Universidad Zaragoza
1542

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CENTRO DE INVESTIGACIÓN Y TECNOLOGÍA
AGROALIMENTARIA DE ARAGÓN

TESIS DOCTORAL

RESPUESTA DEL ARROZ A LA FERTILIZACIÓN CON PURÍN PORCINO EN CONDICIONES MEDITERRÁNEAS

Beatriz Moreno García

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RESPUESTA DEL ARROZ A LA FERTILIZACIÓN CON PURÍN PORCINO EN CONDICIONES MEDITERRÁNEAS

Memoria presentada por: **Beatriz Moreno García**

En satisfacción de los requisitos necesarios para optar al grado de doctor

Directora: Dolores Quílez Sáez de Viteri

Zaragoza, Marzo de 2017

A mis padres

“Cuando lleguemos a ese río, ya hablaremos de ese puente”

Julio César, -Comentarios sobre la guerra de las Galias-

AGRADECIMIENTOS

Durante los años de realización de esta Tesis Doctoral, he recibido la ayuda de muchas personas que, de un modo u otro, han contribuido a la consecución de este trabajo, a las cuales me gustaría expresar mi más sincero agradecimiento.

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RESUMEN

La producción porcina ha experimentado un incremento importante en los últimos años en el Nordeste de España, donde se concentra el 50% de la producción española (1ª de la UE). Tradicionalmente, el purín porcino (Pig Slurry, PS) se ha aplicado a cultivos como cereales de invierno o maíz, pero debido al incremento de la producción de purines debe incluirse en los planes de fertilización de otros cultivos como el arroz. Por ello, el objetivo principal de este trabajo ha sido evaluar distintas estrategias de fertilización con PS en cultivo de arroz inundado en el nordeste español para evaluar si los purines de porcino pueden sustituir a los fertilizantes minerales (M) con los mismos resultados agronómicos y económicos y sin ocasionar un mayor impacto ambiental.

Para ello, se llevó a cabo un ensayo experimental en la localidad de Villanueva de Sigena (Huesca) durante 3 años consecutivos (2011-2013) en el que se evaluaron 3 estrategias de fertilización: 2 dosis de PS en fondo equivalentes a 170 y 120 kg $N\text{-}NH_4^+\cdot ha^{-1}$, y una dosis de fertilizante mineral (M) en fondo de 120 kg $N\text{-}NH_4^+\cdot ha^{-1}$; las 3 complementadas con fertilizante mineral en cobertera. Los resultados mostraron que la aplicación de PS permite conseguir rendimientos máximos aplicando una única dosis en fondo (170 kg $N\text{-}NH_4^+\cdot ha^{-1}$) o una dosis más baja (120 kg $N\text{-}NH_4^+\cdot ha^{-1}$) complementada con fertilizante mineral en cobertera. Además, su aplicación no produce efectos negativos en la germinación de la semilla, la presencia de malas hierbas, plagas o enfermedades, el rendimiento en molino o la calidad del grano. El equivalente mineral del N contenido en el purín fue del 92 % del contenido de N amoniacal aplicado y de un 57 % del contenido de N total aplicado, indicando que el cultivo aprovecha el N amoniacal contenido en el purín tan eficientemente como el del fertilizante mineral. Sin embargo, el N orgánico no es aprovechable durante el año de aplicación y por tanto, las dosis de PS se tienen que ajustar teniendo en cuenta el contenido de N amoniacal para evitar una reducción del rendimiento.

Las emisiones de gases de efecto invernadero (GEIs) se cuantificaron en el ensayo de Villanueva de Sigena durante el año 2013 y en un segundo ensayo en la localidad de Grañén (Huesca), durante el año 2014. En ambos ensayos, las medidas de GEIs se realizaron en tratamientos con purín y mineral fertilizados con dosis de N consideradas óptimas [120 kg $N\cdot ha^{-1}$, (PS o M) en fondo complementado con 60 kg $N\cdot ha^{-1}$ en cobertera o 170 kg $N\cdot ha^{-1}$ (PS o M) en fondo] y en un control sin aplicación de N. La parcela de Villanueva de Sigena había sido cultivada con arroz los 3 años previos al ensayo, sin realizar la técnica del fangueo, mientras que la parcela de Grañén llevaba cultivándose con arroz más de 15 años realizando la labor de fangueo; además, el contenido de materia orgánica del suelo era mayor en Grañén (2.06%) que en Villanueva de Sigena (1.01%). La aplicación de PS en fondo a dosis equivalentes a las de N mineral (120 kg N·ha^{-1} en Villanueva de Sigena y 170 kg N·ha^{-1} en Grañén) no produjo un incremento de las emisiones de GEIs ni del factor de emisión de N_2O en ninguna de las dos localidades. Sin embargo, en el suelo de Villanueva de Sigena, con un contenido menor de materia orgánica, la aplicación de una dosis alta de PS en fondo (170 kg N·ha^{-1}) aumentó las emisiones de metano en comparación con los tratamientos de dosis más baja en fondo, indicando que una estrategia para la mitigación de las emisiones de GEIs es la aplicación de dosis moderadas de PS en fondo (≈120 kg N·ha^{-1}) complementadas con N mineral en cobertera.

Para el ajuste del N mineral en cobertera, se estudió la utilidad de la información derivada de imágenes multiespectrales, tomadas en la fase de panícula en zurrón, como herramienta de ayuda a la decisión. Este objetivo se desarrolló en el ensayo de Villanueva de Sigena. Las relaciones entre el rendimiento y siete índices de vegetación mostraron diferencias entre años y entre tipo de fertilización (PS vs. M), es decir, la respuesta espectral del cultivo en parcelas fertilizadas con PS fue diferente a la de las parcelas fertilizadas con N mineral; lo que obligó a trabajar con valores relativos con respecto a parcelas sobrefertilizadas para cada año y tipo de fertilización. El índice $\text{gMCARI}_{\text{NIR}}$, propuesto en este estudio, que considera la respuesta espectral en las bandas del verde, rojo e infrarrojo cercano, mostró la mejor relación con el rendimiento, y permitió la elaboración de dos herramientas con un alto porcentaje de éxito (87.5 %) en la recomendación de N en su evaluación preliminar. El análisis económico y la evaluación del exceso de N mostraron que el uso de estas herramientas es una opción tanto económica como medioambientalmente favorable frente a la fertilización habitual sin ningún tipo de recomendación.

A partir de los resultados obtenidos en esta tesis, se puede concluir que el purín porcino puede y debe integrarse en los planes de fertilización del cultivo de arroz en la zona del nordeste español. La mejor estrategia es la aplicación de dosis moderadas en fondo (≈120 kg N·ha^{-1}) complementadas con N mineral en cobertera, permitiendo conseguir rendimientos máximos sin un aumento de las emisiones de GEIs. El uso de información multiespectral es una herramienta prometedora para el ajuste de estas dosis de N complementarias en cobertera.

SUMMARY

Northeast Spain has experienced a great increase in pig production over the last few years and now concentrates one half of Spain’s pig production (1st in EU). Pig slurry (PS) has been applied traditionally to winter cereals (barley, wheat) and maize crops; however, PS fertilization should be extended to alternative crops such as rice due to the increase in PS generation. In this context, the main objective of this work was the study of the agronomic, environmental and economic viability of different strategies of fertilization with PS as a substitute to mineral fertilizers (M) in flooded rice systems in Northeast Spain.

To fulfill this objective, a field experiment was conducted at Villanueva de Sigena (Huesca) during three consecutive years (2011-2013) with three fertilization strategies: two PS and one mineral. The fertilization strategies were: two rates of PS equivalent to 170 kg NH_4^+-N·ha^{-1} and 120 kg NH_4^+-N·ha^{-1}, which were compared to a mineral treatment with a rate of 120 kg NH_4^+-N·ha^{-1}, all three applied before seeding and combined with mineral N at topdressing. Pig slurry fertilization with a single 170 kg NH_4^+-N·ha^{-1} rate before seeding or a rate of 120 kg NH_4^+-N·ha^{-1} before seeding complemented with mineral N at topdressing ensured maximum rice yield. Moreover, PS fertilization did not affect seed germination, the presence of weeds, pests and diseases, head rice yield or grain quality. Nitrogen fertilizer replacement value of PS was 92% of the ammonium-N applied with PS and 57 % of the total N applied with PS, showing that rice used PS ammonium-N as efficiently as the ammonium-N provided by mineral fertilizers. However, organic N is not taken by the crop during the year of application and thus, PS rates should be established considering the ammonium-N content in PS to avoid yield impairment.

Greenhouse gases (GHG) emissions were quantified at the Villanueva de Sigena experiment in 2013 and in another experimental field at Grañén (Huesca) in 2014. GHG fluxes were quantified in PS and mineral plots with N rates near the N optimum [120 kg N·ha^{-1} (PS or M) before seeding + 60 kg N·ha^{-1} at topdressing or 170 kg N·ha^{-1} (PS or M) before seeding] and in a control plot with no N application. The field at Villanueva de Sigena had been cultivated for rice in the previous three years with no puddling practices, while the field at Grañén had been cropped to rice for more than 15 years with puddling tillage practices. Additionally, organic matter content was higher in Grañén (2.06%) than in Villanueva de Sigena (1.01%). GHG emissions and fertilizer-

induced N_2O emission factor were not increased by the application of PS at the same N rate of mineral fertilizer before seeding (120 kg $N·ha^{-1}$ at Villanueva de Sigena and 170 kg $N·ha^{-1}$ at Grañén) in both sites. However, at Villanueva de Sigena (soil with lower organic matter content), the higher PS rate applied before seeding (170 kg $N·ha^{-1}$) increased methane emissions compared to the treatments with lower N rate before seeding. Thus, a strategy for GHG emissions mitigation is the application of moderate PS rates before seeding (≈120 kg $N·ha^{-1}$) complemented with mineral N at topdressing.

The information derived from multispectral images taken at the rice booting stage was evaluated as a decision making tool in the adjustment of N topdressing. This objective was developed in the Villanueva de Sigena experiment. The relationships between yield and seven vegetation indices (VIs) showed differences between years and type of fertilization (PS or. M), i.e., the spectral response of rice in PS plots was different from that in M plots. Therefore, the VIs were used as relative values to the maxima for each year and type of fertilizer. The index $gMCARI_{NIR}$, proposed in this study, that includes information in the green, red and near-infrared wavelengths, presented the strongest relationship with yield and allowed the construction of two approaches for N recommendation with a high percentage of success (87.5 %) in the preliminary evaluation. The economic analysis and the evaluation of N excess highlighted that these approaches provide a useful tool for N recommendation, economically and environmentally favorable when compared to the usual practices with no advice.

On the basis of the results obtained in this thesis, it can be concluded that PS should be integrated into N fertilization plans for rice in Northeast Spain. The best strategy is the application of moderate rates before seeding (≈120 kg $N·ha^{-1}$) complemented with mineral N at topdressing, reaching maximum yields without an increase in GHG emissions. The use of multispectral information is a promising tool for the adjustment of complementary topdressing N rates.

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CAPÍTULO 1

INTRODUCCIÓN GENERAL

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1.1. IMPORTANCIA DEL SECTOR PORCINO

España es el primer productor de porcino de la Unión Europea, con un 19 % de la producción total y con un censo para 2015 de 28 millones de cabezas (EUROSTAT, 2015). Más de la mitad de la producción del país (51 %) se concentra en el nordeste español (Aragón y Cataluña). El porcino en Aragón representa el 24 % del porcino de España (MAGRAMA, 2015a) y ha tenido un crecimiento continuado durante los últimos años, casi duplicándose en los últimos 15 años (Figura 1.1); sin embargo, el crecimiento en España no ha sido tan acusado y la mayor parte del aumento del censo se ha debido al incremento en Aragón (Figura 1.1) (MAGRAMA, 2015a).

El sector porcino en Aragón representa el 35 % de la Producción Final Agraria, con una diferencia considerable respecto al valor que representa a nivel nacional (13 %) (DGA, 2015). Por otro lado, representa el 59 % con respecto a la Producción Final Agraria Ganadera. La distribución del censo porcino no es uniforme, concentrándose el 49 % de la producción en la provincia de Huesca (MAGRAMA, 2015a).

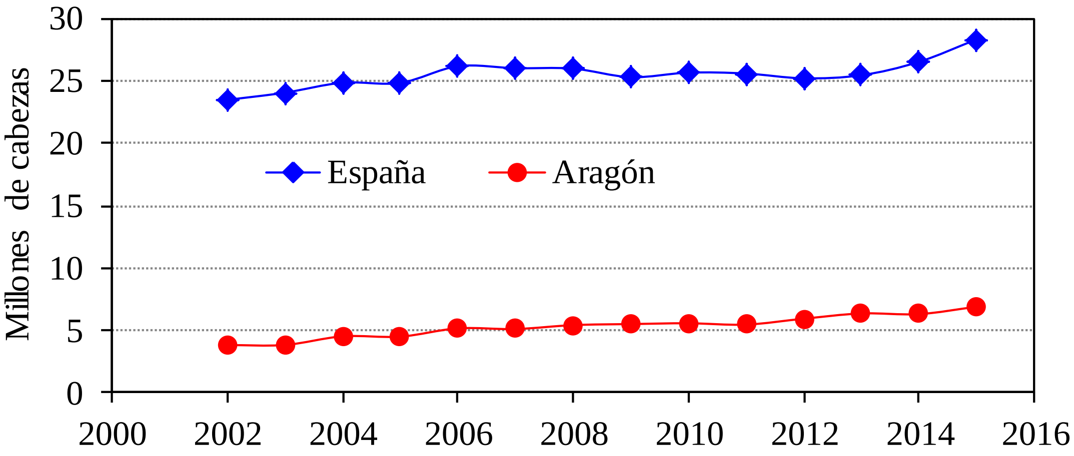

Figura 1.1. Evolución de los censos porcinos en millones de cabezas en Aragón y España. Fuente: MAGRAMA. Encuestas ganaderas 2002-2015. Ganado Porcino.

El aumento del censo porcino lleva asociado un aumento de la producción de purín; la estimación de la producción de purín porcino en Aragón en el año 2015 fue de 11 millones de m^3, lo que supone 40000 toneladas de nitrógeno (N) (BOE, 2000; MAGRAMA, 2015a). La aplicación de purín como fertilizante agrícola, en dosis agronómicas y medioambientales adecuadas, es el método más económico para su gestión, constituyendo un claro ejemplo de reciclaje de nutrientes. Sin embargo, su aplicación como fertilizante debe limitarse a zonas cercanas al lugar de su producción para que resulte rentable (Iguácel y Yagüe, 2007). Además, la aplicación se tiene que hacer de forma racional para evitar problemas de contaminación por nitratos de las aguas superficiales y subterráneas (Daudén y Quílez, 2004) o contaminación atmosférica debido a la emisión de gases de efecto invernadero (Win et al., 2014) y amoniaco (Yagüe y Bosch-Serra, 2013), cumpliendo así con las Directivas a nivel europeo, entre las que podemos destacar la Directiva de protección de las aguas contra la contaminación producida por los nitratos procedentes de fuentes agrarias (EU, 1991), la directiva Marco del Agua (EU, 2000) o la Directiva de techos de emisión de contaminantes atmosféricos (EU, 2016).

Tradicionalmente, el purín se ha aplicado a cultivos como el maíz o cereales de invierno, pero con el incremento de la producción porcina se tiene que extender a otros cultivos, como el arroz, sustentándose además en que en Aragón existe una coincidencia espacial entre las zonas con mayor densidad de granjas de porcino y las zonas en las que se cultiva tradicionalmente el arroz (Figura 1.2).

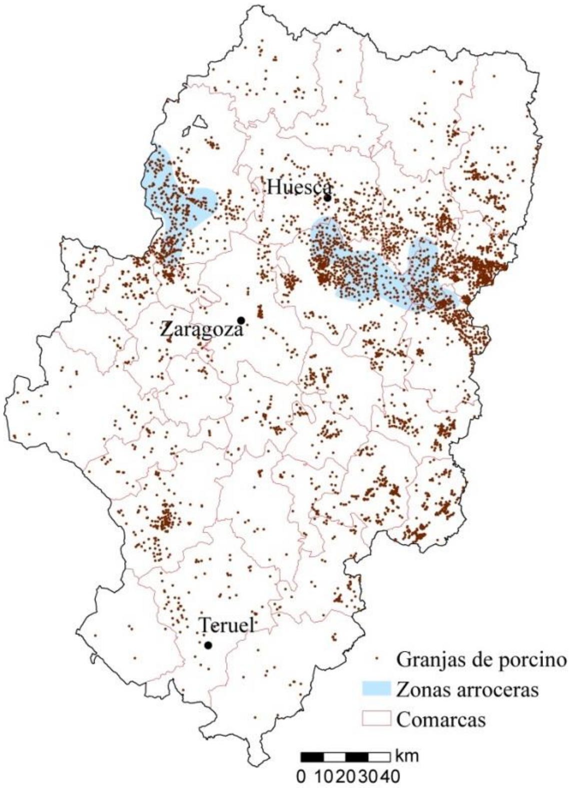

Figura 1.2. Granjas de porcino en la Comunidad Autónoma de Aragón y delimitación de las zonas arroceras en el año 2015. Fuente: datos PAC y Censos Ganaderos (2015) suministrados por el Departamento de Desarrollo Rural y Sostenibilidad del Gobierno de Aragón.

1.2. EL CULTIVO DEL ARROZ

El arroz es uno de los cultivos más importantes en el mundo y constituye el alimento básico para casi la mitad de la población mundial (Mohanty, 2013). La superficie total cultivada en 2014 fue de aproximadamente 163 millones de hectáreas, con una producción de 740 millones de toneladas, localizándose casi un 90 % de esta producción en Asia. En Europa, el arroz se cultiva principalmente en países mediterráneos, con una producción total de 4.7 millones de toneladas. En España, en el año 2014 se cultivaron 110400 ha con una producción de 860000 t, representando un 18 % de la producción total europea (FAOSTAT, 2014). La zona del nordeste español (Aragón y Cataluña) concentra el 25 % de la superficie cultivada de arroz del país (MAGRAMA, 2015b).

En Aragón, el arroz se cultiva principalmente en las comarcas de Los Monegros, Cinca Medio (Figura 1.3) y Bajo Cinca en Huesca y en la comarca de Las Cinco Villas en Zaragoza (Figura 1.2). La mayor parte de las parcelas donde se cultiva se localizan en suelos salino-sódicos de baja permeabilidad donde el cultivo de arroz inundado es la única opción (Blanco, 2014). Además, es habitual realizar la técnica del fangueo, que consiste en labrar el terreno inundado con un tractor de ruedas de hierro, de manera que la paja y los restos de la cosecha anterior son incorporados al suelo. Mediante esta técnica, se compacta el suelo y se crea la denominada suela de fangueo, impermeabilizando el suelo y reduciendo así las pérdidas por percolación profunda. La impermeabilidad de esta capa aumenta a medida que la parcela ha sido cultivada con arroz y se ha realizado fangueo durante años consecutivos (Janssen and Lennartz, 2007).

Figura 1.3. Parcelas de arroz junto a granja de porcino de cebo en la comarca del Cinca Medio.

1.3. CONDICIONES DEL SUELO EN ARROZALES INUNDADOS

El 95 % de la producción mundial de arroz tiene lugar en suelos inundados durante al menos parte de la campaña del cultivo (Buresh et al., 2008). La inundación del suelo determina claramente los procesos químicos y microbiológicos que van a tener lugar en el suelo, así como la nutrición de la planta.

1.3.1. Cambios en el perfil de suelo

En el momento en que el arrozal se inunda, el aporte de oxígeno (O_2) al suelo se reduce drásticamente debido a la baja difusividad en agua en comparación con el aire. Como consecuencia de la disminución del intercambio gaseoso entre aire y suelo, el aporte de oxígeno no puede cubrir la demanda de los organismos aerobios; por lo tanto, estos organismos son desplazados por organismos facultativos y anaerobios, que usan compuestos oxidados como aceptores de electrones en el proceso de respiración (Buresh et al., 2008).

En el perfil del suelo, se van a diferenciar claramente dos zonas: una fina capa aerobia en la zona superficial del suelo y en la zona alrededor de las raíces y otra capa anaerobia por debajo (Figura 1.4) (Buresh et al., 2008; Tinarelli, 1989).

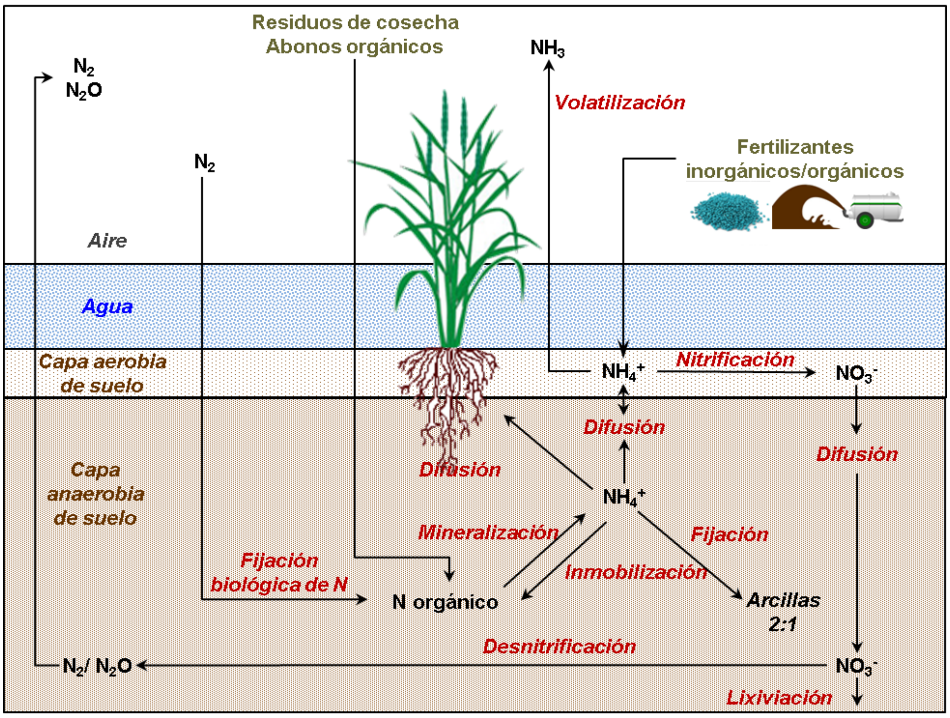

Figura 1.4. Esquema de las transformaciones de N en un suelo inundado (adaptado de Buresh et al., 2008).

Las plantas adaptadas a suelos inundados, como el arroz, han desarrollados mecanismos que permiten el transporte del O_2 desde las partes aéreas de la planta hacia la zona de las raíces (Tinarelli, 1989).

1.3.2. Cambios en la dinámica del N

El nitrógeno disponible para el cultivo puede provenir de los fertilizantes inorgánicos y orgánicos aplicados al suelo, de la materia orgánica, del N mineral presente en el suelo y del N atmosférico fijado por los microorganismos (Figura 1.4). La inundación de un suelo modifica los procesos de transformación de N, así como la proporción de las distintas formas de N (Figura 1.4). Mientras que el nitrato (NO_3^-) es la forma de N inorgánico dominante en suelos aireados, el amonio (NH_4^+) es la forma más estable en suelos sumergidos, ya que la nitrificación está inhibida en estos suelos. La planta de arroz está adaptada a vivir en condiciones reductoras en las que la forma de N inorgánico es el NH_4^+. Como ya se ha explicado anteriormente, el O_2 es transportado desde las partes aéreas de la planta hasta las raíces; es allí en torno a los ápices radiculares donde se forma una zona de oxígeno que oxida diferentes compuestos, entre ellos el amonio. Mediante este proceso en el que se produce la nitrificación del amonio, la planta de arroz puede vivir en condiciones reductoras (Tinarelli, 1989).

En la capa aerobia de un suelo sumergido, se puede producir la transformación de NH_4^+ a NO_3^-. Este nitrato formado se puede desplazar a la zona anaerobia y ser desnitrificado junto con el nitrato ya presente antes de la inundación (Figura 1.4; Buresh et al., 2008).

La descomposición de la materia orgánica también se va a ver fuertemente influenciada por las condiciones creadas durante la inundación. La descomposición de la materia orgánica en un suelo inundado difiere con respecto a un suelo aireado en dos aspectos: es más lenta, y los productos finales son diferentes. Mientras que en un suelo aireado, los principales productos finales son dióxido de carbono (CO_2), NO_3^-, sulfato (SO_4^-) y residuos resistentes como humus; en suelos inundados, los principales productos son CO_2, hidrogeno (H_2), metano (CH_4), NH_4^+, aminas, mercaptanos, ácido sulfhídrico (H_2S) y residuos parcialmente humificados (Ponnamperuma, 1972).

1.4. EMISIONES DE GASES DE EFECTO INVERNADERO EN ARROZALES

A nivel global, el sector agrícola es el principal productor de emisiones antropogénicas de metano (CH_4) y óxido nitroso (N_2O) (Ciais et al., 2013), contribuyendo aproximadamente a un 10-12 % de las emisiones totales de gases de efecto invernadero (Smith et al., 2014). En España, en 2014, la contribución de este sector a las emisiones de gases de efecto invernadero (GEIs) fue de un 11 % (MAGRAMA, 2016).

Los campos de arroz, debido a las condiciones de inundación, contribuyen de manera significativa al calentamiento global. En concreto, el 11 % de las emisiones antropogénicas de CH_4 a nivel mundial son debidas al cultivo del arroz (Ciais et al., 2013).

En los arrozales inundados se emiten N_2O y CH_4 dependiendo fundamentalmente de los periodos de inundación-seca. Cuando el terreno está inundado, debido a las condiciones reductoras, puede tener lugar el proceso de desnitrificación mediante el cual los microorganismos desnitrificadores usan el NO_3^- como aceptor de electrones reduciéndolo a óxidos de nitrógeno o N_2 (Figura 1.4) durante el proceso de respiración. Por otro lado, si el NO_3^- no está presente, los óxidos de nitrógeno como nitrito (NO_2^-) u óxido nitroso (N_2O) pueden servir como aceptores de electrones (Coyne, 2008). A pesar de que el N_2O es un potente gas de efecto invernadero (265 veces más potente que el CO_2) (Myhre et al., 2013), su contribución al calentamiento global durante el periodo de inundación no es importante, puesto que en condiciones anaerobias estrictas, la desnitrificación se completa a N_2 (Cameron et al., 2013; Coyne, 2008). Por el contario, durante los periodos de inundación, el CH_4 contribuye de manera significativa al calentamiento global, ya que se produce mediante el proceso de metanogénesis en el que tiene lugar la fermentación anaerobia de la materia orgánica (Ponnamperuma, 1972). En los periodos de seca, la situación pasa a ser la contraria; se producen picos de emisión de óxido nitroso asociados a los procesos de nitrificación y desnitrificación; sin embargo, las emisiones de metano disminuyen drásticamente (Lagomarsino et al., 2016). De manera global, las emisiones de metano son responsables de casi el 90 % del potencial de calentamiento global (Global Warming Potential, GWP) (Linquist et al., 2012b) de los campos de arroz inundados. A pesar de ello, es necesario el estudio de ambos gases de manera conjunta, puesto que las prácticas encaminadas a mitigar uno de los dos gases, aumentan las emisiones de otro y viceversa.

Las emisiones de GEIs de los campos de arroz se pueden ver afectadas por la fertilización (Linquist et al., 2012a). Cuando la fertilización mineral se sustituye por fertilización orgánica, los procesos de desnitrificación y metanogénesis se pueden ver favorecidos por el aporte adicional de carbono (C). Algunos estudios han demostrado que la aplicación de paja como enmienda orgánica en el cultivo de arroz inundado produce un aumento de las emisiones de metano (Bronson et al., 1997; Schütz et al., 1989) debido al aporte de C; sin embargo, en el caso de las emisiones de N_2O parece que el aporte de C puede promover el paso final en la desnitrificación, disminuyendo las emisiones de N_2O y desplazando la reacción hacia la formación de N_2 (Bronson et al., 1997; Wang et al., 2011). Puesto que las distintas enmiendas orgánicas tienen diferentes composiciones en cuanto a concentración de C, el efecto observado dependerá en gran medida del producto aplicado.

1.5. USO DE INFORMACIÓN ESPECTRAL COMO AYUDA AL MANEJO DE LA FERTILIZACIÓN

Uno de los problemas al que nos enfrentamos a la hora de establecer las dosis de N adecuadas para cada cultivo es que éstas dependen del rendimiento potencial y éste a su vez está influenciado en gran medida por las condiciones particulares de cada año. Como consecuencia, se tiende a sobrefertilizar con N con el objetivo de asegurar que se cubran las necesidades de N del cultivo sin que el rendimiento final se vea comprometido. El uso de información espectral obtenida mediante imágenes aéreas, de satélite o dispositivos como medidores de clorofila, Greenseker o radiómetros puede servir como herramienta para detectar el estado nutricional del cultivo y por lo tanto, como ayuda a la decisión de la fertilización durante la campaña, evitando así los problemas asociados a dosis excesivas de N. Con la información espectral proporcionada en las diferentes bandas del espectro, se pueden calcular índices de vegetación, los cuales han sido relacionados con parámetros agronómicos como el rendimiento (Gilabert y Meliá, 1990; Maresma et al., 2016), el índice de área foliar (Leaf Area Index, LAI) (Li et al., 2015; Haboudane et al., 2004) o la biomasa (Cao et al., 2013; Gnyp et al., 2014), tanto en arroz, como en otros cereales.

Para el cultivo de arroz, algunos autores han obtenido modelos que permiten el cálculo de las necesidades de N en cobertera a partir de estas relaciones (Xue et al., 2014; Xue and Yang, 2008; Yao et al., 2012), habiendo sido desarrollados fundamentalmente en Asia. En condiciones mediterráneas, destacan los trabajos de Gilabert y Meliá (1990) y Casanova et al. (1998) y recientemente a través del proyecto ERMES, investigadores de España, Grecia e Italia están haciendo un esfuerzo importante para generar herramientas de soporte agronómico para los agricultores utilizando información espectral, estimando variables como índice de área foliar (LAI) o contenido de N de las hojas (Campos-Taberner et al., 2016; Confalonieri et al., 2015). En nuestras condiciones, se necesita generar más información que lleve al desarrollo de nuevas aproximaciones que mejoren las recomendaciones de N en cobertera, en especial a nivel regional, con el uso de imágenes aéreas de alta resolución que cubran grandes áreas y a través de un sistema económicamente viable para pequeños agricultores y también para grandes explotaciones.

1.6. FERTILIZACIÓN DEL ARROZ CON PURÍN PORCINO

Existen trabajos en zonas mediterráneas que han evaluado la aplicación de purín porcino a cultivos como el maíz (Berenguer et al., 2008; Yagüe and Quílez, 2010a) o los cereales de invierno (Yagüe y Quílez, 2010b; Bosch-Serra et al., 2015), obteniéndose los mismos rendimientos que cuando se aplican fertilizantes minerales. Sin embargo, como se ha explicado anteriormente, las condiciones del cultivo del arroz son muy diferentes a las de otros cultivos. Por ejemplo, cuando el purín se aplica al maíz en primavera, los procesos de mineralización del N orgánico y nitrificación del amonio están favorecidos, quedando disponible N mineral para el cultivo, pero al mismo tiempo, también existe el riesgo de lavado de nitrato en el caso de que se produzcan fuertes lluvias. Por ello, la práctica más recomendada es aplicar dosis de N bajas en presiembra y complementar en cobertera (Berenguer et al., 2008; Yagüe y Quílez, 2010a). En el caso del cultivo del arroz, las parcelas se inundan tras la aplicación del fertilizante, que se aplica en forma amoniacal. Debido a las condiciones reductoras, el proceso de nitrificación no tiene lugar, por tanto, se evitan en gran medida las pérdidas asociadas a los procesos de nitrificación-lixiviación. Por otro lado, la mineralización del N orgánico es más lenta (Aulakh et al., 2000). Por lo tanto, la aplicación de purín porcino al arroz inundado se debe estudiar de manera independiente a otros cultivos.

Numerosos trabajos han estudiado la fertilización nitrogenada en arrozales inundados, la mayoría de ellos en Asia, donde las prácticas agronómicas y la climatología son diferentes a las de España; además, muchos de ellos se han centrado en la aplicación de fertilizantes minerales (Haefele et al., 2008; Sun et al., 2012). Existen estudios que han evaluado el valor fertilizante de productos orgánicos como la gallinaza (Gu et al., 2009; Myint et al., 2010), estiércol de vaca (Myint et al., 2010) o estiércol de porcino sólido (Pan et al., 2009). Sin embargo, estos productos presentan composiciones diferentes al purín porcino, cuya concentración en Carbono (C) es baja y en el que la mayor parte del N se encuentra en forma amoniacal, directamente disponible para el cultivo. Sí que existen trabajos sobre la aplicación de purín de cerdo digerido mediante un proceso anaerobio (Anaeobically Digested Pig Slurry, ADPS). En estos estudios, se han obtenido rendimientos iguales o mayores en parcelas tratadas con ADPS en comparación con un tratamiento mineral con la misma dosis de N (Lu et al., 2012; Chen et al., 2013), pero no se ha evaluado la eficiencia de uso del N contenido en este producto. Para avanzar en la mejora de la fertilización del arroz inundado con purín porcino, es necesario generar conocimiento sobre la dinámica del N del purín y la disponibilidad del N en este sistema de cultivo y hacer una evaluación de la eficiencia de uso del N en distintas estrategias de fertilización.

Por otro lado, existen numerosos estudios que han evaluado el efecto de enmiendas orgánicas sobre las emisiones de gases de efecto invernadero en sistemas de arroz inundado, la mayoría focalizados en el efecto de la incorporación de la paja sobre las emisiones de metano (Sanchis et al., 2012); sin embargo, la composición del purín porcino es muy diferente a la de la paja y por tanto, los resultados obtenidos para este producto no pueden ser extrapolados al purín porcino. Algunos estudios han evaluado el efecto de la aplicación de ADPS, muy parecido en composición al purín porcino, comparativamente con la fertilización mineral en las emisiones de GEIs de arrozales inundados de Asia, obteniendo resultados contradictorios. Huang et al. (2014) encontró emisiones de metano significativamente más altas en los tratamientos en los que se aplicó ADPS en comparación con la fertilización mineral; sin embargo, en los estudios de Sasada et al. (2011) y Win et al. (2014), la aplicación de ADPS no produjo un incremento significativo de las emisiones de GEIs, aunque en el estudio de Win et al.

(2014) las emisiones de CH_4 acumuladas fueron más altas (no significativo) en comparación con el tratamiento de la misma dosis de fertilizante mineral. El único estudio que ha evaluado, en una estación de cultivo, las emisiones de GEIs en la fertilización con purín porcino en condiciones mediterráneas (Maris et al., 2016), ha concluido que la aplicación de purín porcino a dosis agronómicas no produce un aumento del potencial de calentamiento global con respecto al tratamiento con la misma dosis de N mineral. El trabajo de Maris et al. (2016), se realizó sobre un único tipo de suelo y manejo y en esta tesis se pretende avanzar en los resultados de ese trabajo evaluando otros tipos de suelo-manejo.

En cuanto al uso de información espectral como herramienta de ayuda al ajuste de la fertilización nitrogenada, un aspecto que no se ha encontrado en ninguno de los trabajos revisados es la respuesta espectral comparativa en parcelas fertilizadas con fertilizantes minerales y productos orgánicos (como el purín porcino). Hay antecedentes de diferencias en la respuesta espectral entre la fertilización orgánica y la mineral en otros cultivos, como maíz (Yang et al. 2002) o trigo (Zhao et al., 2015). La evaluación de la respuesta espectral en la fertilización con purín porcino es esencial para desarrollar estrategias óptimas de ayuda a la fertilización que incluyan a este producto.

Por tanto, para que el purín sea integrado de forma óptima en los planes de fertilización del arroz en la zona del nordeste español, es necesario llevar a cabo un estudio global en el que se evalúe su valor fertilizante, así como otros aspectos que se puedan ver afectados por su aplicación, como la presencia de malas hierbas, plagas y enfermedades, la calidad del grano o las emisiones de gases de efecto invernadero. Por otro lado, es importante estudiar la posibilidad del uso de información espectral como herramienta de ayuda a la toma de decisiones dentro de estos planes de fertilización, tanto para la fertilización con purín porcino como para la fertilización mineral.

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CAPÍTULO 2

OBJETIVOS Y ESTRUCTURA DE LA TESIS

CAPÍTULO 2. OBJETIVOS Y ESTRUCTURA DE LA TESIS

2.1. OBJETIVOS DE LA TESIS

El objetivo principal de la presente tesis doctoral es evaluar distintas estrategias de aplicación de purín porcino en fondo en un cultivo de arroz inundado en el nordeste español. Se pretende evaluar la mejor estrategia para la sustitución de los fertilizantes minerales por purín porcino consiguiendo una producción óptima técnica y económica sin un aumento del impacto ambiental.

Para la consecución de este objetivo se han llevado a cabo dos ensayos experimentales entre los años 2011-2014 en las localidades de Villanueva de Sigena y Grañén en la provincia de Huesca.

Los objetivos específicos planteados son los siguientes:

1. Evaluar dos estrategias de fertilización con purín porcino en fondo en comparación con la aplicación de fertilizante mineral, analizando la respuesta agronómica, la eficiencia del uso del N y la calidad del grano.
2. Estudiar el efecto de la fertilización con purín porcino en comparación con la fertilización mineral sobre las emisiones de gases de efecto invernadero en dos tipos de suelo con distintas condiciones de manejo.
3. Evaluar la utilidad del uso de imágenes multiespectrales para estimar las necesidades de N del cultivo en cobertera, y desarrollar modelos de recomendación de N en cobertera, incluyendo una evaluación de su viabilidad económica y sus externalidades.

2.2. ESTRUCTURA DE LA TESIS

La tesis se estructura en 6 capítulos:

- **Capítulo 1. Introducción general.**

En este capítulo se presenta la problemática de la generación de purín porcino en España y Aragón y la necesidad de extender su aplicación a cultivos como el arroz. Se describen brevemente las condiciones de cultivo del arroz inundado y se hace una revisión bibliográfica de los estudios que hay en el contexto internacional y en condiciones mediterráneas sobre la fertilización con purín porcino de este cultivo.

- **Capítulo 2. Objetivos y estructura de la tesis.**

  Se plantea el objetivo principal y los objetivos específicos de la tesis.

- **Capítulo 3. Response of paddy rice to fertilization with pig slurry in northeast Spain: strategies to optimize nitrogen use efficiency.**

En este capítulo se evalúan 3 estrategias de fertilización: una dosis de purín porcino en fondo que cubre las necesidades del cultivo (170 kg N-$NH_4^+ \cdot ha^{-1}$), una dosis de purín porcino en fondo inferior a las necesidades del cultivo (120 kg N-$NH_4^+ \cdot ha^{-1}$) y una dosis equivalente a esta última aplicada con fertilizante mineral; todas ellas complementadas con N mineral en cobertera. Se hace una valoración de la respuesta agronómica del cultivo, las eficiencias del uso del N y se realiza un balance de N. Además se estudian otros aspectos como la presencia de malas hierbas, plagas y enfermedades y la calidad del grano.

El contenido de este capítulo ha sido publicado en la revista Field Crops Research:

- Moreno-García, B., Guillén, M., Quílez, D. Response of paddy rice to fertilisation with pig slurry in northeast Spain: Strategies to optimise nitrogen use efficiency. Field Crops Research, 208: 44-54. http://www.sciencedirect.com/science/article/pii/S0378429017301788

- **Capítulo 4. Greenhouse gas emissions as affected by fertilization type (pig slurry vs mineral) and soil management in flooded rice systems in northeast Spain.**

En este capítulo se cuantifican las emisiones de metano, óxido nitroso y dióxido de carbono bajo diferentes estrategias de fertilización con purín porcino en comparación con la fertilización mineral en dos ensayos con diferentes tipos de suelo y manejo de la parcela. Se cuantifican las emisiones de gases de efecto invernadero (GEIs), se calcula el potencial de calentamiento global (GWP) en valor absoluto y relativizado por el rendimiento (GWP_Y); y en el caso del óxido nitroso, se calcula el factor de emisión inducido por la aplicación de fertilizante.

La información de emisión de GEIs en el año 2012 en la parcela de Villanueva de Sigena, no se ha incluido en esta memoria de tesis por haber sido incluida comparativamente con la de un ensayo similar fertilizado con gallinaza en el delta del Ebro en la memoria de tesis de Stefania Maris (Universidad de Lleida, Diciembre de 2015). Esta información se encuentra publicada en:

- Maris, S.C., Teira-Esmatges, M.R., Bosch-Serra, A.D., Moreno-García, B., Català, M.M., 2016. Effect of fertilising with pig slurry and chicken manure on GHG emissions from Mediterranean paddies. Science of the Total Environment, 569-570: 306-320.

- **Capítulo 5. Multispectral information for topdressing N recommendation in rice under two fertilization strategies, organic and mineral, in mediterranean conditions.**

En este capítulo se evalúa la utilidad del uso de imágenes multiespectrales para estimar las necesidades de aplicación de N al cultivo de arroz en cobertera. Para ello, se establecen relaciones entre diferentes índices de vegetación y rendimiento, estudiando posibles diferencias entre parcelas fertilizadas con purín porcino y con fertilizante mineral en estas relaciones. Por otro lado, se construyen 2 herramientas para la predicción de la fertilización en cobertera basadas en la información anterior y se analizan distintos escenarios de aplicación de N con la información proporcionada por las herramientas, que se evalúan económica y ambientalmente (exceso de N).

