta biomasa puede ser compostada, puede añadirse a sistemas de digestión anaerobia o puede incorporarse al suelo. Las lentejas de agua pueden utilizarse como suplemento en la alimentación del ganado ya que son ricas en proteínas y vitaminas o pueden añadirse a sistemas de digestión anaerobia para aumentar la producción de metano (Zubair *et al.*, 2020). Las microalgas pueden utilizar eficientemente los nutrientes y tienen un gran potencial como materias primas para producir biocombustibles (Zubair *et al.*, 2020).

Aunque existen numerosas técnicas de procesamiento para convertir el estiércol en productos con propiedades mejoradas, el uso de estas técnicas sigue siendo bastante limitado (Foged *et al.*, 2011). En Europa, de todos los subproductos de origen ganadero producidos, únicamente al 3,1% se les realizan tratamientos de separación, al 6,4% tratamientos anaeróbicos, y al 0,7% y 0,8% se les realizan tratamientos a la fracción sólida y a la fracción líquida, respectivamente (Foged *et al.*, 2011). Es necesario disponer de tecnologías que nos permitan llevar a cabo distintos tratamientos de los subproductos para no tener que transportarlos a otros lugares para su aplicación. Por ejemplo, en la CAPV las explotaciones avícolas tienen que transportar la gallinaza a grandes distancias (Gobierno Vasco, 2008). Sin embargo, esta circunstancia está cambiando ya que hay explotaciones innovadoras que instalan secaderos para reducir el volumen final de la gallinaza y revalorizar su valor fertilizante (Rosa *et al.*, 2019).

8.2 Beneficios y riesgos de la aplicación de subproductos orgánicos de origen ganadero

Boghal *et al.* (2019) indican que las aplicaciones repetidas de purín durante largos periodos de tiempo (hasta 20 años de aplicaciones) aumentan los valores de carbono orgánico del suelo (COS) y el funcionamiento biológico y físico del suelo, aunque lo hacen a un menor nivel que las aplicaciones comparables de estiércol. Domingo Olivé *et al.* (2016), bajo condiciones de clima mediterráneo, mencionan que los valores de COS aumentan significativamente tras 12 años de aplicaciones de estiércol vacuno, pero no lo hacen tras aplicaciones de purín porcino.

El aumento de las reservas de COS es un aspecto importante en las estrategias a larga escala de mitigación del cambio climático, ya que el C puede almacenarse en los suelos durante un largo periodo (Guenet *et al.*, 2020). Diacono y Montemurro (2011) observaron aumentos entre el 20 y 90% del COS inicial (estando la mayoría de los aumentos en el rango de 20-45%) después de aplicaciones de subproductos orgánicos durante 3-60 años en distintas condiciones agroclimáticas, en comparación con suelos sin fertilizar o con fertilización mineral. Guenet *et al.* (2020) afirman que se puede asumir que los aumentos de las reservas de COS pueden impactar en el ciclo del N y en las emisiones de N_2O ya que los ciclos del C y del N están fuertemente interconectados en los suelos. Un reciente estudio de modelización sugiere que los beneficios de las medidas para aumentar el secuestro del COS podrían verse reducidos por el aumento de las emisiones de N_2O, dependiendo de la rotación de cultivos (Lugato *et al.*, 2018). Algunos subproductos de origen ganadero contienen compuestos nitrogenados fácilmente mineralizables por lo que, con su aplicación, existe el riesgo de aumentar las emisiones de N_2O (Obriot *et al.*, 2016). En este sentido, Shakoor *et al.* (2021) en un meta-análisis llevado a cabo con los datos de 48 publicaciones científicas mostraron que el aporte de gallinaza a los suelos aumentaba considerablemente las emisiones de CO_2, CH_4 y N_2O en comparación con las aplicaciones de estiércol de porcino y vacuno. Sin embargo, el uso de subproductos orgánicos reduce el uso de fertilizantes minerales, reduciendo las emisiones de N_2O asociadas a su producción (Guenet *et al.*, 2020), que precisa del uso de combustibles fósiles para su fabricación como se ha comentado anteriormente. Quemada *et al.* (2020) mencionan que la eficiencia en el uso del N (EUN) se reduce significativamente cuando se aplican subproductos orgánicos de origen ganadero como estiércol y purín. Sin embargo, estas pérdidas estimadas pueden explicarse en parte por la acumulación de N en la MO del suelo, lo que supondría que mediante la aplicación de estos subproductos se está contribuyendo al secuestro de C y a la fertilidad del suelo, tal y como se ha indicado en los estudios ya mencionados en este apartado.

La aplicación de subproductos de origen ganadero en los suelos puede incrementar las concentraciones de metales pesados (Zhen *et al.*, 2020), la presencia de microplásticos (Yan *et al.*, 2020), las resistencias a antibióticos (Liu *et al.*, 2021) o la biodisponibilidad de algunos antibióticos para los cultivos (Zhao *et al.*, 2019). Por otro lado, el Pacto Verde Europeo, a través de la estrategia de la granja a la mesa, tiene como objetivo reducir un 50% el uso de antibióticos en la ganadería (EC, 2020), lo cual redundaría en una menor generación de resistencias a antibióticos en los microorganismos del suelo. Zhen *et al.* (2020) mencionan que en las condiciones de su estudio el aporte de estiércol vacuno y gallinaza no puede superar las 44 toneladas por hectárea y año durante los siguientes 100 años para no superar el contenido de metales pesados en el suelo que pueda comprometer su salud, lo que eventualmente supone un límite a la cantidad de subproductos orgánicos que se pueden aplicar en un determinado suelo en el tiempo.

