Estrategias de fertilización nitrogenada para mejorar la eficiencia de uso del nitrógeno en *Brassica carinata*

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Página de aprobación

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Dedicatoria

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Resumen

El cultivo de *Brassica carinata* A. Braun (carinata) se presenta como una alternativa en sistemas agrícolas de Uruguay. Este trabajo, "Estrategias de fertilización nitrogenada para mejorar la eficiencia de uso del nitrógeno en Brassica carinata" forma parte del creciente interés por optimizar el uso del nitrógeno, con el objetivo de maximizar los rendimientos y minimizar el impacto ambiental. El análisis de la fertilización nitrogenada en cultivos de carinata en Uruguay (2016-2018) estableció puntos de referencia para el rendimiento agronómico, la eficiencia en el uso del nitrógeno (NUE) y la calidad del grano. Modelos de regresión cuantil, con intervalos de confianza generados mediante *bootstrapping*, identificaron las respuestas de rendimiento utilizando curvas de frontera en los percentiles 90, 50 y 10. Los rendimientos de grano alcanzaron un máximo entre 2,5 y 3,5 Mg ha^{-1} con la aplicación de 90-100 kg N ha^{-1}. Carinata demostró una alta capacidad de absorción de nitrógeno (N_{UPT}), que varió entre 1,0 y 1,5 kg N por kg de nitrógeno disponible, sin afectar significativamente el contenido de aceite (455-517 g kg^{-1}) ni el contenido de proteína (192-253 g kg^{-1}). Ensayos posteriores (2019-2020) compararon urea azufrada (0, 90 y 150 kg N ha^{-1}) con fuentes alternativas (ENTEC y Sulfammo-NPRO). ENTEC (150 kg N ha^{-1}) superó al control (práctica comercial 90 kg N ha^{-1}) y, como resultado, los rendimientos se incrementaron en un 70 %, mientras que urea y sulfammo mostraron aumentos del 32 % y 21 %, respectivamente. El estatus de nutrición de nitrógeno (NNI) y las curvas críticas de absorción de nitrógeno fueron cruciales para comprender la NUE. ENTEC 150 mantuvo un NNI > 1 desde BBCH30 hasta BBCH65, lo que redujo la necesidad de aplicaciones fraccionadas y minimizó las pérdidas ambientales de nitrógeno, esenciales para evaluar el potencial de mitigación de gases de efecto invernadero (GHG).

Palabras clave: brassicas, rendimiento agronómico, curva de dilución, índice de nutrición nitrogenada, gases de efecto invernadero

Summary

Brassica carinata A. Braun (carinata) is proposed as an alternative in agricultural systems in Uruguay, due to its potential for biofuel production and its adaptability. This work, “Nitrogen fertilization strategies to improve nitrogen use efficiency in Brassica carinata” is part of the growing interest in optimizing nitrogen use, with the aim of maximizing yields and minimizing environmental impact. The analysis of nitrogen fertilization in carinata crops in Uruguay (2016-2018) established benchmarks for agronomic yield, nitrogen use efficiency (NUE) and grain quality. Quantile regression models with confidence intervals generated by bootstrapping identified yield responses using frontier curves at the 90th, 50th and 10th percentiles. Grain yields peaked between 2.5 and 3.5 Mg ha^{-1} at 90-100 kg N ha^{-1}. Carinata had a high nitrogen uptake capacity (N_{UPT}), ranging from 1.0 to 1.5 kg N per kg available N, without significantly affecting oil content (455-517 g kg^{-1}) or protein content (192-253 g kg^{-1}). Subsequent trials (2019-2020) compared sulphur-containing urea (0, 90 and 150 kg N ha^{-1}) with alternative sources (ENTEC and Sulfammo-NPRO). ENTEC (150 kg N ha^{-1}) outperformed control (commercial practice - 90 kg N ha^{-1}), increasing yields by 70%, while urea and Sulfammo showed increases of 32% and 21%, respectively. Nitrogen nutritional status (NNI) and critical nitrogen uptake curves were critical to understanding NUE. ENTEC 150 maintained NNI > 1 from BBCH30 to BBCH65, reducing the need for split applications and minimizing nitrogen losses to the environment, which is essential for assessing greenhouse gas (GHG) mitigation potential.

Keywords: brassicas, agronomic yield, dilution curve, nitrogen nutrition index, greenhouse gases

1. Introducción

1.1. Bioenergía, biocombustible y biomateriales

El desarrollo de bioenergía tiene como objetivos principales la sustitución de combustibles fósiles y la reducción de las emisiones de gases de efecto invernadero (GHG) (Adler et al., 2012). Este proceso ha tenido un desarrollo exponencial en los últimos años, especialmente en el sector transporte (Popp et al., 2016). Los cultivos generan una fracción ínfima de la demanda mundial de energía (1,5 % energía eléctrica, 3 % generación de calor, 3 % combustible líquidos del sector transportes), aunque su participación está creciendo en los últimos años (Del Grosso et al., 2014). Un 20 % del total de energía con respecto a la consumida por el sector transportes; dentro de este, los biocombustibles líquidos representan un 3-4 % del total del consumo de combustible del transporte carretero (5 % del consumo total de bioenergía) (Popp et al., 2016). A su vez, se han sumado biocombustibles líquidos de alto valor energético, especialmente para el transporte aéreo (Seepaul, Small et al., 2019).

El crecimiento acelerado de la producción de biocombustibles es consecuencia de la promoción de políticas públicas. Un ejemplo son las políticas implementadas en Estados Unidos (EUA) y la Unión Europea (UE). La legislación de EUA ha promovido leyes a nivel federal y estatal, como la *Energy Independence and Security Act* de 2007 (EISA) o la *Renewable Fuel Standard* (RFS), con el objetivo de impulsar la industria de biocombustibles celulósicos (Adler et al., 2012) mediante el encargo anual de grandes cantidades de biocombustibles (Adler et al., 2012).

California Air Resources Board (CARB) adoptó el estándar *Low Carbon Fuel Standard* (LCFS) con el objetivo de reducir las emisiones de GHG de sus análisis de ciclo de vida (LCA, por sus siglas en inglés, *life cycle assessment*) en los combustibles para el transporte en este estado (Adler et al., 2012). El LCA cuantifica el desarrollo sostenible de los diferentes productos y servicios desarrollados evaluando de manera sistémica los impactos ambientales desde la materia prima hasta su el uso final del producto o servicio.

Cada proveedor de combustible debe controlar la intensidad de carbono (kilogramos de carbono equivalente por unidad de producto producida: kg CO2eq/unidad de producto) de su producto, con el objetivo de cumplir con la intensidad de carbono objetivo anual establecida (Adler et al., 2012). La UE, a través de la directiva de calidad de combustible (Directiva 2003/30), coordinó una reducción de la intensidad de emisiones de GHG en los combustibles utilizados en el sector transporte (Del Grosso et al., 2014). El objetivo fue una sustitución del 5,75 % de los combustibles fósiles por biocombustibles, lo que elevó ese valor a 10 % para el año 2020 (Del Grosso et al., 2014).

No obstante, los impulsos en las políticas en bioenergía muchas veces poco tienen que ver con una conciencia real del problema ambiental, sino que más bien responden a intereses y enmascaran subsidios a la actividad agrícola, bajo el pretexto de energía *verde* (Sampaio et al., 2004). Un argumento común entre países y economías para el desarrollo de energías renovables es promover la capacidad de suministro interno de cada país, lo que generó al mismo tiempo un efecto dinamizador de la economía rural (Adler et al., 2018).

1.2. Manejo de cultivos energéticos, emisiones de GHG y estrategias de mitigación

La literatura internacional se ha enfocado en la identificación de fuentes y sumideros de GHG, especialmente de tres de ellos, dióxido de carbono (CO_2), metano (CH_4) y óxido nitroso (N_2O), con el objetivo de identificar estrategias que puedan mitigar su emisión (Ogle et al., 2016). La principal fuente de emisiones de GHG ocurre en el sector energía, con aproximadamente 62 % del total, mientras que el sector agropecuario es responsable de aproximadamente un 15-30 % de las emisiones totales de GHG (Scheer et al., 2014). A pesar de que este porcentaje representa una menor proporción del total, los suelos que se usan para agricultura son responsables de la mayoría (más del 50 %) de las emisiones antropogénicas de óxido nitroso (N_2O), el GHG de mayor potencial de calentamiento (GWP, del inglés *global-warming potential*) según el Intergovernmental Panel on Climate Change (IPCC, 2014). El óxido nitroso (N_2O) en la tropósfera absorbe la radiación térmica terrestre lo que genera el calentamiento de la atmósfera y así el *efecto invernadero* (Del Grosso et al., 2009). El

GWP es 265 veces mayor que una masa igual de CO_2, sobre un horizonte de cien años (IPCC, 2014). Los óxidos de nitrógeno, es decir, de óxido nítrico (NO), dióxido de nitrógeno (NO_2) y N_2O también desempeñan un papel importante en la química del ozono estratosférico (Crutzen, 1970). N_2O es una sustancia que agota el ozono, mientras que el NO contribuye a la formación de ozono troposférico (O_3) y lluvia ácida (Pilegaard, 2013). A pesar de la significativa contribución potencial de este gas (N_2O), en el total del ciclo de vida del biocombustible, fruto de la etapa de producción de la materia prima, la cuantificación de los GHG asociados a esta etapa representa un desafío, debido a que estos pueden variar considerablemente con la posición geográfica y con el manejo (por ejemplo: emisiones de N_2O en diferentes climas o distintas dosis de fertilizantes por manejo) (Adler et al., 2012). El N_2O es producido en los suelos a través de dos procesos bioquímicos, nitrificación y desnitrificación (Khalil et al., 2004). La nitrificación es la oxidación de amonio (NH_4^+) a nitrato (NO_3^-), debido a la acción de microorganismos autotróficos o heterótrofos en condiciones aeróbicas, mientras que la desnitrificación es la reducción de NO_3^- a N_2O/N_2 por microorganismos heterótrofos en condiciones anaeróbicas (Guardia et al., 2018).

El uso de fertilizantes nitrogenados (orgánicos o sintéticos), la inclusión de leguminosas y el riego tienden a aumentar la producción de N_2O y las emisiones superan los niveles basales del suelo en ausencia de esa práctica de manejo, ya que aumentan el N disponible para ser nitrificado o desnitrificado (Del Grosso et al., 2009). La fijación biológica de nitrógeno es la principal fuente natural de N reactivo (N_R). Se considera N_R al N que no es removido en la biomasa cosechada del cultivo, contenido y reciclado en los residuos de los cultivos, o incorporado dentro del suelo a la materia orgánica o *pools* inorgánicos de N; por ende, se escapa de los sumideros de N y de los límites del agroecosistema, lo que genera una cascada de problemas ambientales externos a este (Cassman et al., 2002).

La fijación industrial del N se ha vuelto muy importante en los sistemas agrícolas, debido a que el aumento continuo de la demanda de alimento ha obligado a incrementar las cantidades de fertilizante nitrogenado aplicado, lo que ha alterado el ciclo global del N (Ladha et al., 2005). El N de origen inorgánico aplicado a los cultivos incrementa el *pool* N mineral, que es determinante para desencadenar las emisiones de N_2O; pero existen otros factores que pueden estimular la pérdida de N en forma gaseosa, como el contenido de carbono orgánico del suelo (COS) (suelos con altos niveles de materia orgánica mineralizan N desde la forma orgánica a la forma mineral) (Del Grosso et al., 2009), pH (equilibrio entre el NH_4^+ y el NH_3^+) en la solución del suelo) (Perdomo y Barbazán, 2007), temperatura y humedad (Bouwman et al., 2002). En resumen, si aumenta la disponibilidad de N, independiente de origen, también se incrementa el sustrato para los microorganismos nitrificadores y desnitrificadores]; por ende, las emisiones se ven positivamente estimuladas (Cavigelli et al., 2013; Mosier y Kroeze, 2000).

Las emisiones no solo se producen directamente de los suelos bajo cultivo, sino también indirectamente, ya que, debido a las pérdidas de N del suelo por volatilización, lixiviación o escorrentía, el N_2O puede generarse en otro lugar (Mosier et al., 1998). La agricultura actual, para ser agronómicamente viable, requiere del agregado de fertilizantes nitrogenados. Sin embargo, en términos medios, los cultivos solo son capaces de absorber entre un 30% y un 50 % del N agregado (Cassman et al., 2002; Dobermann, 2005), lo que trasforma al N no absorbido en una fuente potencial de contaminación ambiental (Ladha et al., 2005). Esto explica mayormente la baja eficiencia de uso del nitrógeno (NUE), ya que una fracción del N aplicado se pierde del sistema planta-suelo a través de las vías de volatilización del amoníaco (NH_3^+), lixiviación, nitrificación y desnitrificación (Cameron et al., 2013).

Sin embargo, la buena noticia es que los sistemas de cultivos pueden ser sumidero de CO_2 (Lal, 2004); por ende, mitigar el balance negativo de gases de efecto invernadero. En este sentido, el cambio a sistemas con menores períodos de barbecho y la eliminación de labranza convencional han aumentado los niveles de COS (Ernst et al., 2020; Lal, 2004). A su vez, el uso correcto de prácticas de fertilización puede resultar en un incremento de la productividad primaria neta (PPN) y, con ello, los balances de carbono de los suelos (Mazzilli et al., 2015). Si bien estas prácticas son positivas desde el punto de vista de la conservación del recurso suelo (aumentos en el nivel de COS), los residuos en superficie de los sistemas de labranza cero son fuente de N después de la cosecha, lo que ha aumentado la emisión de N_2O, que es capturado en los inventarios que las naciones deben reportar (Ferrari Machado et al., 2021).

La contabilización de los flujos de GHG del suelo presentan importantes incertidumbres, tanto espaciales como temporales, lo que dificulta la cuantificación de las diferencias incrementales de los insumos aplicados (Gao et al., 2018). Varias estrategias han sido sugeridas para reducir las emisiones de GHG de los suelos cultivados. Las opciones destinadas a reducir las emisiones de un gas de efecto invernadero (por ejemplo, N_2O) probablemente perturben los flujos de los restantes gases de efecto invernadero (Robertson y Vitousek, 2009).

La bibliografía es coincidente en las estrategias necesarias para la mitigación de las emisiones de GHG (en especial, las emisiones de N_2O) destacándose 1) la reducción de la dosis de fertilizante nitrogenado aplicado, 2) la aplicación de precisión en los fertilizantes nitrogenados, 3) el uso de inhibidores de la nitrificación o ureasa 4) el uso de siembra directa (Davis et al., 2013; Erisman et al., 2010; Robertson y Vitousek, 2009; Smith et al., 2008). También es posible que la reducción de la dosis de fertilizante nitrogenado aplicado conduzca a una disminución de las emisiones de N_2O (Bouwman et al., 2002). La estrategia de reducción de las tasas de fertilización parece ser la opción más sencilla; sin embargo, esta práctica puede conducir a una reducción de los rendimientos de los cultivos y, con ello, al ingreso de carbono al suelo y los niveles de COS (Del Grosso et al., 2009; Ogle et al., 2016), además de que esta estrategia solo sería viable en sistemas que fertilizan con mayores niveles que los necesarios (Ogle et al., 2016). Por estos motivos, estrategias de aplicación precisa de fertilizantes (por ejemplo: agricultura de precisión con dosis variable, fraccionamiento de dosis) y uso de inhibidores son consideradas más viables, ya que la disponibilidad de N se daría de manera más sincronizada con la demanda de la planta, lo que resulta en una reducción de la cantidad de N disponible para los procesos microbianos que dan lugar a las emisiones de N_2O (Del Grosso et al., 2009; Ladha et al., 2005).

1.3. Cultivos de brassicas para biocombustibles

Antes de considerar un cultivo agrícola como potencial productor de biocombustibles, hay tres elementos a tener en cuenta: 1) las emisiones de GHG y los insumos energéticos necesarios para la producción, los cuales son variables según tipo de cultivo y región, 2) la utilización de cultivos alimentarios para biocombustibles puede conducir a un incremento de los precios de los alimentos y 3) la demanda por cultivos para biocombustibles puede aumentar la frontera agrícola y con esto las emisiones de GHG por el cambio de uso de la tierra (Miller y Kumar, 2013). Debido a estos tres problemas, cuando se introduce un nuevo cultivo para bioenergía, es necesario reducir daños colaterales de su producción. En este sentido, es sensible el aumento del número de publicaciones científicas destinadas a evaluar la sostenibilidad de los biocombustibles. Para medir dicha sostenibilidad, se sugiere evaluar las emisiones de GHG del ciclo de vida (LCA) y la relación de energía neta entre la producción de energía (por el cultivo) y el aporte de energía de los combustibles fósiles (NER) (Miller y Kumar, 2013).

Los biocombustibles son clasificados principalmente de acuerdo con el tipo de materia prima y tecnología utilizadas para su producción (Serrano-Ruiz y Luque, 2011). A partir de estos criterios se dividen en biocombustibles de primera y segunda generación (Serrano-Ruiz y Luque, 2011). Los biocombustibles de primera generación son aquellos que provienen de materias primas de origen comestible (azúcares, almidones y aceites vegetales comestibles), producidos a través de tecnologías convencionales y bien establecidas, por ejemplo: fermentación y transesterificación (Serrano-Ruiz y Luque, 2011).

Por otra parte, los combustibles de segunda generación son aquellas materias primas que no son de origen comestible. Algunos ejemplos de ellos son cultivos lignocelulósicos y oleaginosas no comestibles (Serrano-Ruiz y Luque, 2011). Muchas tecnologías se encuentran en desarrollo para estos biocombustibles, pero la generación de biodiésel a partir de la hidrogenación es una tecnología destacada y probada en este tipo de biocombustibles (Seepaul et al., 2019). Sin embargo, hay alternativas a la clasificación clásica de combustibles de primera y segunda generación, ya que se puede obtener diferente biodiésel a partir de la misma materia prima; un ejemplo de ello es el diésel renovable derivado de la hidrogenación (HDRD), cuya composición se aproxima más a la del diésel proveniente de combustible fósil, teniendo así mejores propiedades de flujo en frío que el biodiésel (esta excepción es debida principalmente a cambios en los procesos tecnológicos de producción) (Kalnes et al., 2007; Šimáček et al., 2011).

En la Unión Europea ha incrementado el área de colza 2,2 veces entre el año 2003 y 2018 (Ruser et al., 2017). En Europa el cultivo de colza tiene una destacada participación en las rotaciones, representando más del 75 % del total de oleaginosas que se producen en el continente (Carré y Pouzet, 2014), teniendo como líderes a Alemania y Francia, con un promedio de rendimiento en grano de 5,0 Mg ha^{-1} demostrando el alto potencial de rendimiento de colza de tipo invernal, determinante de su liderazgo en estos países (Ruser et al., 2017a). Por varios años, el desarrollo de biocombustibles de primera generación se consideró una de las soluciones principales para mitigar el cambio climático y también aumentar la seguridad energética debido a la sustitución en las importaciones de combustibles fósiles (Farrell et al., 2006). Los ambiciosos objetivos llevadas a cabo tanto en EUA como la UE para el desarrollo de biocombustibles fueron revisados y se establecieron un conjunto de criterios de sostenibilidad (Ben Aoun et al., 2016). A partir del año 2017, la UE determinó que la sustitución de combustibles fósiles por biocombustibles debía permitir al menos un ahorro del 50 % de las emisiones de GHG, sobre la línea base del análisis de ciclo de vida (LCA) (Ben Aoun et al., 2016) y que la energía empleada para la producción de biocombustibles no debe superar el 7 % de la energía total consumida por el sector transporte para ninguno de los países miembros (Ben Aoun et al., 2016).

El análisis del cultivo de la colza como materia prima para biodiésel en Europa incluye la producción de fertilizantes, el uso de agroquímicos (insecticidas, fungicida y herbicidas), el transporte de la materia prima a la planta de producción de biocombustibles y el uso de fertilizantes tienen un elevado porcentaje del total de los gases de efecto invernadero contabilizados en el ciclo de vida para el producto final (75-86 %) (66,7-119,5 g CO_2 totales por unidad de producto MJ fuel $^{-1}$) (Hoefnagels et al., 2010).

Las emisiones de N_2O, asociadas a la fertilización nitrogenada, explican en parte la elevada contribución de la etapa de campo al total de las emisiones computadas al biocombustible (Kaiser et al., 2000; van Groenigen et al., 2010). Por lo tanto, es de suma importancia la relación entre las emisiones de N_2O por unidad de N que se agrega, ya que el rendimiento adicional asociado a ella (unidad de N agregada) debe ser tal que permita disminuir la huella de emisión por unidad de producto derivada (kg rendimiento/kg de N_2O emitidos) (Hoefnagels et al., 2010). En este sentido, el cultivo de colza presenta una baja eficiencia de uso del nitrógeno (NUE) (Hegewald et al., 2016; Rathke et al., 2005; Ruser et al., 2017a).

El cultivo de colza y las brassicas en general requieren altas cantidades de fertilizante nitrogenado para construir biomasa, pero posteriormente bajas cantidades de biomasa son acumuladas en la semilla, presentando así un bajo índice de cosecha (HI) (Hegewald et al., 2016). Sumado a ello, el índice de cosecha de N (NHI) es bajo, quedando altas cantidades de N en la biomasa no cosechada ($N_{SURPLUS}$) ($N_{SURPLUS}$ = N Fertilizado – N absorbido por los órganos cosechados) con valores mayores a 90 kg N ha^{-1} $año^{-1}$, muy superiores a los reportados para trigo de invierno para una misma zona geográfica (40 kg N ha^{-1} $año^{-1}$) (Sieling y Kage, 2010).

El nitrógeno que no es cosechado ($N_{SURPLUS}$) queda susceptible a pérdidas gaseosas o de lixiviación en el medioambiente (Rathke et al., 2005); debido a ello, Alemania, por ejemplo, ha establecido leyes que regulan el uso de fertilizantes N en colza (Sieling y Kage, 2010). Otras zonas productoras de colza denominada Grandes Llanuras del norte integrada por los estados de Alberta, Saskatchewan y Manitoba en Canadá, y Montana, Dakota del Norte y las partes occidentales de Minnesota en EUA siguieron lo hecho en Alemania. Estos estados también requieren de la aplicación de nitrógeno (N) para optimizar el rendimiento y la calidad de los cultivos de colza (Malhi et al., 2005). Tiessen et al. (2006) reporta para la provincia de Manitoba que la sincronización entre la oferta y demanda de N es un punto crítico. Esto se debe a que la probabilidad potencial de pérdida de N aumenta con el tiempo que el fertilizante está en el suelo antes de que el cultivo pueda absorberlo, lo que resulta en mayor probabilidad de pérdida en el caso de que la temperatura y la humedad del suelo aumenten, debido a que la tasa de conversión de NH_4^+ a NO_3^-, lo que incrementa el riesgo de desnitrificación y lixiviación (Tiessen et al., 2006).

1.4. *Brassica carinata* A. Braun: un biomaterial promisorio para Uruguay y el mundo

La creciente demanda de aceites vegetales para usos alimentarios y no alimentarios convive con una disponibilidad limitada de tierras cultivables productivas

(Seepaul et al., 2021). Para cumplir con las demandas industriales sin afectar la capacidad de producir alimentos, es esencial que los programas de mejoramiento desarrollen nuevos y mejores cultivares de semillas oleaginosas con el objetivo de aumentar la producción en las regiones del mundo que no son óptimas para los cultivos de alimentos (Marillia et al., 2014). En el caso particular de las brassicas para biocombustibles, esto es especialmente necesario para crear un sector energético sostenible (Gressel, 2008). En Canadá el principal cultivo de semillas oleaginosas es la colza, pero también es sembrada *Brassica rapa* L. (Marillia et al., 2014) y más recientemente se ha puesto el foco en *Brassica carinata* A. Braun (carinata), comúnmente llamada *mostaza abisinia* o *etíope*, que es un anfidiploide (BBCC, 2n = 34) formado a través de la hibridación interespecífica entre las especies diploides *B. nigra* L. (BB, 2n = 16) y *B. oleracea* L. (CC, 2n = 18) (Axelsson et al., 2012).

Carinata tiene la capacidad de crecer en suelos limitantes para otros cultivos y, por lo tanto, tiene el potencial de contribuir a la expansión de la frontera agrícola sin afectar la producción de cultivos para alimento (Marillia et al., 2014). Características como la alta tolerancia a la sequía y temperaturas elevadas ha impulsado el interés y expansión del cultivo en países como España (Velasco et al., 1999), India (Pan et al., 2012), Italia (Cardone et al., 2003), sureste de EUA (Seepaul et al., 2019), Australia (Zhao et al., 2017), Argentina y Uruguay (Bonansea et al., 2023). Además, de las anteriores características agronómicas deseables, presenta mayor tolerancia a algunas enfermedades como *Leptosphaeria maculans* (Axelsson et al., 2012), resistencia a los áfidos (Axelsson et al., 2012), tamaño de semilla mayor al de colza y tolerancia a la dehiscencia natural (Khedikar et al., 2020).

Comparando con sus parientes cercanos (*B. rapa*, *B. napus* y *B. juncea*), la inclusión de carinata en programas de mejoramiento genético ha sido limitado, debido especialmente al menor rendimiento, la menor calidad del aceite frente a canola y a la falta de disponibilidad de herramientas y recursos genéticos (Zhang et al., 2020). Esto ha cambiado en los últimos años, principalmente, en consecuencia, por el interés por biocombustibles y materias primas bioindustriales, ya que lo que se consideraba una mala calidad de aceite para la elaboración de alimento ahora es una excelente calidad como materia prima para elaborar biocombustible, consecuencia de su composición de ácidos grasos de cadena larga y muy larga, (*e. g.*: erúcico C22:1Δ13, nervónico 24:1Δ15, docosadienoico C22:2Δ5Δ13 (Axelsson et al., 2012). Esta composición ha llevado a que carinata se considere un cultivo adecuado para la producción tanto de etanol como de biodiésel (Bouaid et al., 2009), así como para aplicaciones industriales diversas como biopolímeros, lubricantes, jabones, surfactantes (Pan et al., 2012), biofumigación (Márquez-Lema et al., 2009) y farmacéutica (Axelsson et al., 2012).

Entre los múltiples usos industriales que se reportan para este cultivo (carinata), una utilidad destacada y desarrollada, especialmente en Canadá y EUA, es la producción de biocombustibles para el abastecimiento del mercado del transporte aéreo (Kumar et al., 2020; Mulvaney et al., 2019). Las materias primas utilizadas para la producción de biocombustibles de segunda generación deben ser capaces de utilizar los recursos naturales de manera competitiva, mientras que no deben quitar tierras que se destinan a la producción de alimentos (Mulvaney et al., 2019; Seepaul, Marois, et al., 2019). El aceite extraído de carinata es de alto valor comercial, ya que, a través de procesos como la incorporación de hidrógeno, se puede obtener un biocombustible con propiedades físico-químicas similares a los productos generados a base de petróleo, por ejemplo: *hydrotreated renewable jet fuel* (HRJ) (Cardone et al., 2003; Gesch et al., 2015).

1.5. Manejo de la fertilización nitrogenada para reducir emisiones de GHG

El uso de buenas prácticas de manejo (BPM) tales como la rotación de cultivos y el manejo balanceado de la fertilización nitrogenada pueden conducir a una reducción de las emisiones de N_2O (van Groenigen et al., 2010). Por lo tanto, es de vital importancia, debido a que las entradas de N como fertilizante están fuertemente vinculadas tanto al rendimiento en grano como a las emisiones de N_2O (van Groenigen et al., 2010). Si se relaciona el rendimiento en grano con las emisiones de GHG, es posible que se alcance la viabilidad económica de los cultivos al mismo tiempo que se conserva el medioambiente a través del uso de dosis de nitrógeno equilibradas (Mosier et al., 2006). En estudios agronómicos se usa la eficiencia de uso del nitrógeno (NUE) para optimizar la inversión en fertilizantes, pero, al mismo tiempo, la NUE también sirve como indicador de pérdidas de N y emisiones potenciales de GHG (van Groenigen et al., 2010). Las emisiones de N_2O muestran una relación significativamente negativa con índices agronómicos de NUE, como la recuperación aparente del N (RE_N) (fracción de N aplicado absorbido en la biomasa aérea en la madurez fisiológica en relación con un tratamiento sin fertilización nitrogenada) (van Groenigen et al., 2010). Dentro de las estrategias más promisorias para disminuir las emisiones de N_2O como consecuencia de la fertilización nitrogenada, se encuentra el uso de fertilizantes de eficiencia mejorada (EEF) (Lam et al., 2018; Villar y Guillaumes, 2010). Estos productos incluyen principalmente tres grupos: 1) inhibidores de ureasa (UI), 2) fertilizantes nitrogenados recubiertos (SR) e 3) inhibidores de nitrificación (NI) (Villar y Guillaumes, 2010). En términos generales, los tres grupos buscan, a través de su patrón de liberación retrasada o lenta del N, mejorar la sincronicidad entre la oferta y la demanda de los cultivos (Ladha et al., 2005). Análisis estadísticos (metaanálisis) reportan algunas tendencias en cuanto al uso de los EEF: en general, reducen las emisiones de GHG entre un 5,4 % a 39,8 % para las emisiones de N_2O y entre un 30,7 % a 61,5 % para las emisiones de NH_3^- (Xia et al., 2017) e incrementan la recuperación del N aplicado como fertilizante, entre 16,4 % y 10,2 %, aunque estos valores son más variables entre los diferentes estudios (Sha et al., 2020). En cuanto al rendimiento en grano, modestos incrementos son reportados variando entre 5 % y 10 % (Linquist et al., 2013). Diferentes tipos de EEF tienen distintos desempeños según la variable de interés (Ábalos et al., 2014). La variabilidad en los resultados (especialmente, agronómicos) son la principal razón de que, a pesar de que el uso de EEF se ha duplicado en los últimos años, solo representa un 0,15 % del total de los fertilizantes N usados (Linquist et al., 2013). Otra limitante del uso de esta tecnología es que su inclusión en los esquemas de producción implica incurrir en un costo extra de aproximadamente un 30 % con relación al uso de los fertilizantes convencionales (urea) (Linquist et al., 2013). La elección de una tecnología debería estar guiada por técnicas que puedan medir con la mayor precisión su desempeño agronómico y ambiental (Lemaire y Meynard, 1997). Por lo tanto, el enfoque clásico de NUE no es la mejor herramienta, dado que existen otras más apropiadas relacionadas al índice de nutrición nitrogenada (NNI) (Lemaire et al., 2008). La gestión precisa de la nutrición nitrogenada en los cultivos es crucial para optimizar los rendimientos mientras se minimizan los riesgos ambientales, y durante varias décadas, la curva de dilución crítica de nitrógeno (CriNDC), que vincula la biomasa de la planta (AB) con la concentración de nitrógeno ($\%N_{VEG}$), ha surgido como una herramienta fundamental para evaluar el estado nutricional de las plantas (Ciampitti et al., 2022). Por lo tanto, esta técnica parece alcanzar conclusiones más generales y precisas que el enfoque clásico, lo cual sería crucial para determinar el grado de contribución ambiental de estos nuevos cultivos o biomateriales como el caso de carinata.

A nivel local se cuenta con un protocolo de fertilización para colza-canola utilizando como fuente de N la urea azufrada (urea-S), aplicada de manera fraccionada en dos hojas verdaderas (BBCH21) y luego con base en el estatus nitrogenado de la planta (NNI) en elongación (BBCH30) (Ferreira y Ernst, 2014). Carinata es un nuevo cultivo de invierno que aún no tiene un modelo de fertilización establecido y se desconoce su potencial de emisión de GHG; por lo tanto, avanzar en estos dos puntos mediante el uso de técnicas más precisas es fundamental para determinar el manejo agronómico que maximice su verdadero potencial de contribución ambiental.

2. Hipótesis, objetivos y resultados esperados

2.1. Hipótesis general

La aplicación de diferentes estrategias de fertilización nitrogenada influye significativamente en el desempeño agronómico de *Brassica carinata*, con cambios en el rendimiento, la calidad del grano, la eficiencia en el uso del nitrógeno (NUE) y las emisiones de gases de efecto invernadero (GHG).

2.2. Hipótesis específicas

— La reducción de las pérdidas de nitrógeno en el sistema incrementa la disponibilidad y absorción de nitrógeno (N) por parte del cultivo, lo que incrementa el rendimiento por unidad de N aplicado y la NUE.

— El uso de fertilizantes nitrogenados específicos y su manejo estratégico optimizarán la fertilización de *Brassica carinata*, lo que impacta de forma positiva tanto en la productividad y como en el impacto ambiental.

2.3. Objetivo general

Optimizar las estrategias de fertilización nitrogenada para *Brassica carinata* para maximizar el rendimiento agronómico y la eficiencia en el uso del nitrógeno (NUE) mientras se minimizan las emisiones de GHG.

2.4. Objetivos específicos

— Establecer valores de referencia (*benchmarks*) para el desempeño agronómico y la NUE del cultivo de *Brassica carinata*, considerando dosis y momentos de aplicación de urea-S (40N-0/0-0-6S), con base en ensayos de campo realizados entre 2016 y 2018.

— Evaluar si las fuentes alternativas de nitrógeno (NI y SR) superan el desempeño agronómico y la NUE de urea-S en condiciones de producción comercial, utilizando como marco teórico el índice de nutrición nitrogenada (NNI).

3. Base de referencia para los componentes del uso de nitrógeno de Brassica carinata de nitrógeno en el sur de Sudamérica

El presente estudio investiga el papel de los biocombustibles en la reducción de emisiones de gases de efecto invernadero, centrándose en el óxido nitroso (N_2O) generado principalmente por la fertilización con nitrógeno (N). Se evaluó el cultivo poco explorado de *Brassica carinata*, una planta de invierno con múltiples aplicaciones (cultivo de cobertura, biocombustible para jets y alimentación animal). Mediante experimentos de fertilización en campo realizados entre 2016 y 2018 en el sur de Sudamérica, se utilizó regresión de cuantiles para establecer una línea base del rendimiento de semilla (Y_{SEED}) y de los componentes de la eficiencia de uso del N (NUE).

Los resultados indican que el rendimiento máximo de semilla, para los percentiles 50 y 90, varió entre 2,5 y 3,5 Mg ha^{-1}, con una disponibilidad de N de 150 a 160 kg ha^{-1} (suelo + fertilización). La NUE registrada osciló entre 3 y 13 kg de semilla por kg de N disponible. *Brassica carinata* demostró una alta capacidad de absorción de N (N_{UPT}), alcanzando entre 1,0 y 1,5 kg de N_{UPT} por kg de N disponible. Asimismo, las tasas de fertilización evaluadas no afectaron significativamente las concentraciones de aceite (455 a 517 g kg^{-1}) ni de proteína (192 a 253 g kg^{-1}).

El estudio permitió caracterizar la zona agroclimática en el contexto mundial de producción de carinata, logrando establecer tasas óptimas de fertilización entre 90 y 100 kg N ha^{-1}, que maximizan el rendimiento sin comprometer la NUE. Además, se observaron altos valores de eficiencia en la absorción y utilización del N, fundamentales para reducir el N residual y minimizar pérdidas por desnitrificación, lo que disminuye las emisiones de GHG. Los datos sugieren que la región centro-suramericana posee un potencial para la producción de carinata, lo que garantiza altos rendimientos y calidad de semilla, y beneficios ambientales en la producción de biocombustibles.

3.1. Baseline for *Brassica carinata* components of nitrogen-use efficiency in southern South America

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Baseline for *Brassica carinata* Components of Nitrogen-Use Efficiency in Southern South America

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Abstract: Biofuels play an important role in the reduction of greenhouse gas emissions, but their production results in greenhouse gases such as nitrous oxide (N_2O), mainly from nitrogen (N) fertilization. *Brassica carinata* (carinata) is an unexplored winter crop in the world's main cropping areas, with multiple applications (cover crop, jet biofuel, and animal feed, among others). We analyzed a set of on-farm fertilization experiments (2016–2018) in southern South America using quantile regression to establish a baseline for both seed yield (Y_{SEED}) and the different components of nitrogen-use efficiency (NUE). Maximum Y_{SEED} for the 50th and 90th percentiles ranged from 2.5 to 3.5 Mg ha^{-1}, with a N availability of 150–160 kg ha^{-1} (soil + fertilization). The NUE ranged from 3 to 13 kg seed per kg of N available. Carinata, in the absence of other limiting factors, had a high N uptake (N_{UPT}) capacity (1.0 to 1.5 kg ha^{-1} N_{UPT} per kg ha^{-1} N available [soil + fertilization]). The explored N fertilization rates had no significant influence on oil concentrations (455 to 517 g kg^{-1}) and protein concentrations (192 to 253 g kg^{-1}). The region has a high potential for carinata production, with a high capacity to take up available N.

Keywords: Brassica; nitrogen fertilization; bioenergy crop; seed yield; nitrogen uptake efficiency; nitrogen-utilization efficiency

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1. Introduction

In a globalized commodity and energy market with global climate change driven by greenhouse gas (GHG) emissions, new crops in different regions are being explored for their mitigation potential through bioenergy production. This represents an opportunity for the agricultural sector. However, the sector also accounts for about 14% of anthropogenic GHG emissions [1], and it is responsible for 85% of global nitrous oxide (N_2O) emissions, mainly from soils (due to nitrification and denitrification processes) [2–4]. N_2O is a GHG with a global warming potential (GWP) approximately 265–310 times higher than that of CO_2. Crop field management (nitrogen fertilizer, crop rotation, tillage, etc.) contributes between 42 and 80% to the total emissions from biofuel feedstock production [5], mainly in the form of N_2O emissions, depending on the nitrogen (N) fertilization rate [2,6]. The use of N fertilizers is an essential input to obtain high grain yields from annual crops [7]. However, excessive N application increases potential N_2O emissions [1,6]. This tradeoff between sustainable grain and high-quality production and a cascade of environmental concerns has identified higher N use efficiency (NUE) as an essential requirement for bioenergy production based on annual industrial crops [8].

Brassica carinata A. Brown (carinata), known as "Ethiopian Mustard" or "Abyssinian Mustard" [9], is an unexplored winter crop in the world's main cropping areas, introduced mainly for its oil quality (high proportion of erucic acid, 40–45%) for biofuel production and other industrial applications (e.g., plastics, lubricants, paints, and animal feed) [10–14]. The main regions where carinata research is ongoing are Canada (in the Palliser Triangle of Alberta and Saskatchewan) [15], and the southern region of the USA (Florida, Georgia, Alabama, Tennessee, and Texas) [16]. The average seed yield (Y_{SEED}) for these regions ranged from 2500 kg ha^{-1} [17] to 3500 kg ha^{-1} [18,19]. The nitrogen fertilization rate affects both carinata Y_{SEED} and oil yield (Y_{OIL}) [9,18], and it varies between years, zones, and countries [18,20]. In Canada, no optimum N fertilization rate has been found between 0 and 200 kg N ha^{-1}, suggesting that, for this group of trials, higher doses should be added to find a rate at which yields would maximize or begin to plateau [20], whereas in the southern region of the USA, the maximum Y_{SEED} was achieved with 102 kg N ha^{-1} [18]. (In both zones, Y_{SEED} ranged between 1700 and 3000 kg ha^{-1}.) The high efficiency of soil N uptake has been highlighted [18,20], which could be an advantage in terms of N_2O emissions (less residual N) [21]. Carinata oil concentrations (O_C) ranged from 315 to 485 g kg^{-1}, while protein concentrations (P_C) ranged from 240 to 360 g kg^{-1} and, similar to other oilseed crops, carinata had a negative relationship between O_C and P_C [9,17,20,22], although this relationship depended on the variety [19].

Nitrogen-use efficiency (NUE: Y_{SEED} per unit of total N available; N_{TOTAL} = N fertilizer [N_{FERT}] + soil N [N_{SOIL}]) and agronomic efficiency (AE_N) ([ΔY_{SEED}] per unit of N_{FERT} [ΔN_{FERT}]) [7] decrease when the N_{TOTAL} increases. NUE values for Brassica crops (*B. juncea*, *B. rapa*, and *B. napus*) range between 14 and 21 kg Y_{SEED}/kg N_{TOTAL}, and AE_N ranges between 6 and 26 kg Y_{SEED}/kg N_{FERT}. Variations in both parameters depend mainly on soil-mineralized N supply, Y_{SEED}, and rainfall [20]. Plants respond to total N supply (N_{TOTAL}), which includes both N_{SOIL} and N_{FERT} [23]. On-farm experiments may have different N_{TOTAL} levels due to the local conditions of climate, soil, rotation, and management, thus generating important differences in NUE even though the same species may have been used [24]. To consider soil and plant processes, NUE can be split into two components: (1) N-uptake efficiency (NE_{UPT}), defined as the increment in N uptake (N_{UPT}) by crop per unit of increment in N_{TOTAL}, and (2) N-utilization efficiency (NE_{UTI}), defined as the crop capacity to increment biomass per unit of N_{UPT} [25].

As a new crop in an extensive agricultural system, carinata lacks a N fertilization recommendation, and only a few papers have addressed its nutrient-efficiency parameters. A minimum of S fertilizer could be needed for optimal Y_{SEED} and NUE, as it has been reported for wheat [26], maize [27], and oilseed rape (*B. napus*, *B. rapa*, *B. campestris*, and *B. juncea*) [28]. A N fertilization strategy requires baselines for nutrient-use efficiency components. In the global context, where there is a debate about agricultural contributions to GHG emissions (particularly N_2O) and the mitigation potential achievable through bioenergy production, it is important to not only optimize yields, but also to establish benchmark values for the nutrient-use efficiency of biofuel crops. Increasing the proportion of total N inputs that are removed via seeds or biomass could reduce the amount of reactive N in agricultural soils, thus potentially reducing GHG emissions [8]. The main objective of this study was to determine a baseline for NUE and its components under differential N supplies in soils from the agroclimatic zone of southern South America, using a database covering 3 years of on-farm fertilization experiments (2016–2018).

2. Materials and Methods

2.1. Data Collection, Experiments, and Crop Management

The study area is located in the northwestern part of Uruguay. Seven on-farm experiments were conducted during 3 carinata growing seasons: 2016, 2017, and 2018 (three experiments in the first year and two experiments per year in the second and third years). Each site was carefully selected from commercial fields located within a maximum range of 50 km from the Dr. M.A. Cassinoni Experimental Station (32°23′8.12″ S 58°3′47.61″ W; 61 m above sea level), ensuring similar soil and weather conditions. The soils were set to Typic Argiudolls (USDA Soil Taxonomy), which is considered prime agricultural land in this region (25–50 cm superficial layer [A], with 4.6 to 5.2% of organic matter and 24 to 33% of clay, and a pH between 5.8 and 6.0). Trials were conducted on commercial carinata crops, managed under local farmers' practices, with sowings between 15 May and 15 June at a row spacing of 0.19 m, except for one site in 2018 which was sown at a row spacing of 0.38 m. The harvest was carried out between 28 October and 17 November. All sites were under continuous no-till cropping systems, but with different cropping intensities and crop sequences. The N doses were compared under a nonlimiting supply of P and K. The latter was ensured by applying 46 kg ha^{-1} of P_2O_5 (as triple superphosphate) and 60 kg ha^{-1} of K_2O at the sowing date. In all cases, areas with a low emergence rate were avoided, and the genotype used was Avanza 641 (Nuseed), which was the only commercially available genotype in the region at the time of the experiment.

Treatments were a combination of N and S fertilization rates and application times (BBCH 12 and BBCH 30), which resulted in a total of eight treatments, including a control treatment with no N or S fertilization (Table 1). In this study, the application timing was not analyzed. Treatments were established in a randomized complete-block design with three replications. Each plot was 4 m wide and 10 m long. A combination of urea (46-0-0/0) and ammonium sulfate (21-0-0-24) was used as the N and S sources. In all of the cases, fertilizer was broadcast before a rain event. The phenological stages assessed were two true leaves unfolded (BBCH 12), stem elongation begins (BBCH 30), full flowering (BBCH 65), and harvest maturity.

Table 1. Nitrogen and sulfur fertilization rate by carinata phenological stage.

Treatment	BBCH 12 *		BBCH 30 *		Total	
	N	S	N	S	N	S
	kg ha^{-1}					
control	0	0	0	0	0	0
2	20	0	20	0	40	0
3	20	20	20	0	40	20
4	20	20	60	0	80	20
5	20	20	60	10	80	30
6	80	20	20	10	100	30
7	40	30	80	0	120	30
8	40	30	120	0	160	30

* BBCH12: 2nd true leaf unfolded; BBCH30: Beginning of stem elongation of rosette growth.

Composite soil samples were taken by blocks at the beginning of the experiment at two depths (0–0.2 m and 0.2–0.4 m). Samples were dried at 40 °C for 48 h in a fan-forced dehydrator, and then nitrate (N-NO_3) [29] and ammonium (N-NH_4) content [30] were determined. Aboveground biomass was sampled manually in central rows at harvest maturity (2 m of two adjacent central rows). For the latter, biomass was split into seed and residue. The number of plants harvested was recorded. Aboveground biomass was oven-dried at 60 °C for 72 h. Aboveground dry matter accumulation at harvest (total aboveground biomass: AB_{TOTAL}) and seed yield (Y_{SEED}) were expressed per hectare.

O_C was determined using a nuclear magnetic resonance spectrometer (Oxford 4000 NMR, Oxford Analytical Instruments Ltd., Abingdon, UK). Y_{OIL} was estimated by multiplying Y_{SEED} by O_C. The harvest index (HI) was estimated as the ratio between Y_{SEED} and AB_{TOTAL} at harvest. N concentrations and the total amounts of N uptake were measured in aboveground biomass at harvest (seeds and residues). Each sample was ground and sieved through a 0.1 mm mesh. Aboveground biomass N (g kg^{-1}) and S (g kg^{-1}) concentration were measured using the micro-Kjeldahl method [31]. Total P_C was calculated from seed N concentration (g kg^{-1}) by multiplying the empirical factor 6.25 (based on the assumption that the average grain protein content is about 16% N per unit of weight) [32]. N uptake for each treatment was estimated by multiplying aboveground dry matter by nutrient concentration. Total nitrogen uptake (N_{UPT}) at harvest was calculated as the sum of the amounts of nutrients in the AB_{TOTAL}, i.e., residue and seeds.

2.2. Nutrient-Use Efficiency Indices

We estimated NUE parameters by considering the total N supply [25] ($N_{TOTAL} = N_{SOIL} + N_{FERT}$). N_{SOIL} (kg ha^{-1}) was calculated based on the extractable nitrate (N-NO_3^-) and ammonium (N-NH_4) using soil-sample bulk density, which was estimated from soil texture and soil organic matter [33]. NUE was estimated as Y_{SEED} per unit of N_{TOTAL} available (Equation (1)). NUE comprises two main components: (i) N-uptake efficiency (NE_{UPT}), which is the N_{UPT} by crop per unit of N_{TOTAL} (Equation (2)), and (ii) N-utilization efficiency (NE_{UTI}), which is the increase in Y_{SEED} per unit of increase in N_{UPT} at maturity (Equation (3)) [25]. In addition, we extended the calculation of NE_{UTI} with two components due to their importance in yield, seed nutrition, and industrial quality: (i) N_{SEED} (g kg^{-1}) and (ii) nitrogen harvest index (NHI) (Equation (4)) [24,34–36]. In the second instance, for a more agronomic point of view, we calculated agronomic efficiency (AE_N) and apparent N-use (AppNU) [7,37], taking into account only the N fertilized rate (Equations (5) and (6)).

$$NUE = Y_{SEED}/N_{TOTAL} \quad (1)$$

$$NE_{UPT} = N_{UPT}/N_{TOTAL} \quad (2)$$

$$NE_{UTI} = Y_{SEED}/N_{UPT} \quad (3)$$

$$NHI = N_{SEED}/(N_{SEED} + N \text{ in straw}) \quad (4)$$

$$AE_N = (Y_{SEED/FERT} - Y_{SEED/UNFERTILIZER})/N_{FERT} \quad (5)$$

$$AppNU = (N_{UPT\_FERT} - N_{UPT\_UNFERTILIZED}/N_{FERT}) * 100 \quad (6)$$

2.3. *Data Analysis*

Data was analyzed using generalized linear modeling (GLM) and conditional quantile functions at the 10th, 50th, and 90th percentiles using the quantreg package [38] on the basis of a procedure reported in [39]. Univariate and multivariate models were fitted to evaluate the relationship between the dependent variables (e.g., AB_{TOTAL}, Y_{SEED}, NE_{UTI}, and NHI) and the independent variables (e.g., N_{TOTAL} and N_{UPT}), using the nlraa package of R [40–42]. Different models were evaluated, including linear quantile regression, linear-plateau, exponential, exponential-plateau, and quadratic-plateau [43] for the 10th, 50th, and 90th percentiles. While the 50th percentile represents the median response, we define the 90th percentile as the benchmark to the maximum of NUE components, and the 10th percentile as NUE limited by factors other than N.

After the quantile regression analysis, goodness-of-fit tests were performed as pseudo-R^2, in addition to confidence intervals for each parameter of the models built by bootstrapping [39]. The pseudo-R^2 (Rpseud) metric is a local measure of goodness-of-fit at a particular quantile, obtained through weighted deviations from the model of interest, with a model in which only the intercept appears (null model). The presence of unbalanced treatments (different amounts of combinations between factor levels) prevented us from investigating the interactions between N and S. However, as N rates increased, S rates were also increased as a way to prevent S deficiencies. Due to the limited amount of data for each treatment, the quantile regression models were fitted without fitting different models for different treatments, but they visually showed different treatments. Finally, a model was selected based on Rpseud, the distribution of the errors, and the Akaike information criterion (AIC), the latter of which was chosen for the lowest values. Analyses were carried out in R version 1.4.1106 [44].

2.4. *Climate Characterization*

In the period between the planting and harvesting of the crops in the 2016, 2017, and 2018 seasons, rainfall was 16, 85, and 3% higher, respectively, than the baseline (2002–2015). For the same period, the average temperature was 1 °C and 2 °C below the baseline (2002–2015) for 2016 and 2018, respectively, whereas during 2017, the growing season was 2 °C warmer, particularly during the vegetative period, which shortened the cycle during this phase. In the critical period for Y_{SEED} determination (September and October) [45], rainfall in the 2016 and 2017 growing seasons was 35% below the baseline, whereas in 2017, it was 12% above the baseline (Figure 1). The average temperatures for the 2017 and 2018 growing seasons during the critical period for Y_{SEED} determination were similar to the baseline (18 °C), whereas in the 2016 growing season, it was 1 °C below the baseline. According to these measurements, and despite the differences between years, all of the growing seasons presented an adequate combination of rainfall and temperature for carinata growth. Between August 20 and the end of October, the period in which flowering and an important part of the critical period of yield determination occurred, the minimum temperature was below 0 °C at only 2 times during 2016, which indicates that frosts were not important during the period of our study.

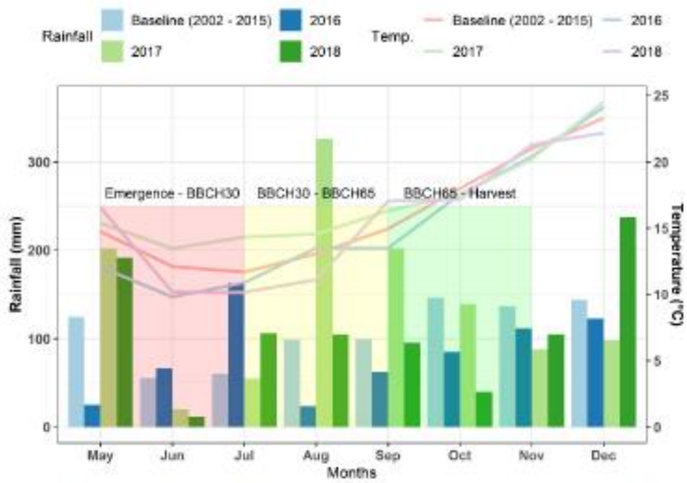

Figure 1. Total monthly rainfall and average monthly temperatures for the 2016, 2017, and 2018 seasons in relation to the baseline (2002–2015). BBCH30: Beginning of stem elongation of rosette growth; BBCH65: Full flowering: 50% of flowers opened.

3. Results

3.1. Crop Productivity

Y_{SEED} ranged from 4.2 Mg ha^{-1} (Max) to 0.6 Mg ha^{-1} (Min), with a mean of 2.3 Mg ha^{-1}, and AB_{TOTAL} ranged from 16.7 Mg ha^{-1} (Max) to 4.3 Mg ha^{-1} (Min), with a mean of 9.7 Mg ha^{-1} (Table 2). The O_C ranged from 556 g kg^{-1} (Max) to 438 g kg^{-1} (Min), with a mean of 488 g kg^{-1}. The P_C ranged from 324 g kg^{-1} (Max) to 186 g kg^{-1} (Min), with a mean of 224 g kg^{-1} (Table 2).

3.2. Nitrogen-Use Efficiency

Adequate N (and S) nutrition increased Y_{SEED}, reaching a maximum of 2.5 and 3.5 Mg ha^{-1} Y_{SEED} for the 50th and 90th percentiles, respectively (Figure 2a,b, Table S1). Maximum Y_{SEED} levels were reached at about 150–160 kg ha^{-1} of N_{TOTAL}, corresponding to 90–100 kg ha^{-1} N_{FERT} for the 50th and 90th percentiles (Figure 2a,b, xs in fitted models 1 and 2, Tables S2 and S3). The highest Y_{SEED} percentile (90th percentile) was approximately between 1.0 and 1.5 Mg ha^{-1} above the median (50th percentile), indicating the importance of other factors not associated with N (and S) availability. There was a linear response for the entire supply range of N_{TOTAL} and N_{FERT} in the 10th percentile, indicating that N nutrition can overcome some of the non-N limiting factors (Figure 2a,b, fitted models 1 and 2, Tables S2 and S3). The mean Y_{SEED} of the control treatment was 1.5 Mg ha^{-1} (SD: 0.49 Mg ha^{-1}), indicating the high environmental potential of the region (soil + climate). NUE and AE_N (b in fitted models 1 and 2, Tables S2 and S3) ranged between 3 and 13 kg ha^{-1} Y_{SEED} per kg ha^{-1} N_{TOTAL} available, whereas the 50th percentile presented an intermediate range between 10–12 kg ha^{-1} Y_{SEED} per kg ha^{-1} N_{TOTAL}.

NUE and AE_N showed little variation for all percentiles (*b* parameter). Only the 10th percentile presented a linear response to increases in N availability (N_{TOTAL} and N_{FERT}), with a constant NUE and AE_N for the N supply range explored.

Table 2. Seed yield (Y_{SEED}), total aboveground biomass (AB_{TOTAL}), harvest index (HI), protein concentration (P_C), and oil concentration (O_C) for all of the sites and years evaluated. Mean, standard deviation (SD), maximum (Max) and minimum (Min) values are presented.

Year		2016			2017		2016	
Farm	**Don Belimirio**	**Don Vital**	**Tres Aguas**	**Las Cumbres**	**La Carolina**	**Don Luis**	**Las Cumbres II**	**Mean**
				Y_{SEED} (Mg ha^{-1})				
Mean	2.5	2.8	1.7	2.6	2.6	2.1	1.7	2.3
SD	0.6	0.8	0.5	0.7	0.6	0.5	0.6	0.6
Max	3.6	4.2	3.0	3.9	3.6	3.1	2.9	3.5
Min	1.3	1.4	1.0	1.4	1.3	1.1	0.6	1.2
				AB_{TOTAL} (Mg ha^{-1})				
Mean	9.1	12.1	6.9	10.9	9.9	9.3	10.0	9.7
SD	1.9	2.7	2.0	2.6	1.9	2.1	2.2	2.2
Max	1.2	16.7	12.0	16.1	14.1	14.2	14.1	12.6
Min	5.3	6.9	4.3	6.1	6.4	6.3	5.0	5.7
				HI				
Mean	0.27	0.23	0.24	0.24	0.26	0.22	0.17	0.23
SD	0.02	0.02	0.02	0.05	0.03	0.02	0.04	0.03
Max	0.31	0.27	0.26	0.41	0.31	0.26	0.26	0.30
Min	0.20	0.19	0.21	0.16	0.15	0.18	0.08	0.17
				P_C (g kg^{-1})				
Mean	209	202	205	227	232	215	278	224
SD	14.2	20.2	10.2	16.6	9.54	11.4	20.1	15
Max	240	241	222	246	252	245	324	253
Min	186	137	183	186	216	196	237	192
				O_C (g kg^{-1})				
Mean	485	478	467	514	503	531	439	488
SD	13.9	10.6	16	12.2	11.9	15.3	31.9	16
Max	514	496	496	535	525	556	496	517
Min	450	460	438	494	483	500	358	455

3.3. *Nitrogen Uptake Efficiency*

NE_{UPT} (kg^{-1} kg^{-1}) decreases with an increase in N_{TOTAL} supply, following the Law of Diminishing Returns (Figure 3a). A similar effect was seen in AppNU (%) for the entire range of N_{FERT} explored (Figure 3b). However, NE_{UPT} and AppNU presented high variability (Figure 3a,b). Carinata presented high N_{UPT} capacity for the entire range of N supply explored (both N_{TOTAL} and N_{FERT}), particularly in the 50th and 90th percentiles (Figure 3a,b). NE_{UPT} was high (1.0 to 1.5 kg ha^{-1} N_{UPT} per kg ha^{-1} N_{TOTAL}) for the range of 100 to 150 kg ha^{-1} N_{TOTAL}, and in the absence of other limiting factors (50th and 90th percentiles) (Figure 3a, fitted model 3, Tables S2 and S3). When considering only the fertilizer effect (N_{FERT}), AppNU (%) was approximately 50% (50th percentile) for all of the N_{FERT} rates explored (Figure 3b, fitted model 4, Tables S2 and S3). However, in the absence of other limiting factors (90th percentile), AppNU (%) was between 80 and 90% of N_{FERT}.

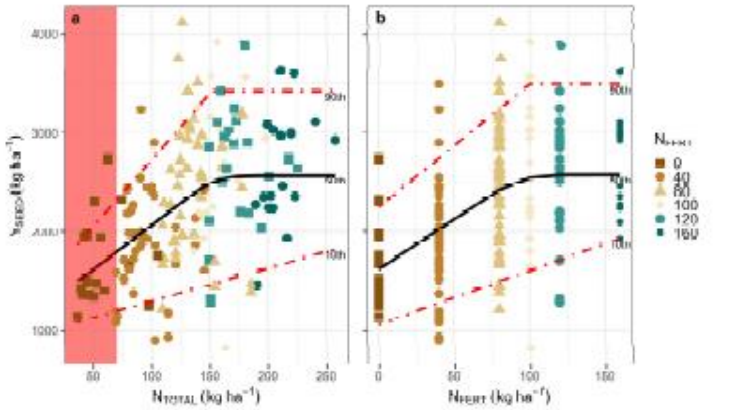

Figure 2. Quantile regression models fitted to different percentiles relating Y_{SEED} in response to N_{TOTAL} (**a**) and N_{FERT} rate (**b**). Each data point is a plot on a farm with fertilization treatment. Red zones represent the N_{SOIL} supply in unfertilized plots (control).

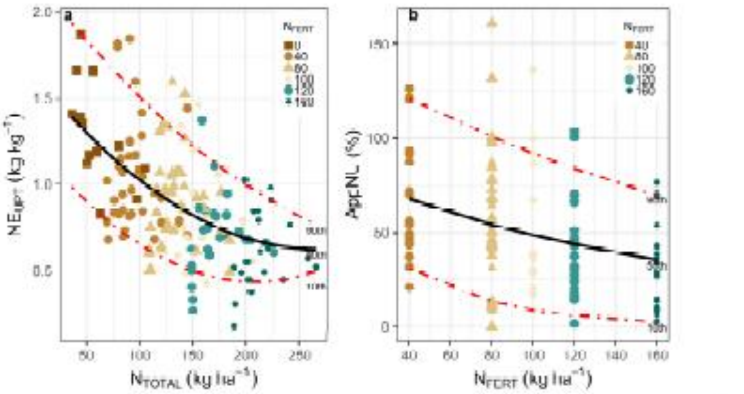

Figure 3. Quantile regression models fitted to different percentiles relating NE_{UPT} (kg kg^{-1}) in response to N_{TOTAL} (**a**) and AppNU (%) in response of N_{FERT} (**b**). Each data point is a plot on a farm with fertilization treatment.

3.4. Nitrogen-Utilization Efficiency

Maximum Y_{SEED} was reached at about 160–180 kg ha^{-1} of N_{UPT}, more than 3 times higher than at low N_{UPT} (Figure 4a, *xs* in fitted model 5, Tables S2 and S3). At these N_{UPT} values (160–180 kg ha^{-1}), Y_{SEED} reached between 3.2 to 3.6 Mg ha^{-1} for the 50th and 90th percentiles, respectively (Figure 4a). NE_{UTI} ranged between 13 kg Y_{SEED} per kg of N_{UPT} (10th percentile) and 16 kg Y_{SEED} per kg of N_{UPT} (50th and 90th percentiles), showing slight variations between percentiles (b in the adjusted model 5, Tables S2 and S3). Considering NE_{UTI} as the result of the relationship between two variables (N_{SEED} and NHI), a higher proportion of plant N in the seed is associated with a higher NE_{UTI}, while a higher concentration of N in the seed is associated with a lower NE_{UTI}.

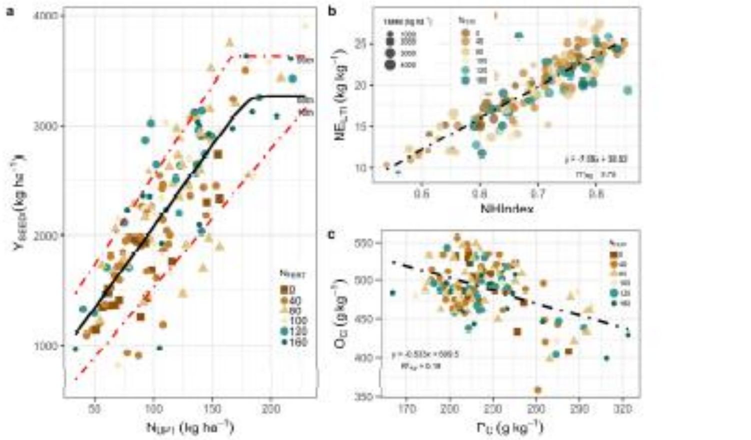

Figure 4. Quantile regression models fitted to different percentiles relating N_{UPT} (kg ha^{-1}) to Y_{SEED} (kg ha^{-1}) (**a**), relationship between NHI and NE_{UTI} (**b**), and relationship between PC and OC (**c**). Each data point is a plot on a farm with fertilization treatment.

We observed that NE_{UTI} increased approximately 7 kg kg^{-1} for each unit of increase in NHI ($p < 0.001$) (Figure 4b), with a low association between NHI and Y_{SEED} ($R^2_{ADJ} = 0.02$). The relationship between P_C and O_C was linear and negative (Figure 4c), where O_C decreased, 0.53 g kg^{-1} per unit of P_C increased ($p < 0.001$), but the data showed a high variability in O_C, mainly in the range of P_C values between 170 and 250 g kg^{-1} (Figure 4c). For the N_{FERT} range explored, no statistically significant relationship was found with either O_C or P_C.

4. Discussion

4.1. Productive Characterization: Comparison with Other Regions of the World

Our results showed that in on-farm experiments, average Y_{SEED} (2.3 Mg ha^{-1} +/− 0.6 SD, including control treatments) (Table 2) was equal to or higher than that found in other carinata-producing areas (USA, Canada, Europe, and Ethiopia) [11,12,22,46,47], which is consistent with recent reports on semi-commercial conditions in the region [48]. In the agroclimatic context of Uruguay, the maximum seed yield was 4.2 Mg ha^{-1} (Table 2), achieving 3.3 Mg ha^{-1} in the 90th percentile, which is higher than the values reported elsewhere [9,11,49–51]. AB_{TOTAL} ranged between 5–16 Mg ha^{-1}, with an average of 10 Mg ha^{-1} (Table 2), similar to the values reported for high productivity regions such as Minnesota, USA (14 Mg ha^{-1}) under spring sowing [12], and Florida, USA (7 Mg ha^{-1}) with fall sowing [52]. HI had a mean value of 0.23 (0.41–0.08) (Table 2), with minimums lower than those reported for Minnesota (0.28 to 0.37) and Florida (0.30 to 0.34) [12,52]. However, HI is expected to be a parameter with low variation (SD = 0.03, Table 2) in the absence of frost during grain filling [37,53] or with excessive growth and lodging [13], as was the case with the Site "Las Cumbres II" (the only site sown at 0.38 cm row spacing) (Table 2). The mean O_C of 488 (SD = 16 g kg^{-1}) (Table 2) and P_C of 224 g kg^{-1} (SD = 15 g kg^{-1}) (Table 2) were higher than in other carinata-producing regions of the world [51]. This implies that, in addition to the higher yield achieved, the production per unit of area of oil and protein is also higher than in other regions (1122 kg oil ha^{-1} and 500 kg protein ha^{-1}), but it could be even higher if future management practices or improved genetics increase the harvest index.

4.2. Nitrogen-Use Efficiency

Carinata responded to N availability (N_{TOTAL} and N_{FERT}) in agreement with reports from other sites [17,46,52]. To reach maximum Y_{SEED} (2.5 to 3.5 Mg ha^{-1} at the 50th and 90th percentiles, respectively) (Figure 2a,b), an uptake of 150–160 kg ha^{-1} N_{TOTAL} at harvest was necessary in these experiments. As N_{SOIL} available at the beginning of the experiment was only about 60 kg ha^{-1} (Figure 2a), N fertilization was a critical component for optimizing the yield in local systems. N_{FERT} rates that maximize Y_{SEED} (90–100 kg ha^{-1} N_{FERT}) were lower or similar to those reported in other locations [9,17,49,51]. However, the attainable Y_{SEED} (90th percentile) was higher for our agroecological zone. NUE and A_{EN} varied between 3–13 kg ha^{-1} Y_{SEED} per kg ha^{-1} N_{TOTAL} (N_{FERT}) available, in concordance with the ranges reported for the USA and Canadian prairies [20,52]. The *b* parameter (NUE and AE_N) for the 50th and 90th percentiles did not differ until the maximum Y_{SEED} was reached. This suggests that, in the presence of nutrient (N)-limited Y_{SEED}, NUE and AE_N remain constant with increasing available N.

However, the parameter *a* was different for the same percentiles, which suggests the existence of other limiting conditions (non-N or S), implying a higher requirement of N for the same maximum Y_{SEED}. At the 10th percentile, both the *a* and *b* parameters were statistically different in relation to the other percentiles. A specific study is necessary to determine the cause of low Y_{SEED} in this percentile (e.g., soil degradation). Considering the typical parameters of the fitted models (*a*, *b*, and *xs* in Table S3), it becomes evident that a higher Y_{SEED} does not necessarily lead to an improvement in NUE. Therefore, it is necessary to identify the factors (e.g., management practices, cropping systems, or breeding) that alter parameters *b* (higher EUN) and *xs* (reduced optimum N), and not only their asymptote (parameter *a*, maximum Y_{SEED}).

4.3. Nitrogen Uptake Efficiency

As with other grain crops, NE_{UPT} and AppNU decreased with an increased N supply (N_{TOTAL} and N_{FERT}) (Figure 3a,b, respectively) [24,54]. However, there was great variability in NE_{UPT} and AppNU, likely as a result of different combinations of crop rotations, previous crops, soil conditions, agronomic management, and climatic conditions, which could not be identified using our dataset.

Different N_{SOIL} availabilities resulted in different N_{TOTAL}, even when the same rate of N_{FERT} was used [24]. Therefore, the crop growth rate could have been affected, resulting in changes in NE_{UPT} and AppNU [55]. In the absence of other limitations (50th and 90th percentiles), N_{TOTAL} uptake was above 0.5 kg kg^{-1} (with maximum values above 1 kg kg^{-1}) for NE_{UPT} and 50% (with maximum values above 100%) for AppNU [22,52]. High N uptake efficiency may be partially explained by N mineralization during the crop cycle (that being ≈30 +/− 3 kg N ha^{-1} if we only consider the mean difference between N_{SOIL} at the beginning of the experiment and the N_{UPT} of the control treatment), an input that was not considered since only the N available at the beginning of each experiment was considered for the calculations [56]. If we consider this contribution, NE_{UPT} would be 20% lower, but it would still have high values.

The results of our on-farm experiments are in agreement with field and greenhouse experiments in Florida, USA [18]. Carinata has been shown to be efficient in N_{TOTAL} uptake (as a "soil N scavenger"), regardless of soil supply [18]. NE_{UPT} and AppNU were higher than the values reported for on-farm canola [37,57]. NE_{UPT} and AppNU were similar to the values reported by on-farm experiments carried out in our agroecological zone (25–70%) in wheat (*Triticum aestivum*) (Santa Fe, Argentina) [26]. In our case, the N surplus, considering the fraction of N not absorbed by the crop and N in crop residues, averaged 50 +/− 4 kg N ha^{-1}, an amount that would not scale the N_2O emissions [21] even if we did not consider that, in our production systems, another crop would be sown immediately after the harvest of this crop [58–60]. Based on our results and previous studies in different regions, it is possible to conclude that carinata could be a useful biofuel feedstock with a low contribution to GHG emissions (N_2O emissions) [5,6], with high N uptake, and leaving little N surplus in the soil [21].

4.4. Nitrogen-Utilization Efficiency, Efficiency Components, and Seed Quality

The N_{UPT} range (160–180 kg N ha^{-1}) that maximized Y_{SEED} (50th and 90th percentiles) was slightly higher than that reported in North America (USA and Canada) (80–160 kg N ha^{-1}) [18,20]. This may be explained by the higher AB_{TOTAL} accumulated at harvest as compared to other studies (5–16 Mg ha^{-1}) [12,61]. Long-term studies are necessary to determine the impact of "N leakage" that the inclusion of carinata in rotations could have, both in economic and environmental terms, as reported for the southern USA and Canada [20,52]. NE_{UTI} levels found in our on-farm trials (13–16 kg of Y_{SEED} per kg^{-1} N_{UPT}) were in a higher range than those values reported for a greenhouse experiment in Florida, USA (6–9 kg of Y_{SEED} per kg^{-1} N_{UPT}) [52] and for winter and spring oilseed rape in the UK (8–12 kg of Y_{SEED} per kg^{-1} N_{UPT}) [62]. Lower NE_{UTI} values were also found for canola in Australia (≈12.5 kg of Y_{SEED} per kg^{-1} N_{UPT}) [63,64]. In comparison with wheat (a common winter crop in our region), carinata has a lower NE_{UTI} [26]. However, carinata grain, due its oil content, has a higher glucose equivalent than wheat grain in terms of the glucose required for synthesis [65,66]. NE_{UTI} was directly related to NHI and inversely related to N_{SEED}, similar to what has been reported for other crops (e.g., maize) (Figure 4b) [34,35]. We found no clear relationship between NHI ($R^2ADJ = 0.02$) and N_{SEED} ($R^2ADJ = 0.01$) and Y_{SEED} (in the explored range of Y_{SEED}). These results are contrary to those reported for both cereals (wheat) [67] and brassicas (*Brassica napus* L.) [68].

The explored N_{FERT} rates did not have a significant influence on the oil or protein concentrations ($p > 0.001$), unlike that which was reported for the USA (North Florida), Canada (Saskatchewan and Alberta) and northern India [51]. A linear and negative relationship between O_C and P_C ($b = -0.533$) (Figure 4c) implied a change of greater magnitude in the protein yield than in the oil yield. However, there was a wide variability between the plots, especially in the range of 170 to 224 g kg^{-1} P_C. Therefore, a greater knowledge of the factors that regulate the concentration and final content of oil and protein in carinata seeds is necessary, for example, climatic conditions during grain filling (water stress, temperature, and supplies of N_{SOIL} and N_{FERT}), genetic factors (varieties vs. hybrids) and management factors (sowing dates).

5. Conclusions

Our work, based on a detailed statistical analysis, allowed us to characterize our agroclimatic zone in the worldwide context of carinata-producing regions, and it provides the first estimation of optimal fertilization rates (90 to 100 kg N ha^{-1}) that maximize Y_{SEED} while not compromising NUE. Maximum Y_{SEED} ranged from 2.5 to 3.5 Mg ha^{-1}, a significantly high value, considering the current reports and the fact that many management practices must be adjusted. NE_{UPT} was above 0.5 kg kg^{-1} (with maximum values above 1 kg kg^{-1}) and 50% (with maximum values above 100%) for AppNU, high values that are fundamental to minimizing the residual N and its potential loss by denitrification as GHGs. We reported high O_C values and a low association with Y_{SEED}, which determines that yield increases are linearly associated with increases in the amount of oil harvested. According to our data, the central region of South America could be a carinata-producing area with high yield potential and high seed quality without compromising the environmental benefit of biofuels.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy13020412/s1, Table S1. Nitrogen use efficiency and variability of *Brassica carinata* A. Braun, constructed from averages for each site per year; Table S2. Summary of goodness-of-fit tests for different efficiency parameters based on the log-likelihood ratio and respective chi-squared tests, and pseudo-R2 measures *; Table S3. Quantile regression models and parameters fitted relating variables of interest for the 10th, 50th and 90th percentile. Values between parentheses are 95% confidence intervals estimated by bootstrap resampling.

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writing—review and editing, O.R.E. and S.R.M. All authors have read and agreed to the published version of the manuscript.

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4. Optimización de la gestión del nitrógeno para obtener biocombustibles de aviación sostenibles: un estudio de caso de *Brassica carinata* en Sudamérica

La optimización del manejo del nitrógeno (N) es fundamental para mejorar la sostenibilidad de la producción de biocombustibles y mitigar su huella de gases de efecto invernadero (GHG). Este estudio demuestra que el uso de fertilizantes de eficiencia mejorada (EEF), específicamente ENTEC 150, aumenta significativamente el rendimiento de semilla en *Brassica carinata* en comparación con prácticas convencionales. Se observó un incremento del 70 % en el rendimiento respecto al tratamiento control, destacando el potencial de los inhibidores de la nitrificación para mejorar el desempeño agronómico, incluso bajo estrés hídrico.

El uso de ENTEC 150 permitió sincronizar la disponibilidad de N con la demanda del cultivo, según lo indicado por el índice de nutrición nitrogenada (NNI), lo que mejoró la producción de biomasa y la eficiencia en el uso del N (NUE) sin necesidad de aplicaciones fraccionadas. A pesar de la variabilidad estacional, el NNI demostró ser una herramienta robusta para evaluar el estado nutricional del cultivo y orientar estrategias de fertilización. ENTEC 150 mantuvo la suficiencia de N (NNI >1) desde la elongación temprana hasta la floración, aumentando la biomasa en un-23–32% y lo que asegura la eficiencia agronómica del N (AE_N) en distintos ambientes. Además, la eficiencia de recuperación del N (RE_N) superó los valores de referencia regionales, resaltando la capacidad de *Brassica carinata* para captar N y reducir pérdidas reactivas.

Estos hallazgos tienen importantes implicaciones ambientales y agronómicas. ENTEC 150 redujo la necesidad de aplicaciones fraccionadas y mejoró la NUE, alineándose con el objetivo de reducir las emisiones de N_2O en sistemas de producción de biocombustibles. Futuras investigaciones deberían integrar el CriNUC en evaluaciones de GHG y desarrollar modelos NNI específicos para mejorar la predicción del rendimiento y la toma de decisiones agronómicas.

4.1. Optimizing Nitrogen Management for Sustainable Aviation Biofuels: a Case Study of *Brassica carinata* in South America

Abstract

Biofuel crops offer climate benefits, but inefficient nitrogen (N) management can increase GHG emissions. We hypothesized enhanced-efficiency fertilizers improve agronomic, environmental, and grain quality performance in *Brassica carinata*. However, conventional N use efficiency metrics are insufficient for quantifying these benefits. For the first time in biofuel crops, we developed a critical N uptake curve tool (CriNUC) to assess agronomic, environmental, and grain quality performance of alternative versus traditional N fertilizer practices. On-farm trials at four *Brassica carinata* sites in northwestern Uruguay (2019–2020) compared alternative N sources (ENTEC, Sulfammo-NPRO) to split-applied Urea-S at 0, 90, and 150 kg N ha^{-1}. ENTEC 150 increased seed yield by 70% over traditional practices ($p<0.05$), reaching 4.71 Mg ha^{-1} while eliminating energy-wasting split applications. This ENTEC 150 superior yield was underpinned by extended time and magnitude above the CriNUC (NNI 1–1.25), resulting in increased N uptake and biomass without affecting N use efficiency components ($p>0.05$). *Brassica carinata* exhibited exceptional N scavenging efficiency (REN>1), reducing reactive N losses, consistent with global findings. Higher nutritional status (ENTEC 150, Urea 150, NNI>1) reduced oil content, N harvest index, and internal N efficiency and increased protein. Our findings highlight NNI and the CriNUC as robust tools for crop N management at the production level. Furthermore, the ENTEC’s stable N harvest index and internal efficiency also revealed an increased N residue, indicating a potential for increased N_2O emissions. Future studies should integrate our CriNUC into GHG assessments to determine *Brassica carinata* true offset potential and biodiesel sustainability.

Keywords: Biofuel; Nitrogen Use Efficiency; Critical Nitrogen Uptake Curve; *Brassica carinata*; Enhanced-Efficiency Fertilizers; Greenhouse Gas Emissions; Nitrogen Nutrition Index (NNI)

Highlights

- CriNUC outperforms NUE in assessing agronomic, grain quality and environmental impacts.
- ENTEC 150 removed split applications, increased yield and maintained grain quality.
- NUE components (RE_N, IE_N) varied yearly; alternative N matched current practices.
- NNI and IE_N assess biofuels and GHG impact, emphasizing residue quality.

4.2. Introduction

The climate benefits of biofuel crops can be offset by substantial GHG emissions during feedstock cultivation (Ogle et al., 2016). Nitrous oxide (N_2O), a potent GHG with a 100-year global warming potential (GWP) 296 times that of carbon dioxide (CO_2), also contributes to stratospheric ozone depletion (Crutzen et al., 2008). N_2O emissions, primarily driven by nitrogen (N) inputs, represent a major share of biofuel's life cycle GHG emissions (Ogle et al., 2016). Fertilization practices account for 42–80% of total GHG emissions from biofuel feedstock production, encompassing both soil emissions and upstream emissions from N fertilizer manufacturing (Adler et al., 2012). Thus, optimizing N management is essential to reducing the GHG footprint of biofuel production (Ogle et al., 2016).

Suboptimal nitrogen (N) management often results in low N use efficiency (NUE), typically around 30–50% (Ábalos et al., 2014). NUE can be assessed through multiple indices (Dobermann, 2005), including the 'difference method', which compares crop yield (Yseed) and total N uptake (N_{UPT}) in fertilized versus unfertilized plots. Agronomic efficiency (AE_N), defined as yield increase per kilogram of applied N, is further dissected into recovery efficiency (RE_N; kg N_{UPT} per kg applied N) and internal efficiency (IE_N; kg Yseed per kg N applied) (Ciampitti & Vyn, 2012; Dobermann, 2005).

Improving NUE requires strategic fertilization. While reducing N rates can enhance efficiency, it risks yield loss, affecting biofuel feedstock supply (Ogle et al., 2016). Split urea applications offer a conventional approach (Velasco et al., 2012), but enhanced-efficiency fertilizers (EEF) incorporating nitrification inhibitors (NIs), urease inhibitors (UIs), or slow-release polymers (SR) better synchronize N supply with crop demand, reducing losses and reactive N impacts (Caviglia et al., 2014a; Galloway et al., 2003). Meta-analyses indicate EEFs improve yields (Yseed) by 5.7% and increase AE_N and RE_N by 8.0% relative to urea (Linquist et al., 2013; Tedone et al., 2018). However, high costs limit farmer adoption despite the potential to reduce energy-intensive split applications (Ábalos et al., 2014; Linquist et al., 2013; Maaz et al., 2021).

The Nitrogen Nutrition Index (NNI) quantifies crop N status as the ratio of actual shoot N concentration (%N) to the critical %N (%$N_{CRITICAL}$) derived from the N dilution curve (CriNDC), which defines the allometric relationship between %N and aboveground biomass (AB) (Lemaire et al., 2019, 2008; Lemaire & Ciampitti, 2020). NNI values indicate whether biomass growth is N-limited (NNI < 1), optimal (NNI = 1), or excessive (NNI > 1), making it a powerful real-time diagnostic tool for optimizing fertilization (Rodriguez et al., 2024). A well-established co-regulatory relationship exists between NNI and soil nitrate concentration, which dictates the rate of N uptake (N_{UPT}) (Sadras & Lemaire, 2014). Maintaining an adequate crop N status at any given soil nitrate level is key to maximizing N_{UPT} while minimizing environmental N losses (N_R) (Devienne-Barret et al., 2000; Sadras & Lemaire, 2014). Expanding the CriNDC framework to include a critical N uptake curve (CriNUC) enables the determination of critical plant N content (N_{UPT}CRITICAL, kg N ha^{-1}) by integrating CriNDC with AB. This approach enhances agronomic and environmental assessments through metrics like RE_N while accounting for NNI dynamics (Gastal & Lemaire, 2002; Lemaire et al., 2008).

Sampling timing is crucial, particularly around the formation of critical yield components in grain crops (Rodriguez et al., 2024). While NNI can be assessed using multiple samples throughout vegetative stages, directly linking it to yield at any single point remains challenging (Lemaire et al., 2008; Rodriguez et al., 2024). Measurements closer to flowering may improve yield predictions and refine evaluations of diverse N management strategies, including alternative N fertilizer sources (Bohman et al., 2021; Caviglia et al., 2014a; Colnenne et al., 1998).

Brassica carinata A. Braun (carinata) is an emerging biofuel feedstock valued for its industrial byproducts, including high-protein meal (Seepaul et al., 2021).

Cultivated in the southern USA, Canada, Australia, Europe and South America (Uruguay, Brazil, Argentina), it plays a key role in Sustainable Aviation Fuel (SAF) production (Seepaul et al., 2021). Efficient on-farm management is critical to reducing greenhouse gas (GHG) emissions per unit of product, thereby maximizing carbon offset potential, with nitrogen (N) management being a primary driver (van Groenigen et al., 2010). In Uruguay, Yseed is highly sensitive to weather variability, leading to widespread adoption of split N applications using sulfur-enriched urea (Urea-S, 40N-0/0-0-5S) to optimize nutrient availability (Bonansea et al., 2023).

Our hypothesis proposes that quantifying the relationship between the Nitrogen Nutrition Index (NNI) and Nitrogen Use Efficiency (NUE) components provides a robust framework for assessing the agronomic and environmental performance of alternative nitrogen sources, such as nitrification inhibitors (NI) and slow-release fertilizers (SR), in nitrogen-fertilized biofuel crops like Brassica carinata. This framework allows us to evaluate experimental nitrogen treatments comprehensively, considering their impact on key agronomic variables within the context of crop nitrogen status, as expressed by the NNI. Furthermore, it allows us the opportunity to develop a quantitative model linking NNI to NUE components —Agronomic Efficiency of Nitrogen (AE_N), Recovery Efficiency of Nitrogen (RE_N) and Internal Efficiency of Nitrogen (IE_N)— to analyze how varying nitrogen statuses influence productivity, management and decision-making relevance and environmental outcomes. By examining these relationships, the study will identify connections among NNI, NUE components and critical agronomic variables such as oil and protein concentrations. This approach will provide the necessary insights to optimize nitrogen management practices and enhance the sustainability of carinata biofuel production systems, while considering environmental trade-offs.

4.3. Materials and methods

4.3.1. Study sites, Data Collection and Crop Management

The study was conducted in northwestern Uruguay during the 2019 and 2020 growing seasons. Four sites representative of spring *Brassica carinata* cash crop fields were selected: Barrancas (31°48'50.36"S, 57°58'35.85"W) and La Oriental (32°11'19.01"S, 58°3'42.42"W) in 2019, and LO20 (32°11'33.46"S, 58°4'56.22"W) and La Encantada (32°25'6.78"S, 58°0'15.25"W) in 2020 (Figure 1). The region experiences variable temperatures, with an annual mean of 17.7°C, and rainfall ranging from 1200 to 1600 mm. The soils are Typic Argiudolls, known for their high agricultural value, with organic matter content ranging from 4.6% to 5.2%, clay content between 24% and 33% and pH values of 5.8 to 6.0 in the top 25–50 cm, as classified by USDA Soil Taxonomy and described in Bonansea et al. (2023).

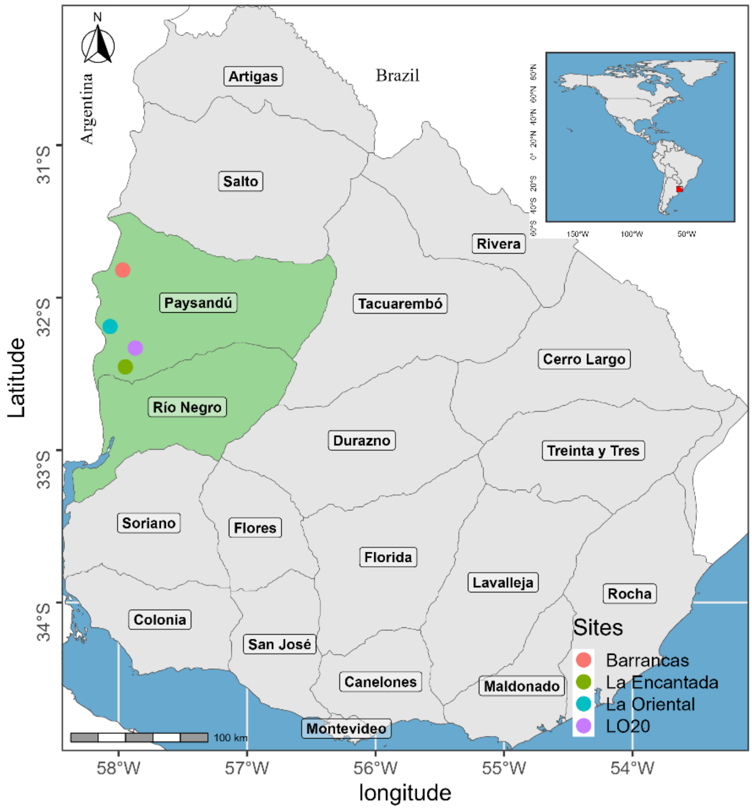

Figure 1

On-farm trial sites in the northwestern zone of Uruguay during the 2019 and 2020 growing seasons, with each trial represented by a point.

Carinata cash crops were managed according to local practices, with a sowing window between May 17 and May 28 and harvest dates between November 9 and 25 and the row spacing was set at 0.19 m. The chosen cultivar, Avanza 641 (Nuseed Company), was the only commercially available option in the region at the time of the experiment. All sites employed continuous no-till cropping systems, with differences in cropping intensity and rotation practices. The trial sites were selected based on optimal seed emergence rates. Basal fertilizer applications included 46 kg ha^{-1} of P_2O_5 and 60 kg ha^{-1} of K_2O to address nutrient deficiencies during the emergence stage. At the two-true leaf stage (BBCH12) (Lancashire et al., 1991), plots received different treatments as detailed in Table 1.

Table 1

Treatments Application and On-Farm Management Practices across Growing Seasons.

	Source – Rate – Time of treatment		
Treatment	BBCH12	BBCH30	N Total
UnFert	0	0	0
CONTROL	70	20	90
Urea 150	70	80	150
ENTEC 90	90		90
ENTEC 150	150		150
SULFAMMO 90	90		90
SULFAMMO 150	150		150

Each site included seven treatments arranged in a randomized complete block design with three replicates. The treatments compared alternative nitrogen sources (ENTEC and Sulfammo-NPRO) to the traditional split application of Urea-S at rates of 0, 90 and 150 kg N ha^{-1}. The plots were 4 m wide and 10 m long. Fertilizers were applied before or immediately after rainfall to ensure proper incorporation into the soil. The on-farm standard for *Brassica carinata* cultivation in Uruguay involves a split application of 90 kg ha^{-1} of N as Urea-S. This was used as the CONTROL treatment, following the guidelines outlined by Laurent et al. (2019). Additionally, an unfertilized N treatment (UnFert) served as a baseline to evaluate environmental yield potential and measure crop response to nitrogen fertilization. Phenological stages were assessed at four key points: two unfolded true leaves (BBCH12), the beginning of stem elongation (BBCH30), full flowering (BBCH65) and harvest maturity, using the BBCH scale described by Lancashire et al. (1991).

Soil sampling began at the emergence stage, with composite samples collected from each block at depths of 0–0.2 m and 0.2–0.4 m. The samples were dehydrated at 40°C for 48 h, and nitrate ($N\text{-}NO_3^-$), ammonium ($N\text{-}NH_4^+$) and organic carbon content were analyzed following standard protocols (Gelderman & Beegle, 2011; Rhine et al., 1998; Walkley & Black, 1934). Additional soil samples were collected at phenological stages BBCH12, BBCH30 and BBCH65, maintaining the same depths. The aboveground biomass (AB) was manually sampled from the central rows at BBCH30, BBCH65 and harvest maturity. At harvest, AB was separated into seeds and residues, and the harvest index (HI) was calculated as the ratio of Yseed to total aboveground biomass ($AB_{HARVEST}$). All AB samples were oven-dried at 60°C for 72 hours, and biomass metrics (Yseed, AB_{BBCH30}, AB_{BBCH65} and $AB_{HARVEST}$) were expressed per hectare. The ground samples were analyzed for nitrogen content using the micro Kjeldahl method. Seed protein concentration [Pc] was estimated from seed nitrogen concentration using a conversion factor of 6.25, assuming 16% nitrogen per unit of protein weight (FAO, 1998). Oil concentration was determined using a nuclear magnetic resonance spectrometer (Oxford 4000 NMR, Oxford Analytical Instruments Ltd., Abingdon, United Kingdom). Nitrogen uptake (N_{UPT}) was calculated by multiplying dry matter by nutrient concentration at each phenological stage. At harvest, the total nitrogen uptake (N_{UPT}HARVEST) was calculated as the sum of the nitrogen in the seeds (N_{SEED}) and residues ($N_{RESIDUE}$).

4.3.2. Agronomic indices of N use efficiency

The framework was based on the methodology proposed by Laurent et al. (2019), with the objective of quantifying the impact of a novel management practice on yield (Yseed) relative to a CONTROL treatment, which represents standard cropping practices or products commonly utilized by farmers. The "difference method", as described by Dobermann (2005), was employed. This approach is cost-effective and straightforward, rendering it well-suited for on-farm research. However, the assessment of nitrogen efficiency necessitates meticulous experimentation, as a multitude of factors—including cropping practices, water management and soil nitrogen mineralization—have the potential to impact the outcomes, particularly in unfertilized treatments (UnFert) and across varying nitrogen application rates.

This method is based on the assumption that the contribution of non-fertilizer nitrogen pools to crop nitrogen uptake (N_{UPT}) is consistent between fertilized and unfertilized treatments (UnFert). Although this assumption may not always be valid in the context of diverse nitrogen application rates (Gastal, Lemaire, & Durand, 2015), it is considered to be applicable within the limited range of nitrogen rates employed in our experiment. Based on this framework, seed yield (Yseed) and nitrogen uptake (N_{UPT}) were calculated for both fertilized (Yseed_Fert and N_{UPT}_Fert) and unfertilized (Yseed_UnFert and N_{UPT}_UnFert) plots.

The agronomic efficiency of N applied (AE_N) measures the increase in seed yield per kilogram of nitrogen applied (NFT), calculated using the following equation:

AE_N = (Yseed_Fert – Yseed_UnFert)/NFT [Eq. 1]

where Yseed_Fert represents the yield in fertilized plots, Yseed_UnFert corresponds to the yield in unfertilized plots, and NFT denotes the amount of nitrogen applied. All values are expressed on a dry weight basis (Ciampitti & Vyn, 2012; Ernst et al., 2020).

The crop recovery efficiency of applied N (RE_N) measures the increase in nitrogen uptake (N_{UPT}) per kg of N applied, calculated as follows:

RE_N = (N_{UPT}_Fert – N_{UPT}_UnFert)/NFT [Eq. 2]

where N_{UPT}_Fert refers to N uptake in fertilized plots, N_{UPT}_UnFert refers to N uptake in unfertilized plots, and NFT is the amount of nitrogen applied, all expressed as kg N_{UPT} per kg NFT on a dry weight basis (Ciampitti & Vyn, 2012).

The internal nitrogen efficiency (IE_N) was determined using the following calculation, defined as the increase in kg of Yseed per kg of nitrogen applied (NFT):

IE_N = Yseed_Fert – Yseed_UnFert / N_{UPT}_Fert – N_{UPT}_UnFert
[Eq. 3]

The nitrogen harvest index (NHI), along with the harvest index (HI), was related to seed and residues N behavior, $AB_{HARVEST}$, and N partitioning. NHI was calculated as:

$NHI = N_{SEED} / N_{SEED} + N_{RESIDUE}$
[Eq. 4]

where $N_{RESIDUE}$ includes all non-seed biomass components (leaves, stems, branches, pods without seeds), and N_{SEED} represents seed nitrogen content (Ciampitti & Vyn, 2012).

Using AB_{BBCH30}, AB_{BBCH65} and tissue N concentration ($\%N_{VEG}$), we computed Nitrogen Nutrition Index (NNI) at BBCH30 and BBCH65 stages (Eq. 7), based on the critical N dilution curve (CriNDC) (Eq. 8) (Gastal et al., 2015b; Lemaire & Ciampitti, 2020). Due to the absence of a CriNDC for carinata, we used the CriNDC for winter oilseed rape, with parameters a = 4.48 and b = -0.25 (Colnenne et al., 1998) (Eq. 8).

Extending this curve, we calculated the critical nitrogen uptake curve (CriNUC) by multiplying CriNDC by AB_{BBCH30} and AB_{BBCH65}, which provided critical N_{UPT} content in plants ($N_{UPT}CRITICAL \sim NNI = 1$) (Eq. 9) (Bohman et al., 2021; Houlès et al., 2007; Lemaire et al., 2008). By integrating NNI into Eq. 9, we generated curves representing varying nitrogen statuses relative to $N_{UPT}CRITICAL$ (-50% ~ NNI = 0.5, -25% ~ NNI = 0.75, +25% ~ NNI = 1.25) (Eq. 10) (Bohman et al., 2021). Additionally, we explored the relationships among NNI values, nitrogen efficiency, agronomic components and quality traits such as oil and protein concentrations (Yin et al., 2018).

$NNI = \%N_{VEG} / \%N_{CRITICAL}$
[Eq. 7]

$\%N_{CRITICAL} = 4.38 * AB^{(-0.25)}$
[Eq. 8]

$N_{UPT}CRITICAL = 10 * 4.38 * AB_{BBCH30/\,BBCH65}^{(1-0.25)}$ [Eq. 9]

$N_{UPT} = NNI * 10 * 4.38 * AB_{BBCH30/BBCH65}^{(1-0.25)}$
[Eq. 10]

4.3.3. Statistical analysis

For the statistical analysis, linear mixed-effects models were used, incorporating a nested trial effect within the growing season. The trial-by-treatment interaction was evaluated, with the block effect included as a random effect. The lme4 package (lmer function) (Bates et al., 2015) was used in R (version 1.4.1106; RStudio Team, 2021). Post-hoc tests for the fixed effect (treatments) were conducted using the emmeans package (Lenth, 2016), contrasting new management practices against the CONTROL treatment (traditional split application of urea at 90 kg ha^{-1} N). This package reduces the risk of false discoveries (Type I errors) in multiple comparisons, providing more reliable results. For all models generated with the different variables (e.g., Yseed), residuals, variance component partitioning and interactions were evaluated. When no significant interaction was observed, a global adjustment of means by treatment was performed and compared, assuming consistent differences across trials. Additionally, confidence intervals from the fitted models were used as a measure of stability for the treatments. Principal component analysis (PCA) was applied to seed quality traits ([Oc] and [Pc]), agronomic, physiological and nitrogen efficiency variables, grouping them into clusters using the K-means technique (Yuan & Yang, 2019). Before performing PCA, outliers were removed using the Interquartile Range (IQR) method, which affected less than 1% of the data, ensuring the integrity of the analysis.

4.4. Results

4.4.1. Climate Characterization

In 2019 and 2020, substantial environmental fluctuations had a considerable impact on the experimental results. The rainfall patterns observed in 2019 exhibited baseline levels throughout the crop cycle. In contrast, the rainfall levels in 2020 exhibited a significant 50% reduction, particularly during the critical period for seed yield (Yseed), which occurred between early and mid-October (Figure 2). Additionally, temperature fluctuations influenced the critical period, with temperatures slightly exceeding baseline levels in both years, particularly towards the end of the growing season (Figure 2). As a result, the severe water limitations that occurred in 2020 became the primary abiotic stressor affecting the trials (Figure 2). Furthermore, a late frost event, defined as a daily minimum temperature below 0°C, occurred in early October between late flowering (BBCH69) and early pod development, thereby exerting additional influence on the trials conducted in 2020 (Figure 2). Among the trials that were affected, the La Encantada trial suffered severe damage from a late frost event, which led to its exclusion from subsequent analyses.

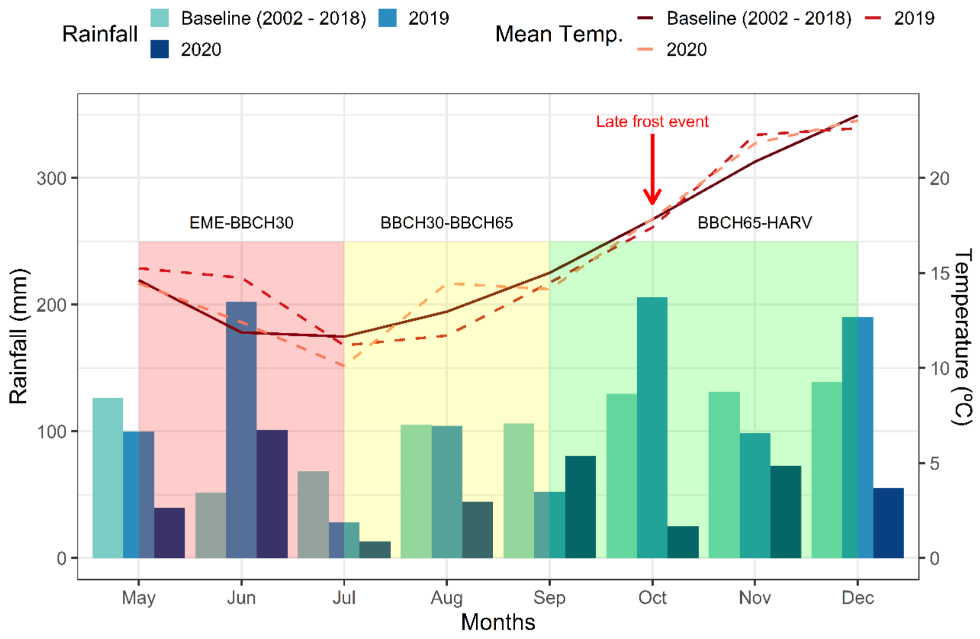

Figure 2

Monthly precipitation and temperature deviations during the experimental period (2019 and 2020 growing seasons) compared to baseline (2002–2018). EME: Emergence; HARV: Harvest.

4.4.2. Nitrogen Nutrition Index, Agronomic Components and Nitrogen Dynamics

ENTEC 150 significantly increased Yseed yields by 70% compared to the CONTROL ($n = 61$, $p < 0.05$) (Figure A). The maximum yield reached 4.71 Mg ha^{-1}, surpassing Uruguay's agroecological benchmark (red dotted line) (Figure 3A).

The NNI values responded to N treatments without trial interaction ($n = 62$, $p < 0.05$) in both stage BBCH30 and BBCH65 (Figure 3A inside, Table 2). At BBCH30, the NNI showed no significant differences among nitrogen treatments, except for the UnFert treatment ($n = 62$, $p < 0.05$) (Figure 3A inset, Table 2). At BBCH65, the ENTEC 150 and Urea 150 treatments maintained sufficient nitrogen status (NNI ≥ 1) (Figure 3A inset, Table 2). These treatments showed increases of 32% and 26%, respectively, compared to the CONTROL ($n = 62$, $p < 0.05$) (Figure 3A inset, Table 2). From the perspective of nitrogen status, both the full application of ENTEC 150 and the split application of Urea 150 (150 kg N ha^{-1}) were effective (Figure 3A inset, Table 2). The NNI values did not correlate with NHI ($n = 62$, $p > 0.05$) (Figure 2B).

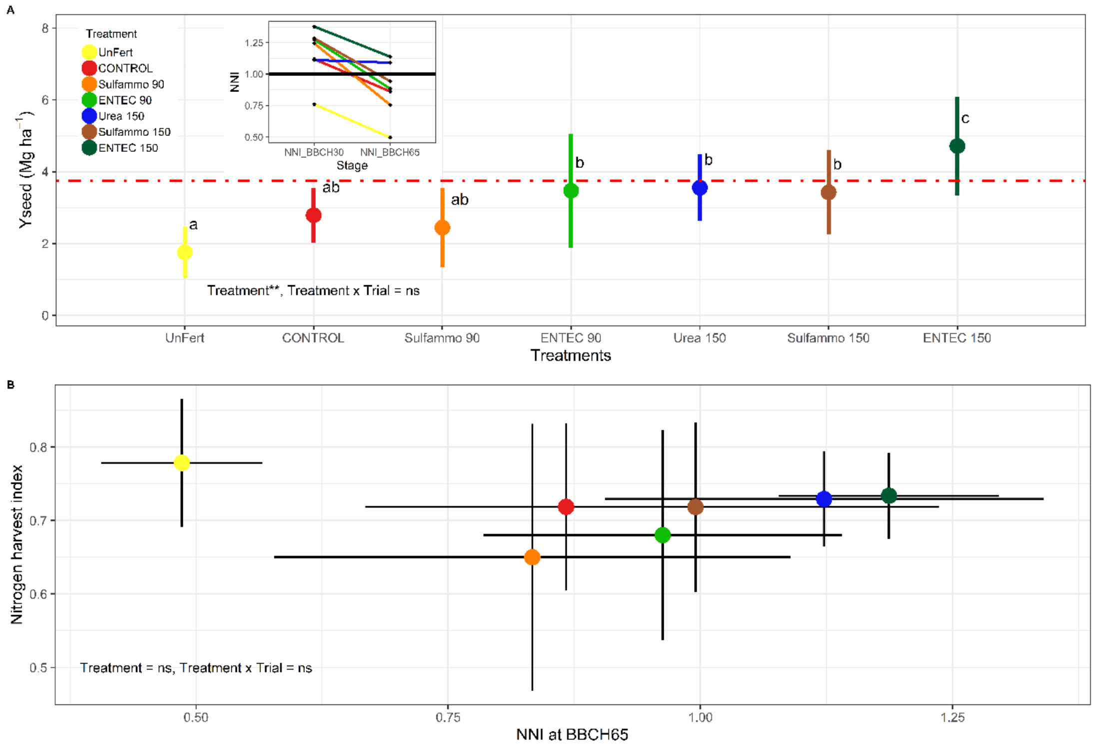

Figure 3

(A) Mean seed yield per treatment across all sites. The red-dot dash lines represent the 90th percentile of reporting for Bonansea et al. 2023. (3A Inset) N nutrition index (NNI) by treatments and stages BBCH30 (elongation) and BBCH65 (full flowering without reaching seed formation). The black solid line indicates NNI = 1, the NNI value above 1 indicates luxury N consumtion, whereas an NNI value below 1 indicates a N deficiency. † '***', '**', '*' and '.' denote significance at the α = 0.001, 0.01, 0.05 and 0.10 levels, respectively. ns indicates a non-significant effect. Yseed values followed by different letters indicate significant differences between treatments according to the post-hoc tests for the fixed effect (treatments). (B) Relationship between NNI at BBCH65 and nitrogen harvest index (NHI) per treatments which mean from all sites.

Table 2

The Aboveground Biomass (AB) and Nitrogen Nutrition Index (NNI) at BBCH30, BBCH65 and Harvest Maturity.

Variables	AB_{BBCH30}	AB_{BBCH65}	$AB_{HARVEST}$	NNI_BBCH30	NNI_BBCH65
	Mg ha^{-1}	Mg ha^{-1}	Mg ha^{-1}	-	-
Variation Source	**Pr(>F)**				
Treatment	**	ns	**	***	***
Treatment x Trial	ns	ns	ns	ns	ns
UnFert	0.513 a	4.39 a	7.82 a	0.76 a	0.48 a
Urea 150	0.725 ab	6.49 ab	15.03 bc	1.12 b	1.12 c
Sulfammo 150	0.732 ab	7.51 b	16.16 bc	1.28 d	0.99 bc
CONTROL	0.733 ab	7.23 ab	14.09 b	1.12 bc	0.86 b
Sulfammo 90	0.817 b	8.42 b	13.27 b	1.25 bcd	0.83 b
ENTEC 90	0.856 b	8.20 b	15.99 bc	1.27 cd	0.96 bc
ENTEC 150	0.902 b	9.07 b	18.67 c	1.37 d	1.18 c

†Values followed by different letters indicate significant differences between treatments according to the post-hoc tests for the fixed effect (treatments).

At BBCH30, AB did not vary significantly among nitrogen treatments, except for the UnFert treatment ($n = 62$, $p < 0.05$) (Table 2). ENTEC 150 showed a 23% higher value (0.9 Mg ha^{-1}), while Urea 150 was 1% lower (0.7 Mg ha^{-1}) compared to the CONTROL (Table 2). At BBCH65, AB also did not vary significantly among nitrogen treatments, except for UnFert ($n = 62$, $p < 0.05$) (Table 2). However, ENTEC 150 maintained a 25% higher value (9 Mg ha^{-1}), whereas Urea 150 was similar to the CONTROL (6.5 Mg ha^{-1}) (Table 2). At harvest, AB from ENTEC 150 was the only treatment that significantly differed from the CONTROL ($n = 62$, $p < 0.05$) (Table 2).

Table 3

Agronomic Components of Seed Yield and Nitrogen Parameters.

Parameters	Yseed	HI	Seed Weight	Pod m^{-2}	Seed/Pod	NHI	$N_{STUBBLE}$
	mg ha^{-1}	-	mg^{-1}	-	-	-	kg ha^{-1}
Variation Source							
Treatment	***	ns	ns	***	ns	ns	***
Treatment x Trial	ns	ns	ns	ns	ns	ns	ns
Tretatments							
UnFert	1.75 a	0.22 a	3.47 a	4153 a	12.1 a	0.78 a	16.9 a
CONTROL	2.78 ab	0.20 a	3.93 a	7487 b	9.9 a	0.71 a	45.3 b
Sulfammo 90	2.44 ab	0.18 a	3.82 a	7128 ab	9.6 a	0.65 a	54.7 bc
ENTEC 90	3.47 b	0.21 a	3.9 a	10189 bc	9.1 a	0.68 a	61.9 bc
Urea 150	3.55 b	0.23 a	3.63 a	9099 bc	11.1 a	0.72 a	64.2 bc
Sulfammo 150	3.42 b	0.21 a	3.58 a	10885 cd	9.1 a	0.71 a	57.5 bc
ENTEC 150	4.71 c	0.25 a	4.02 a	13397 d	9.5 a	0.73 a	75.3 c

†Values followed by different letters indicate significant differences between treatments according to the post-hoc tests for the fixed effect (treatments).

4.4.3. Agronomic Efficiency of Applied Nitrogen

The agronomic efficiency of applied nitrogen (AE_N) showed significant variability between years (2019: 0.66-33 kg kg^{-1}; 2020: 4-14 kg kg^{-1}) and between sites ($n = 49$, $p < 0.05$) (Figure 4). However, AE_N was not significantly affected by N treatments ($n = 49$, $p > 0.05$), probably due to the high variability within sites, as indicated by the shaded areas of the regression (Figure 4). Similarly, no significant relationship was observed between AE_N and the nitrogen nutrition index (NNI) ($n = 49$, $p > 0.05$) (Figure 4). However, higher AE_N values were generally associated with higher NNI values (Figure 4). ENTEC 150 consistently outperformed other treatments across years and sites, with confidence intervals remaining consistently positive (data not shown). Its stability and superior performance relative to on-farm practice standards (CONTROL) were particularly remarkable, with increases of 80% at Barrancas, 70% at La Oriental and 66% at LO20 (range: 14-28 kg kg^{-1}). In addition, ENTEC 150 exceeded the AE_N benchmark at all sites within the Uruguayan agroecological zone (Figure 4).

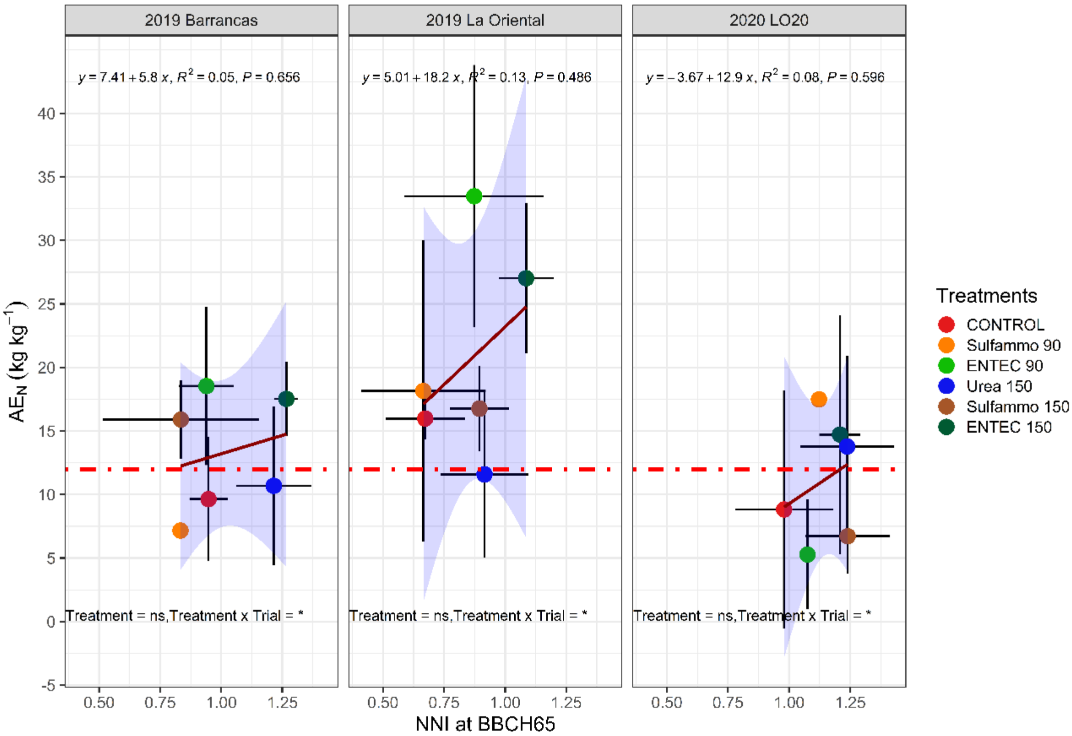

Figure 4

Relationship between Nitrogen Nutrition Index (NNI) at BBCH65 and Agronomic Efficiency of Applied N (AE_N). Each point represents the mean value for the N treatment. The red dashed lines indicate the 90th percentile reported by Bonansea et al. (2022). Shaded areas represent 95% confidence bands of linear model fits. † '***', '**', '*' and '.' denote significance at the α = 0.001, 0.01, 0.05 and 0.10 levels, respectively. ns indicates a non-significant effect (p > 0.05).

Table 4

Nitrogen Uptake (N_{UPT}) at Different Growth Stages (BBCH30, BBCH65 and Harvest) Across Treatments and Sites.

Parameters	N_{UPT}_BBCH30	N_{UPT}_BBCH65		N_{UPT}_HARVEST	
	kg ha^{-1}	kg ha^{-1}		kg ha-1	
Variation Source					
Treatment	***	**		***	
Treatment x Trial	ns	ns		*	
Tretatments/Sites			Barrancas	La Oriental	LO20
UnFert	20.1 a	58.7 a	103.4 a	71 a	63.7 a
CONTROL	40.9 b	159.5 b	202.3 abc	138.9 ab	143.9 ab
Sulfammo 90	49 bc	153.1 b	151.3 ab	150.4 ab	162.8 abc
ENTEC 90	50.7 bc	175 bc	246.5 bcd	234 bc	143.3 ab
Urea 150	39.2 bc	189.4 bc	292.2 cd	173.7 ab	202.2 bc
Sulfammo 150	45.4 bc	180.6 bc	265.9 cd	188.5 b	150.1 ab
ENTEC 150	59.6 c	235 c	312.9 d	300.3 c	261.5 c

†Values followed by different letters indicate significant differences between treatments according to the post-hoc tests for the fixed effect (treatments).

4.4.4. Recovery Efficiency of Applied Nitrogen

Carinata exhibited a high capacity for nitrogen uptake, with RE_N values ranging from 0.5 to 1.8 kg kg^{-1}, despite significant differences between growing season conditions (Figure 5A). RE_N was not significantly affected by nitrogen treatments or NNI ($n = 49$, $p > 0.05$), likely due to the high variability within sites, as indicated by the shaded areas in the regression (Figure 5A). ENTEC 150 consistently showed higher RE_N values compared to the CONTROL treatment, although the differences were not statistically significant. In Barrancas, RE_N increased by 27%, while in La Oriental, the increase was 102%. At LO20, RE_N was 48% higher with ENTEC 150. Across all sites, the RE_N values ranged from 1.3 to 1.5 kg kg^{-1}. These values indicate that the nitrogen uptake of carinata exceeded the amount of nitrogen applied (NFT). In addition, ENTEC 150 was the only treatment that maintained sufficient nitrogen status (NNI ≥ 1) at all sites (Figure 5A). It also (ENTEC 150) was the only treatment at all sites that exceeded the benchmark RE_N in the Uruguayan agroecological zone (Figure 5A). Figure 5B and its inset show the relationship between aboveground biomass (AB), $\%N_{VEG}$ and NNI values. N_{UPT} and AB showed a non-linear relationship at constant NNI levels, with N_{UPT} being limited when $\%N_{VEG}$ fell below $\%N_{CRITICAL}$

(NNI < 1), which directly affected AB production. This was evident at BBCH30, where the unfertilized treatment (UnFert) had the lowest values for N_{UPT}, $\%N_{VEG}$, NNI and aboveground biomass (AB_{BBCH30}) (n = 63, $p < 0.01$) (Figure 5B inset and Table 4). Conversely, all N treatments at BBCH30 maintained above $\%N_{CRITICAL}$ (NNI ≥ 1), avoiding limitations in AB production at this stage (Figure 5B inset).

At BBCH65, the unfertilized treatment reached only 50% of N_{UPT}CRITICAL (NNI = 0.50), corresponding to 58 kg N_{UPT} ha^{-1} (n = 63, $p < 0.01$) (Figure 5B and Table 4). Nitrogen treatments showed significant differences (n = 63, $p < 0.01$) with ENTEC 150 outperforming the CONTROL treatment. ENTEC 150 maintained N_{UPT} values ranging from N_{UPT}CRITICAL (NNI = 1) to 25% above N_{UPT}CRITICAL (NNI = 1.25), avoiding limitations in AB production at this stage (AB_{BBCH65}). This corresponded to an average N_{UPT} of 235 kg ha^{-1}, 47% higher than the CONTROL treatment (Figure 5B and Table 4). Other treatments showed smaller increases compared to CONTROL: Urea 150 (18%), Sulfammo 150 (13%), ENTEC 90 (10%) and Sulfammo 90 (-4%) (Figure 5B and Table 4). These results align with the lower $\%N_{VEG}$, $\%N_{CRITICAL}$ and NNI values observed (Figure 5B and Table 4). Adequate nitrogen status at BBCH30 (NNI ≥ 1) does not necessarily ensure its maintenance at BBCH65. This was particularly evident with lower nitrogen rates (CONTROL treatment) or when alternative sources, such as Sulfammo-NPRO, were used (Figure 5B).

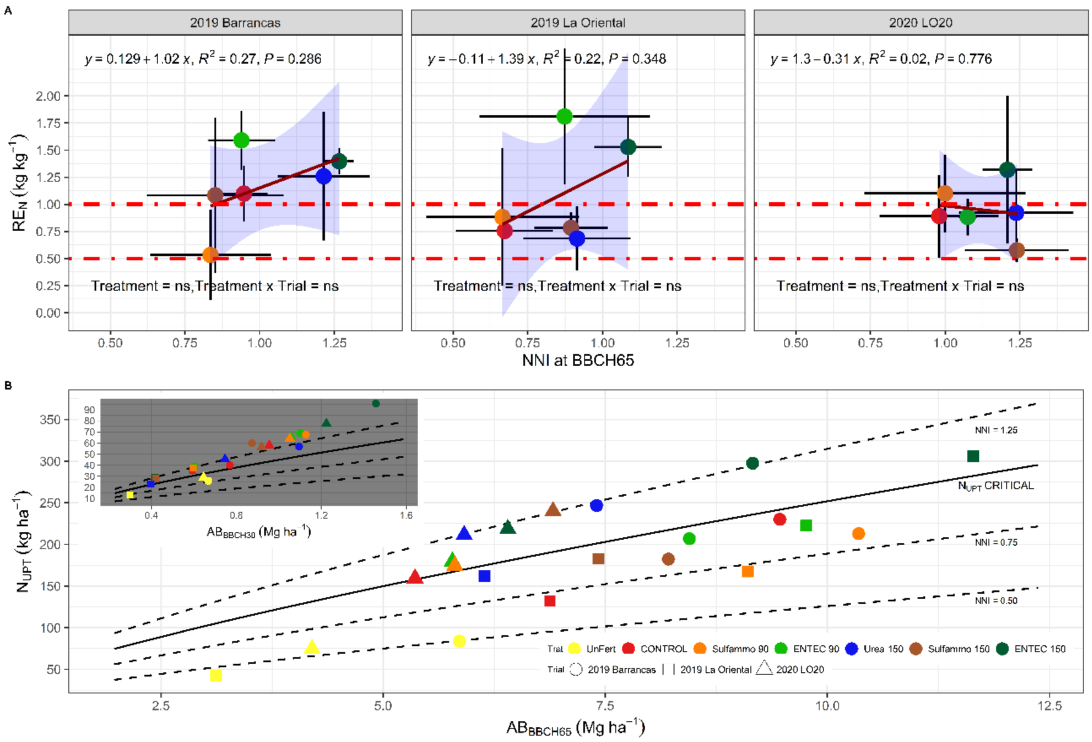

Figure 5

(A) Relationship between N nutrition index (NNI) at BBCH65 and recovery efficiency of applied N (RE_N) in where each point shown or the mean value for the N treatment. The red-dashed lines are the 90th percentile of reporting for Bonansea et al. 2023. P > 0.05 indicates a non-significant effect. Shaded areas represent 95% confidence bands of linear model fits (A). † '***', '**', '*' and '.' denote significance at the α = 0.001, 0.01, 0.05 and 0.10 levels, respectively. ns indicates a non-significant effect (p > 0.05). (B) Quantitative theoretical relationships between plant N uptake (N_{UPT}) with whole plant dry matter biomass at BBCH30 (B inset) and BBCH65. The points represent mean values per site of treatments. The solid line represents the critical N uptake curve (CriNUC) (Eqn. 9) (B inset and B). The black dashed lines show the value for N_{UPT} (Eqn. 10) at a constant N nutrition index (NNI) value as indicated in the figure (e.g., NNI equal to 0.50, 0.75, or 1.25).

4.4.5. Internal Nitrogen Efficiency (IE_N)

The relationship between the Nitrogen Nutrition Index (NNI) and Internal Efficiency of Nitrogen (IE_N) varied considerably across experimental sites and years

(Figure 6). No statistically significant relationships were observed between NNI and IE_N, indicating that NNI may not be a reliable predictor of IE_N under the experimental conditions evaluated (Figure 6). The IE_N was not significantly affected by the different nitrogen treatments and showed high variability across sites and years, ranging from 5 to 25 kg kg^{-1} (Figure 6 and Table 4). Most treatments in this trial did not exceed the benchmark values across Uruguay's agroecological zone (red dotted line), with the exception of La Oriental in 2019 (Figure 6).

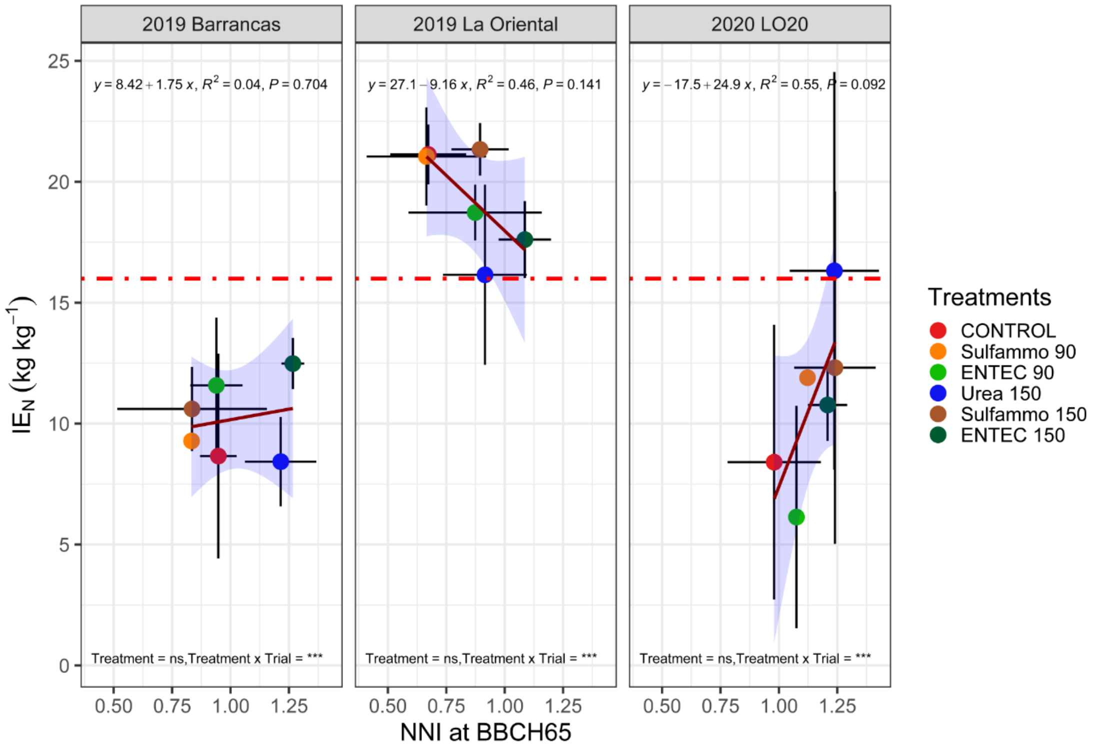

Figure 6

(A) Relationship between N nutrition index (NNI) at BBCH65 and N internal efficiency of N (IEN), where each point shows the mean value for the treatments. The red-dashed lines are the 90th percentile of reporting for Bonansea et al. (2023). P > 0.05 is indicating a non-significant effect. Shaded areas represent 95% confidence bands of linear model fits (A). † '***', '**', '*' and '.' denote significance at the α = 0.001, 0.01, 0.05 and 0.10 levels, respectively. ns indicates a non-significant effect (p > 0.05).

4.4.6. Relationship between quality, agronomy and N efficiency components

The concentrations of oil and protein exhibited a strong negative correlation, as indicated by their opposite angles in the biplot (Figure 7). Cluster three, which includes [Pc], NNI at BBCH65 and NFT, exhibited a negative correlation with cluster one, composed of [Oc], IEN and NHI, as evidenced by the small angles within each cluster and opposite angles between clusters (Figure 7). Seed yield (Yseed) and aboveground biomass at harvest ($AB_{HARVEST}$) demonstrated no correlation with either [Oc] or [Pc], as illustrated by right angles in the biplot (Figure 7). The concentrations of oil and protein were ([Pc] and [Oc]) not significantly affected by the different nitrogen treatments, except for UnFert ($n = 53$, $p < 0.05$) (Table 5). In general, and as shown in Figure 7 and Table 5, treatments with higher nutritional status (ENTEC 150 and Urea 150, NNI > 1) had lower oil content and higher protein content in the grain (Table 5).

Table 5

Effect of Nitrogen Treatments on Quality Grain.

Parameters	[Oc]	[Pc]
	g kg^{-1}	g kg^{-1}
Variation Source		
Treatment	*	***
Treatment x Trial	ns	ns
Tretatments		
ENTEC 150	395 a	294 b
Sulfammo 90	416 ab	267 b
Urea 150	421 ab	278 b
Sulfammo 150	425 ab	267 b
ENTEC 90	426 ab	271 b
CONTROL	427 ab	264 b
UnFert	457 b	227 a

†Values followed by different letters indicate significant differences between treatments according to the post-hoc tests for the fixed effect (treatments).

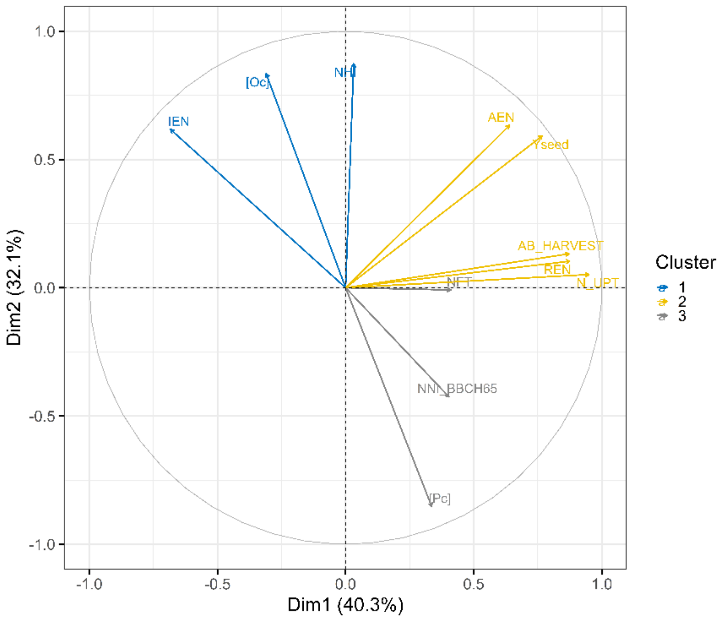

Figure 7

Principal component analysis biplot (PC1 and PC2) and K-means cluster analysis were performed for the different variables.

4.5. Discussion

The combined effects of water deficit and late frosts resulted in increased variability of the measured variables. However, the analyzed variables were not found to be statistically affected by the trial effect (location nested in year), although the magnitude of the recorded values in 2020 was found to be lower. Therefore, the absence of statistical differences in certain variables may be attributed to the influence of climate in 2020.

4.5.1. Agronomic Performance, Nitrogen Nutrition Index and Nitrogen Dynamics

The alternative nitrogen source, ENTEC 150 (source ENTEC at 150 kg ha^{-1} rate), exhibited a 70% higher yield seed compared to the current on-farm management practice (CONTROL) within the same agroecological zone. At the same rate (150 kg ha^{-1}), the response from the other sources was 30% higher than that of the CONTROL, but 50% lower than that of ENTEC 150. Moreover, this treatment (ENTEC 150) demonstrated a consistent Yseed, even in years with water limitations that constrained current on-farm practices (74% relative to the CONTROL in 2020 with low confidence intervals). These findings align with those of various meta-analyses, which have demonstrated that nitrification inhibitors, particularly 3,4-dimethylpyrazole phosphate (ENTEC), have the most favorable outcomes (Ábalos et al., 2014; Linquist et al., 2013). However, these meta-analyses report more modest responses, with improvements ranging from 5 to 10%. Nevertheless, the discrepancies are contingent upon the specific CONTROL treatment selected. In this instance, it is evident that the recommended average nitrogen rate reported by Bonansea et al. (2023) is inadequate to achieve high yields across all sites.

The estimation of crop nitrogen status (NNI) during the growing season can serve as a critical indicator for the monitoring of crop performance. In this study, the nitrogen nutrition index (NNI) ranged from 0.49 to 1.37, with the influence of nitrogen treatments evident but the growing season proving inconsequential. The range exhibited by Carinata was comparable to that observed in other crops, including corn (0.29–1.45), annual ryegrass (0.4–1.6), spring wheat (0.34–1.43), durum wheat (0.25–1.5) and rapeseed (0.75–1.3) (Dordas, 2011; Ferreira & Ernst, 2014). However, NNI was found to be an inadequate predictor of seed yield (Yseed) ($R^2 = 0.25–0.28$, $p < 0.05$). NNI values around anthesis provided superior predictions for Yseed, corroborating the findings reported by Rodriguez et al. (2024) for major crops.

NNI provides a representative sample of the crop's nitrogen status at the time of measurement. However, factors such as soil mineralization, fertilizer application timing and crop nitrogen uptake dynamics render direct yield predictions from NNI measurements challenging (Lemaire et al., 2008). Notwithstanding this limitation, the NNI proved an effective means of capturing the nutritional trajectories of different treatments. The application of ENTEC (NI) at a rate of 150 kg ha^{-1} resulted in the synchronization of plant nitrogen demand with the supply of fertilizer nitrogen, maintaining sufficiency (NNI > 1) from the elongation stage (BBCH30) to full flowering (BBCH65), with no growth restrictions. The NNI values for ENTEC 150 were observed to be 13–37% above the sufficiency threshold (NNI = 1.13–1.37), which correlated with a 23–32% increase in biomass production across all growth stages (BBCH30 to harvest). This synchronization likely supported nitrogen demands during critical periods for grain set, thereby enhancing grain yield (Lemaire et al., 2008). Furthermore, ENTEC 150 obviated the necessity for energy-intensive split applications, as required by Urea 150 and CONTROL, while maintaining or increasing grain yields. This approach is aligned with the objective of reducing yield-scaled N_2O emissions, thereby ensuring that biofuel production contributes to greenhouse gas (GHG) mitigation in comparison to fossil fuels (Del Grosso et al., 2009; van Groenigen et al., 2010).

<u>4.5.2. Components of Nitrogen Use Efficiency</u>

The agronomic efficiency of nitrogen (AE_N) represents a key metric for the evaluation of the effectiveness of nitrogen fertilizers in crops. Although previous studies have indicated an inverse correlation between AE_N and NNI (Dordas, 2011), this study found no significant relationship (Figure 4). This indicates that NNI is an unreliable predictor of AE_N under the conditions evaluated. Moreover, no discernible differences in AE_N were observed among the different N treatments (sources and rates) tested, suggesting that applications up to 150 kg ha^{-1} of N can be employed without negatively impacting AE_N.

The dashed red line in Figure 4 represents the benchmark AE_N reported by Bonansea et al. (2023). Most data points are situated above this benchmark, indicating that the treatments generally achieved AE_N values that can be considered acceptable. Although AE_N was not significantly affected by different treatments, ENTEC 150 demonstrated high stability and superior performance compared to current on-farm practices (CONTROL), with increases of 80% at Barrancas, 70% at La Oriental and 66% at LO20 (range: 14–28 kg kg^{-1}). However, it is possible that these results have been influenced by climatic conditions, particularly in 2020, and the narrow range of nitrogen application rates. Furthermore, the excessive vegetative growth of the AVANZA 641 variety, and the associated lodging observed in some trials, may have reduced the potential to translate NNI differences into seed yield (Yseed), thereby affecting AE_N (Caviglia et al., 2014b; Khan et al., 2017).

As illustrated in Figure 5A, the RE_N range reported by Bonansea et al. (2023) for the same agroecological zone (dashed red lines) demonstrates that the majority of data points exceed the specified threshold, indicating a remarkably high recovery efficiency across treatments ($RE_N > 1$). This underscores carinata's exceptional nitrogen scavenging efficiency (Seepaul et al., 2019), a pivotal factor in curbing reactive nitrogen losses (N_R) as greenhouse gases (GHGs) or through alternative pathways, even under disparate growing season conditions (Guzman-Bustamante et al., 2019; Udvardi et al., 2021).

Furthermore, the results demonstrate that NNI at BBCH65 is an unreliable predictor of RE_N in the analyzed experiments (Figure 5A). This finding contrasts with the results of studies on winter wheat, flax and barley, which have shown that higher yields are typically associated with improved nitrogen exploitation and decreased RE_N as NNI increases (Dordas, 2011). However, the absence of significant differences between treatments and the lack of correlation between NNI at BBCH65 and RE_N may indicate that treatments like ENTEC 150 can achieve higher seed yields (Yseed) while maintaining sufficient NNI levels without compromising RE_N (Figure 3A, 3A inset and 5A). Indeed, ENTEC 150 sustained recovery levels above the applied N rate (NFT) (RE_N = 1.3–1.5 kg kg^{-1}) (Figure 5A).

The limited predictive power of NNI for RE_N and other variables (e.g., Yseed or AE_N) may be influenced by high nitrogen application rates and soil nitrogen supply in South American conditions. Excessive nitrogen levels have been demonstrated to promote lodging by increasing plant height and reducing structural integrity, as evidenced by alterations in lignin, cellulose content, stem diameter and basal internode wall thickness (Khan et al., 2017). Consequently, while rapeseed efficiently absorbs nitrogen, its nitrogen use efficiency (NUE) is relatively low, approximately half that of cereals (Sylvester-Bradley & Kindred, 2009).

Nevertheless, we postulate that NNI could be employed in a different manner to more accurately assess agronomic and environmental outcomes, thereby ensuring that the utilization of biofuels genuinely diminishes GHG emissions in comparison to conventional fossil fuels. This approach may facilitate the identification of management practices at the production level that are not readily discernible through traditional NUE studies. Accordingly, we selected the interpretation of RE_N in terms of environmental impact, specifically potential N losses. This approach has not previously been reported in the evaluation of such biofuels. When nitrogen uptake efficiency is based solely on fertilizer nitrogen inputs (RE_N), it is prone to overestimation (Bohman et al., 2021). Nevertheless, the use of a unfertilized treatment to account for variations in N uptake efficiency resulting from mineralization enables the comparison of treatments and the evaluation of the efficacy of management practices in reducing N losses under specific conditions (Bohman et al., 2021). The accumulated AB at BBCH30 and BBCH65 (AB_{BBCH30} and AB_{BBCH65}) demonstrated a positive correlation with N_{UPT}, which is consistent with the hypothesis that N-dependent growth is a contributing factor.

The UnFert treatments demonstrated reduced aboveground biomass (AB) and nitrogen uptake (N_{UPT}) at both stages, exhibiting values approximately 50% of the critical N_{UPT}CRITICAL threshold. At BBCH30, all nitrogen-treated plots surpassed the N_{UPT}CRITICAL (NNI = 1) threshold. Among the treatments, only ENTEC 150 demonstrated consistent nitrogen sufficiency (NNI $\geq$ 1) across all sites. For grain crops, the timing and duration of nitrogen sufficiency are of critical importance for seed yield (Yseed) (Rodriguez et al., 2024). While deficiencies in vegetative growth (NNI < 1) may not significantly impact seed yield if corrected before anthesis (BBCH65). However, deficiencies occurring later in the reproductive stage can have a severe impact on the number and weight of seeds (Ravier et al., 2017). In wheat, for instance, the restoration of nitrogen nutrition (NNI = 1) prior to anthesis has been demonstrated to prevent yield reductions despite the presence of early vegetative deficiencies (Ravier et al., 2017). Optimal nitrogen sufficiency during the vegetative stages (NNI = 1) maximizes aboveground biomass (AB). However, final yields are contingent upon factors that influence the number and weight of seeds after BBCH65 (Ravier et al., 2017). The results of this study indicate that ENTEC 150 exhibited higher values for $\%N_{VEG}$, N_{UPT} and NNI, which likely contributed to the higher Yseed observed in this treatment. Furthermore, increasing N_{UPT} at comparable AB levels enhances crop resilience to prospective nitrogen deficiencies, reduces reliance on external nitrogen fertilization and mitigates the risk of environmental losses Rodriguez et al. (2024) and Fernandez et al. (2022). In addition, these findings are consistent with those of Plénet & Lemaire (1999), who reported that maintaining a higher NNI near anthesis (1.15–1.20) is essential for maximizing maize yields due to the high nitrogen demand during grain filling and the potential for soil N depletion or reduced root uptake capacity. On the other hand, it is also possible that the observed results may be influenced by differences in sulfur content among nitrogen sources, particularly at higher application rates, which could impact N_{UPT} (Salvagiotti et al., 2009). Further experimentation is required to elucidate the respective contributions of sulfur content and nitrogen inhibitors (UI/NI/SR) to these effects.

The traits that contribute to plant NUE include N uptake (RE_N), assimilation, partitioning, transient storage, as well as N remobilization and utilization (IE_N) in both source and sink organs (Udvardi et al., 2021). The correlation between the nitrogen nutrition index (NNI) and internal efficiency (IE_N) exhibited notable variability across sites and years, with no statistically significant distinction between alternative and traditional N sources, including the current management practice in Uruguay (CONTROL) (Figure 6). Such variation may be attributed to local environmental factors, such as soil and climate conditions, which could potentially attenuate the relationship between NNI and IE_N. For example, the use of N_{UPT} from the unfertilized treatment (UnFert) as an indirect measure of soil N supply under similar climatic conditions (e.g., the 2019 growing season) revealed that Barrancas exhibited a 46% higher N_{UPT} (104 kg ha^{-1} vs. 71 kg ha^{-1}), underscoring the existence of notable site-specific disparities in soil N contribution to Yseed prior to fertilization (Milroy et al., 2019).

N mineralization rates, which are influenced by factors such as soil organic matter, moisture and temperature, also contributed to variations in NUE components, as observed in IE_N values at Barrancas (~10 kg kg^{-1}) versus La Oriental (~20 kg kg^{-1}) (Milroy et al., 2019). In contrast with the findings in barley (*Hordeum vulgare* L.) and winter wheat, this study revealed no inverse correlation between NNI and IE_N, indicating that NNI may not be a reliable predictor of IE_N in the evaluated contexts (Figure 6). It is encouraging to note that there were no differences in IE_N between the alternative and traditional N sources, indicating that there was no negative impact from the alternatives in comparison to the CONTROL treatment (Figure 6). This stability is likely attributable to the absence of alterations in the factors influencing IE_N, such as grain nitrogen concentration (N seed) or harvest index (HI), which remained consistent across treatments (Ladha et al., 2005; Salvagiotti et al., 2009).

The use of N_{UPT}_UnFert to correct soil N contributions assumes that non-fertilizer N pools consistently influence crop N_{UPT} across treatments. However, this assumption may not hold true when considering varying N rates (Gastal, Lemaire, Durand, et al., 2015; Milroy et al., 2019). A more comprehensive view of these findings entails examining IE_N in the context of potential environmental impacts, such as N losses. Oilseed crops such as carinata and canola require substantial nitrogen inputs to support efficient photosynthesis (Hegewald et al., 2016). This often results in high yields but also significant nitrogen surpluses (up to 90 kg N ha^{-1}) and low nitrogen harvest indexes (NHI) (Henke et al., 2007; Sieling & Kage, 2010). Following the flowering stage, the substantial, readily mineralizable crop residues return to the soil, thereby contributing to N_2O emissions, a potent greenhouse gas (Malagoli et al., 2005).

In this study, the ENTEC 150 treatment demonstrated the highest Yseed while also exhibiting elevated NNI, $\%N_{VEG}$, AB and N_{UPT} in comparison to other treatments. However, the HI and NHI remained similar, resulting in augmented $N_{RESIDUE}$ (Table 3). This increase in $N_{RESIDUE}$ may serve as a source of N_2O production, emphasizing the necessity for precise assessments of N_2O emissions to calculate GHG balances for biodiesel production from carinata. Life cycle analyses (LCA) indicate that N_2O emissions constitute 20–40% of the total greenhouse gas (GHG) emissions associated with biodiesel production (Hong, 2012; Dufossé et al., 2013). While alternative nitrogen sources demonstrated no discernible differences from traditional ones, further research is necessary to elucidate genotype-by-environment-by-management (GxExM) interactions, with the aim of optimizing carinata practices, increasing HI and NHI, and reducing high N surpluses.

The positive impact of increased nitrogen (N) rates on oilseed rape seed yield is well-documented. However, higher N fertilizer rates often result in a reduction in oil content (Oc), which is the primary feedstock for advanced renewable fuels (Rathke et al., 2005; Seepaul et al., 2021). As illustrated in Figure 7 and Table 5, there is a strong negative correlation between oil and protein content. The ENTEC 150 treatment, which yielded the highest Yseed, exhibited elevated protein and diminished oil concentrations relative to the current standard (CONTROL), aligning with observations from other carinata-producing regions (Seepaul et al., 2021).

Clusters that diverge from the initial grouping indicate that elevated nitrogen (N) status in near-anthesis stages (NNI > 1) may diminish [Oc], IE_N and NHI (Figure 7), corroborating findings in oilseed species where augmented N status reduces carbohydrate availability for oil synthesis (Rathke et al., 2005, 2006). In addition, IE_N is influenced by the ratio between grain HI and plant $\%N_{VEG}$; therefore, higher $\%N_{VEG}$ concentrations can result in a decrease in IE_N, which may subsequently lead to a reduction in oil outputs and an increase in undesirable emissions, such as N oxides (Ciampitti & Vyn, 2012; Rathke et al., 2006). It is of interest to note that in Uruguay's agroecological zone, comparable or reduced nitrogen application rates have been observed to result in elevated seed yield, accompanied by augmented O_C and slightly diminished Pc (418 *vs*. 390 and 273 *vs.* 316, respectively) (Seepaul et al., 2021). This translates into higher oil and protein yields per hectare, which serves to highlight the region's unique potential for optimizing both productivity and quality.

4.6. Conclusions

Optimizing nitrogen (N) management is fundamental to enhancing the sustainability of biofuel feedstock production while mitigating its greenhouse gas (GHG) footprint. This study demonstrates that the use of enhanced-efficiency fertilizers (EEF), specifically ENTEC 150, significantly improves seed yield (Yseed) in *Brassica carinata* compared to conventional fertilization practices. The observed 70% yield increase relative to the CONTROL treatment highlights the potential of nitrification inhibitors to enhance agronomic performance, despite of the water stress conditions. Moreover, the synchronization of nitrogen availability with crop demand, as indicated by the Nitrogen Nutrition Index (NNI), contributed to improved biomass production and nitrogen use efficiency (NUE) without requiring multiple fertilizer applications.

Despite considerable seasonal variability, our findings underscore the robustness of NNI as a tool for assessing crop N status and guiding fertilization strategies. While NNI alone was a limited predictor of seed yield ($R^2 = 0.25–0.28$, $p < 0.05$), its integration with NUE components provided a more comprehensive assessment of N uptake dynamics. ENTEC 150 maintained N sufficiency (NNI > 1) from early elongation to flowering, ensuring adequate N availability during critical reproductive phases. This resulted in a 23–32% increase in biomass accumulation and stable agronomic efficiency of nitrogen (AE_N) across diverse environments. Furthermore, recovery efficiency of nitrogen (RE_N) exceeded regional benchmarks, reinforcing *B. carinata*'s capacity to optimize N uptake and minimize losses.

These findings have important environmental and agronomic implications. By reducing the need for energy-intensive split applications and enhancing NUE, ENTEC 150 aligns with the broader goal of lowering yield-scaled N_2O emissions from biofuel production systems. Given that fertilization practices account for up to 80% of total GHG emissions in biofuel feedstock cultivation, the adoption of nitrification inhibitors could play a key role in improving the carbon balance of sustainable aviation fuel (SAF) supply chains.

Future research should focus on refining NNI-based models for improved yield prediction and exploring site-specific calibrations to enhance agronomic decision-making. Additionally, economic analyses of EEF adoption should be incorporated to assess its cost-effectiveness at the farm scale. Ultimately, integrating NUE metrics, real-time N status diagnostics and climate-adaptive fertilization strategies will be essential to advancing sustainable *B. carinata* production and optimizing its role in low-carbon biofuel systems.

4.7. References

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5. Discusión general

6.1. Resumen de lo realizado

Como se ha mencionado, los biocombustibles podrían ser claves para reducir las GHE y, por lo tanto, la huella de carbono de las materias primas producidas (Ogle et al., 2016). Sin embargo, las posibilidades de las materias primas vegetales para reemplazar a los combustibles fósiles y mitigar los impactos ambientales siguen siendo inciertas debido a la falta de precisión en la cuantificación de las emisiones de óxido nitroso (N_2O) y al uso variable del grano y la biomasa en la producción de biocombustibles (Adler et al., 2012; Ogle et al., 2016). Si hacemos foco en la emisión de N_2O, la importancia de este gas radica en su alto poder de calentamiento (265-315 GWP) sumado a los daños ocasionados a la capa de ozono (Ogle et al., 2016). Al mismo tiempo, este tiene una estrecha relación con las prácticas de manejo ineficientes de la fertilización con nitrógeno, lo cual coloca a la etapa de producción a campo en un papel clave (Ogle et al., 2016). *Brassica carinata* (carinata) es un nuevo cultivo invernal con múltiples usos (biocombustible para aviones, cobertura de suelos, alimento para animales, aplicaciones industriales y farmacéuticas) (Seepaul et al., 2021). Al ser un cultivo nuevo en la región, carinata carece de un modelo de fertilización, sumado a que se desconoce el potencial de emisión de GHG, especialmente de N_2O. Como parte del objetivo específico 1, nuestro artículo buscó establecer *benchmarks* a través del uso de diferentes percentiles (10, 50, 90) con diferentes técnicas estadísticas (por ejemplo: *bootstrap*) que sirvan en primera instancia para guiar nuestras prácticas de fertilización (momento y dosis) con urea-S (40N-0/0-0-6S) que es la fuente utilizada por los productores de la región. Este enfoque nos permitió observar las diferencias entre los percentiles, lo cual nos da la posibilidad de hipotetizar sobre diferentes aspectos del cultivo que expliquen la diferencia en rendimiento ante el mismo nivel de recursos (nitrógeno en este caso). Para establecer esas *benchmarks*, contábamos con una base de datos con experimentos llevados a cabo en la zona noroeste de Uruguay (Paysandú-Río Negro). Fueron siete experimentos distribuidos a lo largo de tres temporadas de cultivo de carinata: 2016, 2017 y 2018. En el primer año se realizaron tres experimentos, mientras que en el segundo y tercer año se llevaron a cabo dos por temporada. Basándonos en los resultados anteriores y en la bibliografía internacional, hipotetizamos que la inclusión de fertilizantes de solución lenta podría tener ventajas para el rendimiento de carinata y evitar las aplicaciones divididas, lo que genera gastos de carbono extras al mismo tiempo que se mantiene la NUE. Por lo tanto, como se mencionó, con base en los valores generados en el objetivo específico 1, se trabajó en el objetivo específico 2 (segundo artículo). Este consistía en un grupo de experimentos de chacras comerciales (2019-2020), con siete tratamientos aleatorizados usando fuentes alternativas de N (ENTEC/Sulfammo-NPRO) donde se aplicaban en un momento único versus la aplicación tradicional dividida de Urea-s en dosis de 0, 90, 150 kg ha^{-1} N. Este esquema de tratamientos buscó evaluar el paquete tecnológico actual (Urea-S en aplicaciones divididas) vs. fuentes alternativas de N con el fin de generar una recomendación de manejo a nivel de producción única hasta el momento en Uruguay con el uso de estas fuentes. Como se mencionó anteriormente, la etapa a campo tiene salidas no deseadas como las emisiones de GHG, con especial énfasis en N_2O debido a su estrecha relación con la práctica de fertilización nitrogenada; por lo tanto, en paralelo a los objetivos específicos 1 y 2 se generó un tercer objetivo específico: establecer y ajustar un protocolo para medir los tres principales gases de efecto invernadero (dióxido de carbono, metano y óxido nitroso) con el fin de evaluar de manera más precisa la contribución de carinata a la mitigación del cambio climático. Para ello se utilizó un sitio experimental en la estación experimental Mario A. Cassinoni (EEMAC) utilizando el método de cámara de flujo cerrado, con un diseño en bloques completos al azar (DBCA) para cada paquete tecnológico y así proporcionar beneficios netos de mitigación más comprensivos. El objetivo específico 1 fue identificar oportunidades de mejora en las prácticas de manejo con fuentes de nitrógeno convencionales, mientras que el objetivo 2 contrastó esas mejoras con prácticas alternativas, como la fertilización con fuentes de N de liberación lenta y baja pérdida (únicas en Uruguay), con el fin de eliminar las aplicaciones divididas que desperdician energía, sin perder eficiencia del nitrógeno (NUE), rendimiento y calidad de carinata. Además, el objetivo 2 propuso una metodología más adecuada para evaluar los beneficios reales del uso de carinata para compensación de carbono (NNI).

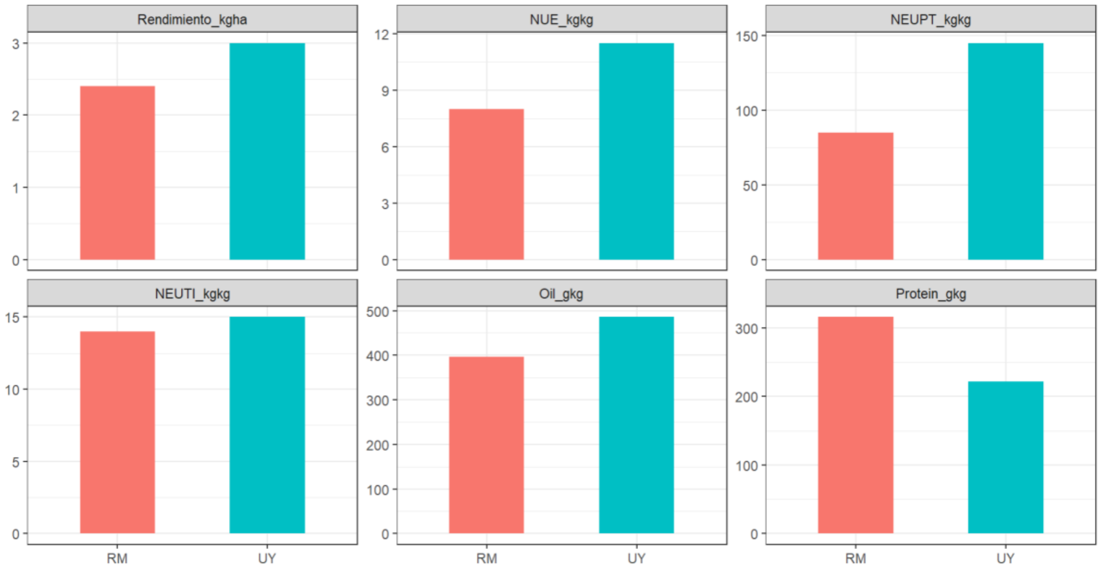
6.2. Discusión de implicancias de los resultados de todo el trabajo

El análisis de experimentos de fertilización en cultivos industriales de carinata entre 2016 y 2018 en Uruguay permitió caracterizar los *benchmarks* en parámetros agronómicos, NUE y calidad. Mediante el uso de regresión cuantílica, se derivaron curvas de frontera (percentil 90), medias (percentil 50) y bajas (percentil 10) de diferentes variables (agronómicas y NUE) en función de variables independientes, como N total (N_{TOTAL} = fertilizante +suelo) y N aplicado N_{FT}. Se ajustaron varios modelos, incluidos regresión cuantílica lineal, lineal-plató, exponencial, exponencial-plató y cuadrático-plató; dentro de estos elegimos el mejor mediante seudo-R^2, además de obtener intervalos de confianza por *bootstrapping* para cada parámetro del percentil ajustado. En términos generales, se obtuvo una excelente estimación de la variación existente bajo condiciones de producción en los diferentes componentes (NUE y sus componentes, agronómicos y calidad), lo que permitió caracterizar la zona agroecológica (Latinoamérica sur, Uruguay) a través de los valores y variabilidad reportados. Se pudieron hipotetizar futuras líneas de investigación.

Como resultado del objetivo específico 1, el rendimiento de grano se maximizó en los percentiles 50 y 90, variando entre 2,5 y 3,5 Mg ha^{-1}, cuyos máximos (plató) se lograron con dosis de 90-100 kg ha^{-1} de urea-S, lo que corresponde a 150-160 kg ha^{-1} de N_{TOTAL} (50-60 kg ha^{-1} aportados por la mineralización del suelo). Si consideramos un valor de insumo dado, en este el valor donde se maximiza el rendimiento en grano (90-100 kg N_{FT} ha^{-1}) existe una diferencia de rendimiento en grano entre el percentil 90 y 50 al menos de 1 Mg ha^{-1} a favor del primero. Esto es evidencia de que existen otros factores que limitan el rendimiento en carinata a nivel comercial en Uruguay que van más allá que la dosis aplicada de nitrógeno (N_{FT}). Algunas hipótesis sobre esto podrían estar relacionadas con la variabilidad espacial y temporal que presentan estos cultivos, en general afectada por las condiciones de siembra (excesiva lluvia o seca, cantidad de rastrojo en superficie), control de malezas, compactación y diferentes tasas de mineralización de nitrógeno del suelo dentro de una misma chacra. Por otro lado, el percentil 10 presentó una respuesta lineal en todo el rango de suministro de N_{TOTAL} y N_{FT}, lo que indica que la mayor oferta de nitrógeno puede superar algunos de los factores limitantes no relacionados con el nitrógeno, pero a costa de una caída en la eficiencia de uso del N (N_{UE}). En relación con lo anterior, la N_{UE} o la eficiencia agronómica (AE_N) (si solo consideramos el N_{FT}) es la pendiente matemática entre el rendimiento en grano (kg ha^{-1}) y la oferta de N_{TOTAL} (kg ha^{-1}) o N_{FT} (kg ha^{-1}), la cual varió entre los diferentes percentiles 3 [3;6] (10.º), 10 [9;12] (50.º) y 13 [12;16] (90.º) kg de semilla por kilogramo de N disponible, siendo muy similar a la AE_N. Estos valores estuvieron dentro de lo reportado previamente en investigaciones a campo en otras zonas productoras de carinata como Estados Unidos (Florida, Minnesota y Dakota del Norte), UE y Canadá. La N_{UE} está compuesta por dos componentes, uno de ellos es la eficiencia de absorción de N ($NE_{UPT\ o}$ RA_N) (kg N absorbido por kg N_{TOTAL} o N_{FT}, respectivamente). Este componente es considerado clave debido a su relación con el N reactivo (N_R), el cual es propenso a ser fuente de N_2O. De acuerdo con lo reportado previamente, carinata mantuvo su fama de *nitrogen scavenger*, ya que varió entre 0,57 [0,55; 0,60] (10.º), 0,88 [0,80; 0,90] (50.º) y 1,31 [1,27; 1,33] (90.º). Independientemente del percentil tomado y la forma de cálculo de la eficiencia de absorción (considerando el N_{TOTAL} o N_{FT}), los valores medios encontrados se ubicaron entre 0,57 a 1,3 kg N absorbido kg^{-1} N disponible [suelo + fertilización]), valores realmente altos si los comparamos con los cultivos de cereales (trigo y cebada). Si consideramos el percentil 90 como situaciones que no tienen otros factores limitantes, excepto el aporte de N, carinata presentó muy alta capacidad de absorción de N, 145 % [134,1; 146,6] del fertilizante aplicado, o sea, una vez y media más de absorción en relación con la dosis aplicada. Esto es reportado con frecuencia para carinata, característica fundamental para una materia prima para biocombustibles, ya que el N que no es absorbido por la biomasa queda expuesto a pérdidas (desnitrificación y nitrificación, lixiviación de nitratos en profundidad), lo que compromete su beneficio ambiental. Por otro lado, el segundo componente de la NUE es la eficiencia de utilización (NE_{UTI}) o eficiencia interna de utilización (IE_N) refiriéndose a la pendiente matemática entre el rendimiento en grano (kg ha^{-1}) y la cantidad de N_{TOTAL} absorbido (kg ha^{-1}) o rendimiento en grano (kg ha^{-1}) por NFT (kg ha^{-1}). Como era de esperar, la NE_{UTI} o IE_N son los componentes de la NUE más conservadores, y por lo tanto, aportan una menor variabilidad a la NUE. Este se encontró dentro del rango reportado en otros lugares productores de carinata, entre 13-16 kg de semilla por kilogramo de N absorbido sin diferencia entre percentiles [11;14] (10.º), [15;16] (50.º) y [15;18] (90.º). Como se mencionó anteriormente, en otros cultivos, como por ejemplo trigo, la NE_{UTI} es un parámetro conservado por las especies, por lo cual era de esperar que no se encontrara diferencia entre los diferentes percentiles. El índice de cosecha de N (NHI), definido como la relación entre el N_{UPT} en el grano y el N_{UPT} total en la AB a cosecha ($AB_{HARVEST}$), se asoció positivamente con la eficiencia en el uso de N (NE_{UTI}); por lo tanto, el aumento en NHI pueden conducir a el aumento en la NE_{UTI}, lo cual podría ser un camino para subir el rendimiento de carinata ante iguales niveles de insumos (NFT), aunque se podría llegar a afectar porcentaje de N en grano (N_{SEED}) lo que resulta en un mayor contenido de proteína ([Pc]). Sin embargo, cuando la carinata creció en condiciones de alta productividad, el NHI no fue asociado negativamente a la caída de rendimiento, lo que indico que a niveles altos de rendimiento en grano el NHI se mantuvo estable. Otro aspecto importante es la caracterización de la calidad del grano que se alcanza en nuestra zona agroecológica, por ejemplo, en cuanto a la concentración de aceite ([Oc]), proteína ([Pc]) y su interacción con la oferta de N. En relación con esto último, ni el NFT (dosis y momento) ni el N_{TOTAL} (suelo + NFT) afectaron de manera significativa las concentraciones de aceite y proteína. Sin embargo, existió una tendencia a que, a mayores ofertas de N, la concentración de aceite tienda a caer y la de proteína a subir, en concordancia con la bibliografía internacional. Los valores en la concentración de aceite se ubicaron entre 455 y 517 g kg^{-1}, mientras que en proteína fue entre 192 y 253 g kg^{-1}. En cuanto a la concentración de aceite, los valores fueron muy superiores a los reportados en otras regiones productoras, mientras que la concentración de proteína fue algo menor. Sin embargo, dado el mayor potencial de rendimiento en grano de la zona, las salidas deseadas se maximizarían tanto en kilogramos de aceite como en proteína por hectárea producida.

Figura 2.1

Comparación de variables agronómicas, eficiencia y calidad entre resto del mundo (RM) y Uruguay (UY).



Nota. NUE = eficiencia de uso del nitrógeno, NE_{UPT} = eficiencia de absorción del nitrógeno, NE_{UTI} = eficiencia de utilización del nitrógeno, Oil = contenido de aceite, protein = contenido de proteína.

El primer artículo permitió caracterizar nuestra zona agroclimática en el contexto mundial de regiones productoras de carinata al mismo tiempo que se generó la primera estimación de dosis óptimas de fertilización nitrogenada usando como fuente urea-S, las cuales maximizan el rendimiento en grano manteniendo la NUE (y sus componentes) además de la calidad de grano. Estos valores ponen a Uruguay y Sudamérica (Brasil, Argentina y Paraguay) como una zona productora altamente calificada para producir carinata de alto valor comercial sin comprometer el medioambiente.

Con base en los resultados obtenidos en el primer artículo, se generó un grupo de experimentos cuya área de estudio fue el noroeste de Uruguay, abarcando los departamentos de Paysandú y Río Negro, durante las temporadas de cultivo 2019 y

2020. Los sitios seleccionados incluyeron Barrancas (31°48'50.36"S, 57°58'35.85"O) y La Oriental (32°11'19.01"S, 58°3'42.42"O) en 2019, así como LO20 (32°11'33.46"S, 58°4'56.22"O) y La Encantada (32°25'6.78"S, 58°0'15.25"O) en 2020. Todos los sitios fueron seleccionados dentro de una zona de un cultivo comercial de cultivo de carinata en Uruguay. Cada sitio tuvo siete tratamientos en un diseño de bloques completos al azar (DBCA) con tres repeticiones, los que incluyen fuentes alternativas de N (ENTEC/Sulfammo-NPRO, con registro de huella de carbono) versus la aplicación fraccionada tradicional de urea-S a dosis de 0, 90 y 150 kg N ha^{-1}. Derivado del primer artículo e investigaciones previas por el grupo del Dr. Mazzilli, la práctica de fertilización comercial para carinata en Uruguay consiste en aplicar 90-100 kg ha^{-1} de N con la fuente urea-S fraccionada en BBCH21 (60-70 kg ha^{-1} N) y el resto de la dosis en BBCH30. Siguiendo el enfoque de Laurent et al. (2019), nosotros consideraremos que el tratamiento control debía ser la práctica actual de los agricultores de carinata en Uruguay y, por lo tanto, el tratamiento por superar además de las *benchmark values* que reportamos en el primer artículo. Además, un tratamiento sin fertilización (UnFert) es utilizado como medida indirecta del potencial ambiental y para evaluar la magnitud de la respuesta a la fertilización nitrogenada. En respuesta a las críticas de los revisores del primer artículo, avanzamos en presentar evidencia de que herramientas como el estatus N en planta con base en curvas de dilución (NNI y CriNDC) y otras relaciones cuantitativas como la curva de absorción crítica de N (CriNUC) son clave para mejorar la eficiencia de los sistemas, reducir emisiones e investigar de una mejor manera el beneficio neto ambiental.

En comparación con los resultados del primer artículo, en términos generales, los fertilizantes de liberación lenta ofrecieron ventajas frente al manejo actual en chacra (CONTROL) en el rendimiento en grano, NUE (y sus componentes) y la calidad de los granos al mismo tiempo que eliminan la necesidad de aplicaciones fraccionadas. En cuanto al rendimiento en grano, ENTEC 150 aumentó significativamente los rendimientos en un 70 % frente al tratamiento control ($n = 61$, $p < 0,05$) (4,71 Mg ha^{-1}), lo que supera los valores reportados para la zona agroecológica de Uruguay (*benchmark values*). El ranking de las otras fuentes y dosis respecto al control fue el siguiente: urea 150 (+32 %), Sulfammo 150 (+21 %), ENTEC 90 (+19 %), Sulfammo

90 (-13 %) y UnFert (-38 %). Desde el punto de vista del estatus nutricional (N_{NI}), tanto la estrategia de aplicación única con la fuente alternativa ENTEC 150 como la aplicación fraccionada de urea 150 (ambos a 150 kg N ha^{-1}) fueron efectivas en mantener un estatus de suficiencia nitrogenada (N_{NI} >1) entre el período que comprende desde elongación (BBCH30) a floración (BBCH65). Esto quiere decir que con el uso de una dosis de 150 kg N ha^{-1} usando una fuente alternativa como ENTEC 150 o fraccionando la fuente tradicional urea-S se puede mantener un estatus de suficiencia (N_{NI} > 1). La AB en elongación (AB_{BBCH30}) no varió significativamente entre los tratamientos, salvo UnFert, pero ENTEC 150 presento un 23 % más de AB, mientras que urea 150 estuvo 1 % por debajo del control, diferencias que se explican claramente por el momento de aplicación de cada tratamiento. En floración, la AB (AB_{BBCH65}) se mantuvo en la misma tendencia, ENTEC 150 siguió manteniendo una AB 25 % superior al control, mientras que urea 150 fue similar. Sin embargo, cuando se midió la AB a cosecha ($AB_{HARVEST}$), ENTEC 150 fue el único tratamiento que se difirió significativamente del control ($n = 61$, $p < 0.05$). Este resultado coincide con estudios previamente reportados, los cuales mostraron que un N_{NI} elevado (N_{NI} > 1) alrededor de antesis (BBCH65) es crucial para maximizar el rendimiento debido a la demanda futura de N que ocurre de antesis en adelante, sumado a la posible disminución de aporte de N del suelo o menor absorción por las raíces (por ejemplo, entre 1,15 y 1,20 para el caso de maíz). Por lo tanto, ENTEC 150, que mantuvo N_{NI} > 1, pudo satisfacer mejor la demanda de N futura del cultivo en comparación con otros tratamientos, lo que resultó en un mayor rendimiento en grano por hectárea.

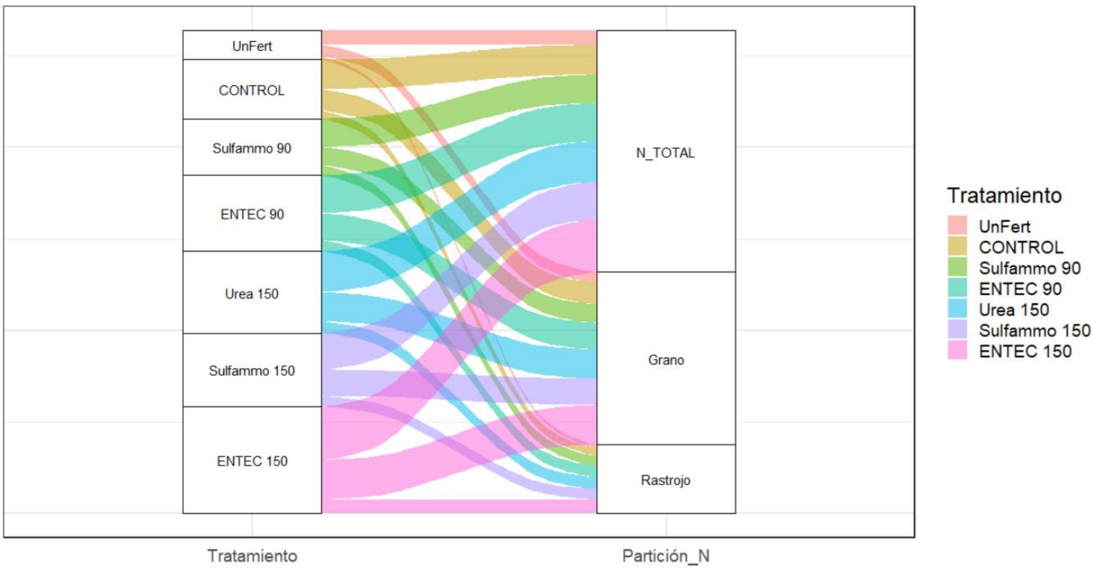
Más allá del rendimiento en grano, la pregunta principal que deberíamos hacernos en la evaluación de un biomaterial con destino a biocombustible es si podemos verificar o cuantificar y posteriormente reportar de una mejor manera (de lo que lo hicimos en el primer artículo) la sincronización de liberación y demanda de N que, en definitiva, es el mecanismo que trae los beneficios de evitar pérdidas de N o de GHG, en especial N_2O. Por lo tanto, comprender los compromisos entre maximizar la producción agronómica y el potencial de pérdida de N al medioambiente es fundamental para interpretar los valores de eficiencia en el uso de nitrógeno (N_{UE}). En este sentido, los N_{NI} y los diferentes componentes de la N_{UE} (NE_{UPT}/RE_N y NE_{UTI}/IE_N)

indican que sí hay ventajas y que el método utilizado (marco teórico del NNI) tiene utilidad para evaluar biocombustibles y su verdadero potencial en la compensación en GHG (*GHG offset*). Específicamente, la eficiencia de recuperación del N (NE_{UPT} o RE_N = kilogramos de N absorbidos por kilogramos de N_{TOTAL} o NFT) es el parámetro clave, debido a la gran influencia que tiene en la variabilidad de los resultados agronómicos y ambientales. Hasta el momento, ningún trabajo a nivel mundial de evaluación de materias primas para biocombustibles ha explorado la posibilidad de desarrollo de relaciones teóricas cuantitativas entre NUE (y sus componentes) bajo el marco teórico del NNI. Por lo tanto, es necesario definir de la mejor manera las conexiones entre NNI, NUE y las pérdidas potenciales de N (ejemplo: N_2O, NOx) para entender su verdadero impacto agronómico y ambiental. El uso de la curva de dilución (CriNDC) y la construcción de la curva crítica de absorción de N (CriNUC) pueden extenderse más allá de una única curva crítica, lo que posibilita definir curvas en cualquier nivel de estado de N de la planta al incluir explícitamente el NNI en su cálculo (INN = 0,5, 0,75, 1 y 1,25). Del uso práctico de este marco, se desprende evaluar e interpretar los diferentes valores de % N_{VEG} e NNI, lo que permite observar las diferentes posiciones logradas frente a la a la curva crítica de N_{UPT} (N_{UPT}CRITICAL ~ NNI = 1). Esto explicita claramente por qué ENTEC 150 logra tener mayores rendimientos en grano, ya que presenta mayores niveles de N_{UPT} ante iguales valores de AB (NNI > 1 tanto en BBCH30 como en BBCH65), lo cual mejora la resiliencia del cultivo ante futuras deficiencias de N, reduce la dependencia de fertilización externa y minimiza las pérdidas ambientales al mismo tiempo que se maximizan las salidas deseadas como grano y aceite. Por otro lado, la eficiencia interna de utilización del N (NE_{UTI} o IE_N = kilogramos de rendimiento o aumento del rendimiento por kilogramo de N_{UPT} procedente del N_{TOTAL} o NFT) está estrechamente relacionada a la calidad final del grano ([Oc] y [Pc]). Análisis de componentes principales (PCA) sugieren que un mejor estado nutricional cerca de la antesis (NNI > 1) está correlacionado negativamente con la IE_N, la concentración de aceite [Oc] y el NHI. Esto coincide con estudios previos en diferentes oleaginosas, donde alto estatus de nutricional cerca de la antesis (NNI > 1) disminuye la disponibilidad de carbohidratos para la síntesis de aceite, sumado a la reducción en IE_N, como resultado de su relación entre el índice de cosecha (HI) y el

% N_{VEG}. Por lo tanto, concentraciones más altas de % N_{VEG} pueden reducir la IE_N. Por lo tanto, como se observó en el primer artículo, menores IE_N conducirán a que menores proporciones de N_{UPT} total se transloquen al grano, lo cual pudo ser demostrado a través de factores como el NNI y IE_N. Estas variables demuestran a su vez que la calidad de los rastrojos ($N_{RESIDUES}$) puede ser un componente importante para elaborar un balance más comprensivo.

Figura 2.2

Distribución de nitrógeno de los diferentes tratamientos y particiones



Como conclusión general, el análisis de los dos experimentos de fertilización nitrogenada en cultivos industriales de *Brassica carinata* (carinata) en Uruguay (2016-2018 y 2019-2020) permitió caracterizar las respuestas agronómicas (rendimiento y calidad de grano) y la NUE bajo diferentes prácticas de fertilización. Los resultados en el primer experimento indican que la aplicación de 90-100 kg ha^{-1} de N maximiza el rendimiento de grano, con diferencias significativas entre percentiles, lo que sugiere que factores adicionales al nitrógeno limitan el rendimiento en nuestros sistemas de producción comercial.

La NUE y sus componentes (NE_{UPT} o RE_N y NE_{UTI} o IE_N) mostraron que carinata tiene una alta capacidad para absorber N más que para utilizar el N absorbido. Esta característica es clave debido a la necesidad de optimizar la captura de N en sistemas agrícolas para reducir las pérdidas al ambiente. Además, se evidenció que, para el rango de dosis utilizadas en el primer conjunto de experimentos, la concentración de aceite y proteína en el grano no fue influenciada fuertemente por el nivel de nitrógeno disponible (NTOTAL o NFT). Sin embargo, existió una tendencia a que el aumento del nitrógeno aplicado (NFT) genere mayores concentraciones de proteína en el grano y, con ello, la disminución del contenido de aceite. El segundo experimento se basó en los resultados del anterior, con foco en el uso fuentes alternativas de N (EEF) empleando el enfoque de estatus nitrogenado (NNI). Demostró que el uso de fertilizantes de liberación controlada, como ENTEC 150, mejora tanto el rendimiento de grano como NUE y sus componentes, lo que supera al manejo comercial actual basado en Urea-S fraccionada (CONTROL). Por lo tanto, el uso de EEF, en especial ENTEC 150 (NI), permitió no solo maximizar la producción agronómica (Yseed), sino que al mismo tiempo mantuvo una muy alta capacidad de absorción de N (NEUPT o RAN), lo que elimina la necesidad de fraccionamiento de aplicaciones de nitrógeno y el gasto de CO_2 sobre el cultivo, algo determinante para el futuro del cultivo como biocombustible.

Los estudios realizados en esta tesis proporcionan una base sólida para el desarrollo de estrategias de manejo de nitrógeno más sostenibles en *Brassica carinata*. La implementación de herramientas como el índice de nitrógeno nutricional (NNI) y la curva crítica de absorción de nitrógeno (CriNUC) ofrecen soluciones prácticas para ajustar las aplicaciones de fertilización según las necesidades reales del cultivo. Esto permite no solo optimizar el uso de nitrógeno, sino también reducir las pérdidas indeseadas de este nutriente en los sistemas agrícolas, con especial atención a la reducción de gases de efecto invernadero (GHG), en particular las emisiones de óxido nitroso (N_2O).

Sin embargo, es crucial avanzar en el perfeccionamiento de los protocolos experimentales que permitan evaluar de forma precisa el efecto individual de los diferentes componentes de las fuentes alternativas de nitrógeno (EEF), ya que existen diferencias sustanciales en el contenido de azufre entre las fuentes de EEF y la urea-S. Si bien nuestros resultados son promisorios sobre el uso de fertilizantes de liberación controlada, presentan barreras de adopción debido a su mayor costo; por lo tanto, comprender el impacto de estas alternativas en el rendimiento y en la sostenibilidad ambiental es un paso necesario para su implementación en gran escala.

Por otra parte, se propone continuar investigando la eficiencia en el uso de nitrógeno (NUE) en relación con el rendimiento y la calidad del grano, pero incorporando la nueva genética de *Brassica carinata*, como la inclusión de los híbridos de reciente liberación. Esto permitirá optimizar y evaluar el rendimiento agronómico, la sostenibilidad ambiental al establecer una plataforma sólida para desarrollar nuevas estrategias de manejo que maximicen el potencial de este cultivo como fuente de biocombustibles en América del Sur, en un marco que considere la interacción genotipo por ambiente por manejo (GxExM).

El potencial de emisiones de GHG, en especial N_2O, aún no ha sido plenamente explorado, ya que *Brassica carinata* es un cultivo emergente y poco estudiado hasta el momento. Este trabajo no solo buscó desarrollar protocolos alternativos de fertilización, sino también estimar las emisiones de (GHG) durante el ciclo del cultivo con el fin de generar un factor de emisión. A través de experimentos de campo realizados en 2019 y 2020, se cuantificaron las emisiones de N_2O, CH_4 y CO_2 mediante el método de cámara de flujo cerrada. Si bien estos resultados quedan fuera del alcance de esta tesis, se prevé su publicación en un artículo independiente que aportará nuevas perspectivas sobre el beneficio neto de mitigación del cambio climático asociado a *Brassica carinata*.

Tal como se destacó en el segundo artículo de esta tesis, el uso de técnicas como el NNI subraya la importancia de la calidad de los residuos vegetales para lograr un balance más comprensivo de compensación de GHG (*GHG offset*). Por lo tanto, ajustar de manera adecuada las prácticas de fertilización nitrogenada (en términos de dosis, fuente, momento y lugar), junto con el mejoramiento genético del índice de cosecha de nitrógeno (NHI), mediante un incremento de N_{EUTI} o IE_N (kg^{-1} de grano por kg^{-1} N_{UPT}) son herramientas clave para aumentar el rendimiento al mismo tiempo que se minimizar las pérdidas de nitrógeno al ambiente.

Finalmente, como se mencionó, es fundamental continuar ampliando el conocimiento sobre la absorción y partición del nitrógeno, así como sobre la contribución de estos procesos a la formación de biomasa y rendimiento, como prerrequisitos para mejorar genéticamente *Brassica carinata*. Desde un enfoque más amplio, a nivel de sistemas de cultivos, la inclusión de oleaginosas invernales (colza, carinata, camelina) en las rotaciones agrícolas en Uruguay ha cobrado mayor relevancia en los últimos años. Por lo tanto, el análisis de la producción de biocombustibles en Uruguay debería prestar especial atención a la introducción de este tipo de cultivos, ya que pueden estar afectando las emisiones de GHG como N_2O y CO_2 del suelo. Sin embargo, los métodos tradicionales, especialmente el método de *tier* 1 del IPCC, tienen limitaciones para capturar con precisión los efectos heredados del manejo de nitrógeno en las rotaciones. Esto se debe a que *tier* 1 asume que todo el nitrógeno aplicado se utiliza en el año, ignorando el *carryover* o herencia de N entre los cultivos de la rotación, lo que puede llevar a subestimar las emisiones de N_2O. Los modelos de *tier* 3, más complejos y basados en procesos, logran estimaciones más precisas al considerar estos efectos heredados. En Uruguay, existen dos estudios reportados sobre el flujo acumulado de GHG en diferentes rotaciones: Salvo Álvarez (2014) y Adler et al. (2018). La actualización de estos datos mediante la inclusión de cultivos alternativos, como las oleaginosas de invierno, resulta esencial para evaluar su impacto real en el balance general de GHG a nivel de sistemas de rotación agrícola.

En resumen, las investigaciones presentadas aquí ofrecen una plataforma robusta para el desarrollo de nuevas estrategias de manejo que equilibren el rendimiento productivo con la sostenibilidad ambiental, donde las bases científicas apunten a un futuro más sostenible para la producción de biocombustibles en la región.

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