- **Capítulo 6. Discusión general y conclusiones**

En este capítulo se discuten los resultados obtenidos en los 3 capítulos anteriores de manera conjunta, se establecen conclusiones y algunas recomendaciones para trabajos futuros.

Cada capítulo tiene un apartado independiente de referencias bibliográficas.

CAPÍTULO 3

RESPONSE OF PADDY RICE TO FERTILIZATION WITH PIG SLURRY IN NORTHEAST SPAIN: STRATEGIES TO OPTIMIZE NITROGEN USE EFFICIENCY

Moreno-García, B., Guillén, M., Quílez, D. Response of paddy rice to fertilisation with pig slurry in northeast Spain: Strategies to optimise nitrogen use efficiency. Field Crops Research, 208: 44-54.

CAPÍTULO 3. RESPONSE OF PADDY RICE TO FERTILIZATION WITH PIG SLURRY IN NORTHEAST SPAIN: STRATEGIES TO OPTIMIZE NITROGEN USE EFFICIENCY

3.1. INTRODUCTION

Spain is the largest pig producer in the European Union (about 28 million heads), with 19% of the total production (EUROSTAT, 2015) and a production of approximately 50 million tons of pig slurry (PS) per year. More than half (51%) of the country’s pig production is concentrated in northeast Spain (Aragon and Catalonia regions, MAGRAMA, 2015a). Traditionally, PS has been applied to field crops, at very high rates in many cases, which may have resulted in negative environmental impacts on the soil, water and atmosphere. Pig slurry has been applied traditionally to winter cereals (barley, wheat) and maize crops. With a growing pig population, pig slurry fertilization should extend to alternative crops such as alfalfa (Salmerón et al., 2010) or rice to recycle more efficiently the increasing amounts of nutrients produced in the farms.

Rice is one of the most important crops in the world. The total cultivated area is about 163 million ha with 88% of this surface in Asia. In Europe, rice is mostly cultivated in Mediterranean countries, with a total harvested area of approximately 642000 ha, and 17% of this area is cultivated in Spain (FAOSTAT, 2014). The rice extension in northeast Spain represents 25% (MAGRAMA, 2015b) of the crop surface in Spain. In order to improve the recycling of nutrients in agriculture, the integration of PS in the N fertilization schedule for this crop is a necessity.

Poor N fertilizer use efficiency is characteristic of paddy rice systems. Low N efficiency is attributed to rapid losses of applied N due to ammonia volatilization, denitrification (Cassman et al., 1998) and nitrate leaching. The particular conditions experienced by a rice crop, i.e., flooding interspersed with periods of drainage, contribute to these losses, especially to gaseous losses due to denitrification (Cai et al., 1997; Wang et al., 2011; Zheng et al., 2000; Zou et al., 2005) and leaching, that can reach 60-70% of the applied N (Tinarelli, 1989).

Different studies in different parts of the world have evaluated the rice response to mineral fertilizers (Xie et al., 2007; Biloni and Bocchi, 2003) or to organic fertilizers such as cow and poultry manure (Myint et al., 2010a, 2010b), anaerobically digested cattle manure, (Nishikawa et al., 2012), anaerobically digested pig slurry (Lu et al., 2012; Zhang et al., 2015), pig manure, (Pan et al., 2009), farmyard manure (Gu et al., 2009) or dairy manure (Xue et al., 2014b). However, most of these studies have not evaluated the fertilizer value of N contained in these organic products for rice, and in some of these studies, even the nutrient composition of the organic fertilizer is unknown. Studies of PS application to rice have focused on gaseous emissions (Maris et al., 2016) or soil properties (Goyal et al., 2006), but studies focus on the fertilizer value of pig slurry N have not been found in the literature and such information is essential for integrating slurry management into the N fertilization schedule of this crop.

In pig saturated areas, a premise is to maximize the amount of PS that can be applied to the crops without adverse environmental effects. In other summer crops, such as maize, presowing application of PS at low rates, complemented with mineral N at side-dressing, is the most efficient strategy for PS fertilization to avoid decreased N efficiencies, N losses to the environment while adhering to EU regulations (Berenguer, 2008; Yagüe and Quílez, 2010). We hypothesize that due to flooding conditions, rice can be fertilized with a single PS application (up to 100% of crop N requirement) before seeding with lower N losses than in other summer crops. In addition, we hypothesize that organic-N is unlikely to be used by the crop during the year of application as anaerobic conditions delay the PS organic-N mineralization process (Aulakh et al., 2000; Ethan, 2015; Olk et al., 2007).

Therefore, the aim of this study was to investigate, in rice flooding irrigation systems in northeast Spain, three fertilization strategies: a PS rate that would cover total crop N requirements (170 kg $N\text{-}NH_4{\cdot}ha^{-1}$), a PS rate with ammonium N content below the N requirements for rice (120 kg $N\text{-}NH_4{\cdot}ha^{-1}$), and a mineral rate of 120 kg $N{\cdot}ha^{-1}$ applied as a mineral fertilizer (M120). The three strategies were complemented with mineral N at topdressing. We evaluated and compared crop response, N use efficiency, rice quality and N budget.

The experimental treatments were established considering the nitrogen recommendation for rice in the area of approximately 170 kg $N{\cdot}ha^{-1}$ (for a potential yield of 7-8 $Mg{\cdot}ha^{-1}$) (Pérez, 2006) and PS rates were established based on ammonium-N content of the PS.

3.2. MATERIALS AND METHODS

3.2.1. Field experiment and design

A field experiment was conducted in Villanueva de Sigena (Huesca) in northeast Spain (41º45’31.87”N, 0º2’18.16”W) (Figure 3.1) on a silty loam textured soil (the soil physicochemical characteristics are in Table 3.1) during three consecutive years (April 2011 to October 2013).

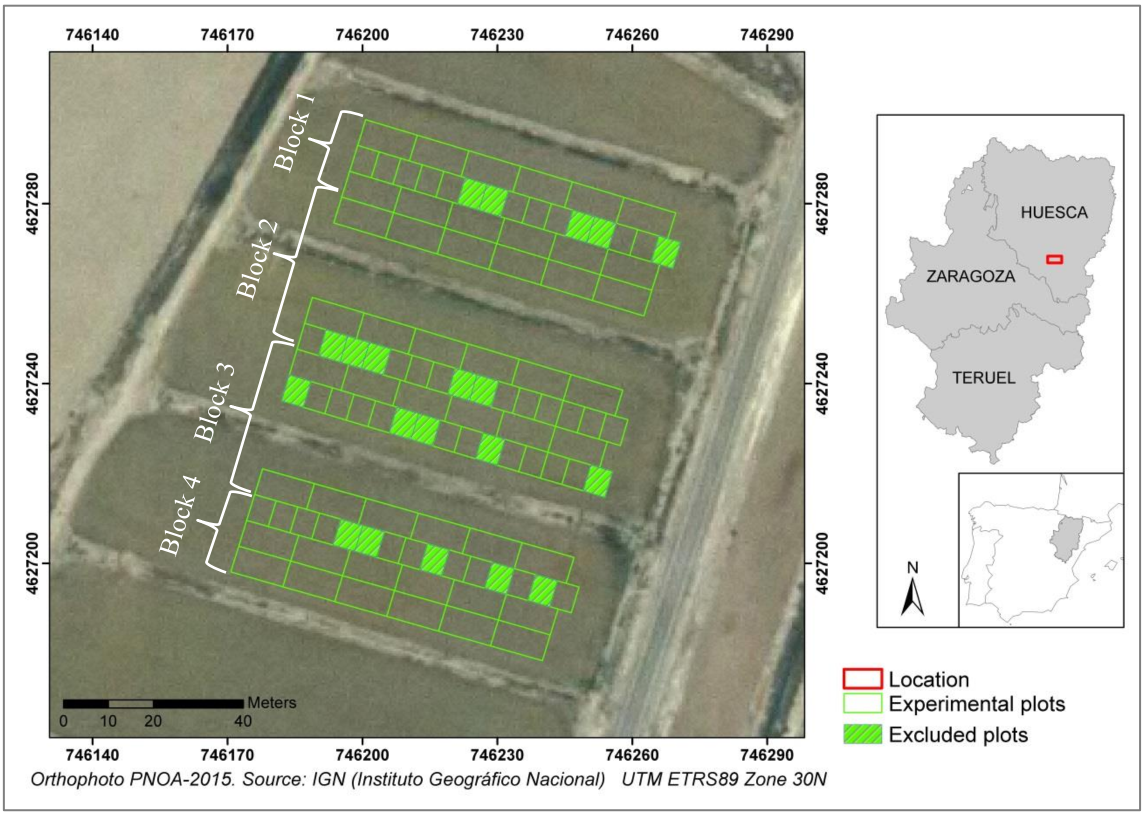

Figure 3.1. Location and experimental design of the field at Villanueva de Sigena.

The climate of the region is semiarid continental Mediterranean, with high temperatures during the summer and low precipitation (15.0ºC annual average temperature and 349 mm annual precipitation, average period 1980-2010). Figure 3.2 shows the average temperatures, rainfall and solar radiation for the three experimental years. The field had been cultivated with rice in 2010 and with barley in previous years.

Table 3.1. Characteristics of the soil at the different depths.

Depth	**pH 1:2.5**	**Ec$_e$**	**CaCO$_3$**	**Organic matter**	**Sand**	**Silt**	**Clay**	**N Kjeldahl**	**P Olsen**	**K (NH$_4$Ac)**
m	*(-)*	*dS·m^{-1}*	*g·kg^{-1} dry soil*					*mg·kg^{-1} dry soil*		
0-0.3	8.5	0.77	290	10.1	134	662	204	800	6	81
0.3-0.6	8.7	0.55	290	4.2	215	627	158	500	2	30
0.6-0.9	8.7	0.59	310	4.2	123	675	202	500	1	15
0.9-1.20	8.4	1.67	290	4.3	67	733	200	600	1	18

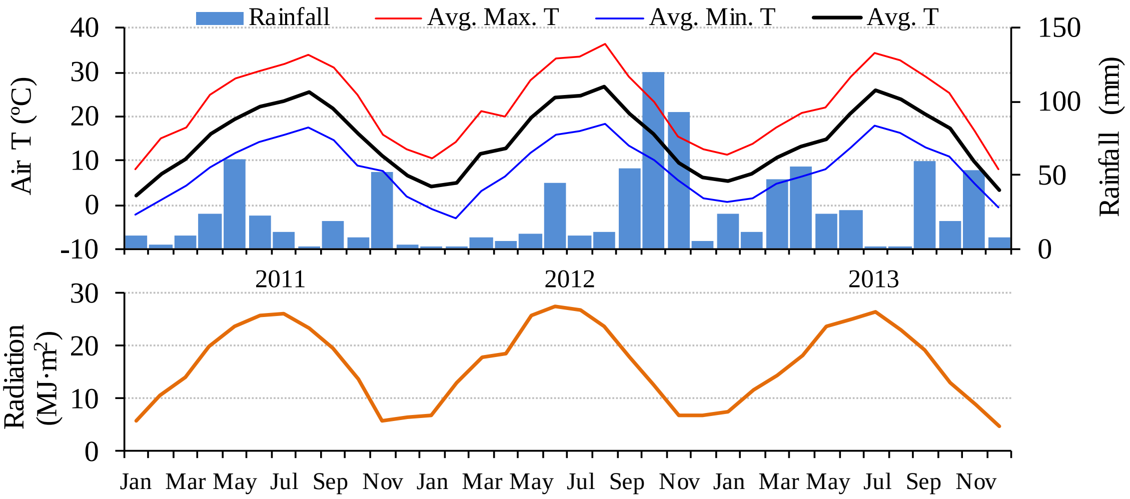

Figure 3.2. Monthly average, average maximum and minimum temperatures (ºC), rainfall (mm) and solar radiation (MJ·m^{-2}) during the three experimental year (2011-2013).

The fertilization strategies were two rates of PS equivalent to 170 kg NH_4^+-N·ha^{-1} (PS170 strategy) and 120 kg NH_4^+-N·ha^{-1} (PS120 strategy) that were compared to a rate of 120 kg NH_4^+-N·ha^{-1} of mineral fertilizer (M120), all three applied before seeding. Theoretically, PS170 rate would cover the whole rice N requirements, whilst PS120 and M120 would need topdressing N in order to achieve maximum yields. PS120 will allow identifying if N provided by mineralization of PS organic-N is used by the crop during the year of application. These strategies of fertilization before seeding were combined with six N rates at topdressing, to obtain the yield response to topdressing N and establish the N rate necessary to reach optimum yields in each strategy. The topdressing mineral N rates (kg N·ha^{-1}) were: 0 (M0), 30 (M30), 60 (M60), 90 (M90), 120 (M120) and 150 (M150) (Table 3.2). The experimental design was a split plot with four replications (Figure 3.1). Additional mineral treatments (Table 3.2) were included to obtain the nitrogen fertilizer replacement value (NFRV) of the two PS strategies. The additional treatments were a control treatment without N application (M0) and 5 rates of mineral N before seeding: 30 kg N·ha^{-1} (M30), 60 kg N·ha^{-1} (M60), 90 kg N·ha^{-1} (M90), 120 kg N·ha^{-1} (M120) and 150 kg N·ha^{-1} (M150). The mineral fertilizer was ammonium sulfate (in 2011 ammonium nitrate was applied by error before seeding). Additional treatments with no N fertilization before seeding and with different N rates at topdressing were included in the experimental design (excluded plots in Figure 3.1); however they were removed from all analyses in the thesis. When an insufficient N rate is applied before seeding, yield potential is significantly reduced with no possibility of recovery with N application at topdressing (Wilson et al., 2001).

Table 3.2. Amount (kg N·ha^{-1}) and timing (BS: Before seeding, TP: topdressing) of the N applied in the different treatments. For PS treatments, amount indicates target N rates.

Pig slurry treatments (PS)			**Mineral treatments (M)**		
	BS	TP		BS	TP
	kg NH_4-N·ha^{-1}	*kg N·ha^{-1}*		*kg N·ha^{-1}*	
PS120M0	120	--	**M120M0**	120	--
PS120M30	120	30	**M120M30**	120	30
PS120M60	120	60	**M120M60**	120	60
PS120M90	120	90	**M120M90**	120	90
PS120M120	120	120	**M120M120**	120	120
PS120M150	120	150			
PS170M0	170	--	**Control (M0)**	--	--
PS170M30	170	30	**M30**	30	--
PS170M60	170	60	**M60**	60	--
PS170M90	170	90	**M90**	90	--
PS170M120	170	120	**M120=M120M0**	120	--
PS170M150	170	150	**M150**	150	--

The experimental plots were 6 m wide by 12 m long for the PS treatments (6 subplots per main plot) and 6 m wide by 5 m long for the mineral treatments (10 subplots) (Figure 3.1; Figure 3.3). The treatments were randomized in the first year (2011) and then repeated in the same plots in the next two years.

Figure 3.3. Villanueva de Sigena field experiment at the tillering phase (21 June 2011).

3.2.2. Agricultural practices

Typical land preparation (two passes of disk plough and two passes of rotavator) was conducted by the farmer in April-May every year before fertilization, seeding and flooding. Puddling was not conducted.

Pig slurry was collected from a fattening farm near the experimental field. The application rates were established according to the ammonium N concentration of the PS, which was measured *in situ* using Quantofix® N-volumeter (Piccinini and Bortone, 1999) and conductimetry (Yagüe and Quílez, 2012) (Figure 3.4a) and slurry samples were collected for further analysis in the laboratory.

Figure 3.4. Analysis of PS ammonium N concentration by Quantofix® and conductimetry (a) and PS application in the experimental plots (b).

The ammonium and total N content in PS ranged between 3 and 3.8 kg N-NH_4·Mg^{-1} and 4.2 and 6.3 kg N·Mg^{-1}, respectively and phosphorus (P) and potassium (K) ranged between 0.7 and 1.8 kg P·Mg^{-1} and 3.1 and 4.1 kg K·Mg^{-1} (Table 3.3).

Pig slurry was band spread on 16 May 2011, 15 May 2012 and 9 May 2013 (Figure 3.4b). On the same days, the basal mineral N fertilizer (ammonium sulfate) was applied to the plots of the mineral treatments at the corresponding rates together with P (100 kg P_2O_5·ha^{-1}) and K (100 kg K_2O·ha^{-1}) to avoid a P or K deficiency. Slurry and mineral fertilizers were then incorporated into the soil with a rotavator in the afternoon of the same day.

Table 3.3. Physico-chemical characteristics of the PS applied each year.

	2011	**2012**	**2013**
Specific weight ***(g·l^{-1})***	1025	1020	1045
Dry matter ***(kg ·Mg^{-1})***	77	37.4	94
Ammonium N ***(kg ·Mg^{-1})***	3.83	3.00	3.05
Total N ***(kg ·Mg^{-1})***	6.27	4.24	5.63
P ***(kg ·Mg^{-1})***	1.40	0.71	1.79
K ***(kg ·Mg^{-1})***	3.50	4.08	3.07

Previous to the PS application, the machinery was calibrated to apply the target N rates; however, applying the proper amounts of PS to reach target rates was difficult as pointed out by Daudén and Quílez (2004). For this reason, in each application, the slurry tank was weighed before and after application to know the actual PS rates (30 to 36 Mg·ha^{-1} for PS120 and 49 to 54 Mg·ha $^{-1}$ for PS170) and the actual N rates (Table 3.4).

Rice (*Oryza sativa* L. spp *Japónica* cv. Guadiamar) was broadcast seeded on 17 May 2011, 16 May 2012 and 15 May 2013 at a seed rate of 180 kg·ha^{-1} and the field was immediately flooded, except in 2013, when flooding preceded rice seeding. Water was continuously flowing in and out of the plot. A water layer of 5 cm was maintained during the first days to improve rice germination, after that, a water layer of 10 cm was maintained until approximately one month before harvest, when field was drained. Moreover, the field was drained for the application of herbicides, pesticides and fungicides, according to habitual practices in the area (twice during the growing season- aprox. between 4-8 days the water inlet was closed). Topdressing N was applied on the water at the end of the tillering stage on 29 June 2011, 4 July 2012 and 29 July 2013 as ammonium sulfate.

Table 3.4. Ammonium and total N applied before seeding and as N topdressing, yield, AE_N (agronomic efficiency) and RE_N (recovery efficiency) calculated considering ammonium nitrogen ($AE_{NH4\text{-}N}$ and $RE_{NH4\text{-}N}$) or total N (AE_{NT} and RE_{NT}), and plant N uptake for the three experimental years and unaccounted N considering PS ammonium-N or total-N for the whole studied period.

	Before seeding NH_4-N *kg·ha^{-1}*			**Before seeding Total N** *kg·ha^{-1}*			**Topdress NH_4-N** *kg·ha^{-1}*	**Yield** *Mg·ha^{-1}*			**AE_{NH4}** *kg·kg^{-1}*		**AE_{NT}** *kg·kg^{-1}*		**RE_{NH4}** *kg·kg^{-1}*		**RE_{NT}** *kg·kg^{-1}*		**Plant N uptake** *kg N·ha^{-1}*			**Unaccounted N** *kg N·ha^{-1}* **2011-2013**	
Treatment	2011	2012	2013	2011	2012	2013	All years	2011	2012	2013	2012	2013	2012	2013	2012	2013	2012	2013	2011	2012	2013	NH_4-N	Total N
M0	0†	0	0	0	0	0	0	1.93	3.72	2.22	--	--	--	--	--	--	--	--	21.6	32.1	19.6	0	0
M30	30†	30	30	30	30	30	0	1.98	2.90	2.36	33.2	26.6	33.2	26.6	0.63	0.21	0.63	0.21	16.7	51.1	25.9	75	75
M60	60†	60	60	60	60	60	0	2.27	4.41	3.20	23.2	26.9	23.2	26.9	0.38	0.29	0.38	0.29	32.4	55.1	36.9	116	116
M90	90†	90	90	90	90	90	0	2.53	5.20	2.50	38.0	20.4	38.0	20.4	0.50	0.22	0.50	0.22	35.9	76.9	39.0	158	158
M120	120†	120	120	120	120	120	0	2.27	5.03	2.67	29.2	21.5	29.2	21.5	0.35	0.20	0.35	0.20	36.8	74.6	44.0	286	286
M150	150†	150	150	150	150	150	0	3.02	6.61	3.03	23.2	19.5	23.2	19.5	0.32	0.23	0.32	0.23	47.6	79.6	54.3	333	333
M120M0	120	120	120	120	120	120	0	3.24	7.22	4.79	29.2	21.5	29.2	21.5	0.35	0.20	0.35	0.20	36.8	74.6	44.0	286	286
M120M30	120	120	120	120	120	120	30	3.78	6.36	5.37	17.6	21.0	17.6	21.0	0.21	0.22	0.21	0.22	39.7	63.6	52.1	345	345
M120M60	120	120	120	120	120	120	60	3.76	8.29	5.24	25.4	16.8	25.4	16.8	0.35	0.17	0.35	0.17	38.8	95.1	49.7	406	406
M120M90	120	120	120	120	120	120	90	4.86	8.32	6.08	21.9	18.4	21.9	18.4	0.29	0.20	0.29	0.20	52.9	92.9	60.7	445	445
M120M120	120	120	120	120	120	120	120	4.79	8.10	5.86	18.3	15.2	18.3	15.2	0.27	0.15	0.27	0.15	52.3	97.5	56.2	558	558
PS120M0	129	91	109	211	129	201	0	4.86	6.14	4.38	26.6	19.9	18.8	10.8	0.35	0.17	0.25	0.09	52.3	64.4	38.3	214	426
PS120M30	129	91	109	211	129	201	30	5.58	6.70	5.38	24.7	22.8	18.8	13.7	0.32	0.25	0.24	0.15	61.6	70.9	53.6	284	496
PS120M60	129	91	109	211	129	201	60	5.59	7.39	5.19	24.2	17.6	19.3	11.4	0.31	0.17	0.25	0.11	63.8	79.0	48.8	363	575
PS120M90	129	91	109	211	129	201	90	5.58	7.57	5.32	21.2	15.6	17.5	10.7	0.27	0.17	0.22	0.12	61.9	80.6	54.4	451	663
PS120M120	129	91	109	211	129	201	120	5.63	8.09	5.81	20.7	15.7	17.6	11.2	0.27	0.16	0.23	0.11	62.1	88.7	56.4	520	732
PS120M150	129	91	109	211	129	201	150	5.94	8.21	5.95	18.6	14.4	16.1	10.6	0.24	0.17	0.21	0.13	67.3	89.6	64.0	597	809
PS170M0	186	152	165	304	215	305	0	6.33	6.62	5.71	18.6	21.4	13.1	11.6	0.26	0.24	0.18	0.13	71.0	72.5	58.5	360	681
PS170M30	186	152	165	304	215	305	30	6.12	7.32	6.42	19.1	21.7	14.2	12.7	0.31	0.26	0.23	0.15	67.7	90.2	69.4	388	709
PS170M60	186	152	165	304	215	305	60	6.29	7.91	5.84	19.3	16.2	14.9	10.0	0.27	0.18	0.21	0.11	71.9	90.1	59.1	491	812
PS170M90	186	152	165	304	215	305	90	5.88	7.11	5.45	13.7	12.8	10.8	8.3	0.20	0.17	0.16	0.11	66.2	81.2	63.8	634	955
PS170M120	186	152	165	304	215	305	120	6.19	7.90	6.58	15.3	15.4	12.4	10.4	0.23	0.21	0.19	0.14	70.7	95.4	79.8	662	983
PS170M150	186	152	165	304	215	305	150	6.37	7.01	6.11	10.7	12.4	8.9	8.6	0.16	0.17	0.13	0.12	74.3	81.3	73.5	727	1048

† NH_4NO_3 was applied in these treatments before seeding instead ammonium sulfate, so half of the N was in the nitric form

3.2.3. Sampling

3.2.3.1. Soil sampling

Soil samples were obtained from each experimental plot (at least two per plot) before fertilization application (27 April 2011, 10 May 2012 and 6 May 2013) and at the end of the experiment (29 October 2013) from 0 to 1.20 m with 0.30 m depth increments (Figure 3.5a). Soil extracts were prepared using 10 g fresh soil (sieved through a 4-mm mesh (Figure 3.5b)) and 30 mL of KCl 2 N solution. Nitrate (AENOR, 1997) and ammonium (AENOR, 2005) concentrations were determined colorimetrically with a continuous flow analyzer (AutoAnalyzer3, Bran+Luebbe, Norderstedt, Germany). Ammonium-N and nitrate-N concentrations in the extracts were converted to mg N·kg dry soil^{-1} considering the volume of KCl added to the samples and the weight of the soil and after that, converted to kg N·ha^{-1} taking into account the depth of the sampled soil (0.30 m) and the soil bulk density.

Figure 3.5. Villanueva de Sigena. Soil sampling before fertilization and seeding (a) and soil sieving (b).

3.2.3.2. Crop sampling

Plant density was determined on 23 June 2011, 4 July 2012 and 23 July 2013 in at least six 0.25 m^2 control areas in the three fertilization strategies.

Weeds (*Cyperaceae*, *Echinochloa*, and broadleaf weeds) density and the number of panicles affected by *Chilo suppressalis* and *Pyricularia oryzae* were determined on 20 September 2011, 25 September 2012 and 24 September 2013 in selected treatments covering all ranges of N application. The treatments were as follows: PS170M0, PS170M90, PS170M150, PS120M0, PS120M90, PS120M150, M0, M150, and M120M30. In each plot, at least two 0.25 m^2 control areas were studied.

Rice was hand-harvested on 20-21 October 2011 and 15-17 October 2012 in two 2 m^2 control areas (total area 4 m^2) in each experimental plot corresponding to the PS treatments and in two 1 m^2 control areas (total area 2 m^2)) in experimental plots corresponding to the mineral treatments. In 2013, a strip, 1.5 m wide and the length of the experimental plot, was mechanically harvested on 25 October (Figure 3.6a). Before harvesting each year, two 0.25 m^2 control areas per plot were hand-harvested to determine the harvest index and the yield components: panicle density, spikelets per panicle and grain weight.

Grain humidity and specific weight were measured (PM-600 grain moisture tester, Keller, Japan) (Figure 3.6b) and yield was adjusted to 140 g·kg^{-1} moisture content. Total N concentrations in rough grain and straw were determined by combustion (Dumas, 1831) (TruSpec CN, LECO, St. Joseph, MI, USA).

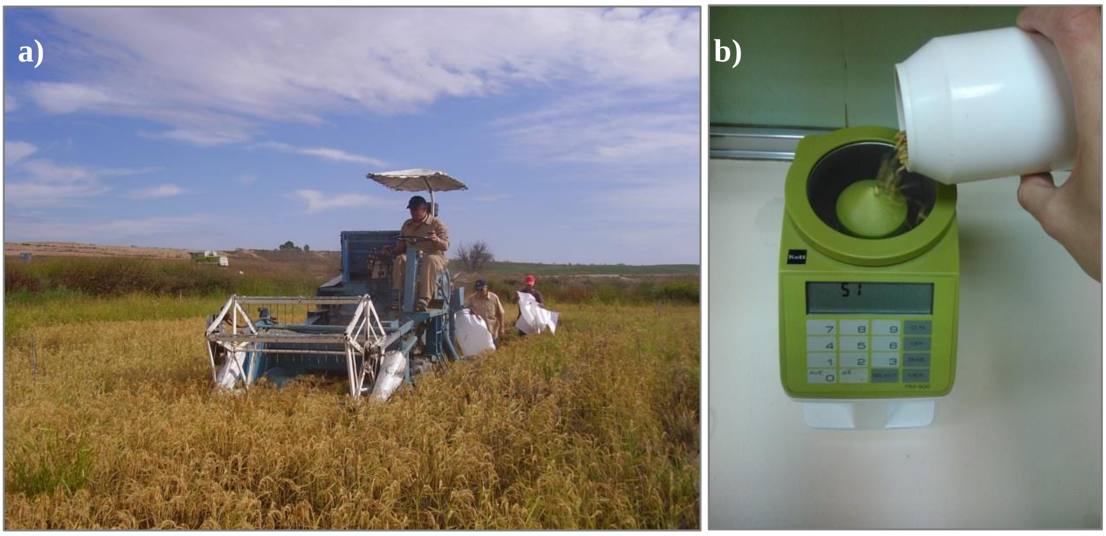

Figure 3.6. Villanueva de Sigena. Rice harvest with a commercial combine (a) and grain humidity measurement with the PM-600 grain moisture tester, Keller, Japan (b).

3.2.3.3. Rice quality

Samples were processed in a laboratory rice huller (PAZ-1-DTA, Zaccaria, Lineira, Brazil) (Figure 3.7) to obtain the head rice yield. 100 grams of rough rice were dehulled to obtain brown rice; then, the brown rice was milled using abrasive stones to obtain white rice. The whole milled grains were separated from the broken grains using a sieve. Finally, imperfect grains (chalky and immature, damaged by fungi or insects and discolored grains) were manually separated to obtain the percentage of the different groups. Head rice yield (%) was calculated by dividing the mass of white rice, once broken and imperfects grains were separated, by the mass of rough rice. The treatments evaluated were PS170M0, PS120M0, PS120M30, M150 and M120M30 (considered *a priori* to be optimum or near-optimum treatments) and PS120M150 and PS170M150 (over-fertilized treatments). The samples were not properly stored in 2011, and the grains broke easily during milling, so the data from 2011 was not considered in the analysis of the results of head rice yield.

Figure 3.7. Milling process in a laboratory rice huller (PAZ-1-DTA, Zaccaria, Lineira, Brazil).

Amylose content (AENOR, 2008) and gel consistency (Cagampang et al., 1973) were evaluated in white rice samples of PS170M0, PS120M30, and M120M30 in 2011 and 2012.

Microbiological contamination of rice grains due to PS application was evaluated in 2012. Different rough rice samples were taken in sterilized bags from PS and mineral plots to evaluate the presence of *Escherichia coli* (ISO, 2005) and *Salmonella spp* (AENOR, 2003) as contamination indicators microorganisms.

3.2.4. Data Analysis

Linear-plateau response equations (Cerrato and Blackmer, 1990 Eq. 3.1) were adjusted to model the response of yield to N rate in the PS120, PS170 and mineral treatments for each of the three years.

$$\begin{cases} If\ N < C;\ \ Y = a + b \cdot N \\ If\ N \geq C;\ \ Y = Y_{max} = a + b \cdot C \end{cases} \qquad \textbf{Eq. 3.1}$$

where Y ($Mg \cdot ha^{-1}$) is the yield; N is the applied nitrogen rate ($kg\ N \cdot ha^{-1}$) (when N was splitting, N is the sum of N applied before seeding and topdressing); a (intercept) is the yield at 0 $kg\ N \cdot ha^{-1}$; b is the increase in yield per unit increase in N; and C is the critical N rate, or N rate above which the maximum yield (Y_{max}) is obtained. For PS treatments only the NH_4^+-N PS content was considered in the calculation of the applied nitrogen.

The nitrogen fertilizer replacement value (NFRV) of the PS applied before seeding in the PS120 and PS170 fertilization strategies was estimated as the mineral N rate that produced the same yield as the PS120M0 and PS170M0 treatments, respectively.

To evaluate nitrogen use efficiency in the different treatments, two ratios were calculated (Ladha et al., 2005). The agronomic efficiency of N was calculated as the yield increment due to the N application divided by the N applied rate (AE_N, Eq. 3.2) and the recovery efficiency of N was calculated as the increment in the N uptake in aboveground biomass due to the N application, divided by the N applied rate (RE_N, Eq. 3.3).

$$AE_N(T)\ (kg \cdot kg^{-1}) = \frac{Y_T - Y_O}{N_T} \qquad \textbf{Eq. 3.2}$$

$$RE_N(T)\ (kg \cdot kg^{-1}) = \frac{UN_T - UN_0}{N_T} \qquad \textbf{Eq. 3.3}$$

where Y_T is the yield ($kg \cdot ha^{-1}$) and UN_T is the plant N uptake ($kg \cdot ha^{-1}$) in the T treatment; N_T is the amount of N applied in the T treatment and Y_0 is the yield ($kg \cdot ha^{-1}$) and UN_0 is the N uptake ($kg \cdot ha^{-1}$) in the control plot (without N application). Both efficiencies were calculated considering both the actual PS ammonium N (AE_{NH4} and

RE_{NH4}) and the actual total N (AE_{NT} and RE_{NT}) rates (Table 3.4) in the denominator (N_T).

A nitrogen budget was established for the three years study period (April 2011-October 2013) for a soil depth 0-1.20 m using Eq. 3.4. The inputs (N_{inputs}) were as follows: soil mineral N at the beginning of the period (N_{is}), mineral N provided by the soil (N_m) and N applied with PS (actual rates, Table 3.4) or M fertilizers (N_f). The outputs ($N_{outputs}$) considered as follows: crop N uptake (N_{upt}) and soil mineral N at the end of the period (N_{fs}). The N_m was estimated as the difference between N inputs and N outputs in the control treatment (M0), assuming that nitrate leaching and atmospheric N losses in this treatment (with low N availability) were negligible (Bhogal et al., 1998) and it was considered to be equal in all treatments.

$$N_{inputs} - N_{outputs} = \left(N_{is} + N_f + N_m\right) - \left(N_{upt} + N_{fs}\right) = \ Unaccounted\ N \qquad \textbf{Eq. 3.4}$$

A positive value of unaccounted N will indicate net N losses form the system. The unaccounted N was calculated considering the ammonium N content of PS and also considering the total N content of PS in N_f.

3.2.5. Statistical Analysis

The effects of the treatments, years and their interaction on the different studied variables were analyzed using analysis of variance through the General Linear Model (GLM) procedure of the SAS 9.4 Software. Multiple comparison among treatments were performed using Tukey multiple range test at p=0.05.

Additionally, correlation coefficient (r) was used to evaluate significant ($p<0.05$) linear relationship between some of the studied variables (yield components with N rate and yield or unaccounted N with N applied).

3.3. RESULTS

3.3.1. Crop response to the different fertilization strategies

Plant density was not significantly affected by the fertilization treatments; however it was significantly affected by the year (Table 3.5). In 2013, plant density was 50% of that in 2011 and the differences were due to strong winds and low temperatures during a period of 30 days after seeding that reduced seed germination.

Table 3.5. Plant density in PS170, PS120 and mineral plots for the three experimental years.

	Plant density ***no. m^{-2}***
Year (Y)	***
2011	103 a
2012	86 b
2013	51 c
Treatment (T)	n.s.
PS170	75
PS120	84
M	81
TxY	n.s.

n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$.
Different letters in the same column indicate significant differences ($p<0.05$) between years.

Echinochloa and broadleaf weed density and plants affected by *Pyricularia* were low and similar between fertilization treatments for the average of the three years (data not shown). *Cyperaceae* density was low in 2011 and significantly higher in 2012 and 2013, but no significant differences were detected between treatments (Table 3.6). The number of plants affected by *Chilo suppressalis* was not very high; however, the over-fertilized treatments were more strongly affected (Figure 3.8), although significant differences were detected only between PS170M150 and the control (Table 3.6).

Table 3.6. *Cyperaceae* and *Chilo Supressalis* in selected treatments during the three years.

	Cyperaceae *Inflorescences·m^{-2}*	**Chilo Suppresalis** *Affected inflorescences·m^{-2}*
Year (Y)	***	**
2011	12.3C	0.1B
2012	120.5 B	6.7 A
2013	309.6 A	6.3 A
Treatment (T)	n.s.	*
M0	177.7	0.8 b
M150	146.0	3.5ab
M120M30	141.7	1.3ab
PS120M0	186.5	2.5ab
PS120M90	123.7	3.0ab
PS120M150	98.8	7.7ab
PS170M0	149.2	3.7ab
PS170M90	147.3	4.7ab
PS170M150	156.5	12.3a
TxY	n.s.	n.s.

n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Different capital letters in the same column indicate significant differences ($p<0.05$) between years. Different lowercase letters in the same column indicate significant differences ($p<0.05$) between treatments.

Figure 3.8. Villanueva de Sigena. Rice lodging in a plot affected by *Chilo Supressalis* (PS170M150 treatment) at harvest time in 2012.

Rice yield for the different treatments and years are shown in Table 3.4. There was a significant yield response to N fertilizer application (Figure 3.9) in the mineral treatments. Critical N rates were established as 177 kg N·ha^{-1} (2012) and 169 kg N·ha^{-1} (2013), with maximum yields of 8235 kg·ha^{-1} (2012) and 5728 kg·ha^{-1} (2013). In 2011 ammonium nitrate (instead ammonium sulfate) was applied before seeding (erroneously), and the maximum yields were not reached in the mineral treatments. Thus, in 2011, the maximum yield in the mineral treatments was 4791 kg·ha^{-1} at the maximum N rate (240 kg N·ha^{-1}), while in the PS treatments, yields reached (Figure 3.9) 5684 kg·ha^{-1} (PS120) and 6195 kg·ha^{-1} (PS170). Hence, NFRV of PS and N use efficiencies were not reliable in 2011 and were not calculated.

In the PS170 strategy, the maximum yield was obtained in all three years (6195 kg·ha^{-1} in 2011, 7312 kg·ha^{-1} in 2012 and 6017 kg·ha^{-1} 2013) without topdressing fertilizer application (Figure 3.9). Thus, the critical values to achieve the maximum yield were 186, 152 and 165 kg N·ha^{-1} for 2011, 2012 and 2013 respectively, i.e., the amount of NH_4^+-N applied with PS (Table 3.4). In the PS120 strategy, topdressings of 35 kg N·ha^{-1} (2011 and 2013) and 88 kg N·ha^{-1} (2012) were necessary to achieve the maximum yield (5684 kg·ha^{-1} in 2011, 7956 kg·ha^{-1} in 2012 and 5567 kg·ha^{-1} in 2013) (Figure 3.9). In PS120 strategy, critical N rates were established as 164 (129 with PS + 35) kg N·ha^{-1} in 2011, 179 (91 with PS + 88) kg N·ha^{-1} in 2012, and 144 (109 with PS + 35) kg N·ha^{-1} in 2013.

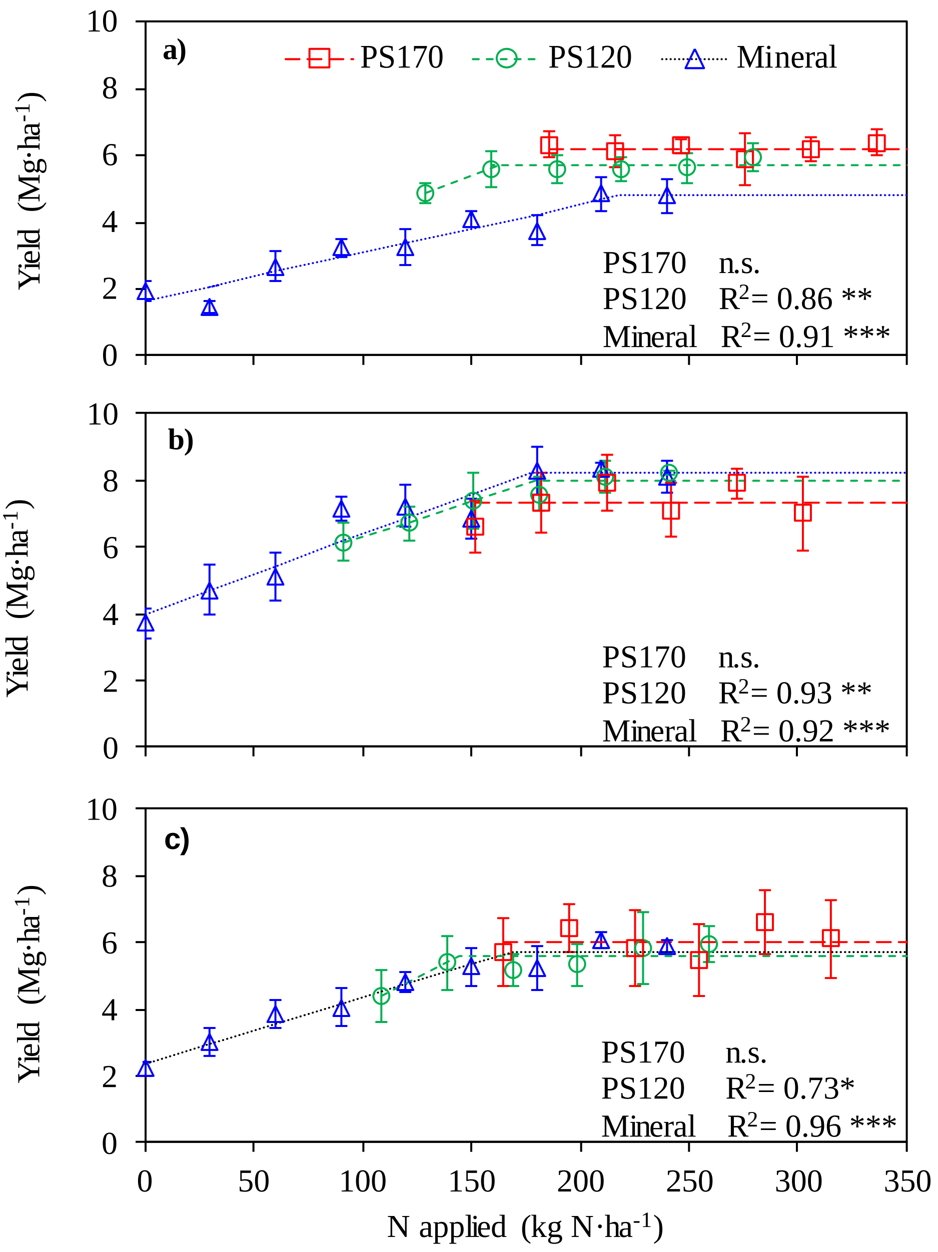

Figure 3.9. Yield response curves to nitrogen (N applied) in the two PS fertilization strategies (PS120 and PS170) and mineral treatments in 2011 (a), 2012 (b) and 2013 (c). Vertical bars represent ±1 standard error. (R^2 denotes the coefficient of determination and the significance of the regressions: n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$. For pig slurry only the NH_4^+-N content has been considered in the calculation of the N applied).

3.3.2. Nitrogen fertilizer replacement value of PS

The nitrogen fertilizer replacement value (NFRV) of the two PS strategies was quantified for 2012 and 2013 (Table 3.7). The NFRV of the PS ammonium N was high, ranging between 72% and 102% of the ammonium-N applied. The NFRV decreased when considering the total N in the PS, ranging from 50% to 69% of the total N applied.

The PS fertilization strategies and the year did not have any effect on NFRV. For the average of the two years (Table 3.7), the NFRV of the lower rate strategy (PS120) represented 96% of the NH_4^+-N applied with the PS (60% of total N in PS), and for the highest rate strategy (PS170), the NFRV was equivalent to 87% of the NH_4^+-N applied with the PS (or 53% of total N applied with the PS).

Table 3.7. Nitrogen fertilizer replacement value (NFRV) for the two PS fertilization strategies (PS120 and PS170) and the percentage that NFRV represents with respect to the amount of the PS ammonium N and the PS total N in 2012 and 2013.

	NFRV *kg N·ha^{-1}*	**PS NH_4^+-N** *kg N·ha^{-1}*	**NFRV/NH_4^+-N** *(%)*	**PS Total N** *kg N·ha^{-1}*	**NFRV/Total N** *(%)*
PS120					
2012	89	91	98%	129	69%
2013	101	109	93%	201	50%
Average	95	100	96%	165	60%
PS170					
2012	110	152	72%	215	51%
2013	168	165	102%	305	55%
Average	139	159	87%	260	53%
Treatment (T)			n.s.		n.s.
Year (Y)			n.s.		n.s.
TxY			n.s.		n.s.

n.s.: not significant.

3.3.3. Yield Components

Panicle density was low in the control treatment (288, 416 and 277 panicles·m^{-2} for 2011, 2012 and 2013, respectively) and reached an average of 536 panicles·m^{-2} (2011), 653 panicles·m^{-2} (2012, Figure 3.10a) and 493 panicles·m^{-2} (2013) at N rates higher than the critical N rate of the corresponding year. Spikelets per panicle behaved in a similar manner, with minimum values for the control treatment (33, 39 and 50 spikelets·panicle^{-1} for 2011, 2012 and 2013, respectively) and reaching average values of 50 spikelets·panicle^{-1} (2011), 53 spikelets·panicle^{-1} (2012, Figure 3.10b) and 74 spikelets·panicle^{-1} (2013) at N rates higher than the critical N rate of the corresponding year. No differences were detected in panicle density and spikelets per panicle between fertilization treatments for treatments with N rates higher than the critical N rate ($p>0.05$), however significant differences were observed between years ($p<0.05$).

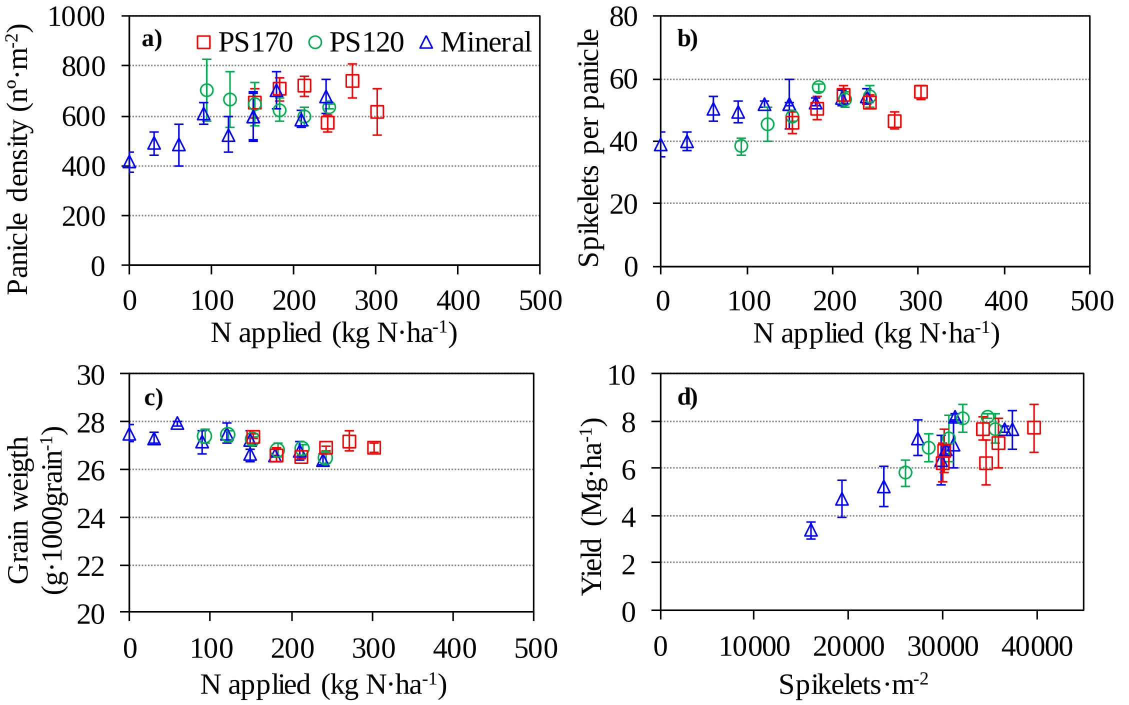

Figure 3.10. Panicle density (a), spikelets per panicle (b) and the grain weight (c) in relation to the amount of N applied and the relationship between grain yield and spikelets per square metre (d) in 2012 in the two PS fertilization strategies (PS120 and PS170) and mineral treatments. Vertical bars represent ± 1 standard error. For pig slurry only the NH_4^+-N content has been considered in the calculation of the N applied.

Panicle density, spikelets per panicle and spikelets per square meter were positively correlated (Table 3.8) with N rate and yield for the three experimental years (2012, Figure 3.10a, b, d). The filled grains percentage and grain weight were not correlated or negatively correlated (Table 3.8) with N rate (2012, Figure 3.10c) and yield, while specific weight was positively correlated (Table 3.8) with both variables in 2011. Finally, harvest index did not show a clear tendency.

Table 3.8. Correlation coefficients (r) between yield components, specific weight and harvest index and N rate and yield. (Data include all treatments).

	N rate			**Yield**		
	2011	**2012**	**2013**	**2011**	**2012**	**2013**
Panicles·m^{-2}	0.86***	0.61**	0.69***	0.95***	0.65**	0.74***
Spikelets·panicle^{-1}	0.89***	0.70***	0.76***	0.92***	0.72***	0.83***
Spikelets·m^{-2}	0.89***	0.83***	0.77***	0.95***	0.84***	0.81***
Filled spikelets (%)	n.s.	n.s.	-0.61**	-0.58**	n.s.	-0.51*
Grain weight	n.s.	-0.68***	-0.78***	n.s.	-0.66***	-0.75***
Specific weight	0.77***	0.47*	n.s.	0.79***	n.s.	n.s.
Harvest index	0.76***	n.s.	n.s.	0.81***	n.s.	-0.44*

n.s.: not significant; * p<0.05; ** p<0.01; ***p<0.001.

3.3.4. Rice quality

No significant differences were observed in head rice yield between evaluated PS treatments (PS120M0, PS120M30, PS120M150, PS170M0 and PS170M150) and mineral treatments (M150 and M120M30). However, significant differences were observed between years. A higher head rice yield occurred in 2013, with lower values of broken and damaged grains (Table 3.9).

Table 3.9. Broken and discarded (chalky, damaged by fungi or insects) grains (% weight over white rice), head rice yield (% weight over rough rice yield) and cooking quality in selected treatments.

	Broken *%*	**Chalky** *%*	**Damaged by fungi or insects** *%*	**Head rice yield** *%*	**Gel consistency** ***mm***	**Amylose content** *%*
Year (Y)	***	n.s.	**	***	*	n.s.
2011					86.67a	8.42
2012	18.29a	6.33	1.54a	54.13b	78.67 b	8.42
2013	7.89 b	5.83	0.74 b	63.60 a		
Treatment (T)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
M150	12.58	6.33	1.02	58.93		
M120M30	14.47	7.13	1.15	57.28	81.84	8.24
P120M0	13.99	5.89	0.98	58.11		
PS120M30	12.45	5.97	1.85	59.20	79.83	8.72
PS120M150	10.53	5.58	1.07	61.01		
PS170M0	15.07	5.43	1.12	58.20	86.34	8.31
PS170M150	12.54	6.23	0.82	59.37		
YxT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Different letters in the same column indicate significant differences ($p<0.05$) between years.

No differences were found between the studied treatments in gel consistency or amylose content (Table 3.9). Gel consistency was significantly different in the two years, but amylose content was not affected by the year.

Escherichia coli and *Salmonella spp* were not detected in any grain samples; consequently, PS can be applied to rice without microbiological contamination concern.

3.3.5. Plant N uptake and N use efficiency

Plant N uptake (Table 3.4) followed a similar pattern to that of rice yield and reached values of 74.3, 97.5 and 79.8 kg·N ha^{-1} in years 2011, 2012 and 2013, respectively.

Table 3.4 shows AE_N (agronomic efficiency) and RE_N (recovery efficiency) values for each treatment considering the PS ammonium N content (AE_{NH4} and RE_{NH4}) and the PS total N content (AE_{NT} and RE_{NT}).

The AE_{NH4} and the RE_{NH4} ranged between 11 and 38 kg·kg^{-1} and between 0.15 and 0.63 kg·kg^{-1}, respectively. For the treatments near the optimum N rate (177 kg N·ha^{-1} in 2012 and 169 kg N·ha^{-1} in 2013), the AE_{NH4} and RE_{NH4} values ranged between 16.7 and 25.4 kg·kg^{-1} and between 0.17 and 0.35 kg·kg^{-1}, respectively (Table 3.4; Figure 3.11). Efficiencies calculated considering PS total N (AE_{NT} and RE_{NT}) were lower than those calculated considering ammonium-N, ranging between 11.6 and 17.6 kg·kg^{-1} and 0.13 and 0.23 kg·kg^{-1} for AE_{NT} and RE_{NT}, respectively, for the treatments near the optimum N rate (Table 3.4).

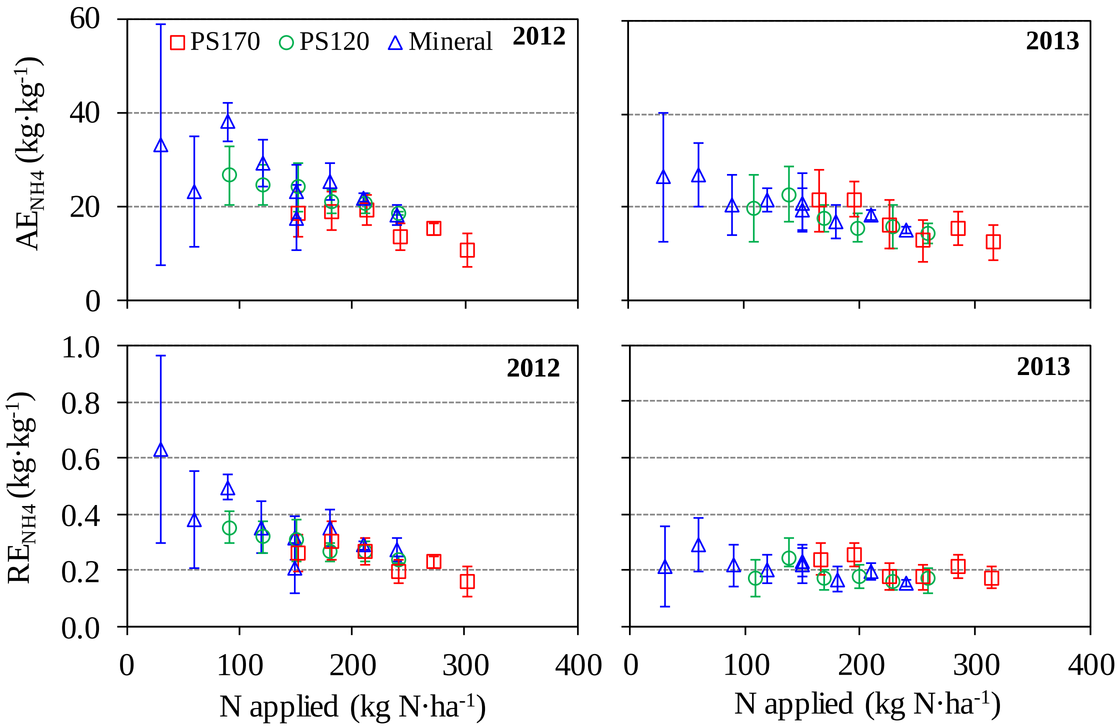

Figure 3.11. Agronomic efficiency (AE_{NH4}) and recovery efficiency (RE_{NH4}) for the two PS fertilization strategies (PS170 and PS120) and mineral treatments in 2012 and 2013 in relation to the amount of total mineral N applied. Vertical bars represent ± 1 standard error. For pig slurry only the NH_4^+-N content has been considered in the calculation of the N applied.

The analysis of variance (Table 3.10) shows that the AE_{NH4} and RE_{NH4} were not significantly different between strategies, neither between topdressing N rates. When efficiencies were calculated considering PS total N content, significant differences were observed between strategies for AE_{NT}, but not for RE_{NT}. The AE_{NT} was the lowest in the PS170 strategy and significantly different to mineral strategy (M120). Efficiencies were significantly affected by the year, being for all cases significantly higher in 2012 than in 2013.

Table 3.10. Analysis of variance of the AE_{NH4}, AE_{NT}, RE_{NH4} and RE_{NT} and N unaccounted considering ammonium N or total N as affected by the fertilization strategy (PS170, PS120 and M120), the N topdressing rate (0, 30, 60, 90, 120 kg N·ha^{-1}), the year (2012 or 2013) and their interactions.

	AE_{NH4} *kg·kg^{-1}*	**AE_{NT}** *kg·kg^{-1}*	**RE_{NH4}** *kg·kg^{-1}*	**RE_{NT}** *kg·kg^{-1}*	**Unaccounted N 2011-2013 (NH_4-N)** *kg N·ha^{-1}*	**Unaccounted N 2011-2013 (Total N)** *kg N·ha^{-1}*
Strategy (S)	n.s.	*	n.s.	n.s.	*	***
PS170	17.3	11.8a	0.23	0.16	507a	828a
PS120	20.9	15.0ab	0.24	0.18	367 b	578 b
M120	20.6	20.6 b	0.24	0.24	408ab	408 c
Topdress (T)	n.s.	n.s.	n.s.	n.s.	***	***
0	22.9	17.5	0.26	0.20	287a	464a
30	21.4	16.6	0.26	0.20	339a	517a
60	19.8	16.2	0.24	0.20	420 b	597 b
90	17.3	14.6	0.22	0.18	510 c	688 c
120	16.8	14.2	0.22	0.18	580 c	758 c
Year (Y)	*	**	**	**		
2012	21.1a	18.1a	0.28a	0.24a		
2013	18.1 b	13.6 b	0.19 b	0.14 b		
SxT	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
SxY	n.s.	n.s.	n.s.	n.s.		
TxY	n.s.	n.s.	n.s.	n.s.		

n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Different letters in the same column indicate significant differences ($p<0.05$) within each factor (S, T or Y).

3.3.6. Nitrogen budget

Soil inorganic N in the 0 to 1.2 m depth profile at the beginning of the experiment (April 2011; Figure 3.12a) was 96 kg N·ha^{-1}. Soil inorganic N was higher in the top layer and decreased with depth at all sampling times. No effects of the fertilization strategies (PS170, PS120 and M120) ($p>0.05$) were detected in soil inorganic N for the second (May 2012; Figure 3.12b) and third (May 2013; Figure 3.12c) sampling times, with average values (0-1.20 m profile) of 179 kg N·ha^{-1} and 142 kg N·ha^{-1}, respectively.

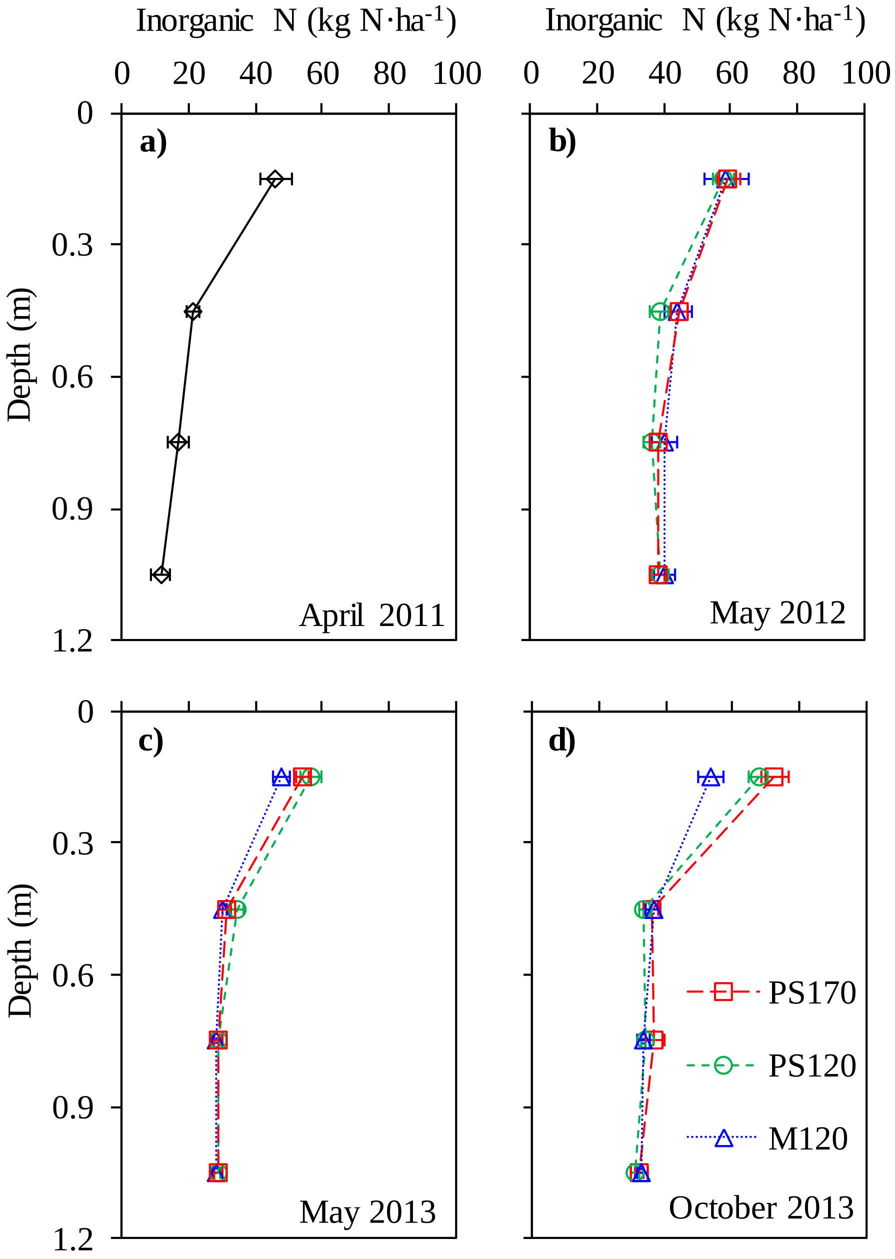

Figure 3.12. Soil inorganic nitrogen (NO_3^--N + NH_4+-N, kg N·ha^{-1}) at different depths before seeding each year (April 2011 (a), May 2012 (b), May 2013 (c)) and at the end of the experiment (October 2013 (c)) in the three fertilization strategies (PS170, PS120 and M120). Horizontal bars represent ± 1 standard error.

However, at the end of the experiment (October 2013), significant differences ($p<0.05$) were detected between the M120 and the two PS strategies at 0-0.3 m soil layer (Figure 3.12d). The graphs in Figure 3.12 show the average line for the main treatments (PS170, PS120 and M120) because no significant differences were detected between the different topdressing levels within each strategy.

Thus, soil inorganic N was independent of the rate of N applied for all depths and dates, except for 0-0.3 m depth in October 2013, even when the N rate was excessive, indicating high N losses in the system in over-fertilized treatments.

Unaccounted N indicated overall high N losses which increased at increasing N fertilization (Table 3.4; Figure 3.13). For optimum treatments, average unaccounted N was 376 kg N·ha^{-1} (Figure 3.13a, vertical arrow) for the whole period (April 2011-October 2013) and reached 496 and 680 kg N·ha^{-1} for PS120 and PS170 strategies, respectively, when total N was considered for the calculation (Figure 3.13b, vertical arrows). These losses represented 70% of the inorganic N applied and reach 79-83 % of the total N applied in the PS strategies.

Unaccounted N for the whole period (2011-2013) (Table 3.10) was significantly affected by the fertilization strategy (PS170, PS120 or M120) and by the topdressing N rate. When ammonium N was considered for the calculation, PS120 and M120 (strategies with the same ammonium N rate applied before seeding) did not differ significantly, hence unaccounted N was not affected by the N fertilization source (PS or mineral fertilizer). Moreover, the unaccounted N was higher when N topdressing rates increasing. When total N was considered for the calculation, unaccounted N differed significantly between the three strategies, and again was higher when N topdressing rates increasing.

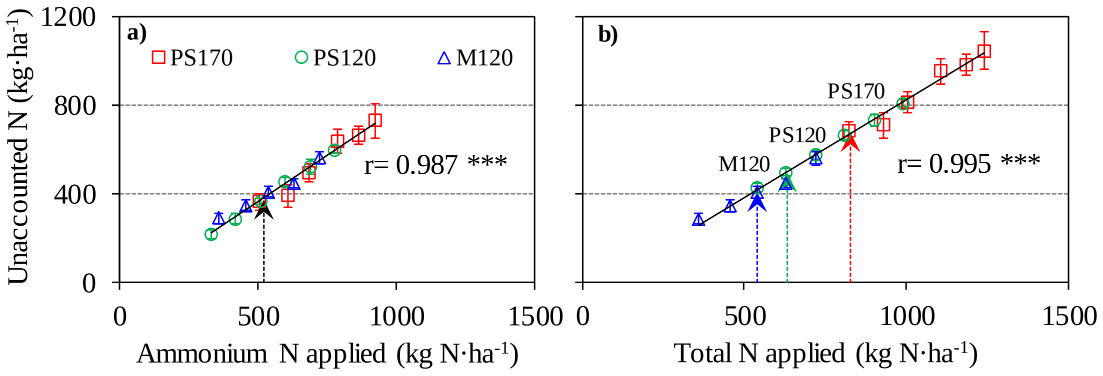

Figure 3.13. Relationship between unaccounted N (kg·ha^{-1}) and ammonium N applied (a) or total N applied (b) in the strategies fertilization (PS170, PS120 and M120) in the entire period (2011-2013). Vertical bars represent ± 1 standard error. Vertical arrows represent optimum N rates. (r denotes the correlation coefficient and its significance: ***$p<0.001$).

3.4. DISCUSSION

3.4.1. Crop response to the different fertilization strategies

The two PS fertilization strategies did not affect rice seed germination when compared to mineral fertilization in any of the three years in this experiment, even at the highest PS rate of 170 kg N·ha^{-1} (Table 3.5). Therefore, if any kind of toxicity was present, it was similar in both the mineral fertilizer and in the PS strategies. However, significant differences were observed between years in plant density that were related to weather conditions after seeding. The low plant density values in 2013 were due to lower temperatures after seeding: the average maximum temperatures in May were 28.4 ºC, 28.1 ºC and 22.1 ºC in 2011, 2012 and 2013, respectively, and the average minimum temperatures were 11.6 ºC, 11.8 ºC and 8 ºC in 2011, 2012 and 2013 respectively (Figure 3.2).

Furthermore, the results suggest that the two PS fertilization strategies did not affect the presence of weeds, pests or diseases, but high N application rates increase the risk for the occurrence of *Chilo* suppressalis (Table 3.6). The cause of the infestation is N excess rather than the application of PS as a fertilizer. For example, the treatment PS170M0, in which the entire N needs of the rice were provided by PS, attained the maximum yield (Figure 3.9) and a high presence of pests was not noted (Table 3.6). It is known that when application is excessive, the crop consumes more nitrogen, the plants are leafy and tall, the green colour is darker and the crop is more exposed to fungi and lodging (Tinarelli, 1989), even though the yield is not increased as happened in the PS170M150 treatment. Therefore, adjusting the N rate in organic and inorganic fertilization is essential for reducing the risk of decreased yield due to pests or diseases.

In the mineral treatments, the rice yield responded to the amount of N applied (Figure 3.9). In 2011, ammonium nitrate was applied before seeding instead ammonium sulfate, and the maximum yield was not reached in the mineral treatments. Ammonium nitrate has 50% of the N in the nitrate form that is likely to be lost after flooding via denitrification (Tinarelli, 1989; Wilson et al., 2001). In 2012, the yields were higher than 8200 kg·ha^{-1}, and the critical N rate was established at 177 kg N·ha^{-1}, slightly higher than the recommended rate of 170 kg N·ha^{-1} (Pérez, 2006). In 2013, the maximum yields were lower than in 2012, below 6000 kg·ha^{-1}, and the critical N rate was similar to that in 2012 (169 kg N·ha^{-1}).

In the two PS fertilization strategies, maximum yields were obtained in all three years (Figure 3.9). The PS170 strategy did not respond to the N applied at topdressing in any of the three years. Other studies have also shown that rice can obtain maximum yields with an adequate N rate before seeding (Bond and Bollich, 2007; Wilson et al, 2001) and without the necessity of applying N at topdressing. However, for the PS120 strategy, topdressing N was necessary to obtain the maximum yield.

There are no studies focused on the use of raw PS in our cultivation conditions. Some studies focused on the application to rice of anaerobically digested pig slurry (ADPS), the outcome of the co-digestion of PS with other organic products, have shown an optimum rice response. Lu et al. (2012) and Chen et al. (2013) reported that yield was not significantly lower in plots fertilized with ADPS than in plots with mineral fertilizers at the same N rates (although N rates were higher than in our work), and Zhang et al. (2015) reported that an effective strategy for using ADPS was application of slurry as basal fertilizer combined with mineral fertilizer at topdressing, but the strategy of an unique basal ADPS application was not assessed.

These high PS rates before seeding cannot be applied in other crops. For example, in maize, low rates of organic or mineral fertilizers before seeding to supply the crop’s N needs at the initial stage and sidedressing applied to reach the target N rate are recommended (Yagüe and Quílez, 2010) to avoid N losses during spring due to nitrification-leaching. However, N is applied in the ammonium form to rice fields, and the fields are flooded immediately after fertilizer application; thus nitrification-leaching losses are avoided. Therefore, rice can be fertilized with only a single application of PS before seeding or by applying most of the required N before seeding, then supplementing with topdressing if necessary.

3.4.2. Nitrogen fertilizer replacement value of PS

Compared to mineral fertilization, the use efficiency of ammonium N in PS was 96% for PS120 and 87% for PS170 and was not significantly different between PS strategies. Considering the total N content of the PS, the efficiency was 60% for PS120 and 53% for PS170 (Table 3.7). The NFRV of PS, thus represented practically all the ammonium-N applied with the PS and only a part of the total-N applied because the PS organic-N, as expected, cannot be used much by the crop in the year of application. These results show that crop uses PS ammonium-N as efficiently as the ammonium provided by mineral fertilization but cannot use the PS organic-N during the year of application (Table 3.7). Therefore, pig slurry rates for rice fertilization should be calculated considering the PS ammonium–N content; if PS total N is considered to calculate the PS rates, N applied will not be sufficient to provide optimum rice yield.

3.4.3. Rice quality

The fertilization strategies did not influence the head rice yield or the cooking quality (Table 3.9). The values for rice cooking quality were similar to those obtained for the Guadiamar variety in the “Arrocera del Pirineo” company (personal communication), where rice from the study area is processed and analyzed.

Head rice yield was significantly higher in 2013 than in 2012 (Table 3.9). Meteorological conditions could be the cause for the higher values in 2013; the crop growth was delayed due to low temperatures in the month of May and flowering occurred later than in 2011 and 2012, and as a result, grain filling occurred later in the year, when the meteorological conditions were different. Temperature is an important factor affecting the filling grain phase (Krishnan et al., 2011; Tinarelli, 1989; Funaba et al. 2006) and this delay might have promoted better grain filling and consequently, a lower number of broken grains. Moreover, significant differences between years for gel consistency were noted. Again, meteorological conditions during grain filling could be the cause of these differences (Lin et al., 2010). Regardless of these differences between years, it can be concluded that fertilization strategies (PS or mineral fertilizer) did not influence head rice yield or cooking quality.

3.4.4. N use efficiency and N budget

For treatments near the optimum N rate, the AE_{NH4} values ranged between 16.7 and 25.4 $kg \cdot kg^{-1}$ (Table 3.4) and were in the range reported in a worldwide study (Ladha et al., 2005) with average AE_N values of 22.0 $kg \cdot kg^{-1}$ for rice. Other studies have also reported similar AE_N values for optimum treatments (Sun et al., 2012; Xie et al., 2007).

The RE_{NH4} values ranged between 0.17 and 0.35 kg·kg^{-1} for treatments near the optimum N rate (Table 3.4) and were lower than the average value of 0.46 kg·kg^{-1} reported by Ladha et al. (2005) for a rice crop. Sun et al. (2012) also showed higher values for the recovery efficiency (48 kg·kg^{-1} for the optimum N treatment) than those obtained in our study. However, other studies have shown similar results to those obtained in the present study; Xie et al. (2007) reported RE_N values around 30 kg·kg^{-1} for optimum treatments and Haefele et al. (2008) shown even lower values (between 13 and 17 kg·kg^{-1}). The low recovery efficiency is related to important N losses in the system.

There were no significant differences in AE_{NH4} or RE_{NH4} between fertilization strategies. PS120 and M120 (strategies with the same ammonium N rate applied before seeding) showed similar values (Table 3.10) and PS170 (strategy with a higher ammonium N rate) showed slightly lower values, although the differences were not significant. Therefore, the results suggest that crop uses the ammonium N contained in PS as efficiently as the provided by the mineral fertilizer. When both efficiencies were calculated considering total N (AE_{NT} and RE_{NT}), the efficiencies values lowered (Table 3.10). PS170 strategy showed the lowest values, PS120 strategy showed higher values than those obtained in the PS170 but lower than in M120, although the differences were only significant for the AE_{NT} (Table 3.10). These results show that the organic N contained in the PS is barely or not available for the crop.

The soil analysis at the 0-0.3 m depth in October 2013 (Figure 3.12) shows mineralization of the organic N in the PS plots; however, this mineralization probably took place after the plot was drained, when crop do not need N and this mineralized N is likely to be lost during the intercrop period. On the previous two sampling dates, May 2012 and May 2013, no significant differences were detected between the three fertilization strategies at any depth ($p>0.05$), indicating that if mineralization and nitrification took place, the mineral N was lost during the period from October to May and it was not available for the next growing season.

The results for the N budget show high N unaccounted values in all treatments, about 70% of the N applied for the optimum treatments (Figure 3.13). Among the most important pathways of these N losses are denitrification, ammonium volatilization, nitrate leaching or immobilization of the inorganic N (Antonopoulos, 2010; Cameron et al., 2013; Cassman et al., 1998; Xu et al., 2012). However, these N losses were independent of the N source when only ammonium N was considered for the calculation, as indicated by the analysis of variance between fertilization strategies, since PS120 and M120 strategies (both with the same ammonium N rate applied) did not differ significantly (Table 3.10). However, when total N was considered for the calculation, the N unaccounted in PS strategies was significantly higher than in M120 strategy.

The N budget results and nitrogen use efficiency results suggest that organic N present in PS is not taken up by rice in flooding conditions during the crop season and hence, it is a source of contamination because this N can be lost in the intercrop period.

Increased N use efficiencies (and lower N losses) could be sought by field-specific N management considering indigenous N resources, plant N status and N organic and inorganic fertilizers (Cassman et al., 1998) and real time N management with lower basal rates and a mineral topdressing complement as necessary (Nishikawa et al., 2012; Xie et al., 2007). It would be relevant in further studies to analyze in detail the effects of field specific N management (including N splitting) on N use efficiency and N losses in the system and effectiveness of tools, such as spectral information, for N recommendations at topdressing (Tubaña et al., 2012; Xie et al., 2007; Xue et al., 2014a).

3.5. CONCLUSIONS

This study shows that PS fertilization with a single rate, equivalent in terms of ammonium-N to crop N requirements (170 kg ammonium N·ha^{-1}), is an alternative to mineral N fertilization and ensures maximum rice yield. The PS fertilization does not affect seed germination, the presence of weeds, pests and diseases nor yield components or grain quality. Nitrogen fertilizer replacement value of PS represents in this experiment 87% (PS170) and 96% (PS120) of the PS ammonium-N applied, indicating that organic N is not taken up much by the crop during the year of application. Therefore, PS rates should be established considering the ammonium-N content of the PS.

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CAPÍTULO 4

GREENHOUSE GAS EMISSIONS AS AFFECTED BY FERTILIZATION TYPE (PIG SLURRY VS MINERAL) AND SOIL MANAGEMENT IN FLOODED RICE SYSTEMS IN NORTHEAST SPAIN

CAPÍTULO 4. GREENHOUSE GAS EMISSIONS AS AFFECTED BY FERTILIZATION TYPE (PIG SLURRY VS MINERAL) AND SOIL MANAGEMENT IN FLOODED RICE SYSTEMS IN NORTHEAST SPAIN

4.1. INTRODUCTION

Agriculture contributes to approximately 10-12 % of the total anthropogenic greenhouse gas (GHG) emissions (Smith et al., 2014); accounting for 60 % and 59 % of the total anthropogenic emissions of methane (CH_4) and nitrous oxide (N_2O), respectively (Ciais et al., 2013). Rice paddies are considered to be responsible of 11 % of the methane anthropogenic emissions (Ciais et al, 2013). Rice paddies also emit N_2O, however, methane emissions contribute to almost 90 % of the global warming potential (GWP) in flooded rice systems (Linquist et al., 2012b). Despite the low contribution of N_2O to GWP, both gases have to be considered together when mitigation practices are developed, since the mitigation practices focus on CH_4 emissions reduction tend to increase N_2O emissions (Cai et al., 1997; Lagomarsino et al., 2016; Zou et al., 2005).

Emission of nitrous oxide (N_2O) to the atmosphere from agricultural soils is mainly related to two biological processes. Nitrous oxide is formed in the nitrification process, as a result of the utilization of nitrite as an electron acceptor by the microorganisms (Wrage et al., 2001); and it is also formed as a result of the reduction of nitrate (NO_3^-) under anaerobic conditions (denitrification process) (Coyne, 2008).

Methane emission is a result of two opposite mechanisms, production and oxidation (Schütz et al., 1989b; Wassmann and Aulakh, 2000). The methane production (methanogenesis) involves the anaerobic degradation of organic matter, while methane oxidation (methanotrophy) is the process of CH_4 oxidation under aerobic conditions (Le Mer and Roger, 2001).

Agricultural soils are also a source of carbon dioxide (CO_2), which is emitted as a result of the decomposition of organic matter (Ponnamperuma, 1972) and respiration processes. However, only agricultural non-CO_2 sources are considered as anthropogenic GHG emissions because the CO_2 emitted is considered neutral due to annual cycles of carbon fixation and oxidation (Smith et al., 2014). Despite CO_2 from agriculture is not considered as anthropogenic GHG emission, practices to increase the soil organic carbon (SOC) diminish the atmospheric CO_2 concentration and thus, mitigate climate change, as well as increase fertility and health of soils (Goyal et al., 2006).

Rice flooded systems should be considered different from other cropping systems because in flooded conditions soil processes are dominated by the anaerobic conditions created under flooding (Buresh et al., 2008; Conrad, 1996) and therefore, denitrification and methanogenesis are two of the main processes taking place.

Fertilization is essential to obtain high rice yields, but fertilization may affect GHG emission (Linquist et al., 2012a). Moreover, when mineral fertilizers are replaced by organic amendments, the additional C source could enhance soil processes such as denitrification and methanogenesis (Coyne, 2008; Neue et al., 1996) and hence, the application of these products could imply higher GHG emissions in comparison to mineral fertilizers.

Studies focus on the influence of organic fertilizers in GHG emissions under flooded rice systems have been carried out in different parts of the word evaluating products such as straw (Bossio et al., 1999; Bronson et al., 1997; Zou et al., 2005), green manure (Aulakh et al., 2001; Denier van der Gon and Neue, 1995), pig manure (Liang et al., 2013), pig slurry (Maris et al., 2016) or anaerobically digested pig slurry (ADPS) (Win et al., 2014). Most of the studies found in the literature have focused on the effects of crop residues management and all of them agree that the incorporation of residues increases methane emissions (Bossio et al., 1999; Schütz et al., 1989a; Zou et al., 2005). However, regarding N_2O, the behavior is the opposite. Different studies have found that nitrous oxide emissions decrease when straw is incorporated to the rice field (Bronson et al., 1997; Wang et al., 2011) and the reason could be that the additional C input may stimulate the last step in the denitrification process, thus promoting N_2 formation rather than N_2O. Pig slurry (PS) is a different amendment, with a very different composition than straw, the C content of the straw is about 30 % (Tinarelli, 1989), while C content in PS is below 5 % (Yagüe, 2006), thus the effect of fertilizing with PS on GHG emissions is expected to be different than that with straw incorporation.

Spain is the largest pig producer in the European Union, with 19% of the total production (EUROSTAT, 2015), and more than half (51%) of the country’s pig production is concentrated in northeast Spain (Aragon and Catalonia regions, MAGRAMA, 2015a). The same region concentrates 25 % of the rice surface in Spain (MAGRAMA, 2015b). Farmers have traditionally fertilized rice with mineral fertilizers (urea and ammonium sulphate), but in the last years, they have started to include PS in the fertilization plans for this crop, initially due to the costs of mineral fertilizers and later because of the pressure to recycle the high amount of PS produced. There are few studies with focus on the effect of PS application to rice, in substitution of mineral fertilizers, on GHG emissions and moreover, results are not consistent. In Asia, Sasada et al. (2011) found no significant differences in CH_4 and N_2O emissions between plots fertilized with chemical fertilizer and with anaerobically digested pig slurry (ADPS). Win et al. (2014) reported cumulative methane emissions for the growing season 1.6 times higher for ADPS fertilized plots than for those with chemical fertilization, but with no significant differences, whilst no differences in N_2O fluxes were found between the two types of fertilizer. Huang et al. (2014) found significant increases in CH_4 emissions applying ADPS. Under Mediterranean rice conditions, only Maris et al. (2016) studied the application of PS to flooded rice and their results show no significant differences in GHG emissions and GWP for PS fertilization compared to ammonium fertilization.

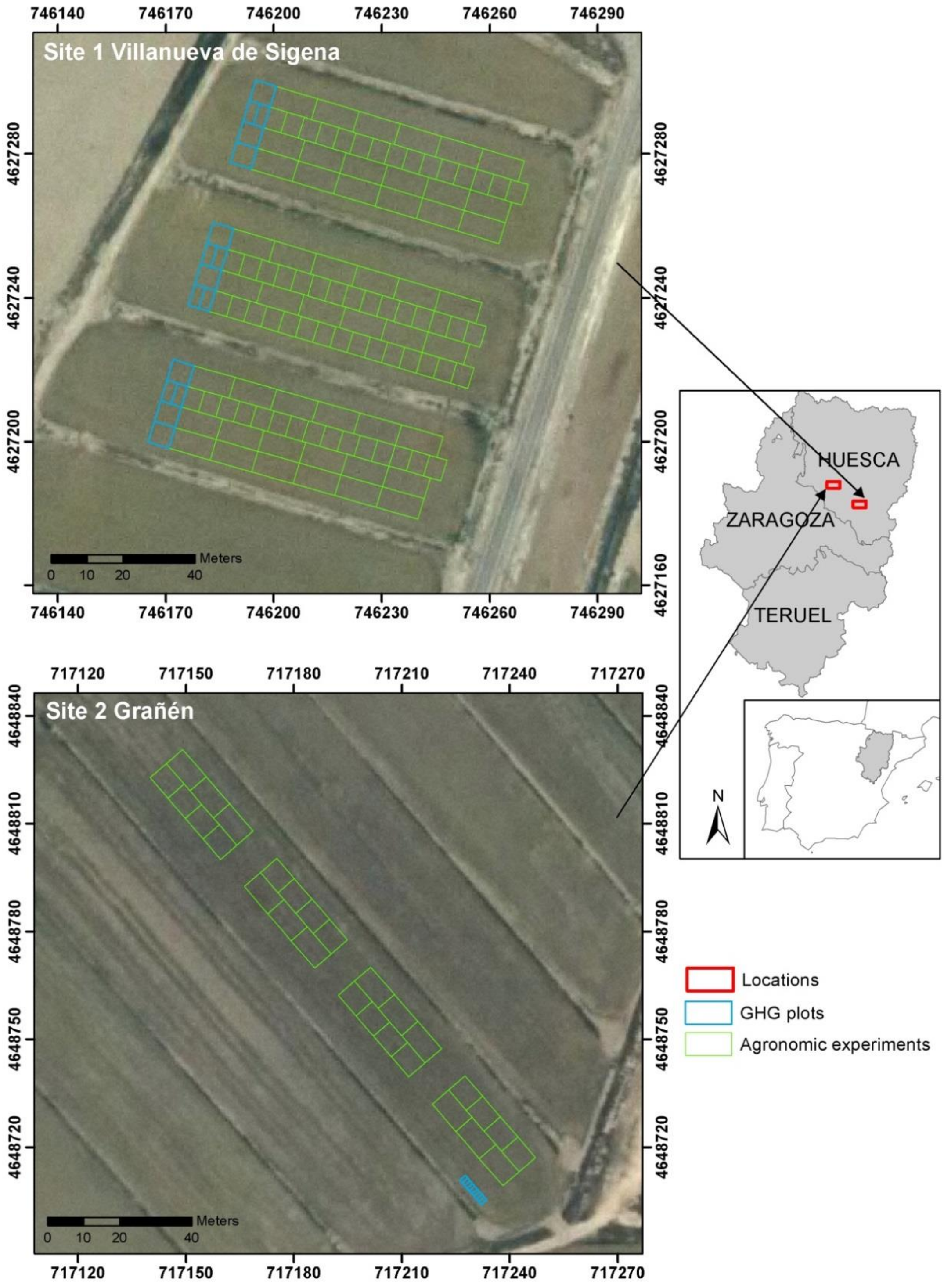
Our objective was to generate information on modification of GHG emissions due to the substitution of mineral fertilizers by PS in Mediterranean flooded rice cultivation conditions under optimal N fertilization. To achieve this objective, CH_4, N_2O and CO_2 emissions from the soil to the atmosphere were quantified during the whole crop season in two different (contrasted) soil types in northeast Spain. We hypothesized that, due to the PS low organic C content, global warming potential (GWP) under PS fertilization will not be higher than that under mineral fertilization.

4.2. MATERIALS AND METHODS

4.2.4. Sites description and experimental design

The study was carried out in two flooded rice fields in northeast Spain (Figure 4.1) with different soil characteristics and crop management practices

Site 1 located at Villanueva de Sigena was sampled in 2013 and site 2 located at Grañén (40 km from site 1) was sampled in 2014. The climate of the two experimental fields is semiarid continental Mediterranean, with high temperatures during the summer and low precipitation. The main climatic characteristics for both sites are detailed in Table 4.1.



Orthophoto PNOA 2015. Source: IGN (Instituto Geográfico Nacional)
UTM ETRS89 Zone 30N

Figure 4.1. Locations and experimental designs of the two experiments at Villanueva de Sigena and Grañen.

Table 4.1. Main site and soil characteristics in the 0-0.30 m soil depth at the beginning of the experiments at the two experimental sites.

Site and soil characteristics	**Site 1** **Villanueva de Sigena**	**Site 2** **Grañén**
Previous years growing rice	3	>15
Puddling	No	Yes
Latitude	41º 45' 31.87" N	41º 57' 29.97" N
Longitude	0º 2' 18.16" W	0º 22' 37.56" W
Elevation *(m)*	250	332
Annual precipitation *(mm)*†	347	334
Mean annual air temperature *(ºC)*†	14.6	13.5
Annual ET_0 *(mm)*†	1201	1194
pH *(1:2.5, water extract)*	8.5	8.3
Electrical conductivity of saturated paste extract (EC_e, $dS \cdot m^{-1}$)	0.8	4.9
Organic matter *(Walkley-Black; % dry matter)*	1.01	2.06
Calcium carbonate eq. *(% dry matter)*	29	24
Kjeldahl N *(% dry matter)*	0.08	0.17
NO_3^- ($mg \cdot kg^{-1}$ *dry soil)*	11.79	14.99
NH_4^+ ($mg \cdot kg^{-1}$ *dry soil)*	6.07	10.87
Olsen P ($mg \cdot kg^{-1}$ *dry soil)*	6	38.2
K *(ammonium acetate extract;* $mg \cdot kg^{-1}$ *dry soil)*	81	224
Na *(ammonium acetate extract;* $mg \cdot kg^{-1}$ *dry soil)*	27	581
Particle size distribution *(%)*		
Sand (2000-50 µm)	13.4	16.4
Silt (50-2 µm)	66.2	54.1
Clay (<2 µm)	20.4	29.5
USDA textural class	Silty loam	Silty clay loam

†Climatic data are average values over the last ten years

Site 1 was cultivated for rice in the previous three years with no puddling practices. Site 2 had been cropped to rice for more than 15 years with puddling tillage practices. In the puddling, plowing and harrowing are carried out at high soil water content with straw incorporation in order to destroy soil aggregates and create an impermeable layer. There are also differences in soil properties between the two sites, organic matter content, salinity ($EC_{e)}$, and the main nutrients content (N, P and K) are higher in site 2 than in site 1 (Table 4.1), and also soil clay content in site 2 is higher than in site 1.

The samplings in site 1 were carried out in the experimental field (Figure 4.1) described in chapter 3 of this document. Four fertilization treatments were selected to evaluate the effect of PS on GHG emissions.

The selected N fertilization treatments were (Table 4.2): a control with no N fertilization (C); M120M60, mineral treatment with a rate of 120 kg NH_4^+-N·ha^{-1} before seeding complemented with 60 kg N·ha^{-1} at topdressing; and 2 PS strategies, PS120M60: with a rate of PS equivalent to 120 kg NH_4^+-N·ha^{-1} before seeding complemented with 60 kg N·ha^{-1} (ammonium sulfate) at topdressing, and PS170M0: with a rate of PS equivalent to 170 kg NH_4^+-N·ha^{-1} before seeding and no topdressing N. In PS120M60, mineral N before seeding is replaced by PS; whilst in PS170M0 treatment, the crop N total requirements are covered by PS before seeding with no N mineral at topdressing.

Small plots specific for GHG emissions measurements were established in the header of the experiment described in chapter 3, in order not to disturb the experimental plots during GHG sampling (Figure 4.1). The experiment was arranged as a randomized block design with four replications and the plot size was 6 m x 6 m for PS plots and 6 m x 3 m for the control and mineral plots.

Table 4.2. N fertilization treatments in the two experimental sites. N rates for PS treatments are the actual N rates applied in the field.

		NH_4^+-N	**Organic N**	**Organic C**	**NH_4^+-N**	**NH_4^+-N**	**Total N**
		kg·ha^{-1}			*kg·ha^{-1}*	*kg·ha^{-1}*	
Site	**Treatments**	Before seeding			Topdressing	Growing season	
1	**Control (C)**	--	--	--	--	--	--
	M120M60	120†	--	--	60†	180	180
	PS120M60	109‡	92	1700	60†	169	261
	PS170M0	165‡	140	2582	--	165	305
2	**Control (C)**	--	--	--	--	--	--
	M170M0	170¥	--	--	--	170	170
	PS170M0	171‡	45	824	--	171	216

† Sulfate ammonium
‡ Pig slurry
¥ Urea

In site 2, sampling was performed in an agronomic experiment, with a split plot design with two main plots corresponding to the following treatments: PS170 with a rate of PS equivalent to 170 kg NH_4^+-N·ha^{-1} before seeding and M170 with a rate of 170 kg NH_4^+-N·ha^{-1} of mineral fertilizer (urea) before seeding. These N rates were combined with 3 different N rates at topdressing (0, 40 and 80 kg N·ha^{-1}). One control plot (C) without N application was added in each of the mineral main plots (M170).

Likewise in site 1, different N treatments were selected in order to evaluate GHG emissions. Selected treatments were (Table 4.2): control (C) with no N fertilization, PS170M0 with a rate of PS equivalent to 170 kg NH_4^+-N·ha^{-1} before seeding and no N at topdressing and M170M0 with a rate of 170 kg NH_4^+-N·ha^{-1} of mineral fertilizer (urea) before seeding and no N at topdressing. Likewise in site 1, small plots specific for GHG emissions measurements were established out of the main experimental design, in order not to disturb the experimental plots during GHG sampling (Figure 4.1). The experiment was arranged as a randomized block design with three replications and the plot size was 2 m x 1 m.

In both experiments, the selected treatments were considered to be optimum or near optimum N treatments (Moreno-García et al., 2017).

Pig slurry was collected from the closest fattening farm to each experimental field. PS application rates were established according to PS ammonium N concentration measured *in situ* by Quantofix® N-volumeter (Piccinini and Bortone, 1999) and conductimetry (Yagüe and Quílez, 2012). Pig slurry was band spread on soil surface; although machinery was calibrated before application in order to apply target rates, the slurry tank was weighed before and after application to know the actual PS rates applied (Table 4.2). Slurry samples were collected in the two sites for laboratory characterization (Table 4.3).

Table 4.3. Physico-chemical characteristics of the PS applied at each site.

	Site 1 **Villanueva de Sigena**	**Site 2** **Grañén**
Specific weight *(g·l^{-1})*	1045	1021
Dry matter *(kg·Mg^{-1})*	94	23
Organic C *(kg·Mg^{-1})*	47.63†	9.13
Ammonium N *(kg·Mg^{-1})*	3.05	1.89
Total N *(kg·Mg^{-1})*	5.63	2.39
P *(kg·Mg^{-1})*	1.79	0.13
K *(kg·Mg^{-1})*	3.07	1.68

† Organic C in site 1 was not measured and was estimated based on the C/N ratio obtained in site 2.

On the same day of PS application, the basal mineral N was applied to the mineral treatments together with P (100 kg P_2O_5·ha^{-1}) and K (100 kg K_2O·ha^{-1}) to ensure that these two nutrients were not limiting. Slurry and mineral fertilizers were incorporated into the soil in the afternoon of the same day.

For both experimental fields, typical land preparation was carried out by the farmer on April before fertilization, seeding and flooding. In both sites, rice straw and stubbles from the previous crop were incorporated to the soil; in site 2 during the puddling, and in site 1, when dry soil was ploughed. Rice (*Oryza sativa* L. spp *Japónica* cv. Guadiamar) was broadcast seeded once the field was flooded. Water was continuously flowing in and out of the plots. A water layer of 5 cm was maintained during the first days to improve rice germination; after that, a water layer of 10-15 cm was maintained until approximately one month before harvest, when fields were drained. Moreover, the fields were briefly drained for several days for the application of herbicides, pesticides and fungicides, according to habitual practices in the area. Topdressing N was applied on the water at the end of the tillering stage in site 1. The dates of the main labors and gas sampling are summarized in Table 4.4.

Table 4.4. Timing of field labors and gas sampling in each experimental site.

Management practice	**Site 1** **Villanueva de Sigena**	**Site 2** **Grañén**
Spring tillage	April, 2013	April, 2014
N fertilization application	May 9, 2013	May 13, 2014
Flooding	May 12, 2013	May 20, 2014
Seeding	May 15, 2013	May 22, 2014
Seed density *(kg·ha^{-1})*	180	200
Start of gas sampling	May 13, 2013	May 23, 2014
Topdressing fertilization	July 29, 2013	--
Drainage	September 14, 2013	October 1, 2013
Harvest	October 25, 2013	November 13, 2014
End of sampling	November 25, 2013	December 3, 2014

4.2.5. GHG measurements and analyses

The emissions of N_2O, CH_4 and CO_2 from the soil to the atmosphere were measured using the static no vented-chamber method (Figure 4.2a).

At the beginning of each experiment, one (site 1) or two (site 2) polyvinyl chloride collars (19.5 cm inner diameter) were inserted in each plot into the soil to a depth of 13 cm. Chambers, 37 cm height, were fitted into the collars at the time of sampling. Rice plants were cut inside the base since only emissions from the soil were measured.

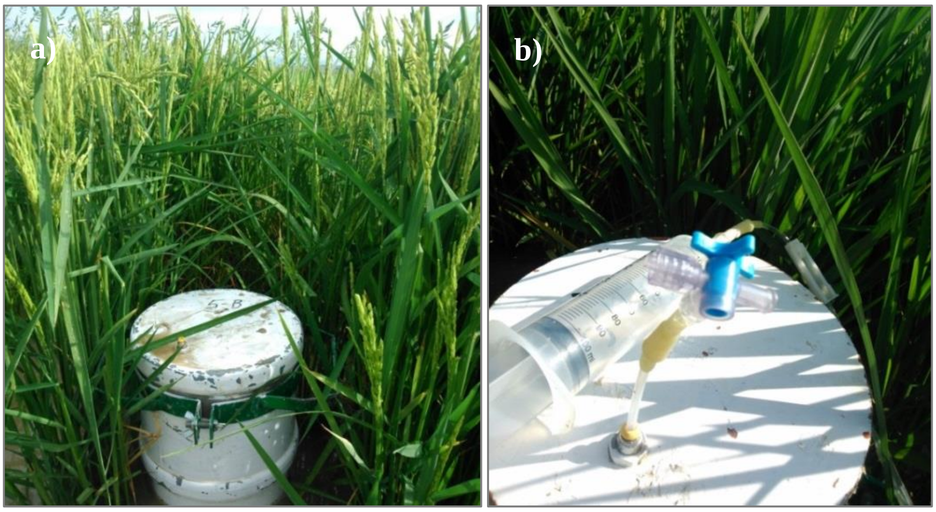


Figure 4.2. Soil gas sampling: the static closed chamber (a), a detailed of the syringe connected to the three-way key to withdraw the air sample (b).

The mechanisms of CH_4 emissions in a paddy soil are well known; there are three pathways: the diffusion through the soil and water, the ebullition as bubbles and the transport through the plant (Khalil and Shearer, 2006; Wassmann and Aulakh, 2000; Wassmann et al., 1993). The diffusion through the water is the least important process because of the low solubility of methane in water (Wassman et al., 1993). The ebullition has been reported to be the most important pathway during the vegetative period when plants are still below the water surface; while transport through the plant has been found to be more important as rice grows and become tall (Holzapfel-Pschorn and Seiler, 1986; Schütz et al., 1989b; Wassmann et al., 1996). Therefore, since in our study, plants were cut inside the collars inserted into the soil, ebullition was the main CH_4 emission pathway as there were no plants to mediate the transport, as happens early in the season. Similarly, nitrous oxide and carbon dioxide were emitted through ebullition and diffusion mechanisms; however, for these gases, the opportunities for the diffusion are higher than for methane due to their high solubility (20 times higher than solubility of methane) (Yu et al., 1997; ILO, 2016).

Samples were taken through a three-way valve placed on the top of the chamber and adjusted with a metal fitting. Gas samplings were performed every 7-10 days or sometimes more frequently when fields were eventually drained. Air samples were obtained through a Teflon ® tube connected to the three-way valve and into a 100 mL propylene syringe, adapted with a valve, at 0, 15, 30 and 45 min after closing the chamber (Figure 4.2b). The air inside the chamber was mixed by filling and emptying the syringe three times before withdrawing the sample. Once the sample was taken, the valve connected to the syringe was closed.

In site 1, 100 ml air samples were taken from the individual chamber installed in each plot; in site 2 (two chambers per plot), syringes were filled with 50 ml from each of the two chambers in each plot (composite sample). Replicate chambers are required to achieve reliable emission rates; however, a high number of gas samples can exceed analytical capacities; therefore, the methodology of composite samples was proposed to overcome this challenge (Arias-Navarro et al., 2013). In both sites, duplicate 100 mL syringes were taken in each plot per sampling time. Samples were transported to the laboratory and the concentrations of N_2O, CH_4 and CO_2 were quantified using the photoacoustic technique (Innova 1412i Photoacoustic Multigas Monitor, Figure 4.3). Average GHG concentrations in duplicates were used for the mass flux calculations (except if some issue was detected in the analyses).

Soil temperature in the uppermost 0.05 m and floodwater depth (to quantify chamber headspace volume) were measured at each sampling date.

Figure 4.3. Innova 1412i Photoacoustic Multigas Monitor, used to quantify the gas concentrations.

Emissions fluxes were calculated using the linear increase of the concentration inside the chamber over time, considering the headspace volume of chamber and correcting the values for air temperature obtained from the closest meteorological station to the experimental sites.

4.2.6. Soil and plant sampling and analyses

Soil (0-0.10m) was sampled in the 80 % of GHG sampling dates and moisture content and nitrate and ammonium concentration were determined. Soil extracts were prepared using 10 g fresh soil and 30 mL of KCl 2 N solution. Nitrate (AENOR, 1997) and ammonium (AENOR, 2005) concentrations were determined by colorimetry with a continuous flow analyzer (AutoAnalyzer3, Bran+Luebbe, Norderstedt, Germany).

Harvesting was carried out with a commercial combine on October 25, 2013 in site 1 and manually on November 13, 2014 in site 2. Grain humidity was measured (PM-600 grain moisture tester, Keller, Japan) and yield was adjusted to 14 % grain moisture content.

4.2.7. Calculations and statistical analysis

The cumulative emissions of N_2O, CH_4 and CO_2 for the studied period were quantified by integrating the emissions over time.

Despite large quantities of CO_2 are exchanged between the atmosphere and agricultural lands, the net flux is estimated to be approximately balanced, and it is estimated to contribute less than 1 % to the global warming potential; therefore, only agricultural non-CO_2 sources are reported as anthropogenic GHG emissions (Smith et al., 2007; 2014). For this reason, carbon dioxide flux was not considered in the calculation of global warming potential (GWP). The GWP of N_2O and CH_4 emissions as CO_2 equivalents (CO_2 eq) over a 100 year horizon time was calculated using a radiative forcing potential relative to CO_2 of 265 for N_2O and 28 for CH_4 (Myhre et al., 2013), by using this equation:

$$GWP(kg\ CO_2 - eq \cdot ha^{-1}) = Cumulative\ N_2O\ emissions * 265 + Cumulative\ CH_4\ emissions * 28$$

The yield-scaled GWP (GWP_Y) was calculated as the ratio between GWP (kg CO_2 eq·ha^{-1}) and the grain yield (kg grain yield·ha^{-1}). The yield values used for calculating the GWP_Y were the average values of each treatment of the agronomic experiments; these values were considered more representative of yield since experimental plots for GHG sampling were small-sized.

Fertilizer-induced N_2O emission factor (EF) was calculated as the difference between the N_2O emissions during the studied period from the fertilized plots and the control plot divided by the amount of the N applied in the fertilized plots. For pig slurry treatments, EF was calculated considering slurry ammonium N and total N concentrations.

Effects of treatments and sampling dates on GHG fluxes and soil ammonium and nitrate concentrations were evaluated by repeated measures analysis of variance (ANOVA). Effect of treatments on GHG cumulative emissions, GWP, yield, yield-scaled GWP and EF was evaluated by analysis of variance. When the analysis was significant, comparison among the treatment means were performed using Tukey multiple range test at $p=0.05$ (SAS 9.4 Software). When necessary, data were log transformed prior to analysis in order to fulfill ANOVA assumptions (homogeneity of variance and normality). A student-t test ($p=0.05$) was carried out to determine whether emission factors (EF) were significantly different than 0.

Relation between GHG fluxes and soil temperature, soil moisture and soil ammonium and nitrate concentrations was tested by correlation analysis, when necessary regression and multiple regression models were used.

4.3. RESULTS

4.3.1. Meteorological conditions and drainage of the plots

Rainfall and air temperature during the sampling period are reported in Figure 4.4a and Figure 4.5a and were obtained from the closest meteorological station to the experimental site (Red SIAR, site 1 Alcolea de Cinca Station and site 2 Grañén Station). Both sites show similar meteorological conditions, with low precipitation during the summer and high temperatures. The average air temperature in August was 23.9 ºC and 22.9 ºC in site 1 and site 2, respectively. The soil temperatures measured at the sampling dates were similar to the daily average air temperatures. In both sites, high rainfall events during fall were registered.

Puddling was conducted in site 2 in contrast to site 1. When puddling is conducted, an impermeable layer (plough pan) which stops percolation is created. This plough pan controls vertical water losses towards the subsoil and it has been found to be less permeable for the older and more developed paddy soils as compared to young paddy fields (Janssen and Lennartz, 2007; Lennartz et al., 2009). This fact combined with the different soil particle size distribution, site 2 has a higher clay percentage than site 1 (Table 4.1), are responsible for the differences in the drainage speed once irrigation stopped (final drainage to harvest). Site 1 drained rapidly, whilst site 2 remained flooded much longer after irrigation was stopped. In site 1, water inflow was closed on September 14, 2013 and the floodwater disappeared one week later (Figure 4.4a); however, in site 2, the water inflow was closed on October 1, 2014 and the field remained flooded for almost 1 month; also, a 40 mm rainfall event on November 29, 2014, flooded again the field (Figure 4.5a).

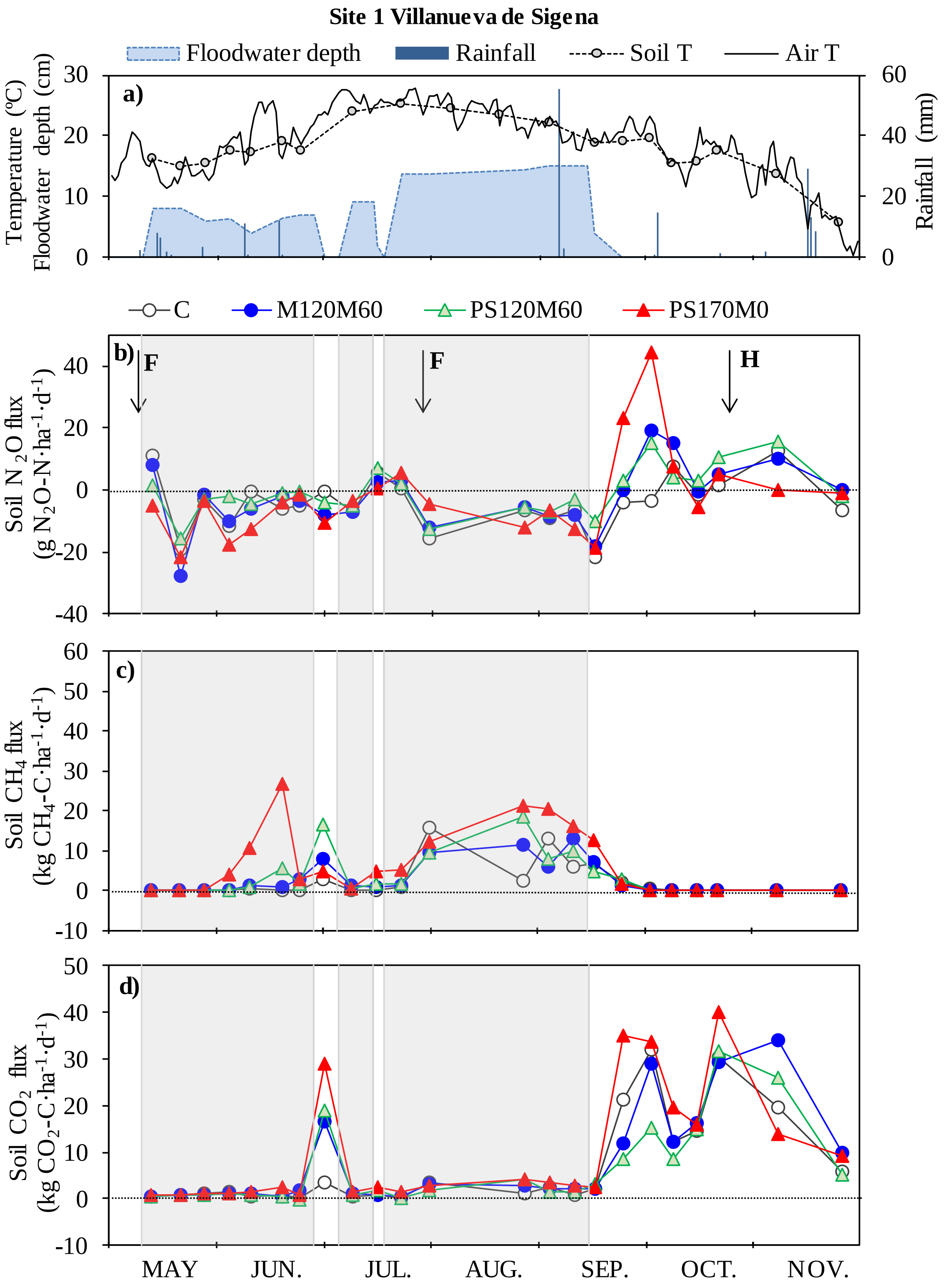


Figure 4.4. Site 1 (Villanueva de Sigena). Soil and air temperature, rainfall and floodwater depth during the studied period (a), N_2O (b), CH_4 (c) and CO_2 (d) emissions as affected by the fertilization treatment. Vertical arrows indicate the dates of fertilization applications (F) and harvest (H). The grey shaded areas represent the periods in which the water inflow to the plot was open. Note that once water was stopped, the field remained flooded for several days.

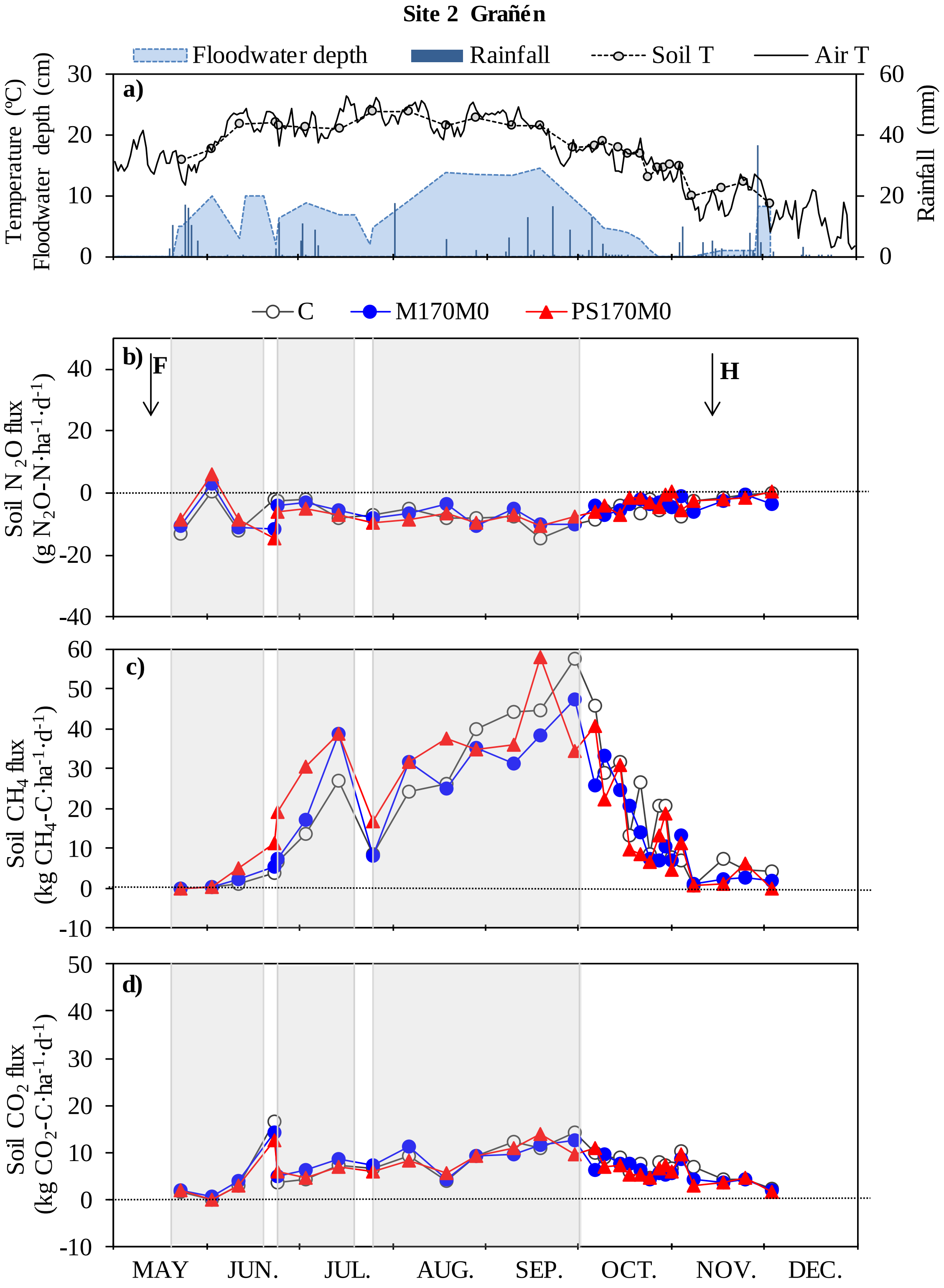


Figure 4.5. Site 2 (Grañén). Soil and air temperature, rainfall and floodwater depth during the studied period (a), N_2O (b), CH_4 (c) and CO_2 (d) emissions as affected by the fertilization treatments. Vertical arrows indicate the dates of fertilization applications (F) and harvest (H). The grey shaded areas represent the periods in which the water inflow to the plot was open. Note that once water was stopped, the field remained flooded for several days.

4.3.2. Nitrous oxide fluxes, cumulative emissions and N emission factor

At site 1, N_2O fluxes ranged from -27.5 to 44.4 g N_2O-N $ha^{-1}\cdot day^{-1}$ and the different N treatments showed similar patterns (Figure 4.4b). No differences between fertilization treatments were noted in the mean values of N_2O fluxes (Table 4.5). The fluxes were negative or close to 0 until the final drainage of the plot, when N_2O emissions became positive in some cases (Figure 4.4b).

When integrating all the sampling period, N_2O cumulative emissions were not significantly different between fertilization treatments (Table 4.5).

The emission factor (EF) ranged between 0.12 % (PS170M0) and 0.39 % (PS120M60) of the N applied when PS ammonium N was considered for the calculation (Table 4.5, Figure 4.6) and from 0.07 to 0.14 % when PS total N was considered. No significant differences in EF between fertilization treatments were found. Moreover, the student t-test showed that EF values were not different than 0 in any of the fertilization treatments ($p>0.05$).

At site 2, N_2O fluxes ranged between -14.7 and 6.3 g N_2O-N $ha^{-1}\cdot day^{-1}$ and as happened at site 1, the different N treatments showed similar patterns (Figure 4.5b) and average fluxes were not affected by treatments (Table 4.6). Although it was observed an increase of N_2O fluxes after the drainage to harvest (the fluxes were negative but lower in absolute value, i.e. consumption of N_2O was lower than previously), the fluxes remained negative for the whole studied period unlike site 1 (Figure 4.5b).

Nitrous oxide cumulative emissions in the study period, negative for the three treatments, did not show significant differences among fertilization treatments in the same manner as in site 1 (Table 4.6).

Significant differences were not detected either in the EF of the two fertilization treatments (M170M0 and PS170M0) with a value of 0.06 % of the N applied (Table 4.6, Figure 4.6). This value decreased to 0.05 % when total N concentration was considered for the PS treatment. As in site 1, values did not differ from 0 ($p>0.05$) in any of the studied treatments.

Table 4.5. Site 1 (Villanueva de Sigena). Average soil (0-10 cm) mineral nitrogen concentration (NO_3^--N and NH_4^+-N), average fluxes of N_2O, CH_4 and CO_2, cumulative N_2O, CH_4 and CO_2 emissions during the studied period, Global Warming Potential (GWP), Grain yield (at 14 % moisture content), Yield scaled GWP (GWP_Y), emission factor for the N applied (EF) (for PS treatments considering slurry ammonium N content (NH_4-N) and total N content (NT)) for the different N fertilization treatments, indicating the effects of Treatment (T), date of sampling (D) and their interaction (T x D).

	Soil Nitrogen		**Gas Fluxes**			**Cumulative emissions**			**GWP**	**Grain yield**	**GWP_Y**	**EF NH_4-N**	**EF NT**
	NO_3^--N	**NH_4^+-N**	**N_2O-N**	**CH_4-C**	**CO_2-C**	**N_2O-N**	**CH_4-C**	**CO_2-C**					
	$mg \cdot kg$ dry $soil^{-1}$		*$g \cdot ha^{-1} \cdot d^{-1}$*	*$kg \cdot ha^{-1} \cdot d^{-1}$*		*$kg \cdot ha^{-1}$*	*$kg \cdot ha^{-1}$*		*$Mg\ CO_2$-eq$\cdot$ $\cdot ha^{-1}$*	*$kg \cdot ha^{-1}$*	*$kg\ CO_2\ eq \cdot$ $\cdot kg\ grain^{-1}$*	*%*	
Treatment (T)	n.s.	***	n.s	***	n.s.	n.s.	n.s.	n.s.	n.s.	**	n.s.	n.s.	n.s.
Control (C)	3.22	8.60 b	-3.92	2.47 b	6.76	-0.71	549.1	1571.0	20.24	2220b	9.12		
M120M60	3.55	13.09 a	-2.33	2.99 b	7.87	-0.45	665.1	1959.1	24.69	5241 a	4.71	0.14	0.14
PS120M60	3.45	11.51 a	-0.45	3.52 b	6.52	-0.04	869.9	1619.6	32.52	5187 a	6.27	0.39	0.25
PS170M0	3.66	12.80 a	-3.24	6.31 a	9.15	-0.51	1336.4	2036.1	49.77	5705 a	8.72	0.12	0.07
Date (D)	***	***	***	***	***								
T x D	**	**	n.s.	n.s.	n.s.								

n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Different letters in the same column indicate significant differences ($p<0.05$) between treatments.

Table 4.6. Site 2 (Grañén). Average soil (0-10 cm) mineral nitrogen concentration (NO_3^--N and NH_4^+-N), average fluxes of N_2O, CH_4 and CO_2, cumulative N_2O, CH_4 and CO_2 emissions during the studied period, Global Warming Potential (GWP), Grain yield (at 14 % moisture content), Yield scaled GWP (GWP_Y), emission factor for the N applied (EF) (for PS treatments considering slurry ammonium N content (NH_4-N) and total N content (NT)) for the different N fertilization treatments, indicating the effects of Treatment (T), date of sampling (D) and their interaction (T x D).

	Soil Nitrogen		**Gas Fluxes**			**Cumulative emissions**			**GWP**	**Grain yield**	**GWP_Y**	**EF NH_4-N**	**EF NT**
	NO_3^--N	**NH_4^+-N**	**N_2O-N**	**CH_4-C**	**CO_2-C**	**N_2O-N**	**CH_4-C**	**CO_2-C**					
	$mg \cdot kg$ dry soil^{-1}		*$g \cdot ha^{-1} \cdot d^{-1}$*	*$kg \cdot ha^{-1} \cdot d^{-1}$*		*$kg \cdot ha^{-1}$*	*$kg \cdot ha^{-1}$*		*Mg CO_2-eq$\cdot$ $\cdot ha^{-1}$*	*$kg \cdot ha^{-1}$*	*kg CO_2 eq$\cdot$ $\cdot$kg grain^{-1}*	*%*	
Treatment (T)	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.
Control (C)	27.89	10.49	-5.45	18.81	7.04	-1.20	3986.8	1329.1	148.60	5766ab	25.77		
M170M0	32.64	10.58	-5.20	16.43	6.68	-1.09	3701.9	1330.7	137.99	4554 b	30.30	0.06	0.06
PS170M0	33.53	10.19	-5.05	18.92	6.45	-1.10	4326.3	1254.1	161.34	7012a	23.01	0.06	0.05
Date (D)	***	***	***	***	***								
T x D	***	n.s.	n.s.	n.s.	n.s.								

n.s.: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$.

Different letters in the same column indicate significant differences ($p<0.05$) between treatments.

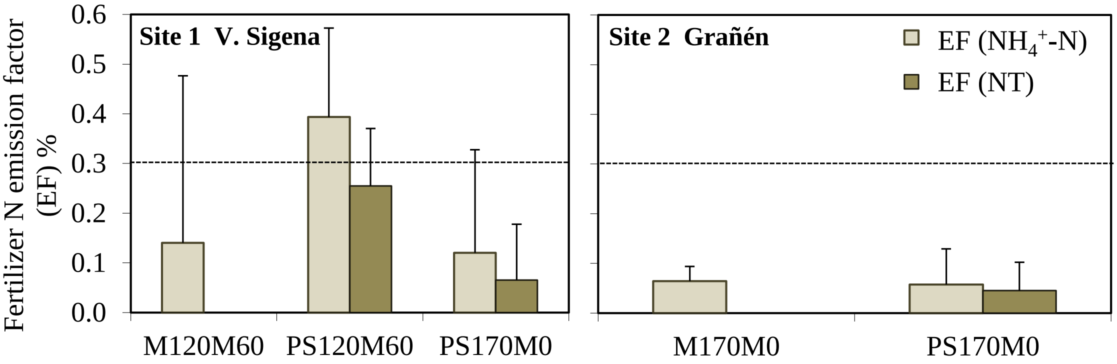


Figure 4.6. Emission factor of the N applied as a fertilizer for the studied period in site 1 and site 2. Error bars represent the standard error of the four replicates (site 1) or three replicates (site 2). Dash line at 0.3 % represents the default value adopted by de IPCC for rice systems (IPCC, 2006). For the PS treatments, EF has been calculated considering the slurry N-NH_4 content and the total N content.

4.3.3. Methane fluxes and cumulative emissions

At site 1, CH_4 fluxes varied from -0.1 to 26.6 kg CH_4-C $ha^{-1}\cdot day^{-1}$ (Figure 4.4c). N fertilization affected the average CH_4 fluxes for the studied period, with a greater mean CH_4 flux for the PS170M0 treatment (Table 4.5). However, no differences in CH_4 fluxes between the M120M60 and PS120M60 (treatments with equivalent N rates applied before seeding) were found.

Overall, the highest emissions were observed at the end of the August (at heading stage); but for the PS170M0 treatment, high emissions were also observed at the beginning of the season (with a peak on June 19). Also, an emission peak occurred in all treatments during a short drainage of the experiment for an herbicide treatment (July 1). Once the field was drained, CH_4 emissions decrease dramatically reaching values lower than 100 g CH_4-C $ha^{-1}\cdot day^{-1}$ (Figure 4.4c).

CH_4 cumulative emissions in the study period in site 1 did not show significant differences among the fertilization treatments (Table 4.5), but the PS170M0 treatment showed higher (although not significant) CH_4 emission than the other three treatments.

At site 2, CH_4 fluxes were higher than at site 1. The values ranged from 0.01 to 57.8 kg CH_4-C $ha^{-1}\cdot day^{-1}$ (Figure 4.5c). The CH_4 fluxes increased over time during the growing season except during a brief drainage episode (July 25). Maximum CH_4 fluxes were reached on late September, during the rice ripening phase and just before the plot was drained. CH_4 emissions decreased after the beginning of final drainage; however, in contrast to site 1, CH_4 fluxes decreased slower and did not reach values lower than 1 kg CH_4-C $ha^{-1}\cdot day^{-1}$. Contrary to site 1 results, N treatments had no significant effect on the average CH_4 fluxes (Table 4.6) and neither on cumulative CH_4 emission during the sampling period (Table 4.6).

4.3.4. Carbon dioxide fluxes and cumulative emissions

In site 1, CO_2 fluxes ranged between -0.10 and 40 kg CO_2-C $ha^{-1}\cdot day^{-1}$ (Figure 4.4d). The average CO_2 fluxes did not show differences among N fertilization treatments; however a higher (although no significant) average flux was observed in PS170M0 treatment (Table 4.6). The evolution of the CO_2 over time (Figure 4.4d) shows CO_2 emissions lower than 5 kg CO_2-C $ha^{-1}\cdot day^{-1}$ until plot was drained to harvest (with the exception of an emission peak on July 1 during a short drainage). Once the field was drainage on mid-September, CO_2 emissions increased rapidly.

At site 2, CO_2 emissions varied from -0.10 to 16.7 kg CO_2-C $ha^{-1}\cdot day^{-1}$ (Figure 4.5d) and N fertilization treatments did not affect the average CO_2 flux (Table 4.6). During the flooded period, CO_2 fluxes in site 2 were higher than in site 1, with a small emission peak during a short drainage period. CO_2 fluxes decreased slightly in site 2 when field was drained for harvest in contraposition to site 1 where CO_2 fluxes increased rapidly after drainage started.

CO_2 cumulative emissions for the studied period did not show significant differences among fertilization treatments neither in site 1 (Table 4.5) nor in site 2 (Table 4.6).

4.3.5. Soil ammonium and nitrate concentration

At site 1, soil (0-0.1 m) nitrate concentration ranged from 0.1 to 18.4 mg NO_3^--N·kg dry $soil^{-1}$ (Figure 4.7a). At the beginning of the experiment, when the plot was flooded, the nitrate content decreased reaching values lower than 1 $mg\cdot kg^{-1}$. In early-June, there was an increment in all treatments associated to a shallow floodwater depth (Figure 4.4a), but immediately, the nitrate content lowered again. After that, the values remained below 1 mg NO_3^--N·kg dry $soil^{-1}$ until the field was drained (Sept. 14), then nitrate content started to increase steadily (Figure 4.7a). Average soil nitrate concentrations during the studied period did not show significant differences between N fertilization treatments (Table 4.5).

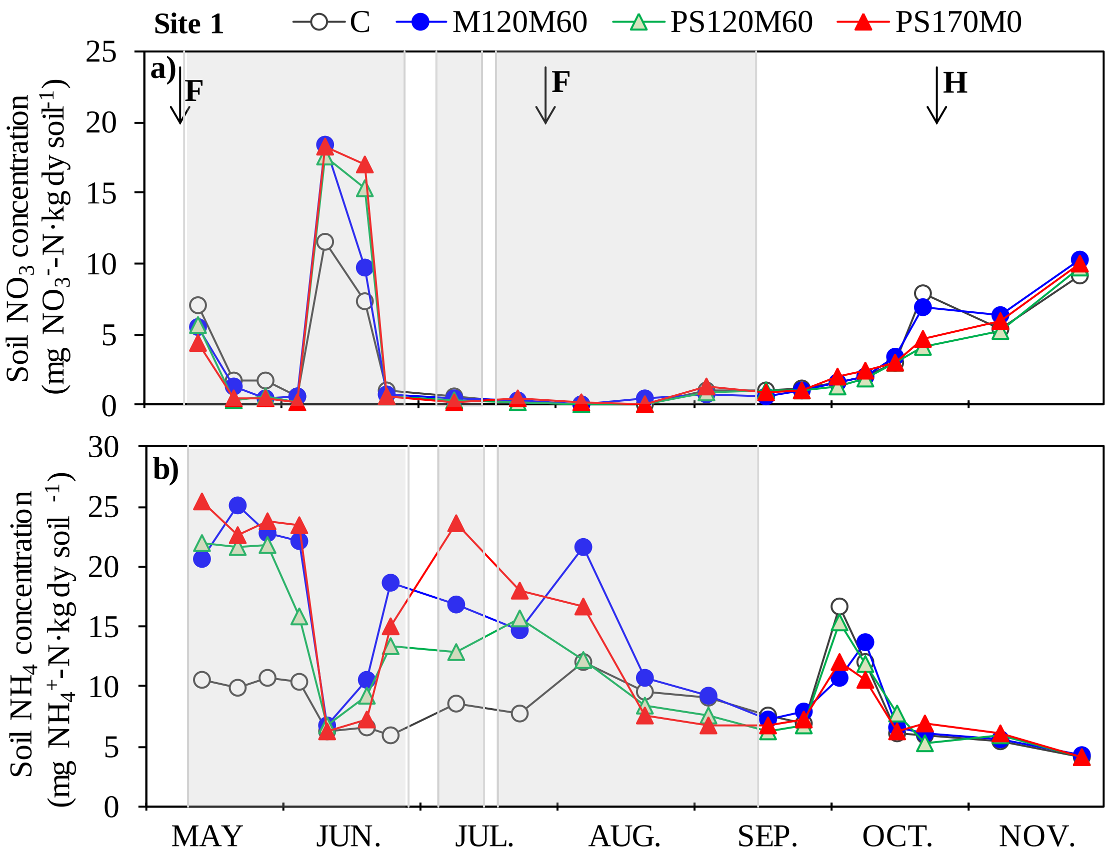


Figure 4.7. Site 1 (Villanueva de Sigena). Soil (0-10 cm) nitrate (a) and ammonium concentration (b) during the studied period as affected by the fertilization treatment. Vertical arrows indicate the dates of fertilization applications (F) and harvest (H). The grey shaded areas represent the periods in which the water inflow to the plot was open.

At site 1, soil ammonium concentration ranged between 4.1 and 25.4 kg NH_4^+-N·kg dry soil^{-1} (Figure 4.7b), and was affected by the fertilization treatments (Table 4.5): average ammonium concentration value in the control treatment presented significant differences with the other three treatments M120M60, PS120M60 and PS170M0. Soil ammonium concentration was high in the first sampling dates in treatments with N application (M120M60, PS120M60, PS170M0), then decreased in early-June (Figure 4.7b) at the same time that nitrate concentration increased (Figure 4.7a) and after that, ammonium increased again at the same time that nitrate decreased. From July until the plot was drained, ammonium concentration decreased gradually. Once the field was drained, a slightly increase was observed, but again NH_4 lowered (Figure 4.7b), coinciding with an increasing of nitrate concentration (Figure 4.7a).

A significant treatment (T) x date (D) interaction was observed for both ammonium and nitrate concentration (Table 4.5). This interaction was due to the oscillations in the ammonium and nitrate concentration values over time, rather than to a differential effect of the treatment on the N mineral concentration in the soil depending on the date of sampling. Thus, ANOVA for each date is not shown because it does not provide any additional information.

At site 2, soil (0-0.10 m) nitrate concentration ranged from 4.5 to 125.7 mg NO_3-N·kg dry soil^{-1} (Figure 4.8a). Nitrate concentrations were high in the first date in M170M0 and PS170M0 treatments, but decreased rapidly. After that and during the whole studied period, soil nitrate concentrations showed fluctuations (varying between 4.5 and 62.5 mg NO_3-N·kg dry soil^{-1}). Significant differences among N fertilization treatments were not found in the average values of soil nitrate concentration (Table 4.6). A significant treatment (T) x date (D) interaction was also observed for nitrate concentration (Table 4.6); but likewise in site 1, this interaction was no qualitative and due to the oscillations in the nitrate concentration over time.

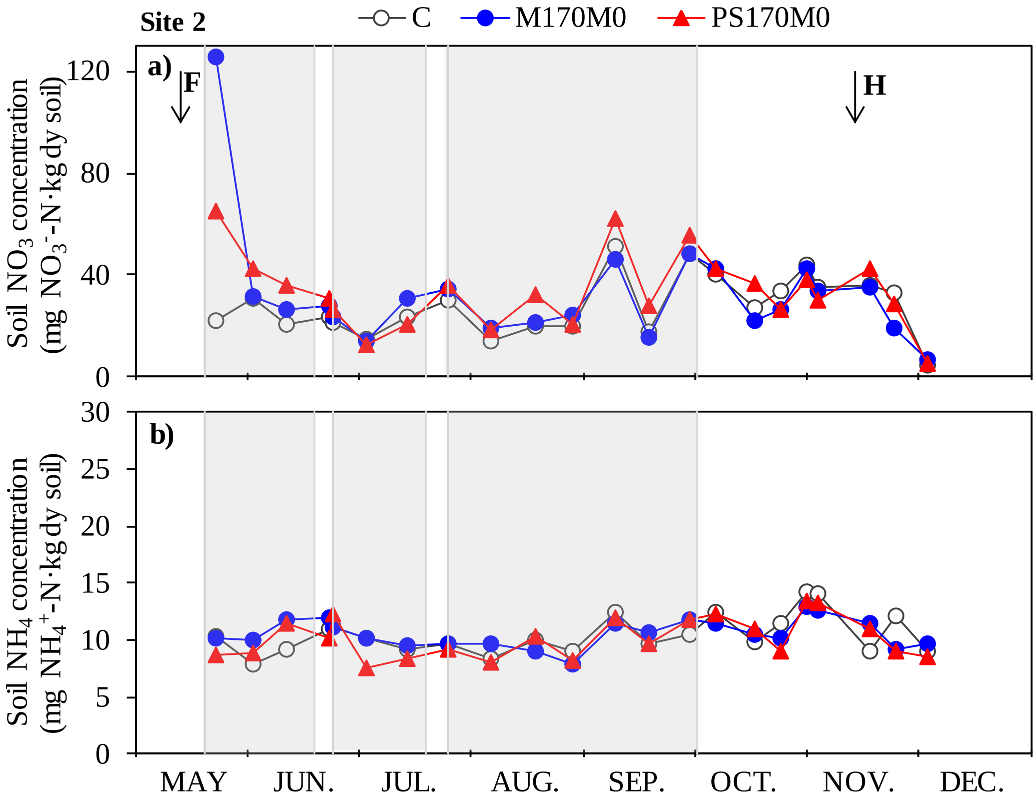


Figure 4.8. Site 2 (Grañén). Soil (0-10 cm) nitrate (a) and ammonium concentration (b) during the studied period as affected by the fertilization treatment. Vertical arrows indicate the dates of fertilization applications (F) and harvest (H). The grey shaded areas represent the periods in which the water inflow to the plot was open.

At site 2, soil ammonium concentration varied between 7.6 to 14.2 mg NH_4-N·kg dry soil^{-1} (Figure 4.8b). The Figure 4.8b shows oscillations over time, but the three treatments showed the same pattern. No significant differences between N fertilization treatments were observed in the average soil ammonium concentrations (Table 4.6).

4.3.6. Relationships between gas fluxes and soil parameters

At site 1, it was a significant negative relationship between N_2O and CO_2 fluxes and soil moisture for moisture values lower than a critical value; however, for moisture content higher than this critical value, no significant relationship was observed (Figure 4.9). Moisture content critical value was estimated to be approximately equal for both gasses (28.5 % for N_2O and 28.1 % for CO_2).

Multiple regressions of N_2O and CO_2 fluxes versus the variables: soil moisture, soil temperature, nitrate and ammonium concentration were clearly dependent on moisture content and the effect of the other variables did not provide additional significant information.

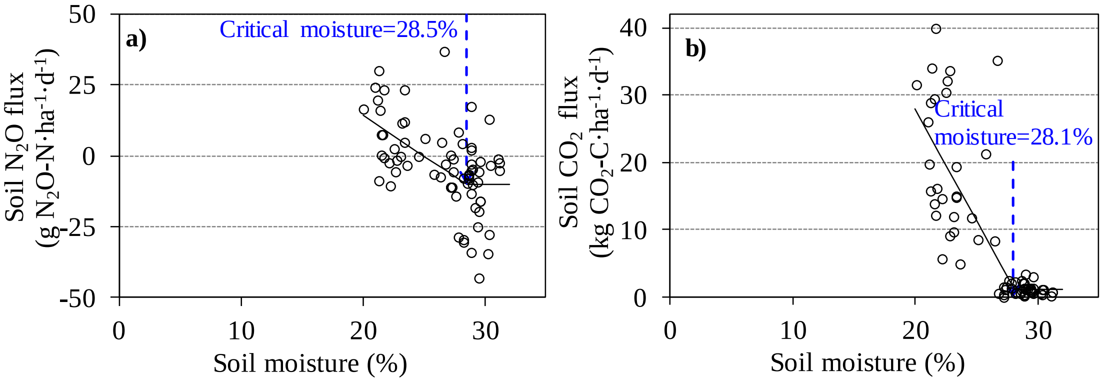


Figure 4.9. Site 1 (Villanueva de Sigena). Relationships between soil N_2O (a) and CO_2 (b) fluxes and soil moisture content. Points represent average values of each treatment for each date.

In contraposition, CH_4 fluxes showed no significant relationship with soil moisture content (data not shown). However, a significant relationship was observed between CH_4 fluxes and soil temperature (Figure 4.10).

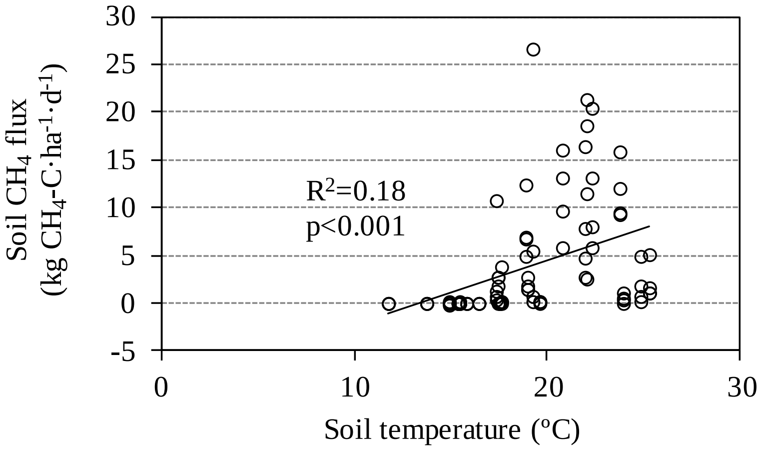


Figure 4.10. Site 1 (Villanueva de Sigena). Relationships between soil CH_4 flux and soil temperature. Points represent average values of each treatment for each date.

At site 2, N_2O, CO_2 (Figure 4.11) and CH_4 fluxes did not show significant relationships with soil moisture content.

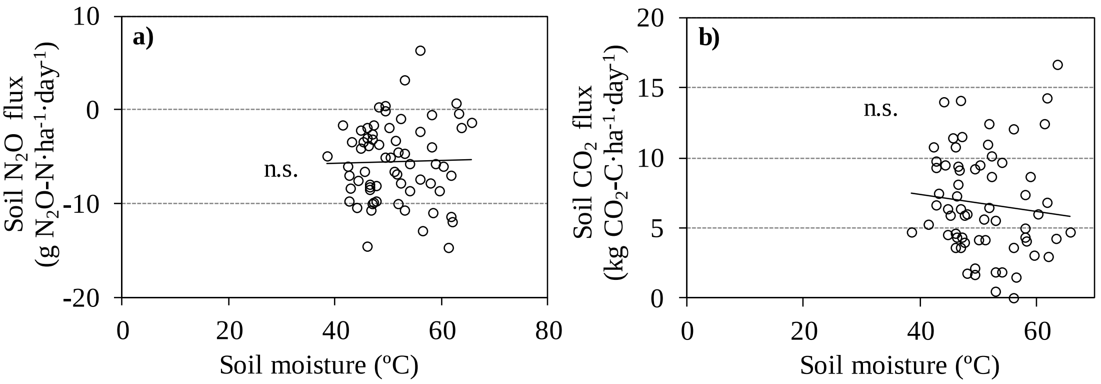


Figure 4.11. Site 2 (Grañén). Relationships between soil N_2O (a) and CO_2 (b) fluxes and soil moisture content. Points represent average values of each treatment for each date. Note that the scale of x-axis is different from Figure 4.9.

However, significant relationships were found between the three gasses and soil temperature (Figure 4.12). The relationship was negative for the N_2O (Figure 4.12a) and positive for CH_4 and CO_2 (Figure 4.12b and c)

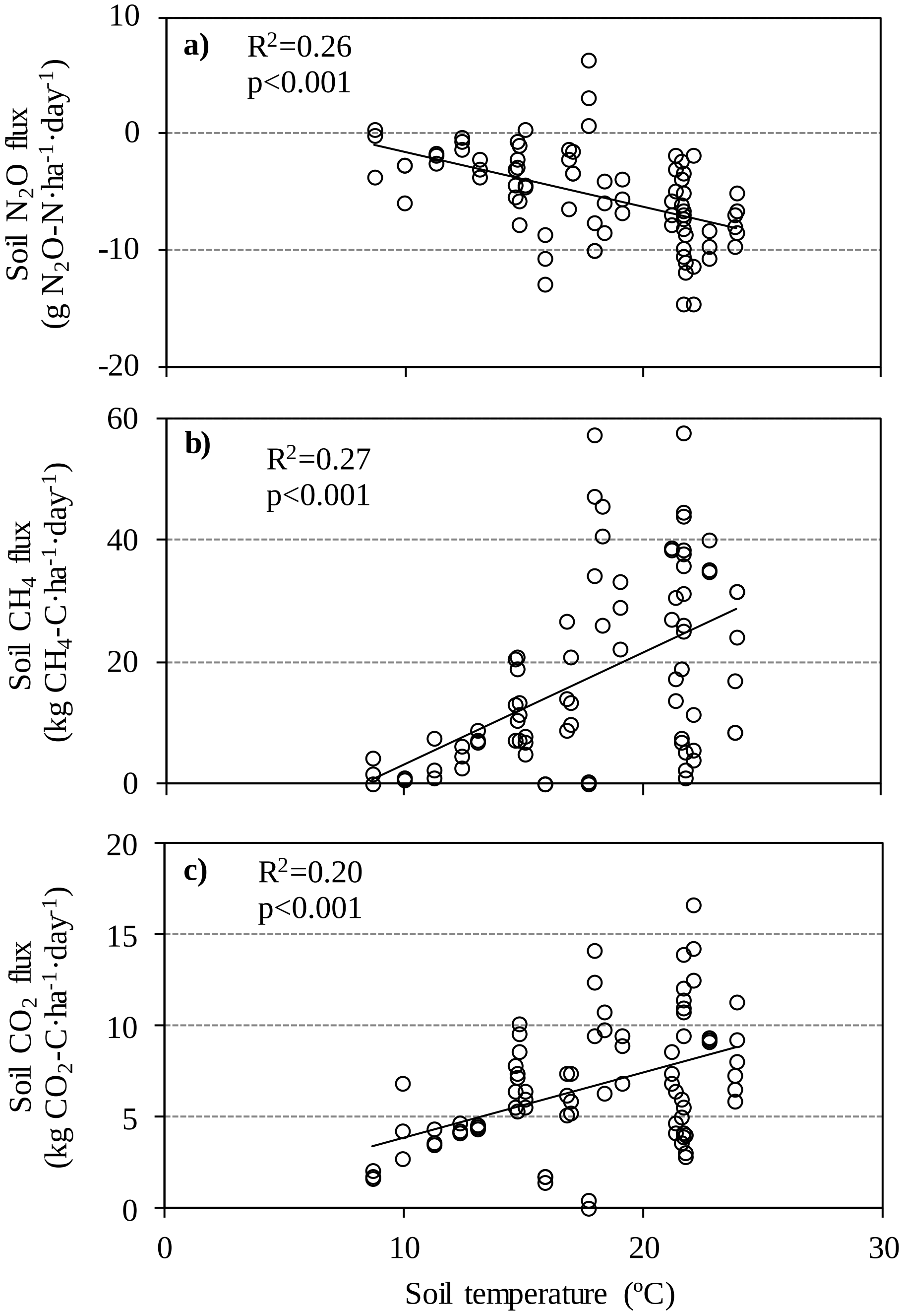


Figure 4.12. Site 2 (Grañen). Linear relationships between soil N_2O (a), CH_4 (b) and CO_2 (c) fluxes and soil temperature in site 2. Points represent average values of each treatment for each date.

4.3.7. Global warming potential (GWP) and yield-scaled GWP (GWP_Y)

In site 1, although GWP did not show significant differences among treatments (Table 4.5, Figure 4.13), the PS170M0 treatment showed higher GWP than the other three treatments. Grain yield for the control treatment was lower and significantly different from the other treatments (Table 4.5). When GWP was relativized considering grain yield (yield-scaled GWP, GWP_Y), significant differences among N fertilization treatments were not found; but the control treatment presented higher GWP_Y than PS170M0 (Table 4.5, Figure 4.13).

In site 2, GWP was higher than in site 1 due to the higher fluxes of methane, but there were not observed significant differences between the treatments (Table 4.6, Figure 4.13). Despite the control treatment did not receive N, grain yield in this treatment was not significantly different from the PS170M0 treatment (Table 4.6) because of the high N content in the soil at the beginning of the experiment (Table 4.1). However, yield in the M170M0 treatment was lower and significantly different than in the PS170M0, due to high *Pyricularia* affection in M170M0. GWP_Y was the highest for M170M0 (30 units) due to the low grain yield in this treatment and the lowest for PS170M0 (23 units), but no significant differences were found among treatments (Table 4.6, Figure 4.13).

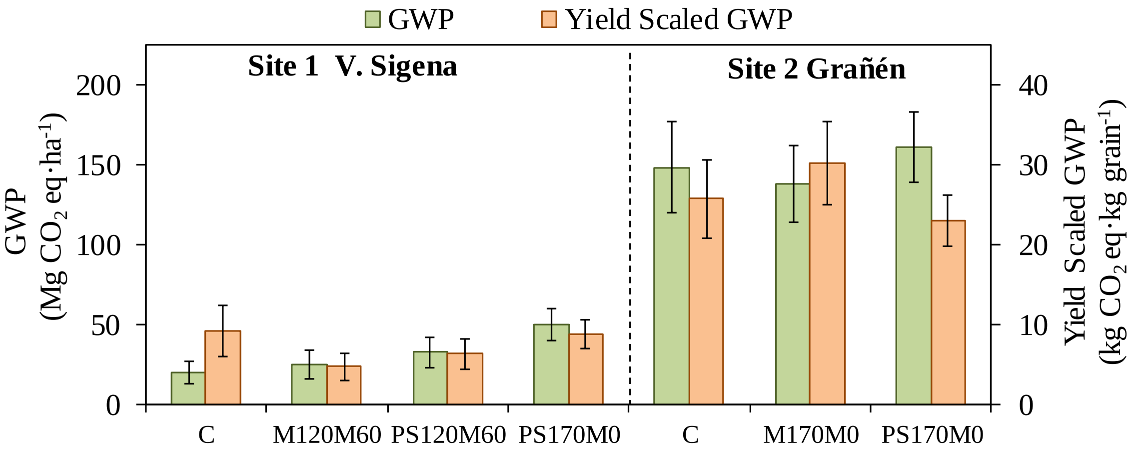


Figure 4.13. Global warming potential (GWP) of CH_4 and N_2O emissions and yield-scaled GWP during the studied period in the different treatments in site 1 and site 2. Error bars represent the standard error of the four replicates (site 1) or three replicates (site 2).

4.4. DISCUSSION

4.4.1. Nitrous oxide emissions, N soil concentration and N emission factor

N_2O emissions ranged from -27.5 to 44.4 g N_2O-N $ha^{-1}\cdot day^{-1}$ in site 1 (Figure 4.4b) and from -14.7 to 6.3 g N_2O-N $ha^{-1}\cdot day^{-1}$ in site 2 (Figure 4.5b).

In site 1, there was N_2O consumption during the crop season until the field was drained (Figure 4.4b). Many researchers have reported consumption of N_2O in rice under flooded conditions (Berger et al., 2013; Ferré et al., 2012; Win et al., 2014) and the reason is that, although nitrate is usually the starting point of denitrification, other nitrogen oxides (NO_2^-, NO, N_2O) can serve as terminal electron acceptors for denitrifying bacteria in the lack of nitrate (Coyne, 2008; Kögel-Knabner et al., 2010) (Eq. 4.1). Although nitrification may take place in oxidized zones, the nitrate formed can be rapidly reduced in adjacent anaerobic soil zones (Buresh et al., 2008), and under saturated conditions (>80 % water filled pore space- WFPS), most gas flux produced in the denitrification process occurs as N_2 (Coyne, 2008; Davidson et al., 1986).

$$2\ NO_3^- \rightarrow 2\ NO_2^- \rightarrow 2\ NO \rightarrow N_2O \rightarrow N_2 \qquad \textbf{Eq. 4.1}$$

Once the field was drained (Sept. 14), N_2O emissions increased rapidly and reached positive values (Figure 4.4b). The formation of N_2O in the soil is due to both nitrification and denitrification processes. On one hand, the increase in the O_2 concentrations leads to the nitrification process, which can produce N_2O as a result of the utilization of NO_2^- (an intermediary compound in the nitrification process) as an electron acceptor by the nitrifiers organisms under oxygen limiting conditions (Nitrifier denitrification) (Poth and Focht, 1985; Wrage et al., 2011). On the other hand, the nitrate formed in the nitrification process can be lost in the denitrification process. When soil starts drying and O_2 penetrates into the soil, the denitrification process can stop in N_2O (3rd step of reaction in Eq. 4.1) since the enzymes that operate in the early part of the reaction are less sensitive to the availability of O_2 than the reductase enzyme that operates in the last step (N_2O to N_2); thus in presence of oxygen, N_2O is produced and at low O_2 concentrations, N_2 is produced (Cameron et al., 2013). Moreover, the N_2O emissions following soil drainage could be due to release of dissolved and entrapped N_2O formed before drainage (Buresh et al., 2008; Cai et al., 1997). Increase of N_2O fluxes after draining plots is well known and it has been reported by many other authors (Cai et al., 1997; Lagomarsino et al., 2016; Pittelkow et al., 2013; Wang et al., 2011).

The N_2O flux pattern in site 1 was related to soil nitrate and ammonium concentrations changes (Figure 4.7a and b). During the flooded period, soil nitrate was very low, with the exception of a short period in June. Because of the lack of nitrate, denitrifying organisms used N_2O as an electron acceptor resulting in N_2O consumption. After the field was drained, the nitrate concentration increased steadily due to nitrification process, which could be the cause of the increase in N_2O fluxes. The increase in the nitrate concentration during June was associated to a reduction in the floodwater depth in order to improve rice seedling growth (Figure 4.4a); the shallow floodwater during consecutive days could have promoted O_2 diffusion to the soil and hence, nitrification. This matches with the decrease in soil ammonium concentration, at the same time that nitrate increased (Figure 4.7a and b). After that, nitrate concentration dramatically dropped probably because of denitrifying process, and the ammonium concentration increased. The cause of the increment in soil ammonium concentration might be the release of the ammonium fixed in the clay minerals. Different studies have reported that after flooding, anaerobic conditions promote the temporary fixation of NH_4^+ in the interlayers or clay minerals (Schneiders and Scherer, 1998; Zhang and Scherer, 1999; 2000) because of a net increase in the negative surface charge of the clay (Stucki et al., 1984). The fixed ammonium is released influenced by the concentration of the ammonium in the soil solution (Mengel et al., 1990; Schneiders and Scherer, 1998). In our study, part of the ammonium from the fertilizer applied before flooding could have fixed in the soil as ammonium non-exchangeable, and the decrease in the exchangeable ammonium due to nitrification may have promoted the release of the fixed ammonium. At the end of the crop season, when the plot was drained, it was observed an increase in the soil ammonium content probably due to ammonification, but rapidly the ammonium content decreased at the same time than the nitrate increased, verifying that nitrification took place.

In site 2, the N_2O fluxes were negative for the whole period, but values were less negative (close to 0) after the plot was drained (Figure 4.5b). In site 2, the field kept flooded during more days than in site 1 (Figure 4.4a and Figure 4.5a), the floodwater only disappear for a few days, but the soil kept saturated and even a very thin water layer (several millimeters) remained over the soil surface. These findings agree with the study reported by Iida et al. (2007), who found that N_2O emission can be mitigated considerably by even a thin film of floodwater on paddy fields.

In this case, the relationship between N_2O flux pattern and soil mineral N pattern was not clear. Soil ammonium concentration was similar between treatments, even at the beginning of the crop season (Figure 4.8b). The ammonium N content at the beginning of the experiment in site 2 was higher than in site 1 (Table 4.1), and the soil had also higher SOM content which could have provided ammonium N through mineralization. Under this high ammonium N concentration in the soil, the ammonium N applied with the fertilizers could have been fixed in the clays promoted by the high concentration of the ammonium in the soil solution, and hence, differences between treatments were not detected. Grain yield showed that N was not limiting even in the control treatment, since rice yield in this treatment did not differ from the rest of treatments (Table 4.6).

Soil nitrate concentrations oscillated over time without significant differences between treatments (Figure 4.8a and Table 4.6), and were higher than in site 1 and also higher than values found in the literature for flooded soils (Aulakh et al., 2001; Wang et al., 2011), since in general nitrate disappears within a few days of submergence (Ponnamperuma, 1972). The consumption of the N_2O can be explained under the absence of nitrate (Coyne, 2008; Kögel-Knabner et al., 2010), and hence, the high nitrate concentrations do not match with N_2O fluxes. Bad samples storage may be a reason why we found these high nitrate concentration values. Although samples were kept refrigerated until the analysis, it is possible that an undetected refrigeration problem promoted soil N transformations.

In both sites, the cumulative N_2O emissions were negative ranging between -0.04 and -1.10 kg $N \cdot ha^{-1} \cdot season^{-1}$ (Table 4.5 and Table 4.6). Simmonds et al. (2015) also found negative values of cumulative N_2O emissions in a field study conducted in California with the lowest value of -0.19 kg $N \cdot ha^{-1} \cdot season^{-1}$.

The fertilizer N emission factor (EF) considering the total N applied was lower than 0.3 % in site 1 (Figure 4.6, Table 4.5) and lower than 0.1 % in site 2 (Figure 4.6, Table 4.6). These values are similar or lower to those reported in the literature for rice fields. The IPCC default value for flooded rice fields is 0.3 % (0-0.6%) (IPCC, 2006) and it was based on a review by Akiyama et al. (2005) considering organic and inorganic fertilization. Linquist et al. (2012a) analyzed N_2O emissions from rice systems fertilized with inorganic fertilizers and reported an average fertilizer-induced emission factor of 0.22 %. Cayuela et al. (2017) obtained an average value of 0.19 % studying Mediterranean rice systems and found flooded systems (rice fields) to have the lowest EF, associated with anaerobic conditions favoring complete denitrification to N_2. The studies evaluated in these reviews usually consider only the rice crop season rather than calculating an EF for the whole year. In our study, the EF was also calculated during the crop season and hence, values are comparable to those in the bibliography; however, it is important to point out that these N losses only represent the growing season and hence the emission factor could be underestimated.

Despite EF values obtained in our study were higher than 0, the cumulative N_2O emissions were not significantly different from the control treatment (Table 4.5 and Table 4.6), and the EF did not differ from 0 in any of the fertilization treatments (student t-test, $p>0.05$) what means that there was not an increment on the N losses in the form of N_2O due to N fertilization. The most important result to highlight is that PS fertilization did not increase N losses as N_2O form compared to inorganic fertilization.

Other researchers have studied the effect of organic and chemical fertilization on N_2O emissions from rice fields. Liang et al. (2013) and Zhou et al. (2017) found an increase of nitrous oxide emissions in plots fertilized with pig manure compared to those fertilized with chemical fertilizer. However, other studies have reported lower N_2O losses from plots fertilized with straw or green manure than those observed in plots fertilized with mineral fertilizers (Aulakh et al., 2001; Bronson et al., 1997; Wang et al., 2011). A decrease in the N_2O emissions could be attributed to the organic C substrates applied with these amendments, which may enhance soil denitrifier activity and efficiency, and the final reduction of N_2O to N_2 by the denitrification process (Qin et al., 2010; Wang et al., 2011). Moreover, decomposition of organic fertilizer with high C/N, such as straw or green manure, may increase the net immobilization of N and reduce the available N for nitrification-denitrification (Bronson et al., 1997; Wang et al., 2011). Therefore, the observed effect will depend on the type and composition of the organic amendment. Pig slurry with low C content would be not expected to have a strong influence on nitrous oxide emissions in comparison to synthetic N. In our study, significant differences in the mean values of N_2O fluxes or in the cumulative N_2O emissions were not found between the treatments fertilized with chemical fertilizer and the PS (with low C content) as we hypothesized. Sasada et al. (2011) and Win et al. (2014) found similar results in rice experiments with anaerobically digested pig slurry (ADPS) in Japan, where no differences in N_2O emissions were found between ADPS and chemical fertilizers. Win et al. (2014) determined the ADPS rates according to ammonium N content, as in our study, and thus the total N in ADPS plots was higher than in chemical plots, but this additional N caused no additional N_2O emissions, in agreement with our study.

4.4.2. Methane emissions

CH_4 emissions ranged between -0.1 and 26.6 kg CH_4-C $ha^{-1}\cdot day^{-1}$ and from 0.01 to 57.8 kg CH_4-C $ha^{-1}\cdot day^{-1}$ in site 1 and site 2, respectively. (Figure 4.4c and Figure 4.5c).

CH_4 is the typical end product of the anaerobic degradation of organic matter (Neue et al., 1996; Ponnamperuma, 1972; Wassmann et al., 1993). Organic matter applied to the fields such as rice straw, soil organic matter (SOM) and organic matter supplied from rice plants (exudates and sloughed tissues) are the carbon sources for CH_4 emitted (Kimura et al., 2004; Watanabe et al., 1999). Likewise, organic C contained in PS (Table 4.3) is an additional source of carbon.

In the present study, at site 1, the average CH_4 flux was significantly higher for the PS170M0 treatment (Table 4.5). This treatment showed an emission peak during the first weeks after flooding; moreover, another peak was observed in all treatments later in the season. The results suggest that the additional C source applied in the PS170M0 plots could have promoted higher emissions early in the season. Our results agree with those of Wassman et al. (1996) in a rice field experiment in Philipines comparing organic fertilization (straw) and urea. They found an early season peak in the plots with high C input (straw) but not in the urea fertilized; later in the season, another peak was observed in all plots, suggesting that C-source for the methanogens was the autochthonous material instead of the straw-C incorporated before flooding. Neue (1996) found that early in the season, organic amendments provide substrates for methanogenesis, whilst root exudates become more important at the later growth stages. However, in our study, the PS120M60 treatment did not show higher CH_4 emissions than M120M60 (Table 4.5) even though an additional C source was applied to the soil

(Table 4.2); thus the organic C input in PS120M60 was not enough to increase the CH_4 emissions.

Moreover, an emission peak was observed in site 1 during a short drainage on July in all treatments (Figure 4.4c); other researchers have also reported methane emission peaks immediately after the drainage of a plot (Denier van der Gon et al., 1996; Pittelkow, 2013) because of the release of the CH_4 trapped in the soil through the macropores. Denier van der Gon et al. (1996) in Philippine rice paddies also found an increase of CH_4 emissions at harvesting for 2 to 4 days after the soil surface fell dry and until the soils started to crack. In our study, after the final drainage, CH_4 fluxes lowered and a peak was not observed, but it has to be considered that in site 1 the sampling frequency was not increased at the final drainage and the peak if existed could have been missed. Once the soil is dry, soil promotes aerobic decomposition of organic matter and hence, the final product is not the reduced form of methane.

Methane emissions were higher in site 2 than in site 1. Puddling was performed in site 2 but not in site 1; puddling disperses soil colloids and increases the water to soil ratio, resulting in very low bulk densities, promoting reduction. In contrast, high soil bulk density from less intense field preparation retards organic matter decomposition and reduces the speed of potential redox changes as well as CH_4 formation (Neue, 1997). Moreover, the SOM in site 2 was higher than in site 1 (Table 4.1). Puddling and a higher SOM content could explain the higher CH_4 emissions in site 2.

At site 2 methane fluxes were not significantly affected by N fertilization (Table 4.6), despite the additional C source (Table 4.2) in the PS treatment compared to the mineral treatment. Although organic amendments may increase CH_4 production providing readily mineralizable carbon sources, these changes are more pronounced when organic substrates are added to soils with low organic matter content (Neue, 1997; Win et al., 2014). Soil at site 1 had lower organic matter content than soil at site 2 (Table 4.1); therefore, organic C contained in the PS could have had a stronger influence at site 1 because of the lower SOC content compared to site 2. Moreover, the organic C content in the PS applied to site 1 was higher than in the PS applied to site 2 (Table 4.3). The results suggest that in site 2, emissions were more influenced by SOM and land management (puddling) than for fertilizers C content. These results are in agreement with those reported in the studies by Sasada et al. (2011) and Win et al.

(2014), where differences in the effects of anaerobically digested pig slurry (ADPS) on methane emissions were attributed to the differences in soil C content, suggesting that the application of ADPS might have a higher stimulating effect on CH_4 emissions when soil C content is lower.

In contrast to site 1, CH_4 fluxes did not decrease immediately after the drainage of the plot, but the emissions decreased slowly (Figure 4.5c) and the reason was the difference in the drying speed. The field at site 2 remained flooded during more days than at site 1 (Figure 4.4a and Figure 4.5a) and even when the floodwater disappear, the soil remained always saturated, a very thin water layer was always present over the soil surface, and hence, favorable conditions for methanogenesis were maintained. Even though in site 2 sampling frequency was increased after the drainage of the plot, a CH_4 emission peak due to the release of the trapped CH_4 was not noticed, since soil never dried out.

It is important to be aware that in both sites, additional to N fertilization, rice straw and stubbles from the previous growing season were left on the field and plowed into the paddy soil. This additional C source, for all the treatments, could have promoted methane emissions, since straw incorporation have been reported consistently to increase methane emissions (Sanchis et al., 2012).

The mean daily CH_4 fluxes ranging between 2.5 and 6.3 kg $CH_4\text{-}C \cdot ha^{-1} \cdot day^{-1}$ and the maximum flux (27 kg $CH_4\text{-}C \cdot ha^{-1} \cdot day^{-1}$) reported at site 1, were similar to those reported in the literature for paddy rice fields (Cicerone et al., 1983; Sasada et al., 2011; Schütz et al., 1989a). The cumulative emissions for the season are also in accordance with the values in the review by Sanchis et al. (2012) in studies with straw incorporation. However, the mean daily CH_4 fluxes at site 2, ranging between 16.4 and 18.9 kg $CH_4\text{-}C \cdot ha^{-1} \cdot day^{-1}$ were higher than in site 1 and higher than those reported in the literature, and hence, cumulative CH_4 losses were higher than expected. One of the reasons for these high values could be the photoacoustic spectroscopy (PAS) technique used for the fluxes quantification. Some researchers have evaluated the accuracy of this technique and some concerns have been reported about water vapor and carbon dioxide interferences with N_2O and CH_4 signals. Although the PAS analyzer is prepared to correct these interferences, the accuracy of these corrections has been questioned. While some authors have reported good results when comparing PAS with gases chromatography (GC) (Iqbal et al., 2013; Yamulki and Jarvis 1999), other studies have reported opposite results. Thus, Rosenstock et al. (2013) found the measurements of CH_4 to be dependent on the water vapor of the air sample, concluding that manufacturer calibration for moisture was not sufficient. Tirol-Padre et al. (2013) compared N_2O, CO_2 and CH_4 fluxes measured by GC and PAS from agricultural fields under wheat, rice and maize. They found a large effect of water vapor on the PAS CH_4 readings and concluded that under the humid rice environment, water vapor interference was not fully addressed by the instrument´s internal compensation.

Taking into account the above considerations, we are aware that the CH_4 fluxes could have been overestimated. At site 1, CH_4 emissions were within the range found in the literature for rice fields (at the upper end). However, at site 2, emissions were higher than in site 1 and much greater than the values found in the literature. Even when CH_4 fluxes could have been overestimated, we consider that, the comparative between PS and mineral treatments, which was the main objective of this study, is reliable, as the air humidity in each sampling date was the same for all the treatments.

As we expected, the results suggested that there is not an increase of methane emissions when mineral fertilizers are replaced by PS at the same N rates (PS120M60 vs M120M60 in site 1, PS170M0 vs M170M0 in site 2; Table 4.5 and Table 4.6). Nevertheless, when N rates applied before seeding increase, methane emissions could increase as we observed in the PS170M0 treatment in site 1. Other researchers have reported increase in methane emissions when different organic amendments such as straw (Bossio et al., 1999; Schütz et al., 1989a), green manure (Denier van der Gon and Neue, 1995) or pig manure (Liang et al., 2013) were applied to rice fields in different parts of the world. However, as we explained before, these products have higher C content than PS, which could promote higher methane emissions.

Some other studies have not found an increase of methane emissions when PS (Maris et al., 2016) or ADPS (Sasada et al., 2011) are applied to replace mineral fertilizers (at the same N rates). However, opposite results were reported by Huang et al. (2014) who quantified CH_4 fluxes when mineral fertilizer was replaced by ADPS at different percentages and found significant increases when ADPS application was fractioned (40% base 25% tillering, 35% heading) and no differences when ADPS was applied in a unique application (base fertilization) and replacement percentage was lower than 75%.

In our study, cumulative methane emissions under both kinds of fertilization (mineral and PS) were not significantly higher than in control plots (Table 4.5 and Table 4.6). The studies about effect of N application on GHG emissions found in the literature are contradictory. Some studies suggest that the presence of ammonium in the soil may promote methane emissions due to methane oxidation inhibition because the enzyme that oxides methane (CH_4 monooxygenase) reacts with ammonium instead of methane (Conrad, 1996; Gulledge and Schimel, 1998). However, other studies have reported that N fertilization stimulates CH_4 oxidizing bacteria leading to a reduction in CH_4 emissions (Bodelier and Laanbroek, 2004; Bodelier et al., 2000 a, b). Linquist et al. (2012a) suggested in a review analyzing 24 rice systems that the contradictory reports in the literature may be in part explained by differences in N rates. For medium N rates (average 148 kg N· ha^{-1}) (which are close to optimum N rates), there is little or no net effect of N fertilization on CH_4 emissions. These findings are in agreement with our study since no differences between the control and the N fertilization treatments (optimums) in the CH_4 cumulative emissions were found.

4.4.3. Carbone dioxide emissions

CO_2 emissions varied from 0.10 to 40 kg CO_2-C ha^{-1}·day^{-1} and between -0.10 and 16.7 kg CO_2-C ha^{-1}·day-1 (Figure 4.4d and Figure 4.5d) in site 1 and site 2, respectively and followed an opposite pattern than methane emissions.

At site 1, low emissions were recorded during the flooded period and higher emissions immediately after the drainage of the plot (Figure 4.4d), clearly associated to the aerobic mineralization of organic matter. During the flooded period, CO_2 emissions were also noted, because carbon dioxide is also a product of the anaerobic decomposition of organic matter (Ponnamperuma, 1972); however, the fluxes were much lower than those observed during the drainage period. Unlike CH_4 emissions, significant differences between treatments in the mean CO_2 flux were not observed; nevertheless the mean CO_2 flux for the PS170M0 treatment was higher (not significant) than for the rest of the treatments (Table 4.5); and like in CH_4 emissions, the reason could be the addition of C with PS.

CO_2 emissions measured at site 2 during the flooded period were higher than that measured in site 1. Since CO_2 is also a subproduct of the anaerobic decomposition of organic matter, these higher CO_2 emissions are linked to the higher SOM at site 2 than at site 1, and linked to the higher CH_4 emissions observed at site 2. Similarly to CH_4 emissions, the pattern of the CO_2 fluxes after drainage for harvest was totally different than that observed in site 1. While at site 1 CO_2 emissions increased (Figure 4.4d), at site 2 CO_2 fluxes decreased slowly (Figure 4.5d). We consider that the soil moisture content was responsible of this fact, as the soil did not dried up after drainage at site 2, O_2 diffusion was restricted and hence aerobic decomposition.

As we explained in the materials and methods section, we cut the plants inside the chamber base, therefore, the CO_2 flux is assumed to be the soil respiration. Cumulative CO_2 emissions for the season were similar to those reported by Aulakh et al. (2001) and Iqbal et al. (2009), in experiments without plants.

Since CO_2 fluxes contribute less than 1 % to the global warming potential (GWP) of agriculture (Smith et al., 2007), most of the studies about GHG emissions in paddy fields are focused on CH_4 and N_2O emissions. Although this gas is not important in the contribution to GWP, our results suggest that, in the same manner as methane emissions, the application of PS at the same N rate than mineral fertilizer, does not increase cumulative CO_2 emissions (Table 4.5 and Table 4.6).

4.4.4. Relationships between gas fluxes and soil parameters

In site 1, nitrous oxide and carbon dioxide fluxes were related to soil moisture content (Figure 4.9). Above the critical value, there was no relationship between both variables because soil was saturated; but below the critical value, emissions were higher with decreasing soil moisture content. Rates of denitrification and respiration are strongly influenced by the percentage of water filled pore space (WFPS) (Coyne, 2008; Lin and Doran, 1984); however, the patterns differ depending on whether soil wets or dries and 60 % WFPS appears to be the critical point. Below this point, formed N_2O is due to nitrification; and above this point, importance of denitrification increases and N_2 is formed rather than N_2O as soil become more saturated. The negative relationship found in our study is due to the transition to the saturated to non-saturated zone. When soil is drying after a flooded period, O_2 started to diffuse into the soil, denitrification tend to be incomplete and N_2O is formed rather than N_2; moreover, N_2O due to nitrification is started to appear, as well as CO_2 flux is increasing due to aerobic soil respiration (Coyne, 2008; Lin and Doran, 1984). Although both processes are influenced by temperature (Coyne, 2008; Davidson et al., 1998), the soil moisture content had a stronger influence.

Methane fluxes were not related to soil moisture. As explained before, methane emission is produced under anaerobic conditions, which are obviously related to soil moisture. However, when a linear regression is carried out between both variables, a relationship is not found because methane emissions show a strong seasonal variation. During the flooded period (same soil moisture conditions), we can observed very different flux values. This evolution is more related to the decomposition of organic amendments, SOM, and rice plants exudates (Neue et al., 1996; Neue, 1997). Higher emissions during late growth stages, related to root exudates, coincide in time with the period with the highest temperatures. Therefore, we think that the positive relationship found with the temperature (Figure 4.10) is non-causal. Nevertheless, different studies have reported higher methane emissions with increasing soil temperature (Holzalpfel-Pschorn and Seiler, 1986: Seiler et al., 1984; Schütz et al. 1989a), but these relationships were obtained associated to diurnal temperature variations.

In site 2, emissions (N_2O, CH_4, and CO_2) (Figure 4.11) were not related to soil moisture since the soil was flooded during almost all the studied period and even when water disappeared from the soil surface, soil remained saturated. On the other hand, it was a significant relationship with temperature. The negative relationship found between nitrous oxide emissions and soil temperature (Figure 4.12a) could be explained as N_2O accumulates at lower temperatures (i.e. N_2O/N_2 ratio increases with decreasing temperature) (Lensi and Chalamet, 1982; Keeney et al., 1979); what means the final step of denitrification is inhibited under low temperatures. In our study, we observed N_2O emissions close to 0 at lower temperatures, so it seems that the consumption of N_2O to produce N_2 was inhibited. The increase in CO_2 emissions with increasing temperature (Figure 4.12b) is not expected to be due to aerobic soil respiration because of the saturated conditions, but it seems to be related with the methanogenesis process since carbon dioxide is also a subproduct of this process. Finally, the relationship between CH_4 flux and temperature (Figure 4.12c) was positive like in site 1 and as explained above, we think that the relation is non-causal.

4.4.5. GWP and yield-scaled GWP

GWP was calculated taking account N_2O and CH_4 emissions. Linquist et al. (2012b) indicated that CH_4 emissions accounts for almost 90 % of total global warming potential in flooded rice systems. Indeed, in our study GWP was due to CH_4 emissions, since cumulative N_2O emissions were negative and hence, reduced the potential of methane to increase the GWP.

GWP was not affected by fertilization treatments. When GWP was assessed as a function of crop yield (GWP_Y), although significant differences were not found among N fertilization treatments, the order of treatments in comparison to GWP changed (Table 4.5 and Table 4.6). At site 1, the Control treatment with the lowest GWP, was the treatment with the highest GWP_Y, similar to the PS170M0 treatment; whilst the M120M60 and PS120M60 (same N rates, PS vs mineral) had the lowest values. At site 2, the lowest yield scaled GWP (no significant) occurred at PS170M0 treatment due to a higher grain yield. The results corroborate the need to express the GWP in terms of yield (van Groenigen et al. 2010). As regard to the comparison between PS and mineral fertilization, results suggest that PS fertilization is a good alternative to mineral fertilization, and does not increase GWP_Y.

It is important to mention that in the present study, emissions were measured only during the crop season and one month after harvesting. Despite it is expected that emissions in the intercrop period were not significantly different between N fertilization treatments, further studies should be include measurements during the whole year.

4.5. CONCLUSIONS

The characteristics of the soil and land management have a strong influence on GHG emissions, as methane fluxes are higher in paddy fields with higher organic matter content, or with continuous puddling tillage practices.

The cumulative nitrous oxide emissions during the crop season were negative at both sites, corroborating that under flooded conditions, methane is the main contributor to GWP rather than nitrous oxide. GHG emissions and fertilizer-induced N_2O emission factor were not affected by the application of pig slurry at the same N rate than mineral fertilizer. However, application of high PS rates before seeding to soils with low SOM, might increase methane emissions. Therefore, application of PS before seeding at rates to cover around 70% of crop N needs and N topdressing to complement crop requirements are recommended in order not to increase methane emissions. Taking into account this consideration, PS might be an excellent fertilizer for achieving maximum yields without an increment of yield scaled GWP.

4.6. REFERENCES

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CAPÍTULO 5

MULTISPECTRAL INFORMATION FOR TOPDRESSING N RECOMMENDATION IN RICE UNDER TWO FERTILIZATION STRATEGIES, ORGANIC AND MINERAL, IN MEDITERRANEAN CONDITIONS

CAPÍTULO 5. MULTISPECTRAL INFORMATION FOR TOPDRESSING N RECOMMENDATION IN RICE UNDER TWO FERTILIZATION STRATEGIES, ORGANIC AND MINERAL, IN MEDITERRANEAN CONDITIONS

5.1. INTRODUCTION

Rice (*Oryza Sativa, L.*) is the staple food for nearly half of the word´s seven billion people (Mohanty, 2013). As for other cereals, nitrogen (N) is an essential element that is required to obtain high crop yields. Although N application increases rice productivity, poor N use efficiency (NUE) is characteristic of irrigated rice systems due to rapid losses of applied N, hence, adjusting N rates and time of application is crucial for optimizing NUE and avoiding environmental problems such as emission of greenhouse gases or surface and groundwater nitrate contamination (Cassman et al., 1998).

Although some studies have shown that a single optimum N application before flooding allows to reach maximum yields (Bond and Bollich, 2007; Wilson et al, 2001; Moreno-García et al., 2017), the estimation of the potential yield at the beginning of the season is difficult, since this is greatly influenced by year to year variation in weather conditions (Schlegel et al., 2005) and there is an important risk for over-fertilization and related environmental problems. However, if N rates are reduced before seeding and later complemented with topdressing N, adjusted to the crop N status in that moment, over-fertilization and hence environmental issues could be reduced or even avoided, and NUE could be increased as reported by Nishikawa et al. (2012) and Xie et al. (2007).

In Europe, rice is mostly cultivated in Mediterranean countries, with a total area of approximately 642000 ha – 17% of this area is cultivated in Spain (FAOSTAT, 2014). The rice extension in northeast Spain (Aragon and Catalonia regions) represents 25% (MAGRAMA, 2015b) of the crop surface in the country. Northeast Spain concentrates more than half of the country´s pig production (MAGRAMA, 2015a), making necessary the integration of the pig slurry (PS) in the N fertilization schedule of rice fields. The local farmer´s most common practise is the application of PS or chemical fertilizers before seeding (≈70% of crop N requirements) complemented with mineral N-topdressing at the end of the tillering stage or during the stem elongation.

However, they apply the N at mid-season without taking into consideration the crop N status at that moment.

Once of the most common method used for real time N management in cereals is the chlorophyll meter. It has been used successfully to monitor leaf nitrogen status and guide N fertilization during the crop season in different cereals (Denuit et al., 2002; Isla et al., 2012) including rice (Peng et al., 1996; Yao et al., 2012). The method implies to measure at leaf level and thus, it is time consuming for large-scale applications. Crop canopy sensors, like Greenseker or Crop circle, measure canopy reflectance, reducing the measurement time and being more suitable for field applications; nevertheless, it is still necessary to go inside the field and perform the reflectance measurements in different areas to obtain a representative value. Aerial or satellite remote sensing images offer other possibilities since they allow covering large areas quickly.

Canopy spectral information offers the possibility to obtain vegetation indices (VIs). The VIs combine data from two or more spectral bands and are intended to enhance the vegetation signal, while minimizing solar irradiance and soil background effects (Jackson and Huete, 1991). The Normalized Difference Vegetation Index (NDVI) that integrates information in the red (R) and near infrared (NIR) bands, is one of the most widely applied VIs. It has been related to leaf area index (LAI) (Casanova et al., 1998; Li et al., 2015), biomass (Gnyp et al., 2014) or yield (Gilabert and Meliá, 1990; Sultana et al., 2014; Quarmby et al., 1993). However, for high chlorophyll (Chl) concentration or large vegetation coverage values, NDVI losses sensitivity and saturates. For this reason, Gitelson et al. (1996) proposed the Green NDVI (GNDVI), that considers the green (G) instead the red (R) band in its formulation, and found that GNDVI was much more sensitive to Chl concentration, in a wide range of Chl variations, than the original NDVI. Different researchers have found good relationships between GNDVI and different agronomic parameters such as yield (Cao et al., 2015) or the fraction of photosynthetically active radiation (FPAR) (Tan et al., 2013).

Other indices have been formulated looking for a response to chlorophyll and N content variations. One example is the Modified Chlorophyll Absorption in Reflectance Index (MCARI) that was designed to be responsive to chlorophyll variation (Daughtry et al., 2000); however, despite no NIR band was considered in its formulation, MCARI has a great potential to predict LAI (Haboudane et al., 2004). Later, this index has been modified by different authors integrating the NIR wavelength to increase the sensitivity to LAI and aboveground biomass changes (Haboudane et al., 2004; Cao et al., 2013) and denoted as MCARI1.

The simple ratios between bands have also shown to be related to agronomic parameters like yield. For example, the ratios of the near-infrared reflectance (NIR) to red (R) reflectance (RVI=NIR/R) or of the near-infrared reflectance (NIR) to green (G) reflectance (GRVI=NIR/G) have shown good relationships with yield (Wang et al., 2010; Wang et al., 2014).

Different authors have proposed approaches for determining the crop N needs in season using the relationships between canopy reflectance measurements and different agronomic parameters. Lukina et al. (2001) and Raun et al. (2002) developed the Nitrogen Fertilizer Optimization Algorithm (NFOA) for winter wheat based on the estimation of the yield potential from spectral measurements obtained by a hand-held multispectral reflectance optical sensor. On the other hand, other studies have developed approaches to estimate N mid-season needs based on leaf area index (LAI) (Wood et al., 2003) or Nitrogen Nutrition Index (NNI) (Denuit et al., 2002).

With regard to rice crop, different authors have demonstrated the usefulness of VIs to be used for crop parameters estimation such as yield (Chang et al., 2005; Gilabert and Meliá, 1990; Tubaña et al., 2012; Wang et al., 2010) or aboveground biomass (Cao et al., 2013). Moreover, these VIs have been used in the development of tools for N topdressing estimation in season. The Nitrogen Fertilizer Optimization Algorithm (NFOA) have been modified and applied to rice using the information provided by different sensors, as spectro-radiometer (Xue and Yang, 2008), Greenseeker (Yao et al., 2012; Xue et al., 2014) or chlorophyll meter (Yao et al., 2012). Approaches based on NNI or LAI have also been used for rice crop (Cheng et al., 2014; Xue and Yang, 2008).

However, most studies focus on the use of canopy reflectance for estimating agronomic parameters or adjusting mid-season N in rice paddy fields have been conducted in Asia. Rice agricultural practices in Asia (Japan, China, Philippines…) are different to those in Mediterranean areas of Europe. Main differences are related to crop implantation, usually transplanted in Asia versus direct-seeded in the Mediterranean area, and meteorological conditions.

Some studies in Mediterranean conditions have been carried out. Gilabert and Meliá (1990) stablished relationships for yield prediction based on VIs obtained from satellite images in Valencia, Spain; while Casanova et al. (1998) estimated LAI and biomass in other area of Spain, the Ebro Delta. Recently, efforts are being made to integrate multispectral information in farm advisory systems. The ERMES project is being developed in Spain, Italy and Greece and has the primary aim of rice yield prediction, based on the compilation of data from remote sensing, in situ data crop and the analysis of these data by using models (Katsantonis et al., 2015). The specific objectives include the support of rice growers for different strategies (fertilization, pesticides) or warning related to biotic and abiotic stress. In the framework of the project, two applications (PocketLAI and PocketN) have been developed for estimating in season LAI, leaf and plant N content from digital photography acquired with commercial smarthphones (Campos-Taberner et al., 2016; Confalonieri et al., 2015). However, more research is needed for Mediterranean conditions; especially studies focus on the development of new approaches or testing the existent ones to estimate the N topdressing needs in season, with particular emphasis on the use of aerial images with high spatial resolution which cover large areas, so that recommendations to different farmers or to large farms are possible.

Moreover, studies focus on the effects of organic fertilizers application, such as PS, on the rice spectral information are lacking. Knowledge of multispectral response sensitivity to differences between mineral and organic fertilization is essential to develop sound approaches for N recommendation. Therefore, the main aim of this study was the evaluation of the utility of multispectral images to estimate topdressing N needs in a paddy rice system in Mediterranean conditions. Different sub-objectives were proposed:

1. To establish relationships between yield and vegetation indices (VIs) derived from aerial multispectral information at the rice booting stage; and evaluate possible differences in these relationships due to organic and mineral fertilization. This objective is covered in Section 5.3.

2. To design and evaluate the agronomic performance of two N topdressing recommendation approaches based in the information obtained in subobjective 1. This objective is covered in Section 5.4.

3. To compare economically different scenarios for N adjustment based in the recommendations approaches from subobjective 2. This objective is covered in Section 5.5.

The chapter, in order to be fully comprehensible, is organized with the following scheme. Firstly, the general description of the field experiment and the spectral information (section 5.2) is presented. The information in section 5.2 gives support to the development of the three subobjectives. Then, the information of specific methodology, results, and discussion is presented grouped for each subobjective:

- Subobjetive 1 (Section 5.3): Spectral information and relation to rice yield.
- Subobjetive 2 (Section 5.4): Tools for topdressing N recommendation.
- Subobjetive 3 (Section 5.5): Economic analysis.

Finally, the conclusions (Section 5.6) and the bibliographic references (section 5.7) are presented jointly for the three subobjectives.

5.2. FIELD EXPERIMENT AND SPECTRAL INFORMATION

5.2.1. Experimental design and agricultural practices

The field experiment was conducted in Villanueva de Sigena (Huesca) in northeast Spain (41º45’31.87”N, 0º2’18.16”W) on a silty loam textured soil (Table 5.1) during two consecutive years, 2012 and 2013 (the same field experiment as in chapter 3). The climate of the region is semiarid continental Mediterranean, with high temperatures during the summer and low precipitation (15.0ºC summer average temperature and 349 mm annual precipitation, average period 1980-2010).

Table 5.1. Characteristics of the soil at the different depths.

Depth	**pH 1:2.5**	**Ec$_e$**	**CaCO$_3$**	**Organic matter**	**Sand**	**Silt**	**Clay**	**N Kjeldahl**	**P Olsen**	**K (NH$_4$Ac)**
m	*(-)*	*dS·m^{-1}*	*g·kg^{-1} dry soil*					*mg·kg^{-1} dry soil*		
0-0.3	8.5	0.77	290	10.1	134	662	204	800	6	81
0.3-0.6	8.7	0.55	290	4.2	215	627	158	500	2	30
0.6-0.9	8.7	0.59	310	4.2	123	675	202	500	1	15
0.9-1.20	8.4	1.67	290	4.3	67	733	200	600	1	18

The experimental design was a split plot with four replications (Figure 5.1). The main plots were assigned to three basal fertilization strategies that consisted in two rates of pig slurry (PS) equivalent to 120 kg NH_4-N· ha^{-1} (PS120) and 170 kg NH_4-N·ha^{-1} (PS170) (Table 5.2, Pig slurry treatments, PS) and a mineral treatment (ammonium sulfate) at different rates (Table 5.2, Mineral treatments, M). Secondary plots included different topdressing N rates applied as ammonium sulfate. The total N rates ranged between 0 and 320 kg N·ha^{-1} and were applied as a basal fertilization or as a combination of basal and topdressing fertilization for a total of 22 treatments (Table 5.2). For PS treatments, only ammonium N content was considered for the calculation of the N rates, because previous findings determined that organic N was not available for the rice crop during the growing season (Moreno-García et al., 2017).

Table 5.2. Amount (kg N·ha^{-1}) and timing (BS: Before seeding, TP: topdressing) of the N applied in the different treatments. For PS treatments, amount indicates target N rates

Pig slurry treatments (PS)			**Mineral treatments (M)**		
	BS	TP		BS	TP
	kg NH$_4$-N·ha^{-1}	*kg N·ha^{-1}*		*kg N·ha^{-1}*	
PS120M0	120	--	**M120M0**	120	--
PS120M30	120	30	**M120M30**	120	30
PS120M60	120	60	**M120M60**	120	60
PS120M90	120	90	**M120M90**	120	90
PS120M120	120	120	**M120M120**	120	120
PS120M150	120	150			
PS170M0	170	--	**Control (M0)**	--	--
PS170M30	170	30	**M30**	30	--
PS170M60	170	60	**M60**	60	--
PS170M90	170	90	**M90**	90	--
PS170M120	170	120	**M120=M120M0**	120	--
PS170M150	170	150	**M150**	150	--

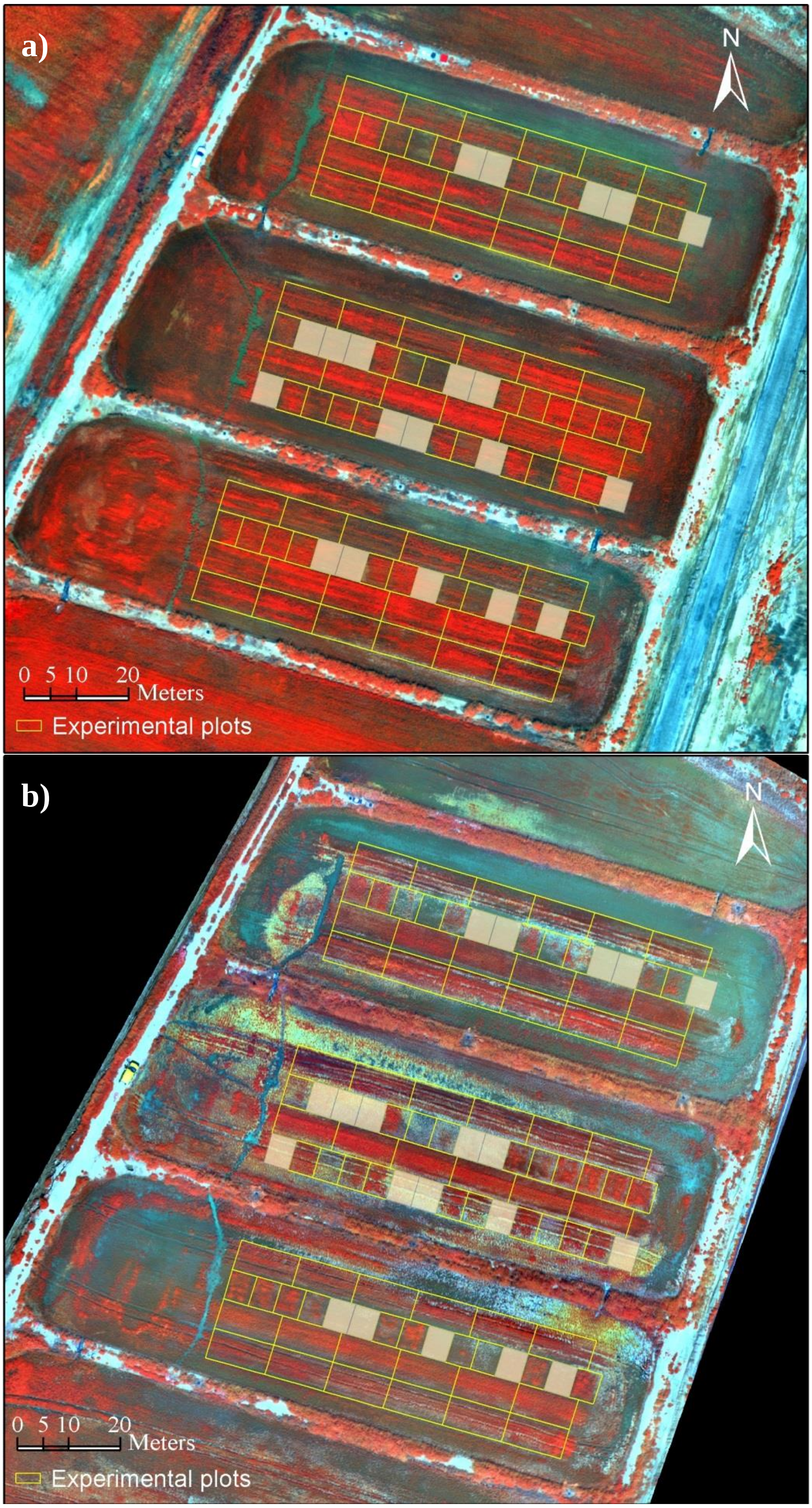

Figure 5.1. Villanueva de Sigena. Experimental design superposed to the images (false color, RGB: NIR/G/B) taken with a multispectral camera at the booting stage, 30 July 2012 (a) and 13 August 2013 (b). Shaded areas represent additional treatments excluded from the analysis according to the explanation in Chapter 3, section 3.2.1.

Pig slurry was band spread on 15 May 2012 and 9 May 2013 and PS rates were established according to the ammonium N concentration of the PS, which was measured in situ by Quantofix® N-volumeter (Piccinini and Bortone, 1999) and conductimetry (Yagüe and Quílez, 2012). Slurry samples were collected for further analysis in the laboratory in order to know actual N rates (Chapter 3, Table 3.4).

On the same days, the basal mineral N fertilizer (ammonium sulfate) was applied to the plots of the M treatments at the corresponding rates together with P (100 kg $P_2O_5 \cdot ha^{-1}$) and K (100 kg $K_2O \cdot ha^{-1}$) to avoid a P or K deficiency. The experimental plots were 6 m wide by 12 m long for the PS treatments and 6 m wide by 5 m long for the M treatments (Figure 5.1).

A japonica rice cultivar, Guadiamar (the most extended in the study area), was broadcast seeded on 16 May 2012 and 15 May 2013 at a seed rate of 180 $kg \cdot ha^{-1}$. In 2012, the field was immediately flooded after seeding; however, in 2013, flooding preceded rice seeding. Topdressing N was applied at the end of the tillering stage, on 4 July 2012 and 29 July 2013, as ammonium sulfate. The field remained flooded until approximately one month before harvest, except for occasional drainages for the application of herbicides, pesticides and fungicides, according to habitual practices in the area.

Rice was hand-harvested on 15-17 October 2012 in two 2 m^2 control areas (total area 4 m^2) in experimental plots corresponding to the PS treatments and in two 1 m^2 control areas (total area 2 m^2) to the M treatments. In 2013 (25 October), a strip 1.5 m wide and the length of the experimental plot was mechanically harvested. Grain humidity (PM-600 grain moisture tester, Keller, Japan) was measured to adjust the yield to 140 $g \cdot kg^{-1}$ moisture content.

5.2.1. Spectral information

Spectral information (Figure 5.1) was taken with a spectral camera from a manned plane (RS Servicios de Teledetección SL). The spectral camera collected data in four wavelengths; centre wavelengths were: band 1 (blue-B): 450 nm, band 2 (green-G): 550 nm, band 3 (red-R): 675 nm and band 4 (near infrared-NIR): 780 nm and the bandwidth was 20 nm (for the 4 bands). The images were collected at solar noon on 30 July 2012 and 13 August 2013 at the rice booting stage, a few days before heading. The images were provided pre-processed by SpecTerra Services: the differential illumination effect corrected, ortho-rectified and mosaicking. The spatial resolution was 0.1 m and the radiometric resolution 16 bits.

Different vegetation indexes (VIs) were selected to evaluate their relation to rice yield and their ability to estimate necessity of additional topdressing N fertilization (Table 5.3). The indices RVI, GRVI, NDVI and GNDVI (Table 5.3) were included because they have consistently shown to be related to agronomic parameters such as yield, and have been used in the development of approaches to recommend N at topdressing in different crops. Furthermore, we have included 3 indices, MCARI1, $\mathrm{MCARI_{NIR}}$ and $\mathrm{gMCARI_{NIR}}$ (Table 5.3), derived from the Modified Chlorophyll Absorption in Reflectance Index (MCARI) (Eq. 5.1) that was originally developed to be responsive to chlorophyll variation (Daughtry et al., 2000).

$$\mathrm{MCARI} = [(\mathrm{R}_{700} - \mathrm{R}_{670}) - 0.2 \cdot (\mathrm{R}_{700} - \mathrm{R}_{550})] \cdot (\mathrm{R}_{700}/\mathrm{R}_{670}) \qquad \textbf{Eq. 5.1}$$

Haboudane et al. (2004) proposed MCARI1 (Eq. 5.2) with suppression of the ratio $\mathrm{R_{700}/R_{670}}$ in order to lower the sensitivity to chlorophyll effects, and replacement of the red-edge wavelength ($\mathrm{R_{700}}$) by the near-infrared wavelength ($\mathrm{R_{800}}$) to increase the sensitivity to green LAI variations.

$$\mathrm{MCARI1} = 1.2 \cdot [2.5 \cdot (\mathrm{R}_{800} - \mathrm{R}_{670}) - 1.3 \cdot (\mathrm{R}_{800} - \mathrm{R}_{550})] \qquad \textbf{Eq. 5.2}$$

We included MCARI1 (Table 5.3) in the study because this index is related to biomass and LAI that are both related to yield.

Cao et al. (2013) modified the MCARI to work with Crop Circle ACS-470 active sensor that provide information in the green (550 ± 20 nm), red edge (730 ± 10 nm) and NIR (>760 nm) wavelengths. The MCARI modified by Cao et al. (2013), also denoted MCARI1 (Eq. 5.3), kept the formula of MCARI, but the $\mathrm{R_{700}}$ and $\mathrm{R_{670}}$ were replaced by $\mathrm{R_{NIR}}$ ($\mathrm{R_{760}}$) and $\mathrm{R_{red\ ege}}$ ($\mathrm{R_{730}}$), respectively.

$$\mathrm{MCARI1} = [\,(\mathrm{R}_{760} - \mathrm{R}_{730}) - 0.2 \cdot (\mathrm{R}_{760} - \mathrm{R}_{550})] \cdot (\mathrm{R}_{760}/\mathrm{R}_{730}) \qquad \textbf{Eq. 5.3}$$

This index had consistent correlations with rice aboveground biomass (R^2= 0.79) and plant N uptake (R^2= 0.83) across growth stages (Cao et al., 2013). In our study, we have adapted that index to the available bands, NIR 780 nm and red 675 nm (Eq. 5.4) and we have denoted the index as $\mathrm{MCARI_{NIR}}$ (Table 5.3).

$$\mathrm{MCARI_{NIR}} = [(\mathrm{R_{780}} - \mathrm{R_{675}}) - 0.2 \cdot (\mathrm{R_{780}} - \mathrm{R_{550}})] \cdot (\mathrm{R_{780}}/\mathrm{R_{675}}) \qquad \textbf{Eq. 5.4}$$

The seventh index is a new index (gMCARI$_{\mathrm{NIR}}$) based on MCARI$_{\mathrm{NIR}}$. The index gMCARI$_{\mathrm{NIR}}$ maintains the difference NIR-R (R_{780}-R_{675}) and the ratio NIR/R (R_{780}/R_{675}) but the difference NIR-G (R_{780}-R_{550}) is replaced by G-R (R_{550}-R_{675}) (Eq. 5.5) (Table 5.3).

$$\mathrm{gMCARI_{NIR}} = [(\mathrm{R_{780}} - \mathrm{R_{675}}) - (\mathrm{R_{550}} - \mathrm{R_{675}})] \cdot (\mathrm{R_{780}}/\mathrm{R_{675}}) \qquad \textbf{Eq. 5.5}$$

Our objective has been to formulate and test an index sensitive to changes in both, biomass and chlorophyll concentration (nitrogen status). The new index keeps the MCARI structure, includes near-infrared wavelength to increase the sensitivity to biomass changes, as it was proposed by Haboudane et al. (2004), and incorporates the green reflectance peak information (G-R) to take account of the sensitivity to chlorophyll concentrations.

Table 5.3. Vegetation indices (VIs) evaluated in this study. Blue-B: 450 nm, green-G: 550 nm, red-R: 675 nm and near infrared-NIR: 780 nm.

Indices (VIs)	**Formula**	**Reference**
RVI Ratio Vegetation Index	NIR/R	Jordan (1969)
GRVI Green Ratio Vegetation Index	NIR/G	Inada (1985)
NDVI Normalized Difference Vegetation Index	(NIR-R)/(NIR+R)	Rouse et al. (1974)
GNDVI Green Normalized Difference Vegetation Index	(NIR-G)/(NIR+G)	Gitelson et al. (1996)
MCARI1 Modified Chlorophyll Absorption in Reflectance Index1	1.2·[2.5·(NIR-R)-1.3·(NIR-G)]	Haboudane et al. (2004)
MCARI$_{\mathrm{NIR}}$ Modified Chlorophyll Absorption in Reflectance Index$_{\mathrm{NIR}}$	[(NIR-R)-0.2·(NIR-G)]·(NIR/R)	Adapted from Cao et al. (2013)
gMCARI$_{\mathrm{NIR}}$ Green peak Modified Chlorophyll Absorption in Reflectance Index$_{\mathrm{NIR}}$	[(NIR-R)-(G-R)]·(NIR/R)	Proposed in this study

The software ArcGIS Desktop 10.3 was used to calculate VIs for each pixel and to extract the average values of the VIs for each plot. The extraction was performed using a mask for each plot, the mask excluded the inner 1 meter from the borders (to avoid edge effects). In 2013, due to adverse meteorological conditions, rice seed germination was hindered and 5 plots were removed from the analyses because of bad nascence.

5.3. SPECTRAL INFORMATION AND RELATION TO RICE YIELD

5.3.1. METHODOLOGY

Linear-plateau equations (Cerrato and Blackmer, 1990 Eq. 5.6) were adjusted to model the response of yield to N rate in the PS and the M treatments for each of the two years.

$$\begin{cases} If\ N < C;\ Y = a + b \cdot N \\ If\ N \geq C;\ Y = Y_{max} = a + b \cdot C \end{cases} \qquad \textbf{Eq. 5.6}$$

where Y ($Mg \cdot ha^{-1}$) is the yield; N is the applied nitrogen rate ($kg\ N \cdot ha^{-1}$) (in some cases this rate is the sum of N applied before seeding and topdressing); a (intercept) is the yield at 0 $kg\ N \cdot ha^{-1}$; b is the increase in yield per unit increase in N; and C is the critical (or optimum) N rate, i.e., the minimum N rate above which the maximum yield (Y_{max}) is obtained. The equations were adjusted with the average yield for each treatment (four replicates). Relative yield (R_yield, Eq. 5.7) was obtained as the ratio between the observed yield in each plot and the adjusted maximum yield (Y_{max}) of that year.

Relative VI (R_VI, Eq. 5.8) was calculated, similarly to R_yield, as the ratio between VI in each plot and the maximum VI. Maximum VI was obtained as the average VI of treatments with N rates equal or higher than the critical rate ($N \geq C$).

$$R\_yield = \frac{Yield_{Plot}}{Maximum\ yield} \qquad \textbf{Eq. 5.7}$$

$$R\_VI = \frac{VI\ value_{Plot}}{Maximum\ VI} \qquad \textbf{Eq. 5.8}$$

Regression analyses were performed to establish the relationships between yield and VIs and between R_yield and R_VIs. The coefficient of determination of those relations was used to evaluate VIs performance.

Differences between years and between PS and M treatments were evaluated by a test of equality of regression lines across groups (SAS 9.4 Software).

5.3.2. RESULTS

The lineal-plateau models of yield response to N applied in years 2012 and 2013 showed no significant differences between M and PS treatments in the two years. Critical N rates were stablished jointly for M and PS treatments at 174 kg N·ha^{-1} in 2012 and 193 kg N·ha^{-1} in 2013 and maximum yields were 7802 kg ha^{-1} and 5942 kg·ha^{-1} in years 2012 and 2013, respectively (Figure 5.2). Yield in year 2013 was low due to adverse meteorological conditions in early season.

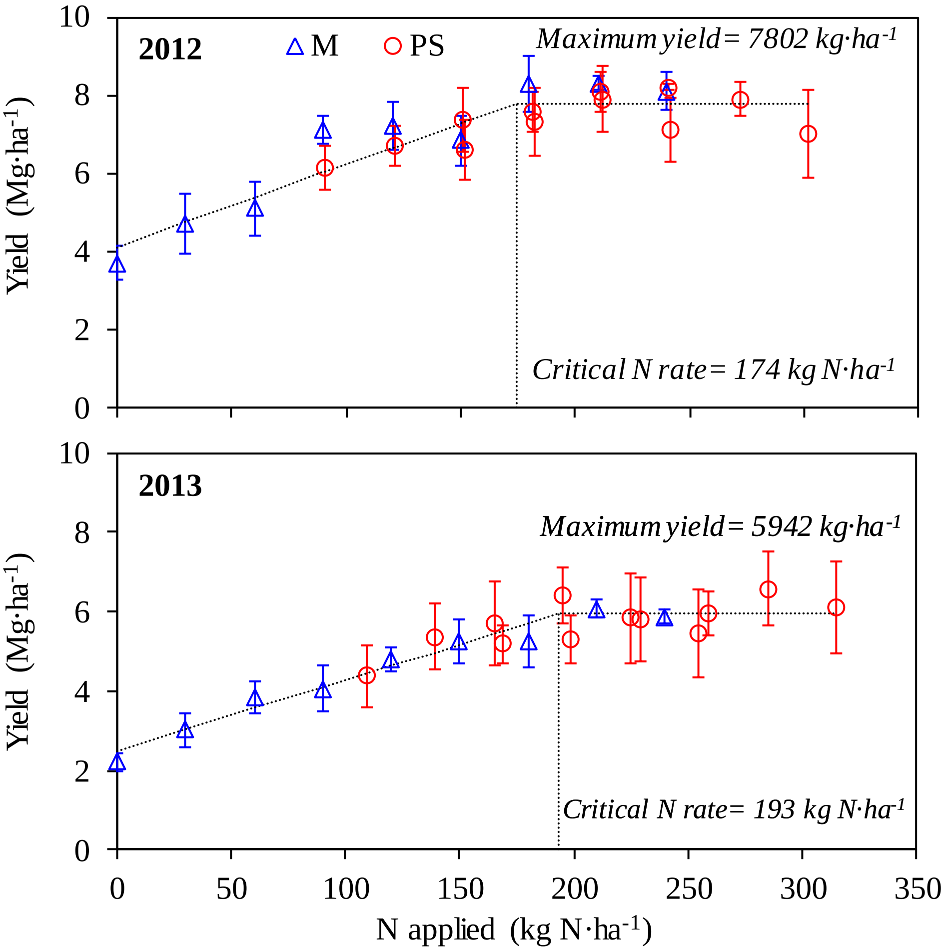

Figure 5.2. Yield response curves to N applied in the PS and M treatments in 2012 and 2013. Vertical bars represent ±1 standard error.

5.3.2.1. Influence of the year and the type of fertilizer

It was observed a significant relation between yield and the seven vegetation indices evaluated (Table 5.4) and all the relations were affected significantly by the year (Table 5.4; and Figure 5.3a for NDVI). Therefore, VIs were relativized by dividing by the maximum VIs of each year (using Eq. 5.8). This process corrected differential effects between years, as for instance differences in crop stage at the date of spectral monitoring. Relationships between yield and R_VIs were significant (Table 5.5) but the significant differences between years persisted (Table 5.5; Figure 5.3b). Thus, yield was also relativized (R_yield) with respect to maximum yield in each year (Eq. 5.7).

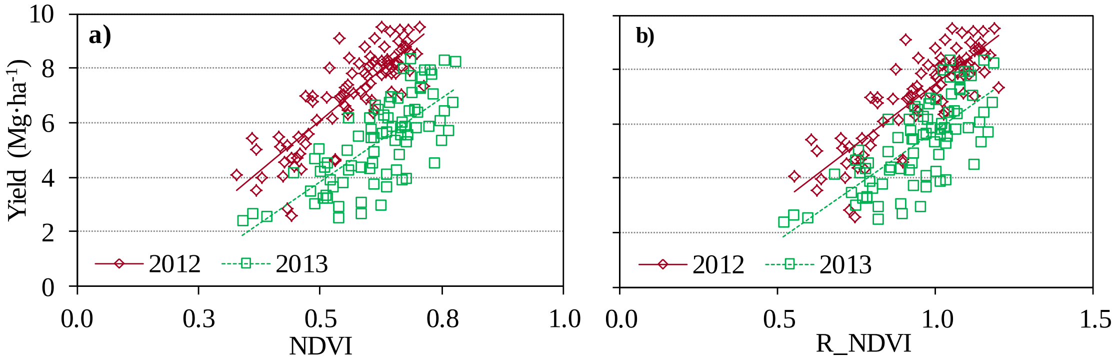

Figure 5.3. Relationship between yield and NDVI (a) and between yield and R_NDVI (relativized to maximum value of each year) (b) in 2012 and 2013 (PS and M treatments pooled data).

Relationships between R_yield and R_VIs did not show significant differences between the two years (Table 5.6, Figure 5.4a). Thus, information of years with different crop development and yield potential could be joint if both, yield and indices, are relativized to maximum values of each year.

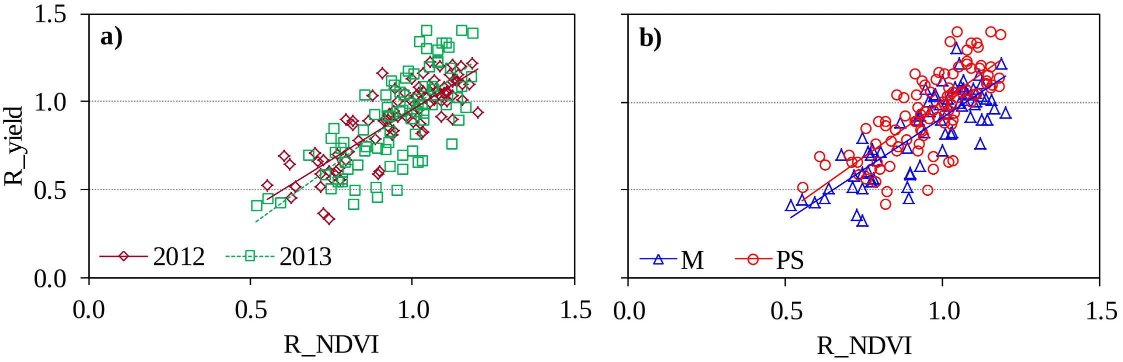

Figure 5.4. Relationship between R_yield and R_NDVI (relativized to maximum values of each year) in 2012 and 2013 (PS and M treatments pooled data) (a) and in PS and M treatments (2012 and 2013 pooled data) (b).

However, the relations R_yield to R_VIs showed significant differences between PS and M treatments (Table 5.6; Figure 5.4b). For instance in the case of NDVI, for the same value of the R_NDVI, PS treatments had a higher expected yield than M treatments (Figure 5.4b), or in other words, PS treatments reached maximum yield with a lower value of R_NDVI than M treatments.

These results suggest that VIs need to be relativized considering PS and M treatments separately. Therefore, R_VIs were recalculated individually for each year, 2012 and 2013, and inside each year individually for PS and M treatments. After this process, the relation between R_yield and R_VIs did not show significant differences between years (Table 5.7, Figure 5.5a) neither between PS and M treatments (Table 5.7, Figure 5.5b).

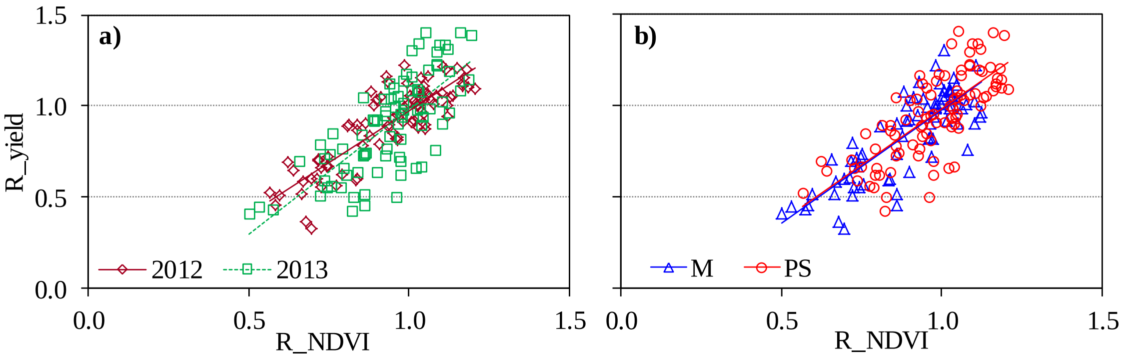

Figure 5.5. Relationship between R_yield (relativized to maximum value of each year) and R_NDVI (relativized to maximum value of each year and inside each year separately for PS and M treatments) for years 2012 and 2013 (PS and M treatments pooled data) (a) and for PS and M treatments (2012 and 2013 pooled data) (b).

Table 5.4. Coefficients of determination (R^2) of the relationship between yield and VIs for years 2012 and 2013, for PS and M treatments; and comparative between years and kinds of fertilizer.

	NDVI	**GNDVI**	**RVI**	**GRVI**	**MCARI1**	**$MCARI_{NIR}$**	**$gMCARI_{NIR}$**
2012	0.71 ***	0.76 ***	0.67 ***	0.73 ***	0.64 ***	0.71 ***	0.75 ***
2013	0.53 ***	0.53 ***	0.54 ***	0.50 ***	0.36 ***	0.55 ***	0.58 ***
PS	0.22 ***	0.30 ***	0.15 ***	0.23 ***	0.45 ***	0.28 ***	0.31 ***
M	0.37 ***	0.48 ***	0.21 ***	0.37 ***	0.57 ***	0.37 ***	0.43 ***
Year	***	***	***	***	***	***	***
Fertilizer	n.s.	n.s.	n.s.	*	***	*	n.s.

n.s: not significant; * $p<0.05$; ***$p<0.001$

Table 5.5. Coefficients of determination (R^2) of the relationship between yield and R_VIs (relativized to maximum value of each year) for years 2012 and 2013, for PS and M treatments; and comparative between years and kinds of fertilizer.

	R_NDVI	**R_GNDVI**	**R_RVI**	**R_GRVI**	**R_MCARI1**	**R_MCARI_{NIR}**	**R_gMCARI_{NIR}**
2012	0.71 ***	0.76 ***	0.67 ***	0.73 ***	0.64 ***	0.71 ***	0.75 ***
2013	0.53 ***	0.53 ***	0.54 ***	0.50 ***	0.36 ***	0.55 ***	0.58 ***
PS	0.45 ***	0.44 ***	0.48 ***	0.42 ***	0.36 ***	0.48 ***	0.48 ***
M	0.57 ***	0.61 ***	0.54 ***	0.59 ***	0.47 ***	0.55 ***	0.58 ***
Year	***	***	***	***	***	***	***
Fertilizer	***	*	*	*	**	*	*

n.s: not significant; * $p<0.05$; ** $p<0.01$; ***$p<0.001$

Table 5.6. Coefficients of determination (R^2) of the relationship between R_yield (relativized to maximum value of each year) and R_VIs (relativized to maximum value of each year) for years 2012 and 2013, for PS and M treatments; and comparative between years and kinds of fertilizer.

	R_NDVI	**R_GNDVI**	**R_RVI**	**R_GRVI**	**R_MCARI1**	**R_MCARI$_{NIR}$**	**R_gMCARI$_{NIR}$**
2012	0.71 ***	0.76 ***	0.67 ***	0.73 ***	0.64 ***	0.71 ***	0.75 ***
2013	0.53 ***	0.53 ***	0.54 ***	0.50 ***	0.36 ***	0.55 ***	0.58 ***
PS	0.57 ***	0.53 ***	0.60 ***	0.50 ***	0.45 ***	0.60 ***	0.60 ***
M	0.67 ***	0.75 ***	0.63 ***	0.73 ***	0.59 ***	0.68 ***	0.73 ***
Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s
Fertilizer	***	**	***	***	***	***	***

n.s: not significant; ** $p<0.01$; ***$p<0.001$

Table 5.7. Coefficients of determination (R^2) of the relationship between R_yield (relativized to maximum value of each year) and R_VIs (relativized to maximum value of each year and inside each year separately for PS and M treatments) for years 2012 and 2013, for PS and M treatments; and comparative between years and kinds of fertilizer.

	R_NDVI	**R_GNDVI**	**R_RVI**	**R_GRVI**	**R_MCARI1**	**R_MCARI$_{NIR}$**	**R_gMCARI$_{NIR}$**
2012	0.74 ***	0.77 ***	0.70 ***	0.74 ***	0.69 ***	0.74 ***	0.77 ***
2013	0.56 ***	0.56 ***	0.56 ***	0.53 ***	0.40 ***	0.58 ***	0.61 ***
PS	0.56 ***	0.53 ***	0.59 ***	0.50 ***	0.44 ***	0.59 ***	0.60 ***
M	0.66 ***	0.75 ***	0.61 ***	0.73 ***	0.57 ***	0.66 ***	0.72 ***
Year	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Fertilizer	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

n.s: not significant; ***$p<0.001$

5.3.2.2. Performance and comparative of the indices

R_yield and the seven R_VIs, for each year individually and for the pooled data of the two years (2012+2013), were significantly related (Table 5.8). Linear models fitted well the relationships between R_yield and R_RVI, R_GRVI, R_NDVI, R_GNDVI and R_MCARI1 (Figure 5.6a-d; Table 5.8) and the residuals from regression were independent and normally distributed. For R_MCARI$_{NIR}$ and R_gMCARI$_{NIR}$ (Figure 5.6e and f), it was observed that the increase in R_yield per unit increased in the indices, decreased as the value of the indices increased, indicating the relation was not linear. Different models were tested and the best fit was obtained with the multiplicative model ($R\_yield = a \cdot R\_VI^{b}$), with residual from regression independent and normally distributed.

Table 5.8. Coefficients of determination (R^2) of the relationships between R_yield and R_VIs for years 2012 and 2013 and the pooled data.

	Model Type†	2012 n=88	2013 n=83	Pooled 2012+2013 n= 171
R_RVI	L	0.70***	0.56***	0.62***
R_GRVI	L	0.74***	0.53***	0.61***
R_NDVI	L	0.74***	0.56***	0.63***
R_GNDVI	L	0.77***	0.56***	0.64***
R_MCARI1	L	0.69***	0.40***	0.52***
R_MCARI$_{NIR}$	M	0.74***	0.58***	0.64***
R_gMCARI$_{NIR}$	M	0.77***	0.61***	0.67***

***p<0.001

†L: Linear, M: multiplicative

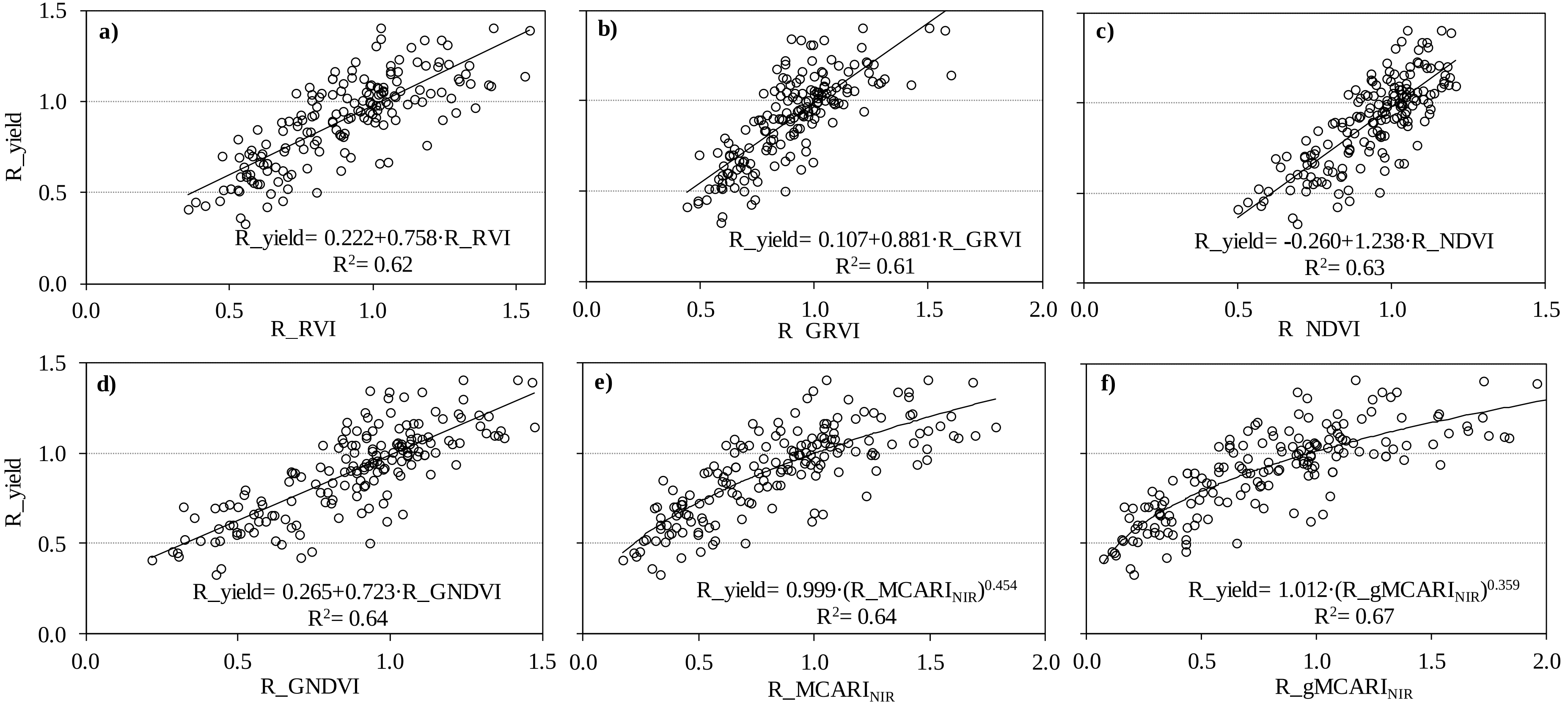

Figure 5.6. Relationship between R_yield and R_RVI (a), R_GRVI (b), R_NDVI (c), R_GNDVI (d), R_MCARI$_{NIR}$ (e), and R_gMCARI$_{NIR}$ (f) for the pooled data of the two years.

The coefficients of determination of the relations between R_yield and R_VIs were used to compare the performance of the indices. Coefficients of determination ranged between 0.40 and 0.77 (Table 5.8); in general, R_NDVI and R_GNDVI improved the relationships in comparison to R_RVI and R_GRVI (all of them including two bands in the calculation). R_gMCARI$_{NIR}$, that includes information of three bands, improved the relationship in comparison to the two bands VIs.

In 2012, R_GNDVI and R_gMCARI$_{NIR}$ explained 77 % of the R_yield variability, performing better than the rest of indices; and for 2013, R_gMCARI$_{NIR}$ was the index that explained the highest percentage (61%) of the R_yield variability (Table 5.8).

When data from both years were pooled; R_gMCARI$_{NIR}$, R_MCARI$_{NIR}$ and R_GNDVI showed the highest coefficients of determination (R^2=0.67, 0.64 and 0.64 respectively), all of them include the green band in their definition (Table 5.8).

R_gMCARI$_{NIR}$ and R_GNDVI were selected for building of the topdressing N recommendation approaches. R_gMCARI$_{NIR}$ was selected because it showed the best relation to R_yield and R_GNDVI was selected versus R_MCARI$_{NIR}$, both with the same strength in their relation to R_yield (Table 5.8), with the aim to test two different functions (linear and multiplicative) and two and three bands VIs. Thus, two different options (two-bands and three-bands VIs) and two different fittings (linear and multiplicative functions) were tested.

5.3.3. DISCUSSION

5.3.3.1. Influence of the year and type of fertilizer

The results show that spectral information absolute values need to be relativized by that of over-fertilized plots to obtain accurate crop’s response estimates (Figure 5.3a). In some studies, relativization processes, such as dividing the VI values by the number of growing degree days (GDD) (Yao et al., 2012) or the number of the days from transplanting (DAT) (Cao et al., 2015; Xue and Yang, 2008), have been included. This kind of relativization has the aim of avoiding differences due to different days of planting, phenology or locations. In our study, we do not find useful this relative values since the aerial images were taken at the same growth stage. Other studies have reported the improvement in yield estimation with relativization of VIs with respect to over-fertilized plots (Xue et al. 2014), obtaining sufficiency indexes. In our study, although the relationships improve when VIs values were relativized by the values in over-fertilization plots (Table 5.5), this relativization was not sufficient to eliminate the differences between years and yields also needed to be relativized to maximum yield of the year (Figure 5.3b and Figure 5.4a). Maximum yields change between years, in this experiment maximum yield in 2013 was about 25 % lower than in 2012, and that change affects the relation between spectral information and yield. Chang et al. (2005) found also differences in the relationships between yield and VIs, at booting stage, between the two different crop seasons of rice fields in Taiwan, and the differences were due to the different grain yields across crop season. They were able to obtain a reliable model for yield prediction splitting first and second crop seasons. Other authors have also obtained good relationships between vegetation index and yield in different years without converting the yield to relative values (Gilabert and Meliá, 1990; Wang et al., 2010; Xue and Yang, 2008; Xue et al., 2014). These findings contrast with the results of this study, where it was not possible to obtain an equation to predict yield absolute values. The variability in yield between years in the study area is high due to two reasons, the area is in the low limit temperature for adequate rice cultivation and the variability in meteorological conditions between years is fairly high.

Furthermore, differences between pig slurry and mineral fertilization were found in the spectral response (Figure 5.4b). Although studies focus on the differences between different types of fertilization in rice fields are lacking, there are some studies in other crops which support these results and could explain some of the possible reasons.

Yang et al. (2002) found differences in hyperspectral responses, using decision-tree models, between maize plots receiving organic manure from those amended with chemical fertilizers. Also, Zhao et al. (2015) measured the canopy apparent photosynthesis (CAP), photosynthetic rate of flag leaves, LAI and yield in a winter wheat experiment in plots fertilized with cow manure, urea and a mixed application. The results showed that during the early growth period, the single application of urea promoted a better development of the crop obtaining higher values of CAP or LAI; however, during the late growth stage, the single application of cow manure and the mixed application delayed the leaf senescence process when compared with the single application of urea. The results suggested that the mixed application of organic and inorganic fertilizers could delay leaf senescence and maintain a better canopy structure and higher photosynthesis capability at the late grain filling stage, which resulted in a higher grain yield. In our study, we have found the same type of behavior than Zhao et al. (2015), i.e. for the same value of R_VIs at booting, PS treatments reached latter a higher yield in comparison to M plots. This better development during the late stage could be attributed to different micronutrients provided by the organic fertilizers. Shahid et al. (2015) reported that manure application increased the concentrations of all the studied micronutrients in soil and hence maintained a positive balance for Fe, Zn and Cu in a long-term experiment in a tropical rice-system. Other authors also have reported a soil fertility improvement due to long-term application of organic fertilizer in rice systems (Dong et al., 2012; Huang et al., 2009).

Other reason that could explain the differences could be the canopy structure. Canopy structure greatly influences the absorption and transmission of solar radiation (Guo et al., 2015; Hu et al., 2011). In our study, the different fertilization (pig slurry versus mineral) could have enhanced a different canopy structure and hence, a different spectral response.

Further studies including canopy structure or plant tissues and soil analysis during the growing season are recommended to evaluate the possible causes of the differences found in this study.

5.3.3.2. Performance and comparative of the indices

R_VIs showed good relationships with R_yield (Table 5.8). Other studies have also found good relationships between these indices and rice yield (Cao et al., 2015; Chang et al., 2005; Gilabert and Meliá, 1990; Harrel et al., 2011; Yao et al., 2012). The coefficients of determination of the obtained relations are in the range reported by the above mentioned studies at the same growth stage (booting). For example, Yao et al. (2012) reported coefficients of determination for the linear relations between yield and NDVI or RVI of 0.59 and 0.73 respectively; and Cao et al. (2015) reported values of 0.56 and 0.41, for NDVI and RVI respectively; in our study the coefficients for the same indices ranged between 0.56 and 0.74.

Concerning to three bands VIs, Cao et al. (2015) showed a strong relation between yield and a modification of MCARI including the NIR wavelength, the coefficient of determination (0.79) was slightly higher than the one reported in this study (between 0.64 and 0.74) for the $MCARI_{NIR}$, with a very similar structure to Cao's MCARI.

R_GNDVI and R_NDVI showed good relationships with R_yield, equal or better than R_RVI and R_GRVI. GNDVI was proposed by Gitelson et al. (1996) to overcome the saturation problem of NDVI for high Chl concentrations or high values of biomass. In the present study, GNDVI performed better than NDVI in 2012, equal in 2013 and better when both years are pooled, thus confirming that GNDVI is more sensitive than NDVI to changes in agronomic parameters, in this case, yield.

One common problem with the two bands VIs is that become saturated under high values of biomass or green LAI. The study reported by Cao et al. (2015) suggests this saturation phenomenon. They observed determination coefficients of the linear relations between rice yield and NDVI and RVI at stem elongation of 0.63 and 0.66, respectively; however, for the rice booting stage, the R^2 decreased to 0.56 and 0.41, and for the heading stage, under high biomass conditions, both indices became saturated and no significant relationship between those two indices and yield existed. Harrell et al. (2011) at the stage of rice panicle differentiation found an even worst relation between yield and NDVI, in a rice field experiment with higher biomass values than in the study reported by Cao et al. (2015), confirming the saturation phenomenon.

Different authors have proposed three-band VIs to tackle this saturation phenomenon. In our study, R_MCARI1 (three bands VI) did not improve the R_yield prediction in comparison with the traditional two bands indices, even the relationships worsened; in contrast to the study reported by Haboudane et al. (2004), where MCARI1 showed to be less sensitive to the saturation phenomenon, although these findings were for different crops than rice (corn, wheat and soybean).

However, R_MCARI_{NIR} and R_gMCARI_{NIR} had a good relationship with R_yield and performed equal or better than the traditional R_NDVI and R_GNDVI. The index proposed in this study (R_gMCARI_{NIR}) was the best for R_yield prediction, improving the relationship obtained with R_MCARI_{NIR}. Therefore, the inclusion of the difference between the response in the green and red bands ("*G-R")* in $gMCARI_{NIR}$ render the index more sensitive to changes in biomass and thus to yield. Both indices were compared and it was observed that gMCARI$_{NIR}$ sensitivity is greater than MCARI$_{NIR}$ sensitivity when VI values are high, i.e. when crop is more developed and vigorous.

Despite the relationships found between R_yield and R_MCARI$_{NIR}$ or R_gMCARI$_{NIR}$ (three bands VI) were stronger than for two bands VIs, there was only a 1-7 % increase in the variability explained by these indices in comparison to two band VIs as R_NDVI and R_GNDVI. These results contrast with the study reported by Cao et al. (2015) where the yield potential variability explained by three bands indices was between 21 and 26 % higher than the variability explained by NDVI or RVI at rice booting stage.

The explanation to those differences in yield prediction improvement with three band indices could be related to potential yield. The highest yield in this study was around 8000 kg·ha^{-1}; however, yields reached 10000 kg·ha^{-1} in the study conducted by Cao et al. (2015) and were much higher than 10000 kg·ha^{-1} for the study conducted by Harrell et al. (2011) (where saturation phenomenon was also observed). Therefore, the saturation phenomenon could be attributed to high biomass conditions in high yielding systems. In these systems, two bands VIs does not perform well, especially at later stages with high biomass conditions; however, three bands VIs are able to overcome this saturation problem.

Thus, in low yielding rice system, as in our systems, two band indices, as NDVI and GNDVI, are no subject to the saturation phenomenon due to low crop biomass. This hypothesis is in agreement with the results of Xue et al. (2014), who found strong relationships between NDVI across the growing season, from tillering to grain filling, and rice yield potential, with yield level lower than 8000 kg·ha^{-1} (similar to the obtained in this study), so that saturation phenomenon due to high biomass conditions was not noticed.

Thus in our study, the best indices to predict R_yield were R_GNDVI, R_MCARI$_{NIR}$ and R_gMCARI$_{NIR}$, all of them incorporate the green band. This supports the findings of Cao et al. (2015) that showed that the best VIs for estimation of response index at harvest (the yield relativized to the yield from the plot with sufficient N) were green band-based VIs.

5.4. TOOLS FOR TOPDRESSING N RECOMMENDATION

5.4.1. METHODOLOGY

The two VIs with the strongest relation to yield were chosen for the elaboration of topdressing N recommendation models. These two indices were GNDVI and $gMCARI_{NIR}$, (see section 5.3.2). Three of the four replicates (75% of the data) of the experiment described in section 5.2.1 were used for establishing the models. The fourth replicate was used for performance evaluation. The replicate used in the evaluation process was not the same for all treatments and was determined by random draw in each treatment.

Two topdressing N recommendation approaches were evaluated, ΔN and RY. Both approaches need of N over-fertilized plots that are used as a specific siteXyear crop potential reference.

5.4.1.1. ΔN approach

The ΔN approach uses vegetation indices as indicators of crop nitrogen status, i.e. as quantifiers of nitrogen nutrition index (NNI). This approach was defined by Denuit et al (2002) using the HydroAgri N-Tester sensor and corrects the readings of the sensor with the readings of the nitrogen over-fertilized plot to obtain relativized VIs (R_VI). This type of approach has been used previously in rice by Chen et al. (2014) or Xue and Yang (2008).

The approach is based on the relationship between the variables ΔN and ΔR_VI (Figure 5.7). ΔN (Eq. 5.9) is the difference between the N applied in the treatment (N_T) and the critical N rate obtained in the yield response curve to N (C, Eq. 5.6); and ΔR_VI (Eq. 5.10) is the difference between R_VI and 1.

$$\Delta N = N_T - C \qquad \textbf{Eq. 5.9}$$

$$\Delta R\_VI = R\_VI - 1 \qquad \textbf{Eq. 5.10}$$

If ΔN estimate from spectral information is positive, topdressing N fertilization is not necessary; and on the other hand, if ΔN estimate is negative, the plot must be fertilized.

The equations adjusted to estimate ΔN from ΔR_GNDVI and ΔR_gMCARI_{NIR} (with the 3 replicates, 75 % of the data) were Eq. 5.11 and Eq. 5.12:

$$\Delta N = 2.843 + 211.491 \cdot \Delta R\_GNDVI \qquad \textbf{Eq. 5.11}$$

$$\Delta N + 200 = 207.291 \cdot (\Delta R\_gMCARI_{NIR} + 1)^{0.517} \qquad \textbf{Eq. 5.12}$$

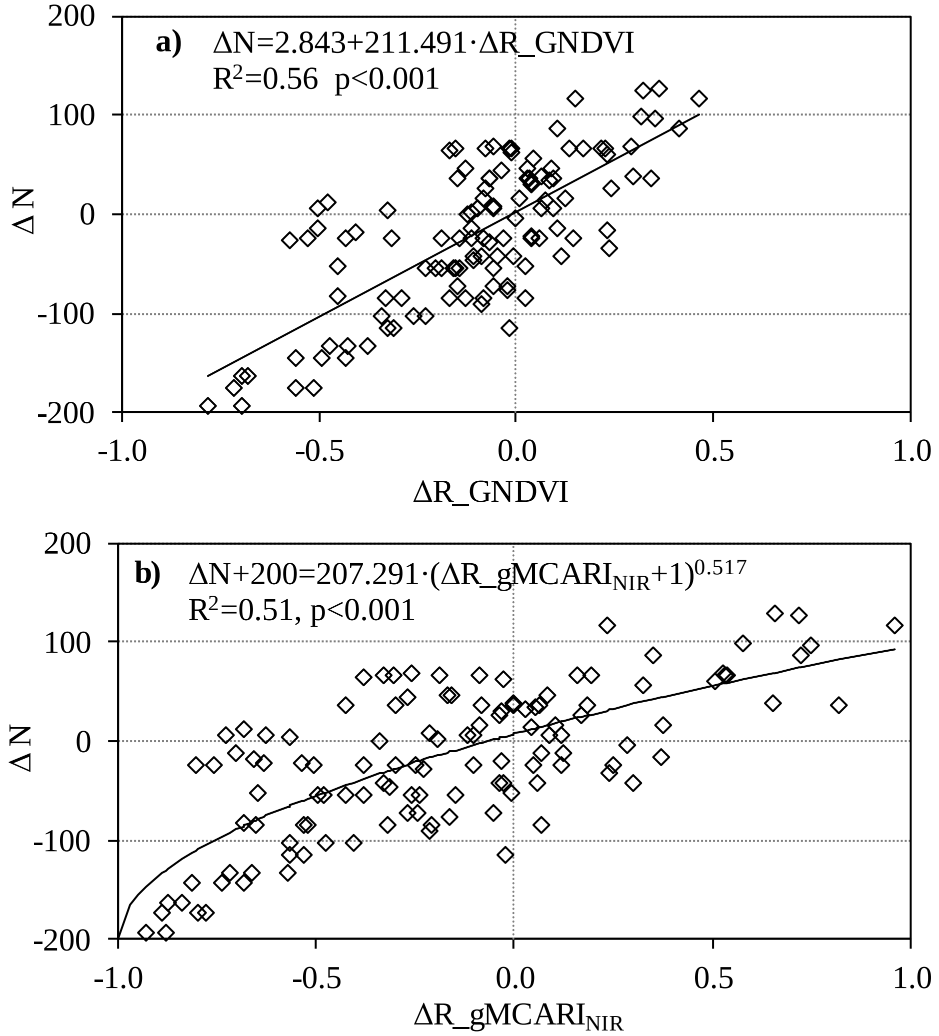

Figure 5.7. Relationship between ΔN (N increased or decreased compared with the optimum treatment) and ΔR_GNDVI (a) or ΔR_gMCARI_{NIR} (b) (R_GNDVI-1 or R_gMCARI_{NIR}-1). (pooled data of the two years, 75 % data).

5.4.1.2. RY approach

The second approach (RY) is based on the relationship between R_yield (Eq. 5.7) and R_VI (Eq. 5.8) (Figure 5.8). If R_yield estimated from R_VI is below 1, the plot must be fertilized; however, if the R_yield estimate is equal or above 1, the plot does not need additional N. This approach does not quantify the N necessity.

The relations between R_yield and R_GNDVI and R_gMCARI$_{NIR}$ (with the 3 replicates, 75 % of the data) were Eq. 5.13 and Eq. 5.14:

$$R\_yield = 0.252 + 0.741 \cdot R\_GNDVI$$ **Eq. 5.13**

$$R\_yield = 1.018 \cdot (R\_gMCARI_{NIR})^{0.365}$$ **Eq. 5.14**

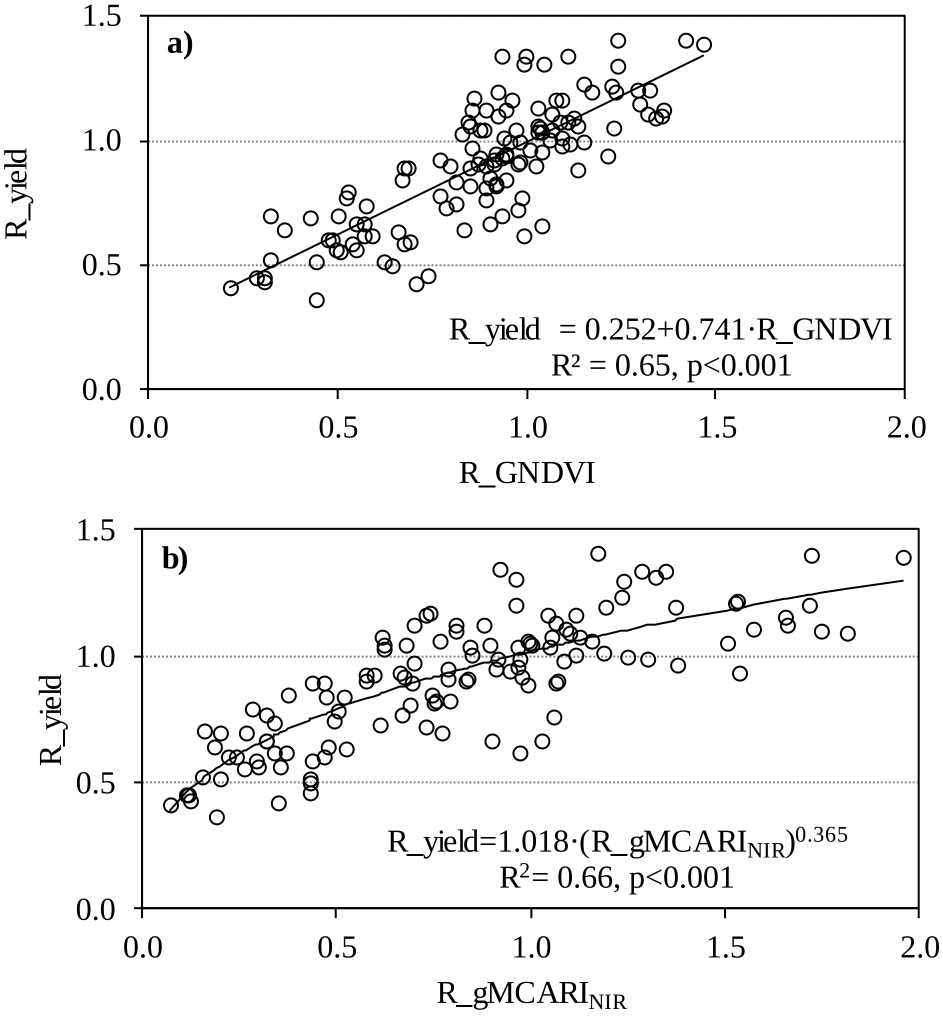

Figure 5.8. Relationship between R_yield and R_GNDVI (a) or R_gMCARI$_{NIR}$ (b) (pooled data of the two years, 75 % data).

5.4.1.3. Validation process

In the validation process, the values of R_GNDVI and R_gMCARI$_{NIR}$ in each year (2012 and 2013) for the validation plots (25% data) were obtained dividing the values obtained in each plot by the values obtained in the over-fertilized plots of the corresponding year (2012 and 2013). Values of VIs for over-fertilized plots were the average VIs of PS120M150 and PS170M150 for PS treatments and the average VIs of M120M90 and M120M120 for mineral treatments (Table 5.2).

For ΔN approach, the values of ΔR_GNDVI (or R_GNDVI–1) and ΔR_gMCARI$_{NIR}$ (or R_gMCARI$_{NIR}$–1) for each of the validation plots were first calculated, and then the values of ΔN were obtained using Eq. 5.11 and Eq. 5.12. If ΔN estimate is positive, topdressing N fertilization would not be needed, while if ΔN estimate is negative, the plot must be fertilized. ΔN gives the N deficit, i.e. gives the amount of N that need to be applied to obtain maximum yield. The performance of the model was evaluated by the percent of success. The sign of ΔN and the actual R_yield were compared in the validation plots and the plots were assigned to one of the following four options.

- ΔN was negative (i.e. the plot would have needed additional N fertilization) and the actual R_yield was below 1, which was a success.
- ΔN was positive (i.e. the plot would have not needed additional N fertilization) and the actual R_yield was equal or higher than 1, which was other success.
- ΔN was negative but the actual R_yield was equal or above 1, which would be a failure (N excess, because the approach would have recommended additional N fertilization, but the plot have reached the optimum yield).
- ΔN was positive, but the actual R_yield was below 1, which would be another failure (N defect, because the approach would have not recommended additional N fertilization, but the plot have not reached the optimum yield).

For RY approach, R_yield estimates for each plot were obtained from R_GNDVI and R_gMCARI$_{NIR}$ using Eq. 5.13 and Eq. 5.14. If R_yield estimate is below 1, the plot must be fertilized, and if R_yield estimate is higher than 1, the plot does not need additional fertilization. These estimates were compared to the actual R_yield in the validation plots and the plots were assigned, as for ΔN approach, to one of the following four options:

- R_yield estimate is below 1 (i.e. the plot would have needed additional N fertilization) and the actual R_yield is below 1, this is a success.
- R_yield estimate is equal of higher than 1 (i.e. the plot would have not needed additional N) and the actual R_yield is equal or higher than 1, which is also a success.
- R_yield estimate is below 1, but the actual R_yield is equal or above 1, which indicates a failure (N excess).
- R_yield estimate is equal of higher than 1, but the actual R_yield is below 1, which would be another failure, in this case by defect (N defect).

For the two approaches, success and failure percentages were calculated considering four strategies:

- The use of GNDVI
- The use of gMCARI$_{NIR}$
- The combination GNDVI & gMCARI$_{NIR}$: the plot would only be fertilized if both VIs recommend additional N fertilization.
- The combination GNDVI OR gMCARI$_{NIR}$: if one of the VIs recommends N fertilization, the plot would be fertilized even if the other index does not recommend N.

The fertilization treatments varied from 0 to 240 kg N·ha^{-1}, and some of the plots presented a high N defect. These plots with high N defect do not represent a real field situation and give an easy success in the validation; therefore, plots with R_yield below 0.7 were eliminated of the validation. The collection of plots for validation (24 in total) represents a real scenario, a situation where plots can be underfertilized but they are near to N optimum

5.4.2. RESULTS

For ΔN approach, $gMCARI_{NIR}$ performed better with an 87.5 % of success compared to GNDVI with 83.3 % success (Table 5.9); the percentage of N excess was lower, but the percentage of N defect was higher than for GNDVI. The combination of both VIs did not improve the ability for N recommendation prediction (Table 5.9). The GNDVI & $gMCARI_{NIR}$ possibility showed the same percentages of success and failure than $gMCARI_{NIR}$, and the GNDVI or $gMCARI_{NIR}$ possibility showed the same results than GNDVI.

For RY approach, the validation shows the same results as for ΔN approach (Table 5.9); both approaches agree in the necessity for fertilization in each of the 24 plot, but ΔN approach has the advantage that also gives the amount of N to be applied.

Table 5.9. Percentage of success and failure (N excess or defect) using the ΔN or the RY approaches (25 % data, excluded plots with R_yield below 0.7, total number of plots used for validation: 24).

APPROACH	SUCCESS	N EXCESS	N DEFECT
ΔN Approach			
GNDVI	83.3	12.5	4.2
$gMCARI_{NIR}$	**87.5**	**4.2**	**8.3**
GNDVI & $gMCARI_{NIR}$	87.5	4.2	8.3
GNDVI OR $gMCARI_{NIR}$	83.3	12.5	4.2
RY Approach			
GNDVI	83.3	12.5	4.2
$gMCARI_{NIR}$	**87.5**	**4.2**	**8.3**
GNDVI & $gMCARI_{NIR}$	87.5	4.2	8.3
GNDVI OR $gMCARI_{NIR}$	83.3	12.5	4.2

Since the combination of VIs did not improve the ability of N recommendation in comparison to using only one VI, $gMCARI_{NIR}$ (the VI with the highest percentage of success) was considered the best option and it was evaluated in the economic analysis.

5.4.3. DISCUSSION

The two evaluated approaches showed success percentages higher than 80 % and reaching 87.5 % for the best options (Table 5.9). Thus, these options confer an advantage against to the application of N topdressing without any advice. ΔN approach showed the same success percentage than the obtained with the RY approach. Both indicates the need (or not) for N at topdressing, but the latter has the disadvantage that a recommended N rate is not obtained. Nevertheless, it is important to point out that this validation process means only a preliminary evaluation of the approaches since it is based on the comparison of the approach´s recommendation and the actual yield harvested in the same experiment where the models (equations) were obtained. The two approaches should be tested further in real field situations, fertilizing the fields following the recommendations of the models and evaluating the yield obtained (Yao et al., 2012; Xue and Yang, 2008; Xue et al., 2014).

Other approaches found in the literature are based on the prediction of yield potential. These methodologies need to make a yield prediction in season to calculate the difference between the yield potential and the yield potential without topdressing fertilization. For instance, Yao et al. (2012) estimated the yield potential without N topdressing and the yield potential which could be achieved if N topdressing was applied (using data from plots with sufficient N application), and once the yield gap was obtained, an agronomic efficiency (AE_N) was used to estimate topdressing N rate. However, the relationships obtained in the present study did not allow predicting an absolute value of yield, just relative values. Therefore, these approaches are not suitable for our data. Meanwhile, the ΔN approach offers the advantage that it can be used when VIs and N applied values are only available.

Most of the times, NDVI is the index that have been used for in-season N recommendations in winter wheat (Raun et al., 2002), corn (Tubaña et al., 2008) or rice (Xue and Yang, 2008; Xue et al., 2014). However, in our study, GNDVI and $gMCARI_{NIR}$ showed relationships slightly better than the obtained with NDVI (Table 5.8), and for this reason these indices were selected for the development of tools for topdressing N recommendations. Nevertheless, if any kind of NDVI product was available (for instance, images provided by satellites), the results of applying the same approach would be presumably similar since there were not important differences in the relationships obtained between R_yield and R_NDVI in comparison to the other selected indices.

On the other hand, in contrast to other studies where only one index was used in the development of these approaches, in our study, the combination of two indices at the same time was tested (the plot is fertilized when both VIs recommend N topdressing or when one of the VIs recommends N fertilization even if the other index does not recommend N). Although these options did not increase the percentage of success under the studied conditions, it should be considered in further studies, because in other situations could increase the percentage of success.

Concerning the best time to obtain the spectral information, different studies have evaluated different growth stages throughout the season. Xue et al. (2014) reported that panicle initiation (PI) was the best among four stages (tillering, PI, heading and filling) for yield prediction. The results reported by Tubaña et al. (2012) indicated that the best period was PI+1 week. On the other hand, Chang et al. (2005) and Liu et al. (2015) found later stages, booting and heading respectively, like the best stages to predict yield. Gilabert and Meliá (1990) reported the ripening phase (too late for correction of N deficits) to be the best stage for yield estimation. In the present study, the images were taken at booting stage so that the crop canopy was fully developed and the interference of water in reflectance measurements was minimized. However, the evaluation of earlier times for images acquisition is needed so that the approach is useful for N recommendation to local farmers, since they usually apply the topdressing N at the end of the tillering or during the stem elongation stage in our study area.

5.5. ECONOMIC ANALYSIS

5.5.1. METHODOLOGY

The best options for N topdressing recommendation, ΔN and RY using gMCARI$_{NIR}$ were economically evaluated (see section 5.4.1).

The net benefit due to N topdressing application was calculated according to Eq. 5.15.

$$Net\ benefit = \left(Y_{N\ top} - Y_{real}\right) \cdot Price_{grain} - Price_{N} \cdot N_{rate} - Cost_{Napplicattion} \qquad \textbf{Eq. 5.15}$$

Where Y$_{Ntop}$ (t·ha^{-1}) is the yield hypothetically reached (according to response equation (Eq. 5.6) if the plot had been fertilized; Y$_{real}$ (t·ha^{-1}) is the actual yield harvested in the plot, Price$_{grain}$ is the rice grain price (€·t^{-1}); Price$_{N}$ is the N (ammonium sulfate) price (€·kg^{-1}); N$_{rate}$ (kg·ha^{-1}) is the N rate established for each scenario; and Cost$_{Napplication}$ (€·ha^{-1}) is the price for N application (gasoil + man labor). Prices of the local market (year 2015) (IAEST, 2015) were considered for the calculation. The net benefit did not include the costs of the images acquisition and the further processing. The net benefit was calculated for each of the 24 plots used in the validation process.

Different scenarios were economically evaluated:

-*Scenario 1- Reference:* All plots are fertilized with a fixed predefined N rate (N_{fix}) without using any recommendation approach. This strategy is currently used by farmers in the study area and will be considered the reference scenario.

-*Scenario 2 (ΔN approach):* Plots are fertilized according to ΔN estimates. Topdressing N is applied to the plots when ΔN is negative. The N rate is given by ΔN, (i.e. if ΔN=-30, N rate will be 30 kg N·ha^{-1}) (Eq. 5.16). But not all plots are fertilized.

$$\begin{cases} If\ \Delta N \geq 0;\ N\ rate = 0 \\ If\ \Delta N < 0;\ N\ rate = |\Delta N| \end{cases} \qquad \textbf{Eq. 5.16}$$

-*Scenario 3* (ΔN approach)*:* Is a variation of strategy 2, but a minimum topdressing N rate (N_m) is established (Eq. 5.17). This option was considered since machinery is not prepared to apply fertilizers at low rates and farmers do not usually go inside the field to apply small N rates.

$$\begin{cases} If\ \Delta N \geq 0;\quad N\ rate = 0 \\ If\ \Delta N < 0; \begin{cases} If\ |\Delta N| \geq N_m;\ N\ rate = |\Delta N| \\ If\ |\Delta N| < N_m;\ N\ rate = N_m \end{cases} \end{cases} \qquad \textbf{Eq. 5.17}$$

-*Scenario 4* (ΔN approach)*:* Is a variation of strategy 2. Plots are fertilized according to ΔN approach establishing a fixed predefined N rate (N_{fix}), i.e. if ΔN estimate is positive, the plots are not fertilized, if the ΔN estimate is negative, the plots are fertilized with N_{fix} (Eq. 5.18).

$$\begin{cases} If\ \Delta N \geq 0;\ N\ rate = 0 \\ If\ \Delta N < 0;\ N\ rate = N_{fix} \end{cases} \qquad \textbf{Eq. 5.18}$$

-*Scenario 5: (RY approach*): Plots are fertilized according to R_yield estimates. If R_yield estimate is below 1, the plot will be fertilized. This approach does not recommend the N rate, and the scenario is evaluated at different fixed predefined N rates (N_{fix}) (Eq. 5.19). Fertilization recommendation by ΔN and RY approaches match in the 24 validation plots of the study, so net benefit and N excess of scenarios 4 and 5 are equal. This situation can be different in further field validations.

$$\begin{cases} If\ R_{yield} \geq 1;\ N\ rate = 0 \\ If\ R_{yield} < 1;\ N\ rate = N_{fix} \end{cases} \qquad \textbf{Eq. 5.19}$$

To consider the environmental impact of overfertilization, N excess was also evaluated. For the plots that would be fertilized according to the different scenarios, N excess was calculated as the difference between the N rate applied and the N rate that would be necessary according to yield response to N equations.

For all scenarios, different predefined N rates (N_{fix}) and minimum N rates (N_m) were evaluated in the range 0-200 kg $N \cdot ha^{-1}$ and the net benefit and the N excess were represented graphically. The net benefit and the N excess are the average of all plots used in the economic analysis (number of plots, 24).

5.5.2. RESULTS

In the framework of the five scenarios analyzed in this work, the net benefit due to topdressing N fertilization ranged between -45 €$\cdot ha^{-1}$ and 120 €$\cdot ha^{-1}$ (the additional benefit if these plots had been fertilized with additional N).

Figure 5.9 shows the net benefit (a) and N excess (b) according to the fixed predefined N rate (N_{fix}) for strategies 1, 4 and 5 or to the minimum N rate (N_m) in the case of strategy 3.

The *Scenario 1*, all plots are fertilized, obtains the lowest net benefit, with a maximum of 75 €$\cdot ha^{-1}$ for a rate of 85 kg $N \cdot ha^{-1}$ (Figure 5.9a), and the highest N excess of the five scenarios reaching 53 kg N excess$\cdot ha^{-1}$ for the rate of maximum net benefit (Figure 5.9b).

Scenario 2, ΔN approach, shows a net benefit of 88 €$\cdot ha^{-1}$ and the N excess was 2.3 kg $N \cdot ha^{-1}$. The net benefit and N excess associated to this strategy have been represented as a horizontal green line in Figure 5.9 for comparative purposes with the rest of strategies.

The *Scenario 3*, ΔN approach establishing a minimum N rate (N_m), shows a higher net benefit than *Scenario 2*, reaching 120 €·ha^{-1} for a N minimum rate of 90 kg N·ha^{-1}. Therefore, the option of establishing a minimum N rate increased the net benefit (32 €·ha^{-1}), but this increase was counterbalanced with an increase in N excess (20 kg·N·ha^{-1} for the highest benefit versus 2.3 kg N·ha^{-1} in *Scenario 2*) (Figure 5.9).

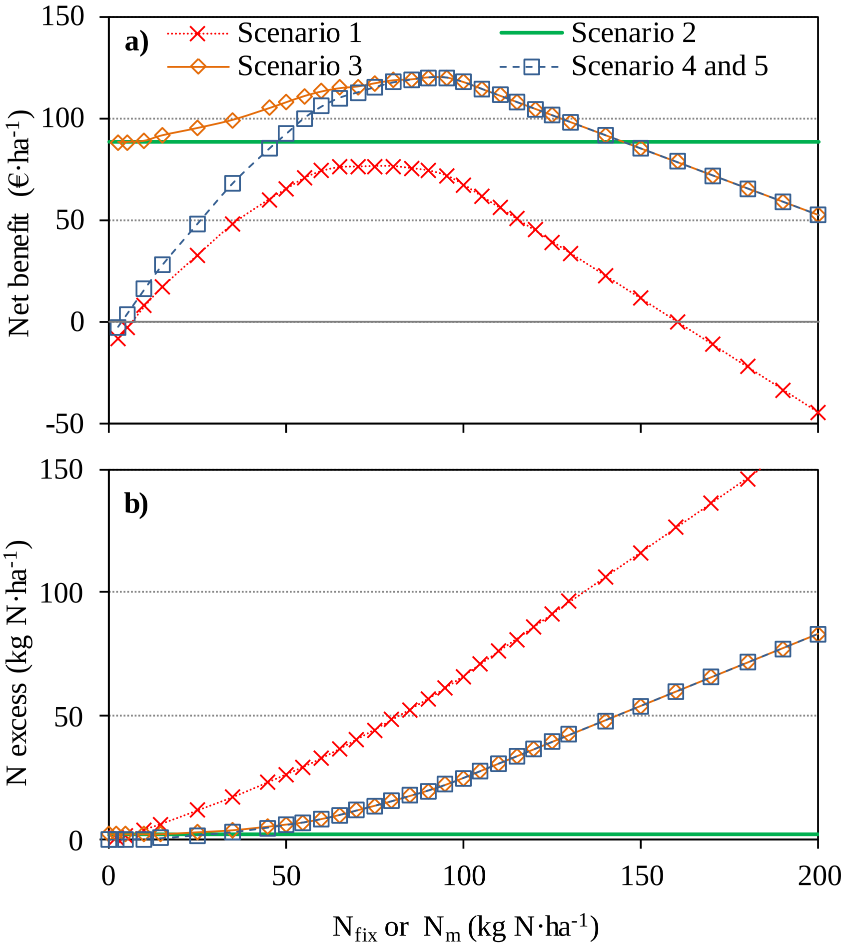

Figure 5.9. Net benefit (€·ha^{-1}) (a) and N excess (b) according to the fixed predefined N rate (N_{fix}, kg N·ha^{-1}) or minimum N rate (N_m, kg N·ha^{-1}). Each point is the average of the 24 plots used in the economic analysis.

The *Strategy 4*, ΔN approach with a fixed predefined N rate (N_{fix}), shows the same maximum net benefit (120 €·ha^{-1}) than *Scenario 3* for a single rate of 90 kg·ha^{-1} (Figure 5.9). In this option, the net benefit dramatically decreases as the N_{fix} decreases; therefore, the election of the optimum N_{fix} rate is a key factor, i.e. if N_{fix} is too low, the net benefit could be dramatically reduced and if N_{fix} is too high the N excess increases drastically. For the optimum N_{fix} rate of this study (90 kg·ha^{-1}), the N excess was 20 kg·N·ha^{-1}, the same than in *Strategy 3*.

Scenario 5, RY approach with a fixed predefined N rate (N_{fix}), shows the same net benefit and N excess curves than *Strategy 4* (120 €·ha^{-1} and 20 kg N excess·ha^{-1} for the optimum N rate: 90 kg N·ha^{-1}) (Figure 5.9). As was explained in section 5.4.2, both approaches ΔN and RY agreed with the fertilization recommendation in each of the 24 validation plots of the study.

5.5.3. DISCUSSION

The results show that *Scenario 1,* in which all plots are fertilized without spectral information consideration, is the worst of the five evaluated scenarios (Figure 5.9). This scenario shows the lowest net benefit and the highest N excess, suggesting that this option is neither economically nor environmentally viable when compared to the other scenarios. Therefore, the use of an approach for in season topdressing N recommendation is clearly necessary.

The ΔN approach seems to be a good strategy. Three possibilities were evaluated: fertilizing with the N rate according to the approach, ΔN, (*Scenario 2*), establishing a minimum N rate, N_m, (*Scenario 3*) or establishing a fixed predefined N rate, N_{fix}, (*Scenario 4*). When a minimum N rate is stablished (*Scenario 3*), the maximum net benefit increases in comparison to *Scenario 2*, the difference is 32 €·ha^{-1}, and the N excess increases in 18 kg N·ha^{-1} (Figure 5.9). On the other hand, when a fixed predefined N rate is established (*Scenario 4*), the maximum net benefit obtained is the same than that obtained in *Scenario 3*. However, in *Scenario 4,* a careful selection of the N_{fix} rate is crucial since the net benefit can dramatically lower with small modifications of the N rate (Figure 5.9); this N rate will depend on the year and it is difficult to estimate. Hence, *Scenario 2 and Scenario 3* seem to be better than *Scenario 4*. *Scenario 3* was considered because farmers do not usually go inside the field to apply small N rates or even machinery is not able to apply small amounts of fertilizer, thus a minimum N rate should be considered. This option allows to increase the net benefit under a large interval of N_m rates (from 0 to 150 kg N·ha^{-1}) (Figure 5.9), in which the net benefit is the same or higher than in *Scenario 2*. Therefore, in *Scenario 3* the net benefit is not at risk; however, the N excess increases as N_m increases; the cost of the risk for contamination, the N excess, has not been considered in the economic analysis and should be incorporated in future works. In Scenario 3, N_m rates between 30 and 90 kg N·ha^{-1} are believed to be optimal considering both economical return and N excess.

Scenario 5, using the RY approach, showed the same net benefit and N excess curves than *Scenario 4* since both approaches agreed in the N recommendation. The RY approach does not recommend the N rate, only recommend or not recommend fertilization; thus, the election of the N_{fix} rate is essential and will depend on the year. The election of a wrong N rate could severely impair the net benefit (Figure 5.9) and therefore, *Scenarios* 2 and 3 (with a recommended N rate provided by ΔN approach) are better options than *Scenario 5*.

The economic analysis suggests that the best option is the ΔN approach, using the recommended N rate (*Strategy 2*) or establishing a minimum N rate *(Strategy 3)* in order to increase the net benefit, although the increase of N excess should be economically evaluated.

These findings should be validated in a different set of field experiments, where some of the plots were fertilized according to the approach´s N recommendation and other plots according to the local practices in order to evaluate the real benefits. Economic evaluation of topdressing N recommendation has been analyzed by Xue and Yang (2008) using a similar approach to the ΔN approach of this work, with the vegetation index NDVI, and showed decreased N losses and increased net benefit for the treatments based on canopy spectra measurements in comparison to the standard N management. Also, Xue et al. (2014) evaluated the Nitrogen Fertilizer Optimization Algorithm (NFOA approach) and found that NFOA could increase rice yield with lower N rates, higher NUE and higher net income than the usual farmers practices.

The inclusion of the cost of the images capture and the further processing is crucial to evaluate whether these tools are economically feasible at regional level, although multispectral information arise as an useful tool for increasing net benefit and decreasing N excess in the agro-systems of Northern Spain.

5.6. CONCLUSIONS

The relationships between yield and the seven VIs evaluated showed significant differences between the two studied years, 2012 and 2013. This was expected, due to the variability in meteorological conditions in the study area that affects greatly rice development and yield. Further differences were detected between PS and M treatments, i.e. fields fertilized with pig slurry had a different spectral response that those fertilized with synthetic fertilizers. The inclusion of overfertilized plots for the two fertilization types and the individual relativization of spectral information for each type of fertilization permits the use of a single equation to explain the relation between VIs and yield in this crop. The differences in spectral response between PS and mineral fertilization should be analyzed in future studies for the elaboration of a sound N recommendation approach.

The best relationships between yield and VIs were obtained with indices including the green band. Additionally, three bands VIs performed better than the traditional two bands VIs, but the improvement was slight in contrast to other studies with higher yields and where saturation of two band indices was observed. The best relationships were obtained with GNDVI, $\mathrm{MCARI}_{\mathrm{NIR}}$ and $\mathrm{gMCARI}_{\mathrm{NIR}}$ (all three including the green band; $\mathrm{MCARI}_{\mathrm{NIR}}$ and $\mathrm{gMCARI}_{\mathrm{NIR}}$ including 3 bands).

The two studied approaches, ΔN and RY, were useful for N recommendation achieving the same success percentage in the validation. The best option was the use of the index $\mathrm{gMCARI}_{\mathrm{NIR}}$ and the combination of VIs did not improve the ability for N recommendation prediction. The ΔN approach gives a recommended N rate unlike the RY approach. This aspect is a clear disadvantage for the RY approach because the election of the N rate is crucial for economic viability since a wrong N rate election could decrease the net benefit as it was shown in the economic analysis. The economic analysis showed that topdressing N recommendations approaches clearly increase the net benefit and lower the N excess in comparison to fertilization without any recommendation. The best option in this study resulted to be the ΔN approach using the recommended N rates or establishing a minimum N rate (optimum minimum N rate between 30 and 90 kg $\mathrm{N \cdot ha^{-1}}$).

Therefore, the use of aerial remote sensing is a promising tool for developing strategies for farmers advising. However, more research is needed, including a validation of this approach to field level, the inclusion of the cost of the recommendation system in the evaluation of the net benefit, and the response of yield to spectral information in earlier crop development stages to adjust as much as possible the N topdressing to the usual practices in the area.

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CAPÍTULO 6

DISCUSIÓN GENERAL Y CONCLUSIONES FINALES

CAPÍTULO 6. DISCUSIÓN GENERAL Y CONCLUSIONES FINALES

6.1. DISCUSIÓN GENERAL

El principal objetivo de esta tesis ha sido profundizar en el comportamiento del purín porcino cuando se aplica como fertilizante sustituyendo a los fertilizantes minerales en cultivo de arroz inundado en la zona del nordeste español. Este estudio se plantea dentro del contexto del aumento de la producción porcina, y como consecuencia el aumento en la generación de purín porcino, en esta zona del país. El uso de este producto como fertilizante permite realizar un reciclado de los nutrientes que contiene, así como evitar los costes económicos y medioambientales asociados a la fabricación de fertilizantes minerales.

En primer lugar, se han evaluado agronómicamente dos estrategias de aplicación de purín porcino en fondo haciendo una valoración de los rendimientos alcanzados, las eficiencias del uso del N, la presencia de malas hierbas, plagas y enfermedades y la calidad del grano; todos estos aspectos se han comparado siempre con la fertilización mineral. En segundo lugar, se ha estudiado el impacto medioambiental a través de la cuantificación de la emisión de gases de efecto invernadero (GEIs) a la atmósfera. Y por último, se ha estudiado la posibilidad del uso de información multiespectral para el ajuste de la fertilización en cobertera, sirviendo así como herramienta de ayuda en la toma de decisiones y evitando los problemas asociados a dosis excesivas que no tienen en cuenta las necesidades reales del cultivo durante cada campaña.

En este apartado se discuten de manera conjunta los resultados obtenidos.

En el ensayo agronómico (Villanueva de Sigena) realizado durante 3 años consecutivos se compararon dos estrategias de aplicación de purín, una dosis de purín porcino en fondo que cubre las necesidades totales del cultivo (170 kg $N\text{-}NH_4^+ \cdot ha^{-1}$) y una dosis de purín en fondo inferior a las necesidades del cultivo (120 kg $N\text{-}NH_4^+ \cdot ha^{-1}$) que se complementa con fertilizante mineral en cobertera. Se ha podido comprobar que la aplicación de purín porcino no afecta a la germinación de la semilla, a la presencia de malas hierbas ni a la afección por plagas o enfermedades, ya que no existieron diferencias significativas entre los tratamientos de fertilización mineral y las estrategias de fertilización con purín. No obstante, los tratamientos con dosis muy altas de N mostraron una mayor afección por *Chilo Supressalis***,** que fue debida al exceso de N y no al tipo de fertilizante, ya que los tratamientos con dosis agronómicas óptimas de purín no mostraron una mayor afección cuando se compararon con las mismas dosis de fertilizante mineral.

Por otro lado, el rendimiento en molino (porcentaje en peso del grano cáscara que es obtenido una vez que el arroz se blanquea y se eliminan los granos imperfectos) fue similar para los tratamientos de purín y los minerales, y la calidad del grano a la cocción, caracterizada por el contenido de amilosa y la consistencia de gel, tampoco se vio afectada por la aplicación de purín. Además el análisis microbiológico del grano indicó que no existió contaminación microbiológica del grano de arroz debido a la aplicación de purín.

Las dos estrategias de aplicación de purín porcino en fondo permitieron alcanzar rendimientos máximos los 3 años de estudio. Para el caso de una dosis de purín porcino equivalente a 170 kg $N\text{-}NH_4^+\cdot ha^{-1}$, se consiguieron rendimientos máximos sin necesidad de aplicación de fertilizante mineral en cobertera; sin embargo, para la estrategia de 120 kg $N\text{-}NH_4^+\cdot ha^{-1}$ fue necesario complementar con fertilizante mineral en cobertera (entre 35 and 88 kg $N\cdot ha^{-1}$) para conseguir rendimientos óptimos. Estos resultados concuerdan con otros trabajos en los que también se consiguieron rendimientos máximos cuando sólo se aplicó N antes de la siembra (Bond and Bollich, 2007; Wilson et al., 2001). El arroz es un cultivo que necesita que se aplique en fondo una proporción importante de sus necesidades totales de N, ya que si la cantidad aplicada es insuficiente, el rendimiento no se puede recuperar aunque se apliquen dosis altas en cobertera (Wilson et al., 2001). Las dos estrategias de fertilización con purín se consideran adecuadas desde el punto de vista agronómico.

En el caso de otros cultivos, como el maíz, en los que se ha estudiado la aplicación de purín porcino, no se pueden aplicar estas dosis altas en fondo, debido a que existe un mayor riesgo de pérdidas asociadas a los procesos de nitrificación-lixiviación. Por ello, la estrategia más recomendada para evitar pérdidas altas de N y cumplir con las directivas europeas es la aplicación de dosis bajas en fondo complementadas con N adicional en cobertera (Berenguer et al., 2008; Yagüe y Quílez, 2010).

El cálculo del equivalente mineral para las dos estrategias de purín porcino estudiadas nos muestra que prácticamente todo el N amoniacal contenido en el purín es aprovechado por el cultivo, obteniéndose como media que el equivalente mineral (es decir la cantidad de N mineral que produce el mismo rendimiento que el rendimiento obtenido con el purín porcino) es un 92 % del N amoniacal aplicado con el purín. Sin embargo, cuando el equivalente mineral se calcula teniendo en cuenta el N total aplicado, el valor medio baja a 57 %. Estos valores nos indican que el N orgánico contenido en el purín no está siendo aprovechado por el cultivo en el año de aplicación.

Los valores de las eficiencias del uso del N calculadas en este estudio [eficiencia agronómica del N (AE_N) y eficiencia aparente de recuperación del N (RE_N)] estuvieron dentro del rango mostrado por otros autores para el cultivo del arroz (Ladha et al., 2005; Xie et al., 2007). Cuando las eficiencias fueron calculadas teniendo en cuenta el contenido de N amoniacal, no existieron diferencias entre los tratamientos de purín y mineral cuando se comparan estrategias de dosis equivalentes aplicadas en fondo (PS120 y M120). Sin embargo, cuando las eficiencias fueron calculadas teniendo en cuenta el contenido de N total del purín, aunque no se observaron diferencias significativas entre las dos estrategias mencionadas, sí hubo una disminución de la eficiencia para el tratamiento de purín, indicando de nuevo que el N aportado en forma orgánica no está siendo aprovechado por el cultivo. Estos resultados nos indican que las dosis de N se deben ajustar teniendo en cuenta el contenido de N amoniacal del purín en vez de su contenido de N total, porque de no ser así, las eficiencias del uso del N y los rendimientos se pueden ver reducidos.

El análisis de N mineral en el suelo nos indica que este N orgánico es una fuente de N no controlado que se puede perder en el periodo intercultivo. Cuando el suelo se muestreó en otoño, después de finalizar el ensayo, se observaron valores de N mineral en los primeros 30 cm de suelo en los tratamientos de purín mayores que en los de mineral; sin embargo, cuando la parcela se muestreó al inicio de la campaña en primavera, nunca se observaron diferencias entre tratamientos de purín y mineral, indicando que ese N mineralizado en otoño una vez que la parcela se drena y las condiciones son favorables para la mineralización, puede salir del sistema, mediante procesos como el lavado, y no ser aprovechado en la siguiente campaña. Por ello, una de las líneas futuras a estudiar podría ser la aplicación de la fase líquida del purín, de tal manera que se minimice la aplicación de N orgánico con el purín, sin aumentar así el riesgo de contaminación debido a la mineralización de ese N orgánico en momentos en los que no es aprovechable.

Aunque los resultados agronómicos mostraron que se pueden conseguir rendimientos máximos aplicando una única dosis de purín en fondo, y que los umbrales de N para conseguir los rendimientos máximos fueron similares en las dos estrategias (solo fondo o fondo+cobertera), el estudio de las emisiones de GEIs apunta a que dosis altas de purín porcino en fondo pueden aumentar las emisiones, en concreto las de metano, y por tanto, el potencial de calentamiento global.

Las emisiones de metano (CH_4) y óxido nitroso (N_2O) estuvieron muy influenciadas por los periodos de inundación-seca. En el caso del N_2O, los flujos fueron negativos siempre que la parcela estuvo inundada. El consumo de N_2O se puede deber a la utilización del mismo como aceptor de electrones en la ausencia de nitrato (Kögel-Knabner et al., 2010). Cuando la parcela se drenó y el suelo se empezó a secar, sí se observaron picos debidos a los procesos de nitrificación y desnitrificación. La comparativa entre los tratamientos de purín y mineral para dosis equivalentes de N no mostró una diferencia en las emisiones de N_2O debido a la aplicación de purín, ni en los flujos medios ni en las emisiones acumuladas durante el periodo estudiado en ninguna de las dos localidades. Aunque el aporte de C al aplicar fertilizantes orgánicos puede tener una influencia en el proceso de desnitrificación (Coyne, 2008), los resultados obtenidos en este estudio apuntan a que las emisiones de óxido nitroso no se ven afectadas en el caso de la aplicación de purín porcino debido a que el contenido de C del purín porcino es bajo.

El factor de emisión de N_2O inducido por la aplicación de fertilizante tampoco se vio modificado por la aplicación de purín porcino en dosis equivalentes a las del fertilizante mineral.

En el caso de las emisiones de metano, el patrón fue opuesto al observado para el óxido nitroso, observándose valores altos de emisión durante el periodo de inundación, debido al proceso de descomposición anaerobia de la materia orgánica (Ponnamepura, 1972), que disminuyeron drásticamente cuando el suelo se comenzó a secar.

En este caso, sí que se observó un aumento significativo en los valores de los flujos medios debido a la aplicación de purín como fertilizante, influenciado por la composición del suelo de cada ensayo. En la localidad en la que el contenido de materia orgánica del suelo era más bajo (Villanueva de Sigena), el tratamiento de purín con dosis de 120 kg $N\text{-}NH_4^+ \cdot ha^{-1}$ aplicada en fondo (PS120M60) no mostró diferencias significativas respecto al tratamiento mineral equivalente (M120M60) ni en los flujos medios ni en el valor de emisión acumulada; sin embargo, el tratamiento de dosis alta de purín porcino en fondo (170 kg $N\text{-}NH_4^+\cdot ha^{-1}$) mostró un flujo medio de metano significativamente más alto que el resto de tratamientos. Las pérdidas de CH_4 acumuladas al final de la campaña también fueron mayores para ese tratamiento aunque no difirieron significativamente de los tratamientos con dosis más baja en fondo. En el caso de Grañén, con un contenido de materia orgánica del suelo (2.06%) más alto que en Villanueva de Sigena (1.01 %), no se observaron diferencias ni en los flujos medios ni en las emisiones acumuladas entre los tratamientos estudiados, a pesar de que la dosis de purín porcino aplicada fue alta (170 kg $N\text{-}NH_4^+\cdot ha^{-1}$). Este diferente comportamiento se puede atribuir a que cuando se aplican enmiendas orgánicas, los cambios observados en las emisiones de GEIs son más acusados cuando se aplican a suelos con bajo contenido de materia orgánica (Neue et al., 1997; Win et al., 2014).

El incremento de las emisiones de metano en cultivo de arroz debido a la incorporación de paja ha sido ampliamente documentado (Sanchis et al., 2012); sin embargo, el contenido de C del purín porcino es bastante inferior al de paja y por ello no se produce un aumento de las emisiones de metano o este es menos acusado.

Los valores del potencial de calentamiento global relativizados al rendimiento (GWP_Y), calculados como la suma de N_2O y CH_4 en términos de CO_2 equivalente y referidos a los kg de grano producido, no mostraron diferencias entre los tratamientos de purín y mineral para dosis equivalentes de N en ninguna de las dos localidades; pero nuevamente, en la localidad con bajo contenido de materia orgánica en el suelo, el tratamiento de dosis más alta de purín en fondo mostró un mayor valor de GWP_Y (aunque no significativo) que los otros dos tratamientos (mineral y purín porcino) en los que se habían aplicado una dosis más baja en fondo y se habían complementado en cobertera.

La influencia de la fertilización del arroz con purín porcino en las emisiones de GEIs ha sido escasamente estudiada. Además, los estudios encontrados se han centrado en la aplicación de purín de cerdo digerido anaeróbicamente (ADPS), y en condiciones diferentes a las mediterráneas (estudios llevados a cabo en Asia), habiéndose obtenido en algunos casos resultados en la línea de los resultados obtenidos en este trabajo, y en otros casos resultados opuestos.

Huang et al. (2014) obtuvo un incremento significativo en las emisiones acumuladas de metano al aplicar ADPS en comparación con las mismas dosis de fertilizante mineral, a diferencia de los resultados obtenidos en este trabajo. Sin embargo, en concordancia con los resultados encontrados en este trabajo, Sasada et al. (2011) y Win et al. (2014) no observaron diferencias significativas en las emisiones de óxido nitroso ni metano cuando se aplicó purín digerido anaeróbicamente (ADPS) en comparación con las mismas dosis de fertilizante mineral. No obstante, en el estudio de Win et al. (2014) se encontraron emisiones de metano mayores (aunque no significativas) cuando se aplicó ADPS, en contraste con los resultados previos del estudio de Sasada et al. (2011) (estudio similar de los mismos autores), donde no se observó un aumento de las emisiones, atribuyendo las diferencias al diferente contenido de materia orgánica de los suelos. En condiciones mediterráneas, el único estudio publicado es el de Maris et al. (2016), que tampoco observó un aumento de las emisiones de óxido nitroso ni de metano debido a la aplicación de purín porcino en comparación con la fertilización mineral.

Por tanto, los resultados de la evaluación de las emisiones de GEIs, sugieren que la mejor estrategia para la fertilización del arroz con purín porcino es la aplicación de dosis en fondo que no cubran completamente las necesidades de cultivo y que sean complementadas en cobertera. Además, esta estrategia permitiría el cumplimiento de la Directiva de protección de las aguas contra la contaminación producida por los nitratos procedentes de fuentes agrarias (EU, 1991), la cual establece que la cantidad de estiércol aplicada a la tierra cada año estará limitada a la cantidad de estiércol o purín que contenga 170 kg $N \cdot ha^{-1}$ en las zonas vulnerables a los nitratos. Si se aplican dosis de 170 kg $N\text{-}NH_4^+ \cdot ha^{-1}$, la dosis de N total superarían el límite de 170 kg $N \cdot ha^{-1}$ impuesto por la Directiva, mientras que la aplicación de dosis más bajas en fondo, que no cubran las necesidades del cultivo, permitiría cumplir con el límite fijado por la Directiva, al mismo tiempo que se evitaría el riesgo de un aumento de las emisiones de GEIs.

Por otro lado, la aplicación de dosis altas en fondo se ha atribuido como una de las causas de bajas eficiencias en el uso del N; sin embargo, la aplicación de dosis más bajas complementadas en cobertera según la demanda del cultivo es una de las estrategias que ha sido relacionada con el aumento de las eficiencias del uso de N (Xie et al., 2007). Una de las herramientas utilizadas como ayuda para el ajuste de las dosis de N en cobertera es el uso de información espectral.

En la presente tesis se ha evaluado la utilidad de la información multiespectral tomada mediante imágenes aéreas, durante la fase de panícula en zurrón, para detectar el estado nutricional del cultivo y por tanto ajustar las dosis de N en cobertera.

En primer lugar, se relacionaron diferentes índices de vegetación (VIs) con el rendimiento en grano, observándose que tanto los valores de VIs como los de rendimiento necesitaban ser relativizados por el máximo de cada año, es decir, es necesario la utilización de parcelas sobrefertilizadas. Por lo tanto, no es posible estimar valores absolutos de rendimiento debido a las grandes diferencias en el rendimiento entre años, causadas principalmente por la variabilidad en las condiciones meteorológicas de la zona. Por otro lado, se observaron diferencias en las relaciones entre VIs y el rendimiento entre los tratamientos de purín y mineral, por lo que fue necesario relativizar los valores de VIs de manera independiente para los tratamientos de purín y mineral utilizando máximos distintos. Estudios llevados a cabo en otro tipo de cultivos también apuntan a que la respuesta espectral pueda ser diferente cuando se aplican fertilizantes orgánicos. Zhao et al. (2015) comparó la fertilización de trigo con urea y estiércol de vaca, obteniendo que la aplicación de estiércol solo o combinado con fertilizante mineral puede conllevar a un peor desarrollo del cultivo durante las primeras fases de desarrollo, reflejándose en valores inferiores de índice de área foliar (LAI); sin embargo, en las últimas fases de desarrollo, se retrasa la senescencia compensándose el menor desarrollo inicial. Las diferencias encontradas en la presente tesis entre purín y mineral son similares a las del estudio mencionado, ya que se ha obtenido el mismo rendimiento para las parcelas de purín y mineral, a pesar de que las parcelas de purín mostraban valores inferiores de VIs en el momento de la toma de imagen, es decir, la información espectral parece indicarnos que el cultivo fertilizado con purín tiene un peor desarrollo, pero finalmente consigue alcanzar el mismo rendimiento que el fertilizado con mineral. Estas diferencias en las relaciones rendimiento-VI necesitan ser estudiadas ya que es imprescindible identificar las causas de las mismas para la elaboración de herramientas robustas de recomendación de N.

Los índices que presentaron las mejores relaciones con el rendimiento fueron GNDVI, $MCARI_{NIR}$ y $gMCARI_{NIR}$ (propuesto en este estudio). Los tres incluyen la banda del verde y los dos últimos incluyen información de 3 bandas. Aunque se observó cierta mejoría en las relaciones entre el rendimiento relativo (R_yield) y los índices relativizados (R_VIs) al utilizar índices de 3 bandas espectrales respecto a los de 2 bandas, la mejora no fue tan acusada como la observada en otros estudios (Cao et al., 2015). La causa es debida a que los índices de vegetación que contienen dos bandas suelen saturar en sistemas de cultivo con altos valores de biomasa y por tanto, de rendimiento (Cao et al., 2015; Harrell et al., 2011). En esos sistemas altamente productivos, se observa una gran mejoría al utilizarse índices de vegetación con 3 bandas. En la zona de estudio, el potencial de producción no es alto y no se observó el fenómeno de saturación en los índices de 2 bandas (NDVI, GNDVI), resultado que concuerda con los observados en el estudio de Xue et al. (2014), en el que los rendimientos fueron similares a los de nuestro ensayo.

Los índices GNDVI y $gMCARI_{NIR}$ se utilizaron en la construcción de dos herramientas para el ajuste de la fertilización. Una de ellas basada en la relación entre la dosis de N aplicada y el valor del índice relativizado (denominada ΔN) y la otra basada en la relación entre R_yield y R_VI (RY).

En el proceso de validación en 24 parcelas del mismo ensayo, las dos herramientas obtuvieron el mismo porcentaje de éxito y de fallos por exceso y defecto en la predicción de la recomendación de N, obteniéndose el mayor porcentaje de éxito (87.5 %) con el índice $gMCARI_{NIR;}$ y la capacidad de predicción no mejoró con el uso combinado de los dos índices. La herramienta ΔN no solo recomienda la necesidad de fertilización, sino que tiene la ventaja sobre la herramienta RY de recomendar la dosis de N que es necesario aplicar.

El análisis económico, así como la evaluación del exceso de N, según diferentes escenarios, mostraron que la utilización de las herramientas propuestas para el ajuste de la fertilización en cobertera es una opción tanto económica como medioambientalmente favorable frente a la fertilización sin ninguna recomendación. La utilización de la herramienta ΔN fertilizando con la dosis recomendada o estableciendo una dosis mínima (N_m) - i.e. siempre que la dosis recomendada sea inferior a N_m, se aplica la dosis mínima N_m- son los mejores escenarios. El escenario que contempla la recomendación de fertilizar o no fertilizar y considera una dosis fija única (N_{fix}) para todas las parcelas no ha sido evaluado como buen escenario, ya que tiene un alto riesgo de producir infra o sobre fertilización. Así, una buena elección de la dosis N_{fix} es crucial, para valores de N_{fix} inferior a la dosis óptima, el beneficio neto disminuye rápidamente, mientras que para valores superiores al óptimo de N_{fix}, el exceso de N aumenta drásticamente.

Esta validación y análisis económico sólo suponen un estudio preliminar. Es necesaria una validación real en campo en la que se utilice la herramienta para la recomendación de la fertilización, y a posteriori después de la cosecha se compare económicamente esta opción frente a la de fertilizar sin ninguna recomendación, tal y como lo han llevado a cabo otros autores obteniendo resultados satisfactorios (Xue et al., 2014; Xue y Yang, 2008). Por otro lado, se considera necesario evaluar imágenes tomadas en fechas más tempranas de desarrollo del cultivo con el objetivo de acercar el momento de la adquisición de la información espectral con el momento habitual de la fertilización de cobertera. Además, sería necesario incluir en el estudio económico los costes asociados a la captura y procesado de imágenes, que no se han tenido en cuenta en este estudio y hacer un análisis económico adicional incluyendo las externalidades ambientales, en este caso definidas por el exceso de N.

Finalmente, a partir de los resultados obtenidos en esta tesis, se puede concluir que la fertilización del arroz inundado con purín porcino en la zona del nordeste español es una alternativa a la fertilización mineral que permite conseguir rendimientos máximos sin que se vean comprometidos otros aspectos como la calidad del grano o la presencia de malas hierbas y enfermedades. No obstante, la aplicación de dosis altas en fondo puede producir un aumento de las emisiones de GEIs, especialmente cuando los suelos tienen bajos contenidos de materia orgánica, y por ello, la opción más recomendable es la aplicación de dosis moderadas en fondo (≈70% de las necesidades del cultivo) complementadas con fertilizante mineral en cobertera. Para el ajuste de estas dosis, el uso de información multiespectral es una herramienta prometedora, en cuyo análisis técnico, económico y ambiental se debe profundizar en investigaciones futuras.

6.2. CONCLUSIONES FINALES

1. La aplicación de purín porcino al cultivo de arroz inundado permitió conseguir rendimientos máximos aplicando una única dosis en fondo (170 kg $N\text{-}NH_4^+ \cdot ha^{-1}$), mientras que la aplicación de una dosis inferior (120 kg $N\text{-}NH_4^+ \cdot ha^{-1}$) necesitó complementarse con fertilizante mineral en cobertera.
2. La aplicación de purín porcino no influyó negativamente en la germinación de la semilla ni en la presencia de malas hierbas, plagas o enfermedades. El rendimiento en molino y la calidad de cocción tampoco se vieron afectados. Además no existió contaminación microbiológica del grano.
3. El equivalente mineral del N aplicado en forma de purín, así como las eficiencias del uso del N indican que el N orgánico del purín no está siendo aprovechado por el cultivo y por tanto, las dosis de purín se deben ajustar teniendo en cuenta sólo el contenido de N amoniacal del purín porcino.
4. El factor de emisión de N_2O inducido por la fertilización no se vio afectado por la sustitución del fertilizante mineral por purín porcino.
5. No se observó una diferencia significativa en las emisiones de ninguno de los GEIs estudiados entre la fertilización mineral y la fertilización con purín porcino para las mismas dosis de N aplicadas en fondo. Sin embargo, en el suelo con bajo contenido de materia orgánica, una dosis alta de purín porcino en fondo incrementó las emisiones de metano. Por lo tanto, la mejor estrategia para conseguir rendimientos óptimos sin un aumento de las emisiones de GEIs es la aplicación de dosis moderadas en fondo (≈70% de las necesidades del cultivo) complementadas con fertilizante mineral en cobertera.
6. Teniendo en cuenta los resultados obtenidos, una de las líneas futuras de investigación sería la aplicación de la fase líquida del purín, de tal manera que se reduzca la cantidad de N orgánico que se aplica con el purín.
7. El uso de imágenes aéreas es una herramienta prometedora para el ajuste de las dosis de N en cobertera.

8. Los índices de vegetación (VIs) estudiados mostraron buenas relaciones con el rendimiento. Sin embargo, las diferencias encontradas entre años así como entre tipo de fertilización (purín y mineral) obligaron a la relativización de los valores de rendimiento y de los VIs. Estas diferencias deben ser analizadas en el futuro con el fin de obtener herramientas sólidas de ajuste de la fertilización.
9. Los índices GNDVI, $MCARI_{NIR}$ y $gMCARI_{NIR}$ fueron los que mostraron mejores relaciones con el rendimiento, permitiendo la elaboración de dos herramientas con un alto porcentaje de éxito en la predicción de la recomendación de N en cobertera. Por otro lado, el análisis económico mostró que el uso de estas herramientas supone una ventaja en comparación con la fertilización sin ningún tipo de recomendación, permitiendo además disminuir el exceso de N. No obstante, es necesaria una validación real en campo de las recomendaciones de las herramientas, el estudio de la toma de imágenes más tempranas, así como la inclusión del coste de la captura y procesado de las imágenes en la valoración económica, lo que determinará si estas herramientas son económicamente viables o no.
10. A partir de los resultados obtenidos en esta tesis, se puede concluir que la fertilización del arroz con purín porcino se puede y debe integrar en los planes de fertilización de las zonas del nordeste español. La mejor estrategia es la aplicación de dosis moderadas en fondo complementadas con fertilizante mineral en cobertera, permitiendo conseguir rendimientos máximos sin un aumento de las emisiones de GEIs. El uso de información multiespectral es una herramienta prometedora para el ajuste de estas dosis complementarias en cobertera.

6.3. REFERENCIAS

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ANEJO. LISTADO DE ABREVIATURAS

ADPS:	Anaerobically Digested Pig Slurry
AE_{NH4}:	Agronomic Efficiency of N, considering only the NH_4^+-N content of pig slurry
AE_{NT}:	Agronomic Efficiency of N, considering the total N content of pig slurry
ANOVA:	Analysis of variance
Avg. Max. T:	Average Maximum Temperature
Avg. Min. T:	Average Minimum Temperature
Avg. T:	Average Temperature
B:	Blue
BS:	Before Seeding
C:	Carbon
CH_4:	Methane
Chl:	Chlorophyll
CO_2 eq:	CO_2 equivalents
CO_2:	Carbon dioxide
ECe:	Electrical Conductivity of saturated paste extract
EF:	Fertilizer-induced N_2O Emission Factor
G:	Green
GC:	Gases Chromatography
GEIs:	Gases de Efecto Invernadero
GHG:	Greenhouse Gas
$gMCARI_{NIR}$:	Green peak Modified Chlorofphyll Absorption in Reflectance $Index_{NIR}$
GNDVI:	Green Normalized Difference Vegetation Index
GRVI:	Green Ratio Vegetation Index
GWP:	Global Warming Potential
GWP_Y:	Yield scaled Global Warming Potential
K:	Potassium
LAI:	Leaf Area Index
M:	Mineral
MCARI:	Modified Chlorophyll Absorption in Reflectance Index
MCARI1:	Modified Chlorofphyll Absorption in Reflectance Index1
$MCARI_{NIR}$:	Modified Chlorofphyll Absorption in Reflectance $Index_{NIR}$
n.s.:	not significant

N:	Nitrogen
N_2O:	Nitrous oxide
NDVI:	Normalized Difference Vegetation Index
N_{fix}:	Fixed predefined N rate for the evaluation of different scenarios in the economic analysis
NFRV:	Nitrogen Fertilizer Replacement Value
NH_4^+:	Ammonium
NH_4^+-N:	Ammonium Nitrogen
NIR:	Near Infrared
N_m:	Mininum N rate for the evaluation of different scenarios in the economic analysis
NNI:	Nitrogen Nutrition Index
NO_3^-:	Nitrate
NO_3^--N:	Nitrate Nitrogen
NT:	Total Nitrogen
NUE:	Nitrogen Use Efficiency
P:	Phosphorus
p:	p-value obtained in the analysis of variance
PAS:	Photoacoustic Spectroscopy
PI:	Panicle Initiation
PS:	Pig Slurry
R:	Red
R_VI:	Relative Vegetation Index
R_yield:	Relative yield
RE_{NH4}:	Recovery Efficiency of N, considering only the NH_4^+-N content of pig slurry
RE_{NT}:	Recovery Efficiency of N, considering the total N content of pig slurry
RVI:	Ratio Vegetation Index
SOC:	Soil Organic Carbon
SOM:	Soil Organic Matter
T:	Temperature
TP:	Topdressing
VIs:	Vegetation Indices
WFPS:	Water Filled Pore Space

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