Pasturas perennes y cultivos de servicio invernal en rotaciones agrícolas: efectos en la dinámica de N y P del sistema

Cristina Margalet Mori Alvez

Doctorado en Ciencias Agrarias

Marzo, 2025

Pasturas perennes y cultivos de servicio invernal en rotaciones agrícolas: efectos en la dinámica de N y P del sistema

Cristina Margalet Mori Alvez

Doctorado en Ciencias Agrarias

Marzo, 2025

Tesis aprobada por el tribunal integrado por la Dra. Virginia Pravia, el Dr. Walter Carciochi y el Dr. Guillermo Siri el 18 de marzo de 2025. Autora: MSc. Cristina Margalet Mori Alvez. Director: Dr. Carlos Perdomo Varela. Codirectora: Dra. Amabelia del Pino Machado.

Agradecimientos

A mis tutores, Dr. Carlos H. Perdomo y Dra. Amabelia del Pino, por su apoyo en diferentes etapas de este proceso de aprendizaje.

A los coautores que, de diversas maneras, contribuyeron a la publicación de los artículos científicos.

A mis compañeros de trabajo de la cátedra de Fertilidad de Suelos y del Departamento de Suelos y Aguas, por su colaboración y apoyo.

A la Facultad de Agronomía de la Universidad de la República, por su respaldo en mi desarrollo académico.

A la Agencia Nacional de Investigación e Innovación, por financiar un proyecto de investigación que hizo posible la realización de esta tesis doctoral.

A la Comisión Académica de Posgrado, por otorgarme una beca que facilitó la realización de mis estudios de doctorado.

Al Organismo Internacional de Energía Atómica, por financiar un proyecto de investigación que cubrió los costos de análisis relacionados con esta tesis doctoral.

A productores y a la Estación Experimental Mario Cassinoni, que abrieron sus porteras para la realización de los ensayos de campo.

A quienes participaron y colaboraron durante extensas jornadas de trabajo en campo y laboratorio, incluyendo ayudantes de proyecto, así como estudiantes de grado y maestría.

Al Dr. Adrian Correndo, profesor asistente del Departamento de Agricultura Vegetal de la Universidad de Guelph (Canadá), por sus valiosas sugerencias y revisiones del artículo «Wheat Performance and Nitrogen Use Efficiency under No-Till in Uruguay: A Comparison of Crop-Pasture and Continuous Cropping Systems».

Al comité de seguimiento, compuesto por el Dr. Miguel Cabrera, la Dra. Soledad Espinoza y el Dr. Guillermo Studdert, por su apoyo a este trabajo de investigación.

Al Ing. Agr. Juan Pablo Viera, por su respaldo en la ejecución de los trabajos de campo, el establecimiento de contacto con productores y su valiosa asesoría en la instalación de los ensayos, especialmente relacionados con el cultivo de lupino.

Al Ing. Agr. Jorge Foderé, representante de la empresa Fadisol SA, por su colaboración mediante el suministro de insumos diversos, como semillas, fertilizantes y herbicidas.

Al productor Henry Rochón, por su asistencia en la siembra y mantenimiento de los ensayos realizados en el experimento ubicado en Ombúes de Lavalle, Colonia, Uruguay.

A mi esposo, a mis padres y hermanas, por su apoyo incondicional y compañía a lo largo de este proceso.

Tabla de contenido

Resumen

El sector agropecuario uruguayo enfrenta desafíos críticos de sostenibilidad ambiental debido a la erosión y pérdida de materia orgánica del suelo (MOS), desbalance de nutrientes y contaminación hídrica, exacerbados por la intensificación y simplificación de los sistemas productivos. Este estudio investiga el impacto de estrategias de diversificación agrícola, como la integración de pasturas perennes y cultivos de servicio (CS), sobre la dinámica de nitrógeno (N) y fósforo (P), la productividad de los cultivos y la sostenibilidad ambiental de los sistemas agrícolas. Se plantean tres objetivos principales: 1) evaluar el efecto de rotaciones cultivo-pastura frente a cultivos continuos en la dinámica de N y el rendimiento de trigo, 2) evaluar el efecto de dos leguminosas invernales (trébol alejandrino y lupino azul) y una gramínea (avena negra) en la residualidad de N en cultivo siguiente de maíz y 3) analizar la capacidad de leguminosas del género *Lupinus* para solubilizar P en suelos de textura, acidez y fertilidad natural contrastantes. La metodología se apoya en experimentos de largo plazo, experimentos en campos de productores y ensayos en invernáculo, combinando tanto técnicas convencionales como isotópicas. Se midieron diferentes parámetros en planta (rendimiento de grano, absorción de nutrientes, biomasa aérea en diferentes componentes de la planta) y en suelo. Los resultados buscaron proporcionar evidencia sobre cómo estas estrategias contribuyen a mejorar la productividad agrícola y la sostenibilidad del sistema para valorar prácticas que favorezcan el ciclaje de nutrientes y la conservación de recursos en Uruguay. Se identifican futuras líneas de investigación enfocadas en comprender los mecanismos que explican las diferencias en la respuesta al agregado de N y en la disponibilidad de P para las plantas. Es fundamental profundizar en los procesos de mineralización e inmovilización, así como en la influencia de la comunidad microbiana sobre el ciclo de nutrientes y la dinámica de la MOS.

Palabras clave: diversificación agrícola, dinámica de nutrientes, fijación de nitrógeno, rotación cultivo pastura, cultivos de servicio

Perennial Pastures and Winter Cover Crops in Agricultural Rotations: Effects on N and P Dynamics in the System

Summary

The Uruguayan agricultural sector faces critical environmental sustainability challenges, including erosion and soil organic matter (SOM) losses, nutrient imbalances, and water contamination. Intensification and simplification of production systems exacerbate these issues. This study investigates the impact of agricultural diversification strategies, such as the integration of perennial pastures and cover crops (CCs), on nitrogen (N) and phosphorus (P) dynamics, crop productivity, and the environmental sustainability of agricultural systems. The research outlines three main objectives: (1) evaluating the effect of crop-pasture rotations compared to continuous cropping on N dynamics and wheat yield; (2) assessing the residual effects of two winter legumes (berseem clover and blue lupine) and one grass (black oat) on the subsequent maize crop; and (3) analyzing the ability of legumes from the Lupinus genus to solubilize P in soils with contrasting textures, acidity, and natural fertility. The methodology integrates long-term experiments, on-farm trials, and greenhouse assays, combining conventional and isotopic techniques. Measurements during the study included plant parameters (grain yield, nutrient uptake, above-ground biomass in different plant components) and soil properties. Results show how these strategies enhance agricultural productivity and system sustainability, fostering practices promoting nutrient cycling and resource conservation in Uruguay. Future research directions prioritize understanding the mechanisms behind differences in plant responses to N inputs and P availability. A deeper exploration of processes such as mineralization and immobilization, alongside the influence of microbial communities on nutrient cycling and SOM dynamics, remains essential.

Keywords: agricultural diversification, nutrient dynamics, nitrogen fixation, crop-pasture rotation, cover crops

1. Introducción general

El sector agropecuario uruguayo enfrenta desafíos significativos en materia de sostenibilidad ambiental, los cuales comprometen los recursos naturales que constituyen su base productiva y ponen en riesgo sus sostenibilidad en el largo plazo. Entre las amenazas más relevantes se encuentran problemas de escala global, como la erosión del suelo (García-Préchac, 2020), la pérdida de materia orgánica (Rubio et al., 2021) y el desbalance de nutrientes (Koritschoner et al., 2023; Lorenz et al., 2019). A estas problemáticas se suman otras de carácter local, como la contaminación de fuentes de agua (Barreto et al., 2017), la acidificación del suelo (Beretta-Blanco et al., 2019) y la disminución de la biodiversidad edáfica (Brazeiro et al., 2020; Céspedes-Payret et al., 2009).

La intensificación del uso del suelo, junto con la simplificación creciente de los sistemas agrícolas (Ernst et al., 2020), ha acentuado las preocupaciones sobre la sostenibilidad y funcionalidad futura de los sistemas de producción actuales. Estos cambios estructurales plantean serias dudas respecto a su capacidad para mantener la resiliencia, adaptarse a las dinámicas del cambio climático, garantizar la provisión de servicios ecosistémicos y preservar la biodiversidad en el largo plazo (Alvarez y Ernst, 2024; Ernst, 2019; Lichtenberg et al., 2017; Paruelo et al., 2024). Asimismo, Uruguay enfrenta el reto de reducir las emisiones de gases de efecto invernadero (GEI) provenientes del sector agropecuario, que constituye una parte significativa de su huella de carbono (C) (Baldassini et al., 2023; Paruelo et al., 2024). Abordar este desafío exige un enfoque integral que combine la conservación de los recursos naturales con estrategias de adaptación a las nuevas condiciones climáticas y de transición hacia un modelo agropecuario más sostenible.

La diversificación de los sistemas de producción agrícola, una práctica con una larga trayectoria, se ha consolidado como una estrategia fundamental para avanzar hacia trayectorias sostenibles en el contexto actual de desafíos ambientales y de intensificación productiva. Esta práctica, promovida activamente en diversas regiones del mundo (Ambrosini et al., 2022; Liebman et al., 2013; Li et al., 2024; Vanino et al., 2022), como en el ámbito local (Alvarez y Ernst, 2024; García-Préchac et al., 2004;

Mazzilli y Ernst, 2019; Pinto et al., 2021;), no solo busca optimizar la producción agrícola, sino también fortalecer la resiliencia y la adaptabilidad de los sistemas agropecuarios frente a los efectos del cambio climático y la explotación creciente de los recursos naturales.