8.3 Uso de sensores de campo en la fertilización nitrogenada

Los resultados del Capítulo 3 se obtuvieron en invernadero, donde las condiciones ambientales son menos cambiantes que en campo y donde la instalación de Rhizons para hacer un seguimiento de la dinámica de la solución del suelo es una práctica sencilla. Las condiciones ambientales en los ensayos de campo son específicas de cada zona y de cada campaña, haciendo que la estimación de los patrones de mineralización de los distintos subproductos orgánicos no sea un proceso sencillo. En este sentido, la evaluación del estado del cultivo, que combina el efecto de distintos factores (*i.e.* el N disponible, el contenido de agua del suelo, el crecimiento de las raíces, el clima o la eficiencia en la absorción del N por parte de las plantas) puede ser de gran interés. Para observar la evolución del cultivo pueden ser muy útiles las tecnologías de la información y la comunicación (TIC) aplicadas en la agricultura. La Agricultura de Precisión se centra en la utilización de distintos tipos de datos obtenidos mediante sensores proximales de campo, drones, y satélites para monitorear el suelo, el agua, los cultivos o los bosques. Los datos obtenidos mediante sensores proximales de campo para evaluar el crecimiento de los cultivos y su estrés, destacaron a principios de los años 90 (Mulla, 2013), y su utilización contribuye a una toma de decisiones más oportuna y precisa.

En el Capítulo 4 de este trabajo se evalúa en trigo el uso de dos sensores de campo (Yara N-Tester^{TM} y RapidScan CS-45) para ajustar la dosis de N en inicio de encañado, momento en el que suele hacerse el mayor aporte de N en el cultivo de trigo en Araba. El diseño de este ensayo de campo simula las prácticas habituales que se llevan a cabo en Araba tanto en los casos en que se aplican subproductos orgánicos en fondo, como cuando se llevan a cabo tratamientos convencionales. Las lecturas normalizadas de ambos sensores de campo, mostradas en el Capítulo 4, revelan que la cantidad de N absorbida por el cultivo hasta inicio de encañado es dependiente de la campaña. Así, el estado del cultivo en inicio de encañado está influenciado por las condiciones ambientales previas

(Lindstrom *et al.*, 1976). Los valores aportados por ambos sensores en inicio de encañado no muestran diferencias entre los tratamientos en fondo con purín y con estiércol dentro de la misma campaña, sugiriendo que el N absorbido por el cultivo proveniente de la mineralización de ambos subproductos hasta inicio de encañado fue similar dentro de cada campaña. Sin embargo, mediante los resultados obtenidos en el Capítulo 3 bajo condiciones de invernadero y en el que se utilizó suelo procedente del campo de ensayo, se puede observar que el N aportado por el purín tras su aplicación, es significativamente superior al aportado por el estiércol. Sin embargo, esta mayor cantidad de N aportada por el purín en el ensayo de invernadero no se ve reflejada en los valores de los sensores en inicio de encañado en el ensayo de campo. En la zona de la Llanada Alavesa el encharcamiento de las parcelas agrícolas es un fenómeno común en los años con precipitaciones intensas durante el invierno, afectando al ahijado del trigo. La etapa de ahijado puede verse retrasada debido a las bajas temperaturas del suelo y a las condiciones anaeróbicas del mismo, ambos factores estando afectados por las precipitaciones (Lindstrom *et al.*, 1976). Por otro lado, el proceso de nitrificación bajo condiciones de encharcamiento se ve reducido, ya que requiere la presencia de oxígeno. Previamente al encharcamiento de las parcelas, el nitrato disponible en la solución del suelo podría lavarse fuera del alcance de las raíces tras las precipitaciones intensas (Basso *et al*., 2016). Por otra parte, el estiércol aplicado en el ensayo de invernadero fue de vacuno de carne y el aplicado en el ensayo de campo de ovino. Aunque la composición de los subproductos de origen ganadero varía significativamente con la especie animal (Whalen *et al.*, 2019), también lo hacen dentro de la misma especie animal (Capítulo 4), haciendo variable su mineralización.

En los tratamientos en los que se aplicó purín en fondo, la dosis óptima de N mineral en inicio de encañado para alcanzar rendimientos máximos fue menor que cuando se aplicó estiércol. Este dato sugiere que el ahorro de fertilizante mineral en el caso del purín se obtiene del N disponible a partir de inicio de encañado, ya que los sensores de campo presentaban los mismos valores en inicio de encañado para ambos subproductos orgánicos. Sin embargo, los resultados obtenidos en el Capítulo 3 bajo condiciones de invernadero, muestran que la disponibilidad de N (tanto amonio como nitrato) en la solución del suelo tras la aplicación de los subproductos era cercana a los 0 mg L^{-1} en inicio de encañado, incluso cuando la dosis aplicada fue de 340 kg N ha^{-1}, que es mucho mayor que la aplicada en campo en los tratamientos con purín en fondo (120 – 192 kg N ha^{-1}). Es probable que los valores de nitrato en la solución del suelo en el ensayo de invernadero (Capítulo 3) sean tan bajos debido a que el cultivo absorbe rápidamente el nitrato que encuentra disponible en la solución del suelo. Por otro lado, algunos estudios muestran que parte del N inmovilizado por los microorganismos del suelo tras la aplicación del purín, se libera meses después, quedando disponible para el cultivo. Jensen *et al.* (2000) mostraron que el 23% del amonio marcado en un purín de vacuno estaba inmovilizado en la biomasa microbiana del suelo tres semanas después de su aplicación en septiembre y que en primavera sólo quedaba el 6% del amonio marcado en la biomasa microbiana. Por otro lado, algunos suelos, debido a su textura y a la mineralogía de sus arcillas, tienen la capacidad de fijar amonio tras su aplicación, y posteriormente liberarlo de forma paulatina durante el periodo de crecimiento del cultivo debido a la demanda de N por parte del mismo (Nieder *et al.*, 2011). El suelo utilizado en este trabajo contenía illitas y esmectitas, ambas arcillas del tipo 2:1, consideradas arcillas con una gran capacidad de intercambio catiónico (Barton y Karathanasis, 2002). Los exudados de las raíces de las plantas, que contienen C, aumenta la actividad de los microorganismos heterótrofos, lo que puede promover la liberación del amonio fijado (Nieder *et al.*, 2011). Finalmente, el purín puede contener compuestos orgánicos de composición recalcitrante, que pueden permanecer en el suelo durante periodos largos de tiempo (Spiegel *et al.*, 2010). Cabe destacar que, a pesar de las diferencias existentes entre el ensayo de invernadero y el de campo, ambos estudios pueden aportar información complementaria cuando se evalúan en conjunto. En el ensayo de invernadero se trabaja en unas condiciones más controladas que las de campo, lo que permite profundizar más en cada uno de los resultados obtenidos, y además ayuda a entender los resultados del ensayo de campo. En los casos en los que se aplicó estiércol en fondo, fue difícil encontrar una relación entre los valores de las herramientas y la dosis óptima a aplicar, ya que hubo una gran variabilidad en la dosis óptima de N entre campañas. La composición de los estiércoles puede ser muy variable dependiendo del contenido de agua que presenten y de la cantidad de cama que se les haya añadido, influyendo en su mineralización (Whalen *et al.*, 2019).