Los sistemas agrícolas de secano en Uruguay han experimentado cambios significativos desde principios del siglo XXI (Arbeletche, 2020; Vassallo, 2013). En sus inicios, estos sistemas se simplificaron priorizando secuencias de cultivos como trigo-soja, lo que redujo la duración de las pasturas perennes, extendió las fases de cultivos anuales y, a su vez, facilitó la expansión del sector agrícola (Arbeletche, 2020; Ernst et al., 2018). Sin embargo, estas transformaciones han tenido impactos negativos en el balance de nitrógeno (N) y la capacidad de secuestro de C del suelo, lo que ha comprometido su calidad y la sostenibilidad de los sistemas productivos (Ernst et al., 2018; Pravia et al., 2019; Rubio et al., 2021). Desde 2015 a la fecha, se ha promovido una mayor integración entre la producción agrícola y ganadera, al gestionarlas como componentes de un sistema productivo unificado (Arbeletche, 2020). Esta nueva estrategia ha reincorporado fases de pastura y ha incrementado la práctica de doble cultivo, lo que favorece la diversidad de cultivos en la rotación y mejora la eficiencia en el uso de los factores productivos, al mismo tiempo que protege a los recursos naturales (Ernst, 2019; Rubio et al., 2025; Xie et al., 2019)

La adopción de un enfoque ecosostenible ha sido retroalimentada por la actualización del marco normativo establecido en Uruguay, en particular por la Ley de Conservación de Suelos y Aguas Superficiales con Fines Agropecuarios (Ley 15.239, Decreto 333/04 y Ley 18.564). Esta legislación, cuya aplicación se volvió obligatoria en 2013 (García-Préchac, 2020), establece directrices específicas para la regulación y el manejo adecuado del uso del suelo. Su implementación ha sido un factor clave en la transición hacia sistemas de producción agrícola más sostenibles, ya que fomenta prácticas que moderan la productividad con la conservación de los recursos naturales (Iglesias Rossini, 2022). La disminución de la materia orgánica y el desequilibrio de nutrientes en el suelo son factores clave que aceleran su deterioro y se erigen como las principales amenazas para la conservación de los recursos naturales y la sostenibilidad de los ecosistemas productivos. Nutrientes clave como el N y el fósforo (P) son esenciales, no solo para mantener la productividad de los cultivos, sino también por su impacto en la capacidad del suelo para almacenar C (Ghaley et al., 2014; Parihar et al., 2020), lo que contribuye así a la sostenibilidad y mitigación del cambio climático.

1.1. Impactos del manejo agrícola y la importancia de la diversificación en los sitemas de producción

En este contexto, el presente estudio hace énfasis en el impacto positivo de las estrategias de diversificación de los sistemas productivos, tales como la integración de pasturas perennes (Ernst y Siri-Prieto, 2009; García-Préchac et al., 2004; Liebman et al., 2013), y en la incorporación de cultivos de servicio anuales invernales (Cafaro La Menza y Carciochi, 2023; Rodriguez et al., 2024) o estivales (Berriel et al., 2020; Berriel y Perdomo, 2023). El impacto de estas medidas de manejo estará determinado, en gran medida, por la cantidad de biomasa aérea y subterránea incorporada (Rubio et al., 2025) y la capacidad y manejo del sistema para retener los residuos generados (Li et al., 2020; Rubio et al., 2025; Tiecher et al., 2020).

En sistemas de rotación cultivo-pastura (CP), el alcance de los beneficios de las pasturas perennes sobre la productividad de los cultivos en la fase agrícola han generado resultados diversos y en muchos casos contrapuestos al compararlos con sistemas de agricultura continua (AC) bajo siembra directa, particularmente en relación con los rendimientos de los cultivos y la eficiencia en el uso del nitrógeno (NUE) (Baiyeri et al., 2019; Dang et al., 2020; Peterson et al., 2020; Pravia et al., 2019). En los sistemas CP, la mejora de la estructura del suelo, el incremento del contenido de materia orgánica (MO) y la mayor actividad microbiana facilitarían un mejor acceso y aprovechamiento del N sobre los cultivos sucesores a la fase de pastura (Ernst et al., 2016; Grahmann et al., 2020). En contraste, los sistemas agrícolas basados en la extracción continua de nutrientes y la falta de diversidad de cultivos tienden a degradar el suelo con el tiempo. Aunque son más simples de gestionar, estos sistemas suelen mostrar una menor eficiencia en el uso del N (Hu et al., 2023). Por lo tanto, incorporar leguminosas en pasturas o como cultivos de cobertura (CC) en las rotaciones, junto con técnicas de siembra directa, se presenta como una estrategia efectiva y prometedora para mantener altos rendimientos de cultivos como el trigo o maíz y optimizar la utilización de N y P desde suelo, de lo reciclado por los residuos y de los fertilizantes. En Uruguay, se ha demostrado consistentemente que los sistemas de rotación CP favorecen la producción agrícola. Además, la mayoría de los experimentos de largo plazo realizados bajo siembra directa han incluido el pastoreo (García-Préchac et al., 2004; Rovira et al., 2020; Rubio et al., 2025; Salvo et al., 2010;), por lo que los posibles efectos adversos del pastoreo ya han sido evaluados en el contexto del sistema completo.

Los cultivos de servicio, entre ellos los CC, son cultivos establecidos con el propósito de mantener el suelo protegido, evitar la erosión, reducir la presión de malezas, minimizar la pérdida de nutrientes por lixiviación y escorrentía, y, en el caso de las leguminosas, aportar N al sistema. Se distinguen de las pasturas porque no generan una renta directa y se cultivan fuera de la temporada habitual dentro de un sistema de siembra de cultivos anuales (Cafaro La Menza y Carciochi, 2023). Los CC se siembran entre dos cultivos de renta y no se incorporan al suelo, a diferencia de los abonos verdes, ni se pastorean, como ocurre con los verdeos, ni se cosechan (Cafaro La Menza y Carciochi, 2023). Además de proteger el suelo del impacto de la gota de lluvia, los CC contribuyen significativamente al aporte de C, un elemento esencial para mantener y mejorar su calidad (Ambrosini et al., 2022; Rubio et al., 2021, 2025; Tiecher et al., 2020). El C desempeña un papel fundamental en la regulación de procesos biológicos, químicos y físicos que sustentan servicios ecosistémicos esenciales, como la capacidad de amortiguación en varios procesos que afectan por ejemplo la reserva de nutrientes, la retención de agua, la filtración de contaminantes, todos ellos imprescindibles para garantizar la sostenibilidad de los sistemas productivos (Reiss y Drinkwater, 2022). Además, los CC facilitan el reciclaje de nutrientes como el N y el P y, en el caso del N, también a través de su fijación biológica. Esto permite reducir la necesidad de fertilizantes comerciales en los cultivos subsiguientes, lo que mejora la eficiencia de uso de los nutrientes y contribuye a una agricultura más sostenible.

El aporte de N mediante la fijación biológica de nitrógeno (FBN) es fundamental para equilibrar los balances de este nutriente en los sistemas de producción actuales. La intensificación agrícola ha incrementado significativamente la extracción de N del suelo, lo que conduce a una mayor dependencia de los fertilizantes nitrogenados en los sistemas agrícolas y aumenta el riesgo de contaminación ambiental (Dabney et al., 2010). La inclusión de leguminosas en las rotaciones agrícolas representa una estrategia eficaz para mitigar este problema, aunque la magnitud de N fijado depende de múltiples factores. Entre ellos, destacan características intrínsecas de cada especie, condiciones climáticas y prácticas de manejo agronómico, así como las interacciones entre estos elementos (Espinoza et al., 2012; Herridge et al., 2022; Ovalle et al., 2010; Peoples et al., 2015). Por ello, resulta imprescindible evaluar la productividad de cada especie bajo diferentes condiciones agroecológicas y niveles de manejo antes de recomendar las prácticas óptimas para su cultivo.

Un aspecto clave al incluir leguminosas con el objetivo de aumentar la contribución neta de N al sistema es evitar posibles desajustes entre la oferta de N derivada de estas fuentes y la demanda del cultivo. Este desajuste, conocido como *asincronía*, puede ocurrir tanto por exceso (oferta > demanda) como por insuficiencia (oferta < demanda). El primero representa un factor potencial de contaminación ambiental (Allar y Maltais-Landry, 2022; Dabney et al., 201). Estudios previos han indicado que los sistemas agrícolas con leguminosas presentan menor probabilidad de asincronía en comparación con los sistemas que dependen exclusivamente de fertilizantes sintéticos. Sin embargo, esta diferencia puede reducirse al implementar buenas prácticas de manejo, como la aplicación fraccionada de fertilizantes, el uso de fertilizantes de liberación lenta o inhibidores de la nitrificación y el laboreo reducido. En tales casos, los sistemas basados en fertilizantes pueden lograr niveles de sincronía comparables o incluso superiores a los observados en sistemas con leguminosas (Crews y Peoples, 2005; Peoples et al.,2015).

Por otro lado, uno de los factores que influye en el rendimiento y la FBN de las leguminosas invernales es la disponibilidad de P en el suelo. Existe una amplia variación en los requerimientos de P entre especies (Bolland y Brennan, 2008; Hallama et al., 2019; Lambers et al., 2006; Pearse et al., 2006; Wang y Lambers, 2020). En Uruguay, las pasturas mixtas de gramíneas y leguminosas han sido tradicionalmente utilizadas en rotaciones agrícolas, donde las leguminosas, como el trébol blanco (*Trifolium repens* L.) y rojo (*Trifolium pratense* L.), presentan altos requerimientos de

P con niveles críticos entre 14 y 18 mg kg^{-1} mediante el método PBray1 (Silveira et al., 2015). Cuando los niveles de P en el suelo disminuyen, estas leguminosas tienden a desaparecer de las mezclas debido a su incapacidad para competir con las gramíneas, que tienen menores requerimientos de P (Del Pino et al., 2016). En contraste, ciertas leguminosas anuales invernales, como especies del género *Lupinus* (*L. albus* y *L. angustifolius*), tienen bajos requerimientos de P (Bolland y Brennan, 2008; Pearse et al., 2006), similares o incluso inferiores a los de cultivos agrícolas como el trigo (*Triticum aestivum* L.) o la canola (*Brassica napus* L.). Además, se ha documentado que estas especies poseen la capacidad de solubilizar formas de P no disponibles en el suelo, lo que aumenta su disponibilidad para las plantas (Kamh et al., 2002; Nuruzzaman et al., 2005; Takahashi, 2015). Este efecto se atribuye a la capacidad de estas plantas para acidificar la rizósfera mediante la excreción de ácidos orgánicos como malato o citrato o la acción de fosfatasas ácidas (Dong et al., 2003; Lambers et al., 2013; Nuruzzaman et al., 2005). Estas modificaciones pueden reducir el pH de la rizósfera, solubilizar formas de P no lábiles, competir por sitios de adsorción de fosfatos o formar quelatos con cationes como Al^{3+}, Fe^{2+} y Ca^{2+} que inmovilizan el P del suelo. Adicionalmente, estas especies pueden aumentar la actividad de fosfatasas y estimular el crecimiento de microorganismos que mineralizan formas orgánicas de P (Hocking, 2001). También podrían reducir la retrogradación de P lábil a formas no lábiles, lo que aumenta así su disponibilidad para cultivos sucesivos (Pypers et al., 2007). En suelos con altas cantidades de P fijado, como los desarrollados sobre basalto y basamento cristalino en Uruguay (Hernández et al., 1995), estas características de *Lupinus* podrían maximizar sus beneficios.