Boghal *et al.* (2019) observaron que la aplicación continuada de subproductos orgánicos (entre los que se encontraban el estiércol y el purín) incrementaba la concentración de los nutrientes N, P, K y Mg en el suelo en un periodo inferior a tres años, permitiendo reducir el uso de fertilizantes minerales. En este sentido, sería de especial interés estudiar el efecto de las aplicaciones a largo plazo de subproductos orgánicos para poder determinar su efecto acumulativo en el tiempo. En un meta-análisis llevado a cabo por Zavattaro *et al.* (2017), con datos obtenidos de aplicaciones de 80 ensayos a largo plazo (10-28 años) de estiércoles y purines en Europa, se concluyó que cuando se aplicaba estiércol o purín bovino los rendimientos de los cultivos eran un 9% más bajos que cuando la fertilización era mineral. Sin embargo, el aporte de estiércol o purín en combinación con fertilizantes minerales incrementaba los rendimientos de los cultivos.

Samborski *et al.* (2009), en su revisión de distintos estudios en los que se han utilizado sensores de campo para hacer recomendaciones de N, concluyeron que la variabilidad climática es uno de los factores que más afectan a las recomendaciones de N. En este sentido, en el Capítulo 4 se observa que el efecto que provocan las condiciones específicas de cada campaña en el rendimiento del trigo es de gran importancia ya que, en la campaña de 2017, aun alcanzando rendimientos inferiores a la media, la cantidad de N necesaria en encañado fue superior. Los resultados obtenidos en el Capítulo 6 explican lo ocurrido en la campaña de 2017, donde el N aplicado en inicio de encañado no empezó a absorberse hasta el estado fenológico de hoja bandera debido a la falta de lluvia. El agua es uno de los aspectos más importantes que deben considerarse al elaborar estrategias de fertilización en sistemas de secano, por su fuerte condicionamiento sobre las respuestas de los cultivos al fertilizante nitrogenado (Colaço y Bramley, 2018). Jørgensen y Jørgensen (2007) identificaron el estrés hídrico como un factor de confusión en los diagnósticos nutricionales de deficiencia nitrogenada mediante sensores. Esto muestra la importancia de tener unas buenas previsiones meteorológicas y de hacer los aportes de fertilizante mineral siguiendo estas previsiones (Ravier *et al.*, 2017a).

Los resultados obtenidos en el Capítulo 6 muestran los beneficios de hacer un seguimiento del estado nutricional nitrogenado del trigo durante su ciclo de cultivo mediante el NDVI (Normalized Differential Vegetation Index), permitiendo detectar si el cultivo está absorbiendo el N aplicado. Ravier *et al.* (2017a) concluyeron que era posible establecer estados nutricionales nitrogenados mínimos para alcanzar rendimientos máximos en el cultivo de trigo mediante el cálculo de INN en estados fenológicos clave de su ciclo de cultivo. Sin embargo, la determinación del INN requiere mediciones destructivas y un tiempo prolongado para la determinación del contenido de N de las plantas y la biomasa del cultivo. La utilización del NDVI para hacer el seguimiento del estado nutricional del cultivo fue validado en el Capítulo 5, donde se observa que este índice es capaz de estimar los valores de NNI de forma positiva y significativa. Esto es relevante puesto que Ravier *et al*. (2017a) han determinado los valores de INN a partir de los cuales el rendimiento no se ve perjudicado. A pesar de que hay numerosos trabajos que estudian diversos índices para llevar a cabo el diagnóstico nutricional nitrogenado (Cao *et al*., 2018; Basso *et al*., 2009; Debaeke *et al*., 2007; Franzen *et al*., 2016), no es fácil encontrar valores adecuados para los distintos momentos del ciclo en los cultivos. La utilización del NDVI permite obtener valores *in situ*, rápidos y no destructivos del estado nutricional nitrogenado del trigo y obtener una mayor representación de la variabilidad que puede existir en cada parcela. Los resultados de Capítulo 6 muestran que el rendimiento del cultivo de trigo no se ve afectado si los valores de NDVI no descienden de 0,7-0,75 en los estados fenológicos de dos nudos, hoja bandera y mitad de floración bajo condiciones de clima mediterráneo húmedo. Estos resultados concuerdan con los resultados obtenidos por Ravier *et al.* (2017a) utilizando el INN. Sin embargo, no es sencillo establecer un valor mínimo de NDVI en inicio de encañado para no comprometer el rendimiento, ya que algunas deficiencias en encañado parece que se pueden remontar si el estado nutricional del cultivo se incrementa para el estado fenológico de dos nudos. Las deficiencias de N tempranas paralizan el ahijado o el crecimiento de los hijuelos en desarrollo, reduciendo el número de hijuelos por m^2 (Jeuffroy y Bouchard, 1999). Sin embargo, esta reducción puede verse compensada por el número de granos por espiga si el cultivo mejora su estado nutricional nitrogenado en los siguientes estados fenológicos (Jeuffroy y Bouchard, 1999). Una carencia en inicio de encañado que se alargue en el tiempo y no se corrija afectará negativamente al rendimiento, ya que se verán afectados tanto el número de hijuelos por m^2 como los granos por espiga. La obtención de valores mínimos de NDVI permitiría determinar si en necesario hacer un aporte extra de N con el fin de corregir las carencias de N detectadas y no comprometer el rendimiento. Bajo las condiciones de clima mediterráneo húmedo en Araba, se ha observado que las aplicaciones de N en hoja bandera son absorbidas por el cultivo siempre que la cantidad de lluvia sea de al menos 20 mm en el período de tiempo comprendido entre una quincena antes y una quincena después de la aplicación de N (Ortuzar-Iragorri *et al.*, 2017).

Weiss (2020) indica que anticipar el rendimiento del cultivo antes de la cosecha es de gran interés para distintos grupos como son agricultores individuales, cooperativas, gobiernos nacionales e instituciones internacionales para reforzar la seguridad alimentaria, y también para las empresas privadas como las aseguradoras de cultivos o empresas comerciales de productos básicos. Los resultados del Capítulo 6 sugieren que mediante el sumatorio de los valores de NDVI obtenidos en inicio de encañado, dos nudos, hoja bandera y mitad de floración se podría estimar el rendimiento del trigo bajo condiciones de clima mediterráneo húmedo.

Los resultados obtenidos en este trabajo han sido calibrados para la variedad de trigo Cezanne, por lo que sería necesario estudiar los valores mínimos de NDVI para otras variedades. Se ha observado que es posible aplicar factores de corrección a los valores de los sensores de campo para normalizar las diferencias entre variedades si fuese necesario (Hoel, 2002). Bonfil (2016) concluye que es posible utilizar la herramienta RapidScan CS-45 para hacer tres y/o cuatro agrupaciones de 13 variedades. En este sentido, sería interesante hacer mediciones con RapidScan CS-45 en las variedades más utilizadas en Araba bajo los mismos regímenes de fertilización y en los estados fenológicos clave, con el objetivo de detectar y cuantificar la posible variabilidad entre ellas.

Colaço y Bramley (2018) mencionan que cuando se utilizan sensores para ajustar la aplicación de N se presta poca atención al suministro de N proveniente del propio suelo, a pesar de que éste es un componente crucial para la respuesta del cultivo al fertilizante. Existen algunos modelos para estimar la tasa de mineralización de N del suelo y predecir el N disponible para el cultivo (Keating *et al.*, 2003; Setiyono *et al.*, 2011), pero rara vez se aplican (Colaço y Bramley, 2018). En este sentido, en el Capítulo 2, en un ensayo llevado a cabo en invernadero, se observa que existe una variabilidad significativa en la liberación de N en 16 suelos agrícolas de Araba. Aun siendo la superficie de cultivos en extensivo de Araba pequeña (53 052 ha^{-1}, Eustat 2013), sus suelos presentan características físico-químicas variables, que se reflejan en los patrones de mineralización de N (Capítulo 2), por lo que sería de interés llevar a cabo ensayos de campo en suelos con distintas características físico-químicas. La mineralización y la disponibilidad del N están controladas por una serie de factores del suelo, como la textura (Sørensen y Jensen 1995a), la actividad microbiana (Bengtsson *et al.*, 2002), la materia orgánica del suelo (Ros, 2012), el pH (Bertrand *et al.*, 2007), o la mineralogía de las arcillas (Cavali *et al.*, 2015).

8.4 Digitalización de la agricultura y agricultura inteligente “Smart Farming”

Los sensores de campo que miden la reflectancia de los cultivos, además de utilizarse de forma manual, pueden utilizarse montados sobre un vehículo. En este sentido, los datos del sensor podrían utilizarse inmediatamente (a tiempo real) para llevar a cabo aplicaciones de N variables “*on-the-go*”, o pueden almacenarse y geolocalizarse para su uso posterior (Antonucci, 2012). Respecto al primer enfoque, Tremblay *et al.*

(2009) comprobaron la utilidad de los sensores Yara N-Sensor/FieldScan® (Yara International ASA, Alemania) y GreenSeeker™ (Ntech Industries - Trimble) instalados sobre un tractor con el objetivo de observar si podían evaluar el estado nitrogenado del maíz y del trigo. Concluyeron que ambos sensores podían diagnosticar el estado nitrogenado de estos cultivos y que sus datos podrían utilizarse para desarrollar algoritmos y hacer aplicaciones variables de N mediante la transmisión de información a un esparcidor equipado con un controlador de velocidad. Tanto Yara (Yara, 2020) como Trimble (Trimble, 2020) hacen recomendaciones de la dosis de fertilizante a aplicar mediante el uso de los datos obtenidos de sus sensores de campo. Cuando se almacenan las lecturas de los sensores, la forma más común de mostrar los datos es en el formato de mapas (Pallottino *et al.*, 2019). En este último enfoque se dispone de una mayor flexibilidad ya que se pueden manipular y procesar los datos para zonificar las parcelas (Pallottino *et al.*, 2019). En este sentido, el objetivo es obtener mapas con unas pocas zonas de gestión diferencial basadas en parámetros de interés para así poder aplicar los tratamientos de manera eficiente. En Araba, un significativo avance en la fertilización del cereal sería la obtención de un mapa de fertilización por parcela estableciendo dos o tres zonas, lo que permitiría ajustar la dosis de N necesaria a cada zona. A la hora de hacer los mapas de recomendación de fertilización nitrogenada, además de la información de la planta disponible a partir de sensores o satélites, se podría añadir un estimador del potencial de mineralización de la materia orgánica del suelo. Sobre todo, si se dispone de variables auxiliares que estiman esta propiedad del suelo (Capítulo 2) que es laboriosa en cuanto a su análisis.

Aun así, aunque los sensores proximales tienen potencial para la evaluación del estado nutricional nitrogenado de los cultivos, las recomendaciones de fertilización nitrogenado basadas en sensores son escasas (Padilla *et al.*, 2018) y no pueden generalizarse con fiabilidad a otras situaciones o condiciones (Diacono, 2013). Según Colaço y Bramley (2018) esto puede ser debido al número limitado de años o de localizaciones en que se desarrolla la experimentación. Por otro lado, para que los agricultores puedan aportar dosis variables de N de forma sencilla, necesitan un equipo avanzado que pueda recibir órdenes de una unidad de control informatizada (Saiz-Rubio y Rovira-Más, 2020).

En la agricultura de precisión, la teledetección ha desempeñado un papel fundamental debido a que los datos de campo se han hecho accesibles gracias a los satélites artificiales. El satélite europeo Sentinel 2, proporciona datos multiespectrales con una resolución de 10 m de píxel para el índice NDVI con una frecuencia de cinco días. Los satélites estadounidenses Landsat 8 son también importantes para proporcionar información agrícola, aunque aportan información cada 8 o 16 días con una precisión de 30 m de pixel. Existen otros satélites como la constelación RapidEye, el sistema GeoEye-1 o el WorldView-3 (Saiz-Rubio y Rovira-Más, 2020). Disponer de una imagen satélite no garantiza que la imagen sea adecuada para su uso. Las condiciones climáticas pueden impedir la visualización de la zona de interés, siendo la presencia de nubes el principal factor limitante.

A medida que los sensores y las máquinas inteligentes se incorporen en las granjas, y los datos agrícolas aumenten en cantidad y alcanEC, los procesos agrícolas se basarán cada vez más en el procesamiento de datos (Wolfert *et al.*, 2017). Los rápidos avances en el Internet de las cosas o *Internet of Things (IoT)* y la computación en nube o *Cloud Computing* están impulsando el fenómeno de lo que se denomina agricultura inteligente o *Smart Farming* (Sundmaeker *et al.*, 2016). Wolfert *et al.* (2017) destacan que aunque la agricultura inteligente está basada en la agricultura de precisión, ésta va más allá, ya que está impulsada por redes interconectadas (IoT), y facilitada por la computación en nube. La agricultura inteligente se engloba en el fenómeno de la utilización de *Big Data*, volúmenes masivos de una gran variedad de datos que pueden ser capturados, analizados y utilizados para la toma de decisiones. Las aplicaciones de *Big Data* en la agricultura incluyen el despliegue de sensores y el análisis de sus datos, modelización predictiva y uso de mejores modelos para gestionar el riesgo de pérdidas de cosechas y para aumentar la eficiencia de los cultivos (Faulkner y Cebul, 2014; Lesser, 2014). En conclusión, mediante el uso de Big Data se puede proporcionar una visión predictiva de los resultados futuros de la agricultura, impulsar las decisiones operacionales en tiempo real y reinventar los procesos comerciales (Devlin, 2012).

Recientemente, muchas empresas han entrado en el campo de la agricultura de precisión o de la agricultura inteligente con el objetivo de adquirir información y utilizar estos datos masivos para la toma de decisiones. Saiz-Rubio y Rovira-Más (2020) enumeran los distintos programas configurados disponibles en el mercado para tratar los datos generados de las explotaciones y mencionan que esto muestra el esfuerzo realizado durante la última década para trasladar los resultados de los ensayos en agricultura de precisión a las granjas comerciales.

La estrategia europea de la granja a la mesa indica que es necesario que todos los agricultores y todas las zonas rurales estén conectados a internet de forma rápida y fiable, permitiendo integrar la agricultura de precisión y la agricultura inteligente. La Comisión Europea tiene la intención de acelerar el despliegue de internet de banda ancha rápida en las zonas rurales para alcanzar el objetivo del 100% de acceso para 2025 (EC, 2020).

8.5 Demanda de un sistema alimentario sostenible

La capacidad del sistema agroalimentario para responder a los desafíos económicos, demográficos y ambientales que se avecinan se ha convertido en un tema de creciente interés (Arnalte-Mur *et al.*, 2020). La agricultura y los sistemas agroalimentarios, que representan la forma en la que producimos y consumimos alimentos, pueden transformarse de forma que contribuyan a la nutrición, a la salud y a preservar el medio ambiente, promoviendo al mismo tiempo la creación de empleo, el crecimiento económico, la estabilidad y el bienestar (FAO, 2020).

La sociedad europea cada vez presta más atención a cuestiones éticas, sostenibles, medioambientales y sanitarias, por lo que busca alimentos con valor añadido. Las personas demandan alimentos frescos, menos transformados, de origen sostenible y de cadenas de suministro cortas (EC, 2020). Este último aspecto se ha puesto más en relieve con la pandemia COVID-19, dando importancia a un sistema alimentario sólido y resiliente, que pueda garantizar a las personas un suministro suficiente de alimentos a precios asequibles (Rowan y Galanakis, 2020).

En este sentido, la estrategia de la granja a la mesa reforzará el marco legislativo sobre indicaciones geográficas (EC, 2020). Además, con miras a aumentar la resiliencia de los sistemas alimentarios locales y regionales, y con el fin de crear cadenas de suministro más cortas, la Comisión Europea apoyará reducir la dependencia del transporte de larga distancia (EC, 2020). En el Capítulo 7 de este trabajo se muestran los resultados relacionados con la estimación del contenido de proteína en grano de trigo para la obtención de harina de fuerza mediante un medidor de clorofila. El ensayo de campo se estableció con las prácticas habituales de fertilización que se llevan a cabo en Araba. En esta zona, debido a los altos rendimientos que suelen obtenerse, no es sencillo obtener valores de proteína altos con la fertilización aplicada. Las variedades comúnmente utilizadas en Araba han sido seleccionadas por sus altas producciones, y no por su alto contenido de proteína en grano. Sin embargo, existe una demanda de harina de calidad en la CAPV, y por eso surgió el proyecto *Euskal Ogia.* Este proyecto tiene como objetivo realizar una prueba de mercado con un pan elaborado a partir de cereal alavés certificable con *Eusko Label*, buscando detectar si el mercado aceptaría un pan diferenciado en origen, producción, trazabilidad y sostenibilidad con respecto al resto de pan habitual en el mercado (HAZI, 2020).

La estimación de contenido de proteína en grano no es un objetivo sencillo, ya que depende de muchos procesos (Colaço y Bramley, 2018) como son la captación, la asimilación, la translocación y la removilización del N (Fuertes-Mendizábal *et al.*, 2012). El N del grano deriva de dos fuentes de N diferentes: el N que es absorbido en el período de post-floración desde el suelo, y el N removilizado al grano, que ha sido previamente acumulado en los órganos vegetativos en el período de pre-floración. Los resultados obtenidos en el Capítulo 7 muestran que los valores del medidor de clorofila Yara N-Tester^{TM} en mitad de floración pueden ayudar a estimar los valores de proteína en grano sólo cuando los rendimientos son inferiores a 8000 kg ha^{-1}. Para estos niveles de rendimiento, los valores del medidor de clorofila deben ser superiores a 700 para lograr harina de fuerza (12,5% de proteína en grano). Como se ha mencionado previamente, mediante el NDVI obtenido por el sensor RapidScan CS-45 podríamos estimar el rendimiento del trigo en mitad de floración mediante el uso de las mediaciones en inicio de encañado, dos nudos, hoja bandera y mitad de floración. Por otro lado, el contenido de proteína en grano no se vio afectado por el tipo de abono de fondo aportado, es decir, la aplicación de purín y estiércol en fondo no se diferenció del tratamiento convencional con 40 kg N ha^{-1} en ahijado. Estos resultados permitirían a los agricultores y cooperativas agrarias contar con herramientas a la hora de tomar decisiones en relación a aplicaciones tardías de N con el objetivo de obtener mejores contenidos de proteína de cara a la venta del grano de trigo. Otra estrategia que no se ha probado en la CAPV, pero que funciona en otros países, es la aplicación foliar de urea posterior a la floración. Así, se ha demostrado que dichas aplicaciones tienen un efecto significativo en el incremento de la proteína, aumentando un 0,8% su concentración por cada 30 kg N ha^{-1} aplicados (Turley *et al.*, 2001), si bien se trata de condiciones edafoclimáticas distintas a las de Araba.

Las lecturas con los medidores de clorofila no son sencillas de tomar, ya que requieren pinzar la última hoja completamente desarrollada de 30 plantas de trigo de la zona a medir. Hansen *et al.* (2003), Pettersson y Eckersten (2007), Söderström *et al.* (2010) y Zhao *et al.* (2020) mostraron resultados prometedores de la estimación de la proteína de los cereales durante su ciclo de cultivo mediante la detección remota y proximal. Sin embargo, en nuestro trabajo observamos que los índices obtenidos mediante el sensor proximal RapidScan CS-45 (NDVI y NDRE) no eran buenos estimadores del contenido de proteína en grano (Capítulo 5).

En general, para mejorar las estimaciones es necesario establecer ensayos con distintas variedades en las que el genotipo determine mayores contenidos de proteína y distintos diseños de fertilización donde se hagan aportes tardíos. De la superficie que se destina en Araba a los cultivos herbáceos, el 45% se encuentra en la Llanada Alavesa y el 32% en Valles Alaveses. En la comarca de Valles Alaveses, las lluvias tardías parecen ser más habituales que en la zona de la Llanada Alavesa, lo cual significaría una mayor probabilidad de aprovechamiento de los aportes tardíos de N. Por ello podría ser una zona interesante para la obtención de granos con altos contenidos de proteína.

Por otro lado, se estima que el mercado de alimentos ecológicos seguirá creciendo, por lo que es preciso continuar fomentando la agricultura ecológica e investigando para mejorar el manejo del cultivo (Muller *et al.*, 2017). La agricultura ecológica se considera una buena opción para mejorar la sostenibilidad de los sistemas alimentarios, ya que no usa fertilizantes y plaguicidas sintéticos, promueve las rotaciones de cultivos y se centra en la fertilidad del suelo cerrando el ciclo de nutrientes. Sin embargo, hoy en día se obtienen menores rendimientos que en los sistemas convencionales (Muller *et al.*, 2017), si bien Ponti *et al.* (2012) mencionaron que los rendimientos en la agricultura ecológica van a aumentando con el tiempo. La UE apoya la transición a este tipo de agricultura y tiene como objetivo que al menos el 25% de las tierras agrícolas de la UE se cultiven bajo el marco de la agricultura ecológica para 2030 (EC, 2020). Los fertilizantes y acondicionadores del suelo permitidos en la agricultura ecológica están recogidos en el Anexo I del Reglamento (CE) no 889/2008. Los subproductos utilizados en este trabajo, los más producidos en la CAPV, están permitidos en el reglamento para la agricultura ecológica siempre y cuando su origen no sea la ganadería intensiva. Ponti *et al.* (2012), en la revisión que llevaron a cabo de distintos ensayos relacionados con la agricultura ecológica, mencionan que los rendimientos obtenidos son un 20% menores que en la agricultura convencional, siendo esta diferencia mayor para los cultivos cerealistas y de patata, y presentando grandes diferencias entre regiones. Shah *et al.* (2017) también observaron que los rendimientos en cereales de invierno en agricultura ecológica son un 30% más bajos que en agricultura convencional, y que estas diferencias son menores en los cereales de primavera. Williams *et al.* (2017) en un ensayo llevado a cabo durante 40 años observaron que las prácticas de la agricultura ecológica mejoraron las propiedades físicas del suelo, lo que conlleva un aumento de la sostenibilidad agrícola. Es de especial interés el estudio a largo plazo de las aplicaciones de los subproductos permitidos para la agricultura ecológica, con el objetivo de evaluar su efecto acumulativo en las propiedades físicas y químicas de los suelos con el consiguiente efecto sobre la liberación de nutrientes y, por tanto, sobre los rendimientos de los cultivos.

Las organizaciones FAO y OMS (2019) mencionan que para reducir el riesgo de enfermedades y el impacto medioambiental del sistema alimentario es necesario dar paso a una dieta más basada en los vegetales con menos carne roja y transformada, y con más frutas y verduras (FAO y OMS, 2019). La estrategia de la granja a la mesa también destaca que las pautas actuales en las dietas son insostenibles, tanto desde la perspectiva de la salud como del medio ambiente. En la Unión Europea, la ingesta media de energía, de carne roja, azúcares, sal y grasas sigue superando las recomendaciones, y el consumo de cereales integrales, frutas y hortalizas, leguminosas y frutos secos es insuficiente (Willet *et al.*, 2019).

En la transición a sistemas alimentarios más sostenibles, la investigación y la innovación son motores fundamentales, desde la producción primaria hasta el consumo. Hemos visto cómo las tecnologías de la información y las comunicaciones pueden facilitar la implantación de la agricultura de precisión y de la agricultura inteligente. Otro enfoque en lo que se refiere a los cereales se orienta hacia la biotecnología, en particular, hacia su aplicación al diseño de plantas que puedan fijar nitrógeno y que ha avanzado gracias a la expresión de las proteínas del sistema nitrogenasa eucariota y a un conocimiento más profundo de las vías de nodulación de las raíces y de los microorganismos diazotrofos de vida libre (Ryu *et al.*, 2019). Durante décadas, los científicos y agrónomos han identificado la fijación biológica de nitrógeno como una fuente alternativa de nitrógeno con menores costos de producción y mayor eficiencia. Esta fijación es una función que ciertas procariotas y arqueas diazotrofas pueden llevar a cabo, reduciendo el nitrógeno atmosférico por medio de la enzima nitrogenasa (Bennett *et al.*, 2020). Bloch *et al.* (2020) concluyen que, aunque se han hecho grandes avances en la ingeniería de los cultivos de cereal tanto para la expresión de la nitrogenasa como para la formación de nódulos, la complejidad de estos procesos hace que el periodo de tiempo necesario para poder incorporarlos a los cereales sea todavía demasiado largo. Por el contrario, los microorganismos diazotrofos inoculantes podrían tener un impacto más inmediato tanto en la industria como en la agricultura. La conjunción de las distintas tecnologías permitirá avanzar hacia la sostenibilidad de los sistemas de producción agraria.

Capítulo 9

Conclusiones

9 Conclusiones

De acuerdo con los objetivos planteados en este trabajo y mediante los resultados obtenidos pueden extraerse las siguientes conclusiones:

1. La heterogeneidad intrínseca del suelo, incluso en áreas geográficas reducidas, origina una alta variabilidad en la dinámica del N_{min} a lo largo del ciclo de cultivo del trigo, lo que dificulta estimar la disponibilidad del N para el cultivo.

2. En los estados fenológicos iniciales, la cantidad de N_{min} en el suelo condiciona el rendimiento del cultivo de trigo. En los estados fenológicos finales, la cantidad de N_{min} en el suelo influye directamente en el contenido de proteína en el grano.

3. Las dinámicas de mineralización de los subproductos orgánicos de origen ganadero aportados al suelo son muy variables, dependiendo de la tipología y composición bioquímica de cada subproducto, lo que conlleva la necesidad de estudiar las cantidades y momentos de aporte de cada fertilizante orgánico de forma individualizada, según el cultivo, climatología, tipo de suelo, etc.

4. El aporte de gallinaza y purín hacen que la disponibilidad de N mineral para el cultivo sea muy alta de forma muy rápida, por lo que su utilización como fertilizante orgánico en cereal en el clima mediterráneo húmedo de la provincia de Araba debe limitarse a cultivos de cereal de primavera o al punto de máxima demanda de N en cultivos de cereal invierno. Es necesario tener siempre en cuenta la proporción de N disponible en la gallinaza y el purín de vacuno, que suele estar principalmente en forma de ácido úrico y de amonio, respectivamente.

5. El estiércol vacuno, con un menor aporte de N mineral que la gallinaza y el purín, es el subproducto ganadero más indicado para la aplicación en fondo a los cultivos de cereal de invierno. Esto es debido a su baja tasa de mineralización, lo que reduce el

riesgo de pérdidas durante el período en que las extracciones de N por el cultivo son bajas.

6. A la dosis baja de N aplicado (170 kg N ha^{-1}), la actividad enzimática ureasa aumenta con la presencia del trigo. La presencia de plantas reduce la concentración de nitrato en la solución del suelo a niveles de no-fertilización desde inicio de ahijado hasta la cosecha, aun cuando la dosis de N aplicada con los subproductos sea alta (340 kg N ha^{-1}).

7. En condiciones de clima mediterráneo húmedo las lecturas normalizadas de los sensores proximales de campo Yara N-Tester™ y RapidScan CS-45 (NDVI y NDRE) permiten ajustar la dosis de N al inicio del encañado del trigo bajo fertilización convencional y fertilización orgánica con purín de vacuno en fondo. No obstante, en subproductos ganaderos con una composición bioquímica más variable y poco N disponible, como el estiércol ovino, la fluctuación en la disponibilidad del N dificulta el establecimiento de relaciones entre las lecturas de los sensores y el rendimiento del cultivo.

8. Los valores absolutos de NDVI obtenidos con el sensor proximal RapidScan CS-45 estiman adecuadamente el índice nutricional nitrogenado (INN) a lo largo del ciclo del cultivo de trigo, mediante la utilización de un único modelo estandarizado para todas las etapas fenológicas.

9. En los estados fenológicos GS32, GS37 y GS65 (*i.e.* aparición del segundo nudo, hoja bandera, y mitad de floración) el rango de valores NDVI umbral del sensor proximal RapidScan CS-45, por encima de los cuales no bajaría el rendimiento del trigo es de 0,70 – 0,75. En el estado fenológico GS30 (*i.e.* inicio de encañado) no es posible establecer dicho umbral debido a que las deficiencias de N en ese momento del ciclo del trigo pueden compensarse en GS32.

10. En periodos de sequía, que ocurren tres de cada diez años en la zona de estudio, la falta de humedad en el suelo hace que las deficiencias de N no puedan ser compensadas a partir del estado fenológico GS30 (*i.e.* inicio de encañado). Por tanto,

es recomendable la aplicación de 40 kg de N ha^{-1} en el estado fenológico GS21 (*i.e.* inicio de ahijado) para la obtención de buenos rendimientos.

11. El sumatorio de los valores NDVI en los estados fenológicos GS30, GS32, GS37 y GS65 (*i.e.* inicio de encañado, aparición del segundo nudo, hoja bandera, y mitad de floración) es un indicador de la variabilidad del rendimiento del cultivo de trigo.

12. Cuando el rendimiento de trigo es inferior a 8.000 kg ha^{-1}, las lecturas obtenidas con el medidor de clorofila Yara N-Tester™ en GS65 (*i.e.* mitad de floración) estiman los valores de proteína en grano. Dichas lecturas deben ser superiores a 700 para alcanzar la cantidad de proteína requerida (12,5%) según el estándar de calidad nutricional para la obtención de harina panificable de fuerza.

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Publicaciones generadas

Yo, Marta Aranguren Rivas, declaro que:

Esta tesis doctoral ha generado cinco artículos ya publicados en revistas indexadas JCR así como dos artículos que actualmente se encuentran bajo revisión:

1. Aranguren M, Aizpurua A, Castellón A, Besga G, Villar N, 2018. Soil properties for predicting soil mineral N dynamics throughout a wheat growing cycle in calcareous soils. *Agronomy*, 8(12), 303 (ver Capítulo 2).
2. Aranguren M, Castellón A, Besga, G, Ojinaga, M, Aizpurua A. Influence of wheat crop on carbon and nitrogen mineralization dynamics after the application of livestock manures. *Geoderma, under revision*. (ver Capítulo 3).
3. Aranguren M, Castellón A, Aizpurua A, 2019. Crop sensor-based in-season nitrogen management of wheat with manure application. *Remote Sensing*, 11(9), 1094 (ver Capítulo 4).
4. Aranguren M, Castellón A, Aizpurua A, 2020. Crop sensor-based non-destructive estimation of nitrogen nutritional status, yield, and grain protein content in wheat. *Agriculture*, 10 (5), 148 (ver Capítulo 5).
5. Aranguren M, Castellón A, Aizpurua A, 2020. Wheat yield estimation with NDVI values using a proximal sensing tool. *Remote Sensing*, 12(17), 2749 (ver Capítulo 6).
6. Aranguren M, Castellón A, Aizpurua A, 2018. Topdressing nitrogen recommendation in wheat after applying organic manures: the use of field diagnostic tools. *Nutrient Cycling in Agroecosystems*, 110, 89 (ver Capítulo 6).
7. Aranguren M, Castellón A, Aizpurua A, 2020. Estimation of the wheat grain protein content using a chlorophyll meter under humid Mediterranean conditions. *European Journal of Agronomy, under revision* (ver Capítulo 7).

De este trabajo son también fruto los siguientes artículos divulgativos publicados en revistas nacionales especializadas en el sector:

1. Aranguren M, Castellón A, Aizpurua A, 2019. Herramientas para ajustar la dosis de fertilizante mineral en cobertera de cereal. *Vida Rural*, 468, 40-44.

2. Aranguren M, Castellón A, Aizpurua A, 2019. Seguimiento del estado nutricional nitrogenado del cultivo de trigo basado en mediciones de NDVI. *Tierras Agricultura*, 280, 76-79.

Asimismo, el presente trabajo ha generado las siguientes comunicaciones a congresos:

1. Aranguren M, Castellón A, Aizpurua A. Monitoring wheat crop N status under humid Mediterranean conditions based on changes in NDVI. Póster. 12th European Conference on Precision Agriculture. Montpellier (Francia), 2019.
2. Aranguren M, Castellón A, Aizpurua A. Use of field diagnostic tools for top dressing nitrogen recommendation when organic manures are applied in humid Mediterranean conditions. Oral Presentation. 14th International Conference on Precision Agriculture. Montreal (Canadá), 2018.
3. Aranguren M, Ojinaga M, Castellón A, Aizpurua A. Nitrate and ammonium dynamic in soil solution after applying animal manure in a wheat greenhouse experiment. Comunicación oral. 17th International RAMIRAN Conference: Sustainable utilisation of manures and residue resources in agriculture. Wexford (Irlanda), 2017.
4. Aranguren M, Castellón A, Aizpurua A. Top dressing nitrogen recommendation in wheat after applying organic manures. Use of field diagnostic tools. Póster. 19th Nitrogen Workshop. Skara (Suecia), 2016.

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