1.2. Hipótesis

En comparación con los sistemas agrícolas simplificados, caracterizados por un bajo número de especies y alta homogeneidad, la diversificación de los sistemas de producción de cereales mediante prácticas de manejo como la incorporación de CC (particularmente leguminosas anuales invernales) o pasturas mixtas perennes (gramíneas y leguminosas) no solo mejora la productividad de los cultivos de renta y la eficiencia en el uso del N, sino que también incrementa el secuestro de C y la disponibilidad de N y P en el suelo. Esta diversificación contribuye a una agricultura más sostenible, tanto en términos agronómicos como ambientales, al promover una mayor eficiencia de aprovechamiento de los recursos, incrementar la productividad, mejorar las propiedades del suelo y mitigar los impactos negativos o adversos al medioambiente.

Predicciones:

1. Impacto en la disponibilidad de N: La inclusión de leguminosas en pasturas mixtas o como CC aumenta la disponibilidad de N en el suelo y mejora su eficiencia de uso por el cultivo sucesor. Sin embargo, la magnitud de este efecto está influenciada influenciada por factores como la proporción de leguminosas al final de la fase de pastura, la especie/s de CC empleada, las prácticas de manejo agronómico, las condiciones edafoclimáticas, etc..
2. Impacto en la disponibilidad de P: La inclusión de lupino blanco y lupino azul como cultivos de servicio incrementa los niveles de P disponible en el suelo, aunque la magnitud de este efecto varía según las especies y su interacción con el tipo de suelo y las condiciones edafoclimáticas. Asimismo, la incorporación de pasturas perennes (mezcla de gramíneas y leguminosas) también contribuye a mejorar la disponibilidad de P en el sistema.
3. Reducción de insumos y balance a largo plazo: A largo plazo, la integración de leguminosos como CC o en pasturas perennes reduce la dependencia de fertilizantes sintéticos de N y P, mejora el balance de N y P en el suelo y favorece una mayor sostenibilidad en los sistemas de producción agrícola.

1.3. Preguntas para responder

Capítulo 1:

1. ¿Cuál es el impacto de integrar pasturas perennes de larga duración en rotaciones agrícolas, tras veintirés años de historia de manejo, sobre la dinámica del N del suelo y la eficiencia en su uso por el cultivo de trigo que sigue a la fase de pastura?

2. ¿Cómo influye el manejo acumulado del suelo durante veintitrés años, bajo un sistema de agricultura continua con rotaciones que incluyen especies C3 y C4, en el desempeño del cultivo de trigo?

3. ¿Cuál de los dos factores tuvo un impacto más significativo en el rendimiento del cultivo de trigo: la rotación de cultivos o el cultivo predecesor?

Capítulo 2:

1. ¿Qué cantidad de N pueden fijar el lupino azul y el trébol alejandrino en cultivos puros, y el lupino azul en mezcla con avena negra (Avena strigosa Schreb.), bajo condiciones edafo-climáticas de Uruguay?

2. ¿Cuál es la disponibilidad del N fijado por estas leguminosas para un cultivo estival posterior? ¿Cómo influye la sincronización entre la liberación de nitrógeno desde el rastrojo y la demanda del cultivo sucesor en la residualidad de N? ¿Qué relevancia tiene esta residualidad en términos de la eficiencia de uso aparente del nitrógeno (EFap)?

3. ¿Cuál de las opciones de CC invernales evaluadas genera mayores beneficios en el rendimiento del cultivo de maíz y en la eficiencia de uso del N, y qué factores determinaron su desempeño superior?

Capítulo 3:

1. ¿Cuál es la magnitud y la relevancia agronómica de la capacidad de las leguminosas invernales del género *Lupinus* para solubilizar P no disponible en el suelo?

2. ¿Qué especie del género *Lupinus* presenta un mejor desempeño en suelos ácidos con baja disponibilidad de P y de qué manera su integración podría contribuir a la sostenibilidad de los sistemas agrícolas?

1.4. Objetivo general

Desarrollar conocimiento sobre la contribución de estrategias de diversificación (cultivos de servicio o pasturas perennes) en la dinámica de N y P en el suelo, con el objetivo de fomentar una producción agrícola más sostenible desde una perspectiva tanto agronómica como ambiental. En definitiva, este estudio busca generar información clave sobre las contribuciones de estas estrategias a la sostenibilidad de los sistemas agrícolas en Uruguay para valorar su adopción como herramientas de diversificación y manejo eficiente de recursos.

1.4.1. Objetivos específicos

Capítulo 1:

El objetivo de este estudio fue evaluar el impacto de sistemas de rotación cultivo-pastura (CP) y de agricultura continua (AC) bajo condiciones de siembra directa en la dinámica de N y en la productividad del trigo en un ambiente de secano. Específicamente, se buscó comprender el papel de la fase de pastura (y el cultivo predecesor) mediante la comparación del rendimiento de trigo en esos dos sistemas: trigo cultivado como el primer cultivo tras la fase de pastura en la rotación CP y trigo cultivado en un sistema continuo de cultivos anuales. El estudio se centró en medir la dinámica de N del suelo (concentración de N mineral en diferentes momentos del ciclo del cultivo de trigo y poscosecha), el rendimiento de grano de trigo (RG), la concentración de proteína en el grano (PG) y la eficiencia en el uso del N (EUN) durante tres temporadas y bajo diferentes niveles de fertilización nitrogenada.

Capítulo 2:

1. Evaluar el efecto de los CC en la disponibilidad de N derivada de la mineralización de sus residuos mediante la medición de cambios en el N mineral del suelo.
2. Analizar el impacto de los CC como cultivos predecesores en el rendimiento de un cultivo de maíz, mediante la medición del rendimiento de grano, la absorción de N y la respuesta relativa en la absorción de N de la planta a la fertilización con N, además de estimar la recuperación de N en el cultivo utilizando un método convencional.
3. Examinar cómo la calidad de los residuos (relación C:N, composición bioquímica) del cultivo precedente afecta la eficiencia en el uso del nitrógeno (NUE) a través de la recuperación de N en el cultivo y en el suelo a partir del

fertilizante aplicado durante la siembra y en otras etapas (V6 y V10) del cultivo de maíz utilizando métodos isotópicos.

Capítulo 3:

Evaluar los cambios en la disponibilidad de fósforo (P) en el suelo y su relación con la acidificación de este, así como determinar qué tipo de lupino tiene una mayor capacidad de solubilización de P y qué combinación suelo-lupino genera el efecto más efectivo en la movilización de P.

1.5. Estructura de la tesis

Entre los diversos enfoques para la diversificación de cultivos en la investigación agrícola, esta tesis evalúa, por un lado, la productividad de trigo en sistemas de producción de cultivo diversificados por la inclusión de pasturas perennes y, por otro, la productividad de maíz en sistemas diversificados por la inclusión de cultivos de servicio invernal predecesores al cultivo de renta.

Esta tesis se estructura en seis capítulos, de los cuales tres corresponden a los estudios principales que conforman el núcleo de la investigación. El capítulo 1 presenta una introducción general que enmarca los objetivos y la relevancia del trabajo. Los siguientes tres capítulos contienen los artículos científicos derivados de la investigación, ya publicados en revistas arbitradas y cada uno precedido por un breve resumen que detalla su contenido y contribución al tema. El capítulo 5 está dedicado a una discusión general que integra los hallazgos de los estudios realizados, mientras que el capítulo 6 resume las principales conclusiones y ofrece una perspectiva sobre las implicancias y posibles líneas futuras de investigación. Finalmente, se incluye una sección de anexos que proporciona material suplementario relacionado con los capítulos específicos de la tesis.

2. Wheat Performance and Nitrogen Use Efficiency under No-Till inUruguay: A Comparison of Crop-Pasture and Continuous Cropping Systems

En este estudio, se analizó la productividad del trigo en sistemas de siembra directa bajo dos esquemas de intensificación similar, pero de diversificación contrastantes: el trigo cultivado como primer cultivo tras una fase de pastura perenne en un sistema de rotación cultivo-pastura (CP) y el trigo en una rotación continua de cultivos anuales (CC).

Se evaluaron variables clave como la concentración de N mineral en el suelo a la siembra y en el estadio Z 2.2 del cultivo de trigo, el rendimiento del grano de trigo (en inglés, WGY), la concentración de proteínas en el grano (en inglés, GPC) y la eficiencia en el uso del nitrógeno (en inglés, NUE). El experimento abarcó tres temporadas agrícolas y consideró cuatro niveles de fertilización nitrogenada.

Los resultados de este estudio de tres años, realizado en suelos uruguayos en un experimento de largo plazo de más de veinte años, revelaron que los cultivos de trigo en el sistema de cultivos continuos (CC) lograron mayores rendimientos que aquellos sembrados como primer cultivo anual después de una fase larga de pastura perenne en el sistema con pasturas (CP) en condiciones de secano. El sistema CC mostró además una mayor absorción de N y mejores índices de eficiencia en el uso del N en comparación con el sistema CP. En el sistema CP, las condiciones adversas del suelo así como una menor demanda de N por parte del cultivo posiblemente limitaron la utilización efectiva del N aplicado en la producción de trigo.

Los resultados evidenciaron que la rotación CC, especialmente cuando incorpora especies C4, promovió una mayor productividad de trigo que en la rotación CP. Este efecto se atribuyó a condiciones edáficas más favorables, derivadas de la inclusión de un cultivo antecesor como la soja de segunda y una secuencia de cultivos que favorecería la formación de materia orgánica particulada (POM) en los primeros centímetros del suelo. En contraste, en el sistema CP, el trigo se estableció tras la finalización de una pastura de larga duración (tres años y medio) con escasa biomasa residual, lo que generó condiciones menos propicias para la acumulación de POM en la superficie del suelo. En este sentido, el pisoteo animal y la escasa cantidad/calidad de biomasa remanente sobre el suelo, que suelen afectar propiedades físicas, serían los factores que explicaron la menor productividad del trigo en CP. Además, el impacto negativo de la compactación del suelo por el pisoteo del ganado acentuó este efecto. En resumen, el estudio subraya que el cultivo antecesor tuvo un mayor impacto en el rendimiento y en la eficiencia en el uso del nitrógeno (NUE) del trigo que el efecto residual esperado de la fase de pastura en el sistema CP, principalmente debido a las diferencias en la cantidad y calidad de los residuos remanentes y a las condiciones físicas del suelo.

La hipótesis de que la integración de pasturas en las rotaciones de cultivos incrementaría los rendimientos del cultivo sucesor, en este caso el trigo, no fue respaldada por los resultados obtenidos. Aunque se esperaban beneficios derivados de una mayor calidad del suelo y una mejor disponibilidad de N, estos no lograron compensar posibles efectos negativos, como los asociados al pisoteo y pastoreo del ganado. Sin embargo, también podrían estar involucrados otros factores, los cuales se analizan en mayor detalle en la discusión general de esta tesis. Lamentablemente, no se cuenta con mediciones directas de variables clave asociadas a estos efectos, como la macroporosidad, la distribución y estabilidad de los agregados o la proporción de la fracción MOP en la MOS, lo que limita nuestra capacidad para evaluar los mecanismos que podrían explicar las diferencias en la productividad del cultivo de trigo entre ambos sistemas. Asimismo, la inclusión de variables relacionadas con propiedades químicas y biológicas del suelo podría ofrecer información adicional para desarrollar hipótesis más sólidas y proponer explicaciones mejor fundamentadas.

Conclusión: El estudio subraya que, aunque las pasturas en las rotaciones de cultivos pueden mejorar la fertilidad del suelo y la calidad del grano, sus impactos negativos en las propiedades físicas y biológicas del suelo pueden limitar los rendimientos y la eficiencia en el uso del N en el corto plazo. Esto destaca la necesidad de investigar estrategias para mitigar estos efectos adversos en sistemas agrícolas sostenibles.

Wheat Performance and Nitrogen Use Efficiency under No-Till in Uruguay: A Comparison of Crop-Pasture and Continuous Cropping Systems

Cristina Mori Alvez[1*], **Oswaldo Ernst Benech**[2], **Pablo González Barrios**[3], **Carlos Perdomo Varela**[1]

[1]Soil and Water Department, Agronomy College, University of the Republic, Av. Garzon 780, CP, Montevideo 12900, Uruguay

[2]Crop Production Department, Agronomy College, University of the Republic, Uruguay

[3]Biometrics, Statistics and Computing Department, Agronomy College, University of the Republic, Av. Garzón 780, CP, Montevideo 12900, Uruguay

*** Correspondence:** Cristina Mori Alvezcristinamori@fagro.edu.uy

Keywords: wheat productivity, N use efficiency, soil N dynamic, no-till systems, residues quality.

Abstract

The shift in Uruguayan agriculture to no-till farming and more intensive agricultural practices, moving from crop-pasture (CP) systems to continuous cropping (CC) rotations, has disrupted biological nitrogen fixation (BNF). However, this change has not reduced the soil organic carbon (SOC) balance under no-till management under diversified cropping sequences that include C4 species, so the impact of this conversion on productivity and sustainability has yet to be significant across the system as a whole. This study, conducted within a long-term experiment (LTE), compared wheat productivity under no-till crop conditions for two systems: the first crop seeded after the perennial pasture phase in CP rotation and wheat in continuous annual cropping (CC rotation) in a rainfed environment. The variables measured included soil nitrogen (N) concentration, wheat grain yield (WGY), grain protein concentration (GPC), and N use efficiency (NUE) by wheat over three seasons. The experimental treatments included four N fertilizer levels evaluated at each rotation system. The comparison between the two rotation systems revealed that CC consistently outperformed CP in terms of WGY, with an average of 2425 vs. 1668 kg ha^{-1}. Grain PC varied between rotations and N rates, with CP generally showing a higher GPC (on average, 10.48 vs. 10.92 %). Nitrate-N levels at tillering correlated positively with WGY and negatively with GPC, but the relationship differed by rotation. Soil NUE indices varied between rotations, with CC generally showing higher efficiencies. The study's findings highlighted the potential of CC rotation, especially when including C4 species in the crop sequence, to achieve higher wheat productivity in the short term due to healthier soil conditions than wheat seeded after post-pasture in CP. Additionally, our study highlights that the effect of the previous crop on yield and NUE in wheat was more relevant than the expected residual effect of the pasture phase in CP, primarily due to the quality of residues and the temporary adverse effects of soil compaction caused by livestock trampling.

2.1 Introduction

Since the beginning of the 21st century, Uruguay has embraced no-till farming and intensified its agricultural practices, increasing reliance on N-based fertilizers (Ernst et al., 2018, 2020; Fassana et al., 2022). This intensification, coupled with a shift from crop-pasture (CP) systems to continuous cropping (CC) rotations, primarily soybean (*Glycine max* L. Merr.), has reduced or eliminated pastures, disrupting the biological nitrogen fixation (BNF) provided by perennial legumes in mixed sward pastures (Lussich Rachetti, 2020). Consequently, this has led to negative N (Quemada and Lassaletta, 2024) and carbon (C) balance compared to previous management practices (Rubio et al., 2021a). The increased use of fertilizers has also heightened the risk of N losses through soil erosion and leaching (Liu et al., 2023). Although this system produces more grain, gaps still exist between the potential and actual wheat yields, which cannot solely be attributed to nutrient deficiencies (Ernst et al., 2018; Hochman and Horan, 2018; Hatfield and Beres, 2019).

There is a growing consensus that crop production intensification should be approached from an ecosystem perspective (Duru et al., 2015; Cassman and Grassini, 2020; Dang et al., 2020). Sustainable intensification, which aims to boost yields from the same land area while reducing environmental impacts, enhancing natural resources, and providing ecosystem services, is increasingly embraced (FAO, 2011). Conservation agriculture, which can support sustainable farming practices, may preserve or enhance soil health by reducing soil disturbance through minimal mechanical tillage, such as no-till. Furthermore, this approach includes two other core crop management principles: implementing crop rotation with a variety of plant species (both annuals and perennials, C3 and C4 species) and maintaining soil cover by retaining residues or using cover crops (FAO, 2011; Griffiths et al., 2022). No-till farming has shown promising effects in specific contexts, such as rainfed agroecosystems in dry climates. However, yield benefits are only realized when combined with the other two conservation agriculture principles (Pittelkow et al., 2015a; Page et al., 2020). When implemented alone, no-till can increase the risk of yield loss for farmers (Pittelkow et al., 2015b; Page et al., 2020). For this reason, the current agricultural system urgently needs a shift towards more sustainable practices, in terms of environmental impact and productivity outcomes, as emphasized by the ecosystem-based approach (FAO, 2011).

In this context, it is reassuring to know that pasture or perennial crops play a significant role in recovering lost functional properties and improving intrinsic soil qualities such as water infiltration, nutrient cycling, and biological diversity (Teague and Kreuter, 2020; Mosier et al., 2021; Rubio et al., 2021b). Long-term experiments in Uruguay, Brazil, Argentina, and the US have indicated that integrated crop-livestock systems based on perennial pastures can sustain crop productivity and climate resilience over the long haul while preserving or increasing soil C storage (Franzluebbers, 2013; Pravia et al., 2019). In Uruguay, incorporating pastures helps maintain soil quality and significantly boosts productivity (Grahmann et al., 2020; Rubio et al., 2021b). For example, CP systems resulted in 19% higher SOC and 14% higher total N levels than CC systems, with wheat yields averaging 1 Mg ha^{-1} higher in CP systems. (Grahmann et al., 2020). In the second study, Rubio et al. (2021)

examined the effects of various long-term cropping systems on maize yield response to soil decompaction through deep tillage and different N fertilization rates (Rubio et al., 2021b). Both short-term remediation strategies failed to mitigate the adverse impacts of soil degradation by CC on corn growth. Nevertheless, the yield of maize grown after mixed pastures (grasses and legumes) failed to accurately represent the overall beneficial impact of CP on soil quality, implying that additional short-term issues related to crop rotation, such as the preceding crop, should be considered (Lollato et al., 2019a; Arnhold et al., 2023). These studies have also shown that pasture significantly helps maintain SOC and N content; however, not all rotation systems converge on improved soil productivity.

The no response in some systems or crops aligns from several works (Franzluebbers and Stuedemann, 2014; Pittelkow et al., 2015a; Lollato et al., 2019a; Dang et al., 2020), asserting that the beneficial impact of no-till management, compared to the conventional tillage, was more pronounced in summer crops than in winter ones and in CC compared to CP rotations (Ernst et al., 2009). These findings suggest that no-till offered a clear advantage for summer crops within CC systems, with a minor influence on winter crop yields and a small positive impact in integrated crop-livestock systems (Dang et al., 2020). Experiments by Salvo et al. (2010) comparing five cropping systems in Uruguay indicated that integrating pastures into rotation did not modify SOC content and its fractions under the no-till system.Ernst and coworkers also noted this phenomenon in the same LTE, attributing it to animals consuming pasture biomass, accounting for 84% of the pasture's dry matter that would otherwise have covered the soil (Ernst et al., 2020). Additionally, annual crops in CC incorporating C4 species rotation under no-till produced more significant crop residues, closely related to C input and soil C sequestration under reduced tillage conditions (Pravia et al., 2019; Baethgen et al., 2021). These adjusted no-till systems that include C4 crops may require increased N inputs, their potential environmental benefits are significant, providing a promising outlook for sustainable farming practices.

Conversely, in Uruguay, no study has compared wheat performance under no-till conditions with equal intensification of the annual cropping phase between wheat seeded after pasture termination (CP rotation) and wheat seeded in continuous annual cropping (CC rotation). The benefits of rotating the annual cropping phase with intensively grazed management in the pasture phase under no-till can be weakened by soil compaction induced by animal trampling, reducing water infiltration, root exploration, and nutrient uptake (Colombi and Keller, 2019; Dang et al., 2020; Shaheb et al., 2021; Stanley et al., 2024). However, livestock treading damage has been reported to have little influence on subsequent crop yields, as the negative impacts are usually limited to shallow depths (less than 0.15 m) and may only persist temporarily, being mitigated by subsequent natural soil processes like wetting/drying cycles or plant root activity (Bell et al., 2011; Stanley et al., 2024). Soil compaction from non-pugged grazing and its recovery follows a cyclical pattern based on earlier studies (Drewry et al., 2008). These studies measured soil compaction in spring and its natural recovery during summer and autumn on soil grazed by dairy cows. The results indicated significant recovery of soil physical properties (macroporosity) in summer and autumn, with less recovery observed in winter. Enhanced recovery of soil physical condition in summer and autumn in temperate environments may be due to the increased natural soil processes mentioned above.

In summary, no-till farming is a practice that has drawn interest for its potential to significantly enhance crop performance and soil health (Baethgen et al., 2021; Romano et al., 2023; Taylor et al., 2024), holding great benefits for the future of agriculture. There are reported contradictory findings between CC and CP under no-till management regarding crop yields and NUE (Baiyeri et al., 2019; Pravia et al., 2019; Dang et al., 2020; Peterson et al., 2020). When pasture rotation enhances soil structure, organic matter content, and microbial activity, wheat might access and utilize better N in CP systems; conversely, agricultural systems with continuous nutrient depletion and lack of crop diversity lead to soil degradation over time and, while easier to manage, may also have lower N efficiency (Hu et al., 2023). Therefore, the incorporation of pasture or cover crops into crop rotations while using no-till techniques presents an optimistic and practical way to maintain wheat output and improve N utilization (Habbib et al., 2017; Dang et al., 2020; Dong and Zeng, 2024; Yin et al., 2024).

In Uruguay, it is well established that crop-pasture rotation systems consistently enhance crop production. Moreover, most LTE conducted under no-till included grazing (García-Préchac et al., 2004; Salvo et al., 2010; Grahmann et al., 2020; Rovira et al., 2020; Baethgen et al., 2021; Rubio et al., 2025). Therefore, the possible adverse effects of grazing have already been considered within the system as a whole. We hypothesized that including pastures in crop rotation would have further beneficial effects on wheat yields (as the first crop post-pasture) due to improved soil quality and greater availability of soil N, despite potential adverse effects such as soil compaction or invasion of weeds caused by cattle trampling and grazing. Then, the novelty of our study lies in evaluating the wheat performance corresponding to the first crop post-pasture compared with a wheat crop in a CC rotation with the equal intensification of the annual cropping phase to CP in a rainfed environment. The study assessed WGY, GPC, and the NUE as affected by the N response trials in wheat seeded under no-till in CP and CC systems in the same year. This allowed us to infer consistencies or dissimilarities between the rotation systems.

2.2. Materials and Methods

2.2.1. Experimental site

The study was conducted in a LTE established in 1993 at the EEMAC Experimental Station near Paysandú, Uruguay (32° 22' 41" South latitude and 58° 02' 50" West longitude). The site is under the influence of a humid subtropical climate (according to the Köppen climate classification), and it is relatively uniform nationwide since Uruguay is located entirely within the temperate zone. The average annual accumulated rainfall is 1300 mm, and the average temperature in the winter and the summer are 12°C and 24°C, respectively. The soil of the experimental area is classified as Typic Argiudoll, according to the USDA Soil Taxonomy, with an A horizon of 18 cm with pH 5.7, clay 290 g kg^{-1}, silt 437 g kg^{-1} and sand 273 g kg^{-1}, located on a slope less than 1%. The soil organic carbon (SOC) and total N at 0 to 15 cm depth were 18.7 g kg^{-1} and 1.6 g kg^{-1}, respectively. The LTE, initiated in 1994, compared four cropping systems under no-till and conventional tillage conditions:

This study evaluated just two no-till cropping systems, CC (i) and CP (ii), because conventional tillage has almost completely disappeared as a tillage system in Uruguay (Table 1). The cropping systems were arranged in non-synchronized randomized replications to ensure the presence of all crop or pasture phases each year, with three replications for CC, and 7 for CP.

Table 1. Crop rotation systems evaluated during the period under study (2014-2017)

System Rotation	Year 1	Year 2	Year 3	Year 4	Year 5	Year 6	Year 7
	3-year rotation			3-year rotation			
CC	**Wheat**†/Soybean 2	Barley/Sorghum	Winter fallow/Soybean 1	Wheat/Soybean 2	Barley/Sorghum	Winter fallow/Soybean 1	
	7-year rotation						
CP	**Wheat**†/Soybean 2	Barley/Sorghum	Winter fallow/Soybean 1	Wheat + PP	PP	PP	PP

CC: Continuous double annual cropping under no-till

CP: The same double annual cropping sequence than CC combined with a long perennial pasture (PP) phase: mixture of birdsfoot trefoil (*Lotus corniculatus* L.), white clover (*Trifolium repens* L.), and tall fescue (*Festuca arundinacea* L.), grazed by dairy cattle at a stocking rate of 23.7 Uruguayan Livestock Units per hectare. Plus sign indicates wheat-pasture consociation. The slash symbol separates the cropping season (winter/summer)

† The wheat crop evaluated in this study

The CP is a 7-year rotation alternating between crop and pasture phases. Pasture yields in the spring, ranged from 1000 to 3500 kg ha^{-1}, depending on the age of the pasture and the proportion of legumes and weeds. Glyphosate herbicide was applied in plots with 3.5-year pastures two months before wheat seeding. On average, the pasture provided 65 kg N ha^{-1} annually, with approximately 90% derived from BNF (data not shown). The grazing criteria were as follows: grazing began when pastures reached 2500 kg of dry matter per hectare, especially in two-year-old pastures. Grazing was also carried out on older pastures (3rd and 4th year), even though their production would have been lower. Grazing ceased when the forage consumption was around 50%, averaging 5 to 7 annual grazing events, and halted if the soil was too wet to avoid animal footprints. The animals withdrew if it rained during grazing.

The grain crops in CP consisted of a succession of wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) as winter crops and fallow winter conditions if the previous crop was sorghum (*Sorghum bicolor* L.) and soybean (*Glycine max* L.) and sorghum as summer crops. In the CC system, an identical grain crop sequence was considered (Figure 1). The soybean crop was defined as a first (Soybean 1) or second (Soybean 2) crop depending on the previous crop´s purpose, i.e., if it was for grain harvest: Soybean 2, and if it was fallow or a cash crop: Soybean 1. As is displayed in Figure 1, while CC had a frequency of C4 crops (sorghum) of 0.14 (or 1 C4 crop every 3. 5 years, Figure 1), under CP rotation, this frequency was 0.07 (or 1 C4 crop every seven years).

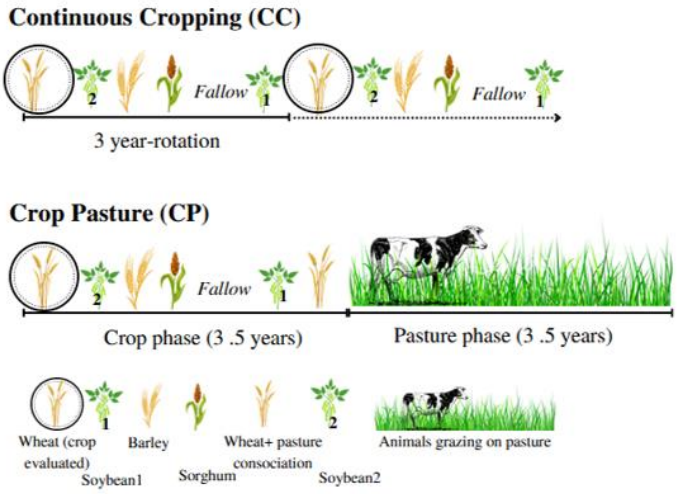


Figure 1. Schematic representation of the treatments: wheat-based rotations evaluated and sequence length for each phase.

2.2.2. Experimental design and treatments management

In this study, we evaluated wheat performance after 20 years (1994-2014) of two contrasting cropping systems under no-till: CP and CC. In the CP system, wheat is the first annual crop after the long perennial pasture phase, making it a reliable indicator for assessing the rotation's carryover effects on such a system. The wheat crop under CP was seeded after 3.5 years of pasture, initially composed of a mix of fescues and legumes (*Lotus corniculatus* L. and *Trifolium repens* L.) but dominated by *Cynodon dactylon* at the termination date, particularly in 2015. Under the CC system, where no pasture phase exists, wheat was seeded following the Soybean 1. The wheat cultivar used was Baguette 501, and the crop was sown at the recommended density on June 27th, May 29th, and May 26th in 2014, 2015, and 2016, respectively.

A completely randomized design trial with three replications was conducted exclusively on the wheat seeded within two plots/year of 10 x 50 m size in 2014, 2015, and 2016, each representing a rotation-tillage combination (CP or CC), as shown in Figure 1. The N response was assessed within each plot, setting four fixed and equidistant N levels (0, 30, 60, and 90 kg N ha^{-1}) as urea. The N factor was an essential aspect of our study, as this source of variation allowed us to infer differences between rotations. Each N rate was split into two equal amounts and applied to the wheat crop at the seeding date and when the wheat reached the tillering phenological stage corresponding to Z2.2 of the Zadoks growth scale. This experimental treatment design was conducted in 2015 and 2016, while in 2014, N was applied only during tillering at two N rates (0 and 30N). Phosphorous was broadcasted without incorporation at 60 kg ha^{-1} of P_2O_5 as triple superphosphate at wheat seeding across the entire experimental area to avoid P limitation in crop growth. The topsoil (0-20 cm) had adequate potassium (0.6 cmol+ kg^{-1}) and a cation exchange capacity (CEC) of 14 cmol+ kg^{-1}, so for each experiment, potassium and CEC values were assumed to be non-limiting (Barbazán et al., 2011). The wheat crop was kept free from weeds, pests, insects, and diseases by applying herbicides, insecticides, and fungicides as needed.

2.2.3. Weather data during the study period (2014-2017)

This research utilized meteorological records from a nearby station at the EEMAC Experimental Station in Paysandú (Latitude: 32° 22' 41" S Longitude: 58° 03' 50" W). These records comprised monthly temperature readings (maximum, minimum, and mean) and monthly precipitation data (accumulated per month). On average, the cumulative precipitation for the wheat-growing season, which extends from May to November, was 542 mm. However, in 2014, 2015, and 2016, the cumulative rainfall recorded was 813 mm, 613 mm, and 512 mm, respectively. While the rainfall exceeded the average in 2014, it was typical in 2015 and 2016. Nonetheless, there were instances of soil water saturation, which were most severe from September to November in 2014 and in August of 2015 (Figure 2A).

In 2014 and 2016, the total rainfall between April and May was significantly higher than the 30-year average, with 298 mm and 726 mm above the average, respectively. However, in 2015, it was 63 mm lower than the 30-year average. In 2016, heavy rainfall occurred 30 days before the wheat seeding, with abundant precipitation over 11 days, ranging from 20 to 188 mm per day. Although the temperature in 2014 was higher than the 30-year average monthly air temperature, and in 2016 was lower, in 2015, it was similar to deviations from the 30-year average, which were relatively small in all three years and thus were unlikely to have a significant impact on crop growth. (Figure 2B). The weather throughout the wheat growing season in 2015 was the most similar to the 30-year average. In 2014, it was a rainy and warm spring, while in 2016, the winter was colder than the two previous wheat seasons, and the three months leading up to the crop harvest were dry.

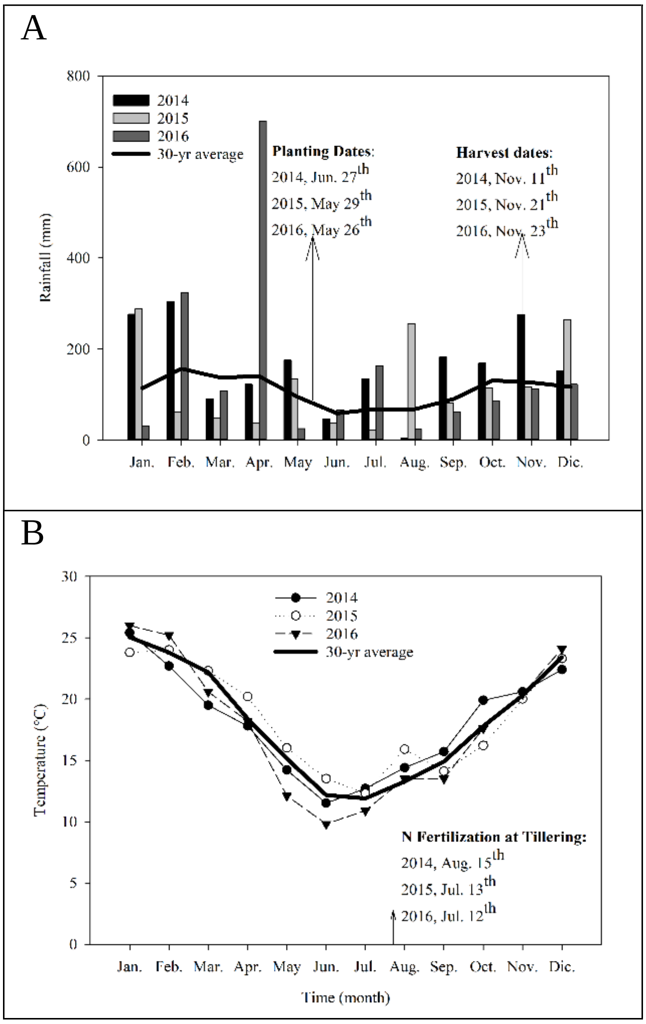


Figure 2. Data on weather from 2014 to 2016 in Paysandú, Uruguay. A) Monthly rainfall for these years and the 30-year rainfall average. B) Monthly temperatures and the 30-year temperature average.

2.2.4. Soil and Plant Sampling

During the three-year study, composite soil samples were collected from each plot at 0 to 20 cm depth to assess the N mineral concentration at two stages of the wheat cycle: seeding and tillering. Additionally, one more sampling was done before the seeding of the succeeding soybean crop in December 2015 and 2016. Fifteen subsamples were taken from each plot, using a sharpened stainless-steel probe with a diameter of 2 cm to obtain the samples at the specified times.

At the end of each wheat growing season (November), plants were sampled in a 1 m-row at physiological maturity to assess biomass yield. The plant samples were separated into grain and stover for individual analysis. Each plot was harvested separately, resulting in three yield replicates per system (CP and CC) and per year of study (2014-2016). The grain harvest area was 1.15 × 4.0 m. Threshing was performed using a stationary machine, and the reported grain yield was corrected to a baseline moisture level of 13.5% using a grain moisture meter to measure the grain moisture content (Model: OHAUS MC2000).

2.2.5. Sample Processing and Analytical Determinations

2.2.5.1. Soil measurements

Before chemical analysis, each soil sample was air-dried and crushed to pass through a 2-mm sieve after removing any visible plant residues. For mineral N, soil extracts were prepared by shaking 10 g of soil with 100 mL of 2 M KCl (Rhine et al., 1998). The concentration of ammonium–N (NH_4–N) and nitrate N (NO_3–N) in soil samples was analyzed by colorimetric determination. The Griess–Ilosvany method (Mulvaney, 2018) was used to determine NO_3–N concentration, while NH_4–N concentration was determined using the colorimetric method based on the Berthelot reaction (Rhine et al., 1998). The N-NO_3 concentration at 0-20 cm of soil depth is used as a parameter for N diagnostic for Uruguayan wheat crops (Rabuffetti, 2017). Other soil subsamples previously air-dried intended for total N and C determination were oven-dried at 40 ◦C, finely ground (< 200 μm), and analyzed by dry combustion (Rayment and Lyons, 2011) using an elemental analyzer (Flash EA 112) coupled to an isotope ratio mass spectrometer (DeltaPLUS, Finnigan MAT, Bremen, Germany).

2.2.5.2. Plant measurements

The plant samples were oven-dried at 65ºC until the mass remained constant. Finally, soil and dried plant materials were ground in a rotary mill (SampleTek Model 200 Vial Rotator, Lincoln, Nebraska). This step transformed the materials into a fine powder, akin to talcum powder, which was a prerequisite for their analysis by mass spectrometry.

Each plant sample was separated into grain and stover to analyze the dry matter and N content in grain and stover (henceforth Nstover) and grain protein concentration (GPC). The GPC values were estimated based on dry grain N concentration data multiplied by 5.7% (Giunta et al., 2021) and reported as a percentage at 13.5% moisture. The total N (TN) concentration (Dumas method; IAEA, 1990) for the soil and plant samples was determined using an elemental analyzer (Flash EA 112, DeltaPLUS, Finnigan MAT, Bremen, Germany).

Our study employed precise plant measurements to determine four Fertilizer-based N-use efficiencies (NUEs). The first of these, agronomic efficiency (AE), was estimated from data gathered in three experiments. This estimation was done using the following equation.

$$\text{AE (kg grain kg N ha}^{-1}) = \frac{\text{Grain yield}_\text{F} - \text{Grain yield}_\text{UF}}{\text{applied N}} \tag{1}$$

Grain yieldF and Grain yieldUF are grain yields of wheat cultivated in plots at a certain level of fertilizer N and in the non-N-fertilized plots, respectively.

Secondly, the RE is the total N amount (grain + stover) difference between crops growing in N-fertilized and non-N-fertilized plots per kg of applied N. This ecophysiological parameter was defined by the Equation 2:

$$\text{RE}\left(\text{kg N uptake kg N ha}^{-1}\right) = \frac{\text{N uptake}_\text{F} - \text{N uptake}_\text{UF}}{\text{applied N}} \tag{2}$$

N uptakeF and N uptakeUF are the total N taken up by plants grown in an N-fertilized plot and a control non-N-fertilized plot, respectively, and N applied is the amount of N fertilizer applied.

The IE is the total grain yield produced per unit of N absorbed. This physiological parameter, also named physiological efficiency (PEN), was estimated with the following equation:

$$\text{IE}\left(\text{kg N grain kg N uptake}^{-1}\right) = \frac{\text{Grain yield}_\text{F} - \text{Grain yield}_\text{UF}}{\text{N uptake}_\text{F} - \text{N uptake}_\text{UF}} \tag{3}$$

The other two indices for assessing NUE in fertilizers were partial factor productivity (PFP) and partial N balance (PNB), neither of which take into account the N supply in the soil, while both fertilizer-based indices mentioned above, RE and AE, consider the background soil N levels by accounting for the N uptake or production in plots that did not receive fertilizer (Mălinaş et al., 2022). The PFP expresses grain yield for each N fertilizer unit used. In contrast, the PNB expresses the grain N uptake for each N fertilizer unit applied.

2.2.6. Statistical analysis

A segmented model by rotation system allowed for assessing the N response on WGY, GPC, soil mineral concentration, and N efficiency indices. This approach was selected due to the lack of degrees of freedom for the rotation factor in the experimental design, making a direct statistical comparison between rotation systems unfeasible. Nevertheless, inferences could be made from the independent N response trials within each rotation system. Additionally, the response of each system to the applied N, the effect of the year, and the interaction between the N rate and year were considered without restrictions. Replicates were nested within the year in the model. Data from trials conducted in 2015 and 2016 were used, as the experimental design in 2014 was different.

$$Y_{ijk} = \mu + B_i + N_j + BN_{ij} + r(B_i)_k + \varepsilon_{ijk}$$

Where:

Y_{ijk} = is the response variable in the ijk-th observation

μ = overall mean

B_i = is the relative effect of the i-th year effect

N_j = is the relative effect of the j-th N fertilization effect

BN_{ij} = is the N fertilization by year interaction

$r(B_i)_k$ = is the k-th replication nested to the year

ε_{ijk} = experimental error

The approach of the model segmented by rotation was represented as follows:

Model CC = Year + Nrate + Year*Nrate + Replicate (year)

Model CP = Year + Nrate + Year*Nrate + Replicate (year)

A Tukey test with a 95% confidence level was employed to compare treatment means within each rotation in the study. The Shapiro-Wilk and Levene tests checked data normality and homogeneity of variance assumptions. For data analysis, we used SAS ® Studio on Demand for Academics (Cary, NC) and R software (version 4.04).

The orthogonal polynomial contrast or comparison analyses tested the response trends of the WGY, GPC, and soil nitrate-N concentration to N applied. To create a predictive model for WGY and GPC, we divided the dataset into two equal subsets for training and validation. We used a linear regression model and SMA regression (package smart in R). Two SMAs (for y against x) were fitted separately for each level of the factor rotation.

2.3. Results

2.3.1. Effects of year and N rate segregated by rotation on soil mineral N

Soil NO_3–N concentration at the 0-20 cm depth at wheat seeding and before N application was not statistically different between years (2014-2016) in CC, averaging 11.5 mg kg^{-1}, while in CP, it was significantly different ($p = 0.0243$), being lowest in 2015 (7.0 mg kg^{-1}) and higher and without differences in 2014 and 2016, with 11.6 and 10.2 mg kg^{-1}, respectively (Supplementary material, Table S1). At tillering (Zadocks 2.2), in 2015 and 2016 and with the four N doses, the effects of year and Nrate on NO_3–N concentration were statistically significant (Table 2). The NO_3–N increase with the Nrate was quadratic in CC and linear in CP. The NO_3–N concentration range varied from 7.2 to 14.6 mg kg^{-1} in CC and 8.0 to 11.8 mg kg^{-1} in CP, yielding the lowest values in 0N and the highest in 60 and 90N treatments. However, the Year × Nrate interaction effect was not statistically significant in either rotation system.

Table 2. Means and standard errors for soil Nitrate–N and Ammonium–N concentration at tillering for wheat crops by experimental year, N rate, and rotation system.

year	N rate	Nitrate–N		Ammonium–N	
		mg kg^{-1}			
		CC	CP	CC	CP
†2014	0	3.3 ± 0.2	4.8 ± 0.7	13.8 ± 1.1	21.7 ± 3.1
	30	3.3 ± 0.2	4.8 ± 0.7	13.8 ± 1.1	21.7 ± 3.1
2015	0	11.3 ± 2.8^{b}	12.8 ± 0.3^{c}	6.3 ± 0.5	5.4 ± 0.3^{b}
	30	18.1 ± 3.8^{a}	14.0 ± 1.8^{bc}	8.5 ± 1.9	6.3 ± 0.9^{b}

	60	21.0 ± 1.1^{a}	18.4 ± 1.2^{a}	10.7 ± 1.4	9.6 ± 1.7^{a}
	90	18.8 ± 2.6^{a}	16.7 ± 2.3^{ab}	9.7 ± 1.7	8.9 ± 1.0^{ab}
2016	0	3.1 ± 0.5^{b}	3.1 ± 0.2	9.7 ± 1.4	8.1 ± 1.0
	30	5.3 ± 0.7^{ab}	4.1 ± 0.3	11.3 ±1.8	7.8 ± 0.6
	60	8.3 ± 1.0^{a}	5.1 ± 0.5	9.1 ± 1.4	11.5 ± 2.1
	90	8.0 ± 1.4^{a}	5.5 ± 0.3	11.3 ± 1.2	11.5 ± 2.2
Variation source		*p*-value			
Year		<0.0001	<0.0001	n.s.	0.0538
Nrate		0.0003	0.0305	n.s.	0.0385
Unfertilized vs fertilized		<0.0001	<0.0196	n.s.	0.0564
Lineal		0.0002	0.0055	n.s.	0.0054
Quadratic		0.0065	n.s.	n.s.	n.s.
Year × Nrate		n.s.	n.s.	n.s.	n.s.
Replication (Year)		0.0019	n.s.	n.s.	n.s.

Different lowercase letters within a column indicate differences among N rates within each year at a 95% confidence level; ns means no significant difference. †File data from 2014 were not included in the ANOVA analysis.

In 2015 and 2016, the N rate had a statistically significant effect on soil NH_4–N at tillering in the CP rotation, while in CC, none of the factors tested showed differences in soil NH_4–N (Table 2). At the seeding of the soybean crop, the succesor crop after wheat (Supplementary material, Table S2), both NH_4^+ and NO_3^- forms of N were higher in the CP system compared to the CC system. The former was the primary N-form in the soil in both systems but generally had a higher NH_4 concentration in CP. Additionally, the year and interaction effects were not significant. In both systems, the NH4–N concentration in all N treatments at tillering was higher than NO_3–N concentration in 2014 and 2016. The higher NH_4–N to NO_3−N ratio in 2014 and 2016 coincided with the heaviest rainfall during the wheat tillering seasons. On average, NH_4–N concentrations were 13.8 and 21.7 mg kg^{-1} across years in CC and CP, respectively (Table 2).

2.3.2. Effects of year and N rate segregated by rotation on physical productivity and quality of wheat

2.3.2.1. Wheat grain yield

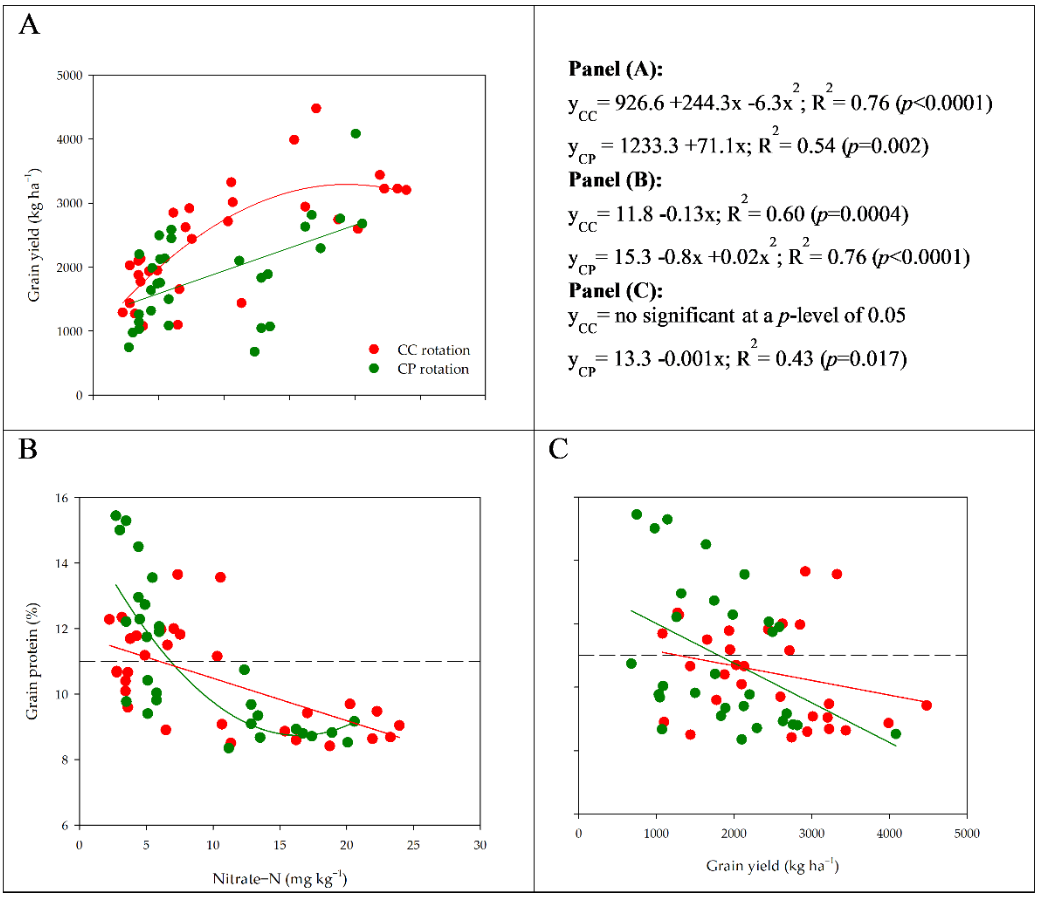
Based on the segmented model by rotation system, the ANOVA analysis revealed that the year (2014-2016) effect did not significantly affect WGY in unfertilized plots (0N). However, considering all N treatments, the ANOVA from 2015 and 2016 data showed that the year and Nrate in both rotations had a statistically significant effect on WGY (Table 3). The lowest yield was observed at 0N, with 1520, and 1080 kg ha^{-1} in CC and CP, respectively, which statistically differed from the rest of the N treatments. The highest yield was registered at 90N and 60N with 3461 and 2630 kg ha^{-1} in CC and CP, respectively.

Table 3. Means and standard errors for wheat grain yield (WGY), grain protein concentration (GPC), and N content in the stover for wheat crops by experimental year, N rate, and rotation system: continuous cropping (CC) and crop-pasture (CP).

year	N rate	WGY		GPC		N Stover content	
		kg ha^{-1}		%		kg N ha^{-1}	
		CC	CP	CC	CP	CC	CP
†2014	0	1769 ± 191	1414 ± 356	10.1 ± 0.3	9.7 ± 0.2	8.0 ± 1.4	9.9 ± 2.1
	30	2011 ± 73	1818 ± 205	10.6 ± 0.1	10.0 ± 0.2	12.1 ± 1.0	14.5 ± 2.1
2015	0	1827 ±567^{c}	1204 ± 358^{c}	8.7 ± 0.1	9.9 ± 0.4^{a}	7.9 ± 3.5^{c}	5.7 ± 2.5^{c}
	30	2945 ± 185^{b}	1822 ± 378^{bc}	9.2 ± 0.3	8.6 ± 0.1^{b}	13.3 ± 3.3^{bc}	10.8 ± 2.2^{bc}
	60	3134 ± 206^{ab}	3157 ± 464^{a}	8.8 ± 0.3	8.8 ± 0.1^{ab}	20.1 ± 4.4^{a}	18.8 ± 3.2^{a}
	90	3891 ± 371^{a}	2442 ± 307^{ab}	9.1 ± 0.2	9.0 ± 0.1^{ab}	18.8 ± 4.9^{ab}	16.3 ± 4.3^{ab}
2016	0	1214 ± 68^{c}	956 ± 115^{c}	12.1±0.2^{ab}	15.3 ± 0.1^{a}	3.8 ± 1.1^{b}	7.7 ± 1.4
	30	1845 ± 96^{bc}	1406 ± 118^{b}	11.5 ± 0.2^{b}	13.2 ± 0.7^{b}	9.1 ± 1.7^{b}	7.0 ± 1.0
	60	2592 ± 80^{ab}	2103 ± 249^{ab}	11.7 ± 0.3^{b}	12.3 ± 0.3^{b}	8.3 ± 1.6^{b}	10.3 ±1.4
	90	3031 ± 149^{a}	2359± 113^{a}	13.1 ± 0.6^{a}	12.5 ± 0.6^{b}	19.1 ± 1.4^{a}	10.5 ±1.0
Variation source		*p*-value					
Year		0.0023	0.0191	<0.0001	<0.0001	0.0023	0.0246
Nrate		0.0001	<0.0001	0.0383	<0.0001	<0.0001	0.0122
	Unfertilized vs fertilized	<0.0001	<0.0001	n.s.	<0.0001	<0.0001	0.0094
	Lineal	<0.0001	<0.0001	n.s.	0.0002	<0.0001	0.0019
	Quadratic	n.s.	0.0406	0.0410	0.0007	n.s.	n.s.
Year × Nrate		n.s.	n.s.	n.s.	0.0213	0.0405	n.s.
Replication (Year)		n.s.	n.s.	n.s.	n.s.	0.0018	n.s.

Different lowercase letters within a column indicate differences among N rates within each year at a 95% confidence level; ns means no significant difference. †File data from 2014 were not included in the ANOVA analysis.

The highest WGY was consistently observed in the CC system in our three-year study. However, the N response tended to be linear with the N rate in CC and quadratic in CP. As there was no significant interaction effect between Year and Nrate, the yield response to N was similar between years at each rotation system. In 2015, the year of higher productive potential, the yield differences between systems increased, with wheat capitalizing better in CC rotations. In the treatments where no N was applied (0 N), WGYs were higher in 2014 and 2015 than in 2016; still, these differences were not statistically significant.



Panel (A):

$y_{CC} = 926.6 + 244.3x - 6.3x^2$; $R^2 = 0.76$ ($p<0.0001$)

$y_{CP} = 1233.3 + 71.1x$; $R^2 = 0.54$ ($p=0.002$)

Panel (B):

$y_{CC} = 11.8 - 0.13x$; $R^2 = 0.60$ ($p=0.0004$)

$y_{CP} = 15.3 - 0.8x + 0.02x^2$; $R^2 = 0.76$ ($p<0.0001$)

Panel (C):

y_{CC} = no significant at a p-level of 0.05

$y_{CP} = 13.3 - 0.001x$; $R^2 = 0.43$ ($p=0.017$)

Figure 3. Data relationship between nitrate-N at tillering and wheat grain yield, (A) nitrate-N and grain protein concentration (B), and wheat grain yield and grain protein concentration (C). This was evaluated for the three experimental years (2014-2016) and the four N rates (0, 30, 60, and 90). The dotted line represents the reference

value of grain protein concentration (11.5 %), which defines the wheat marketing specification in Uruguay.

2.3.2.2. Grain protein concentration and N stover content

The GPC measured in wheat cultivated in unfertilized plots was significantly affected by the year in both rotation systems; its average value was11.2% and ranged between 9.7 and 15.39% in CP, while in CC, it was lower on average at 10.4%, oscillating between 8.7 and 12.1%. The ANOVA, including the four N rate treatments from the experiments of 2015 and 2016, showed that year (p = <0.0001 in both rotations) and Nrate (p = 0.0383 and p < 0.0001 in CC and CP, respectively) had a statistically significant effect on GPC (Table 3). In these two years, the maximum values were registered in the treatments 90N in CC and 0N in CP, averaging 11.1% and 12.6%, respectively.

The GPC values significantly differed between N rates across years (p = 0.0213) only in CP. The analysis of the year × Nrate interaction effect revealed that in 2016, none of the N fertilized treatments enhanced the GPC compared with wheat crops with 0N applied, while in 2015, wheat fertilized with the highest N rates (60 and 90N) were able to reach the GPC achieved by crops non-fertilized. The Nrate effect in CC was consistent across years, showing no N response on GPC values. However, in 2016, the wheat fertilized with the highest dose (90N) reached the maximum values; still, it did not statistically differ from 0N.

Similar to the GPC, the N content in the biomass stover significantly differed between Nrate across years (p=0.0405), but in this case, only in CC. The analysis of this interaction effect revealed that N response on N content in stover differed between years; in 2015, wheat fertilized with high doses of N (60N and 90N) reached higher N values in stover than the unfertilized ones, while in 2016, it was only possible with the maximum N rate (90N). The Nrate effect in CP was consistent across years, showing no N response on Nstover values in 2016; however, in 2015, the wheat fertilized with the medium dose (60N) reached the maximum values, differing from those treatments with 0N applied. At this N rate, WGY was also at its highest. (Table 3).

2.3.3. Relationships between nitrate-N at tillering and grain yield and quality segregated by rotation

Higher levels of nitrate-N at tillering were associated with higher WGY and lower GPC, as shown in Figure 3A and 3B, respectively, for each relationship segregated by rotation. Conversely, when considering a similar nitrate−N level, the increase in N rate did not lead to higher GPC levels because there was no association between N rate and GPC (Table 4). However, there was a high and significant negative correlation between NO_3–N and GPC, suggesting that the soil mineral N available at tillering was a critical factor affecting GPC (Figure 3B). Grain PC variation was negatively associated with NO_3–N measured at tillering; the opposite was the association between NO_3–N concentration and WGY. The relationships between those variables resulted in a distinct relationship between GPC and WGY at each system, which was more robust and significant in CP (Figure 3C).

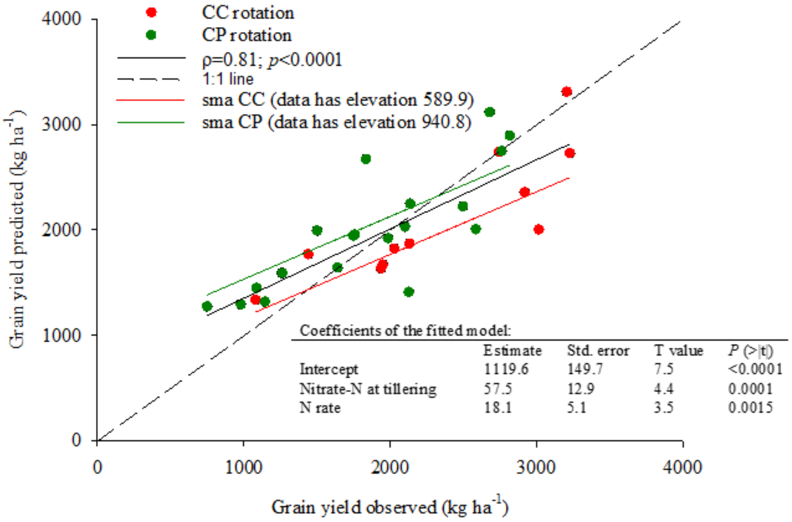
The N effect on GPC was statistically significant in both systems. In 2016, GPC values in wheat growing in CC were similar between unfertilized and fertilized treatments (Table 3). In contrast, in CP, GPC diminished with increased N supply, similar to findings by Bedoussac and Justes (2010). The N response curves for GPC (represented by a positive quadratic response) demonstrate the dilution effect of GPC, which was clearly shown in CP in 2016 at a fertilization rate of 30 and 60 kg N ha^{-1}, attributable to the increase in grain yield per kg of grain N (Table 3).

Table 4. Pearson correlation coefficients (r) within each rotation system: continuous cropping (CC) and crop-pasture (CP) across the three experimental years (2014-2016) and all N rates (0, 30, 60, and 90 kg N ha^{-1}).

Variables		Rotation system			
		CC		CP	
N rate	Nitrate–N_Z22	0.45	*	0.32	
N rate	Grain yield	0.80	***	0.69	***
N rate	Nnuptake_stover	0.66	***	0.46	*
N rate	N uptake crop	0.89	***	0.74	***
N rate	Protein concentration	0.15		-0.15	
Nnitrate_Z22	Grain yield	0.71	***	0.54	**
Nnitrate_Z22	N uptake_stover	0.58	***	0.48	**
Nnitrate_Z22	N uptake crop	0.54	**	0.32	
Nnitrate_Z22	Protein concentration	0.60	***	0.71	***
Grain yield	N uptake_stover	0.70	***	0.66	***
Grain yield	N uptake crop	0.91	***	0.92	***
Grain yield	Protein concentration	-0.26		-0.43	*

Significant at *p <0.05; **p <0.01; ***p <0.001.

A multiple regression model incorporating N rate and NO_3–N at tillering accounted for 68% (p < 0.0001, Figure 4) of the variation in wheat grain yield (WGY). The standardized major axis (SMA) regression test revealed that the best model segregated by rotation had an equal slope (0.59). However, different elevations for each rotation level improved prediction accuracy, explaining 82% of the variation in grain yield for CC and 65% for CP. Nevertheless, the model overestimated low yields in CP and underestimated high yields in CC, likely due to differing relationships between N rate and yield (linear in CC, quadratic in CP). For grain protein content (GPC), only NO_3–N at tillering was significant, explaining 54% ($p < 0.002$) of the variation. The SMA regression test for GPC indicated no significant difference in slopes and elevation.



Coefficients of the fitted model:

	Estimate	Std. error	T value	P (>|t|)
Intercept	1119.6	149.7	7.5	<0.0001
Nitrate-N at tillering	57.5	12.9	4.4	0.0001
N rate	18.1	5.1	3.5	0.0015

Figure 4. Scatterplot of grain yield observed against predicted and included SMAs: fitted separately for each level of the rotation factor (the red line represents CC, and the green line represents CP rotation). The 1:1 line is given.

2.3.4. Effects of year and N rate segregated by rotation on NUE indices

Based on 2015 and 2016 data and considering the three fertilized treatments, the ANOVA showed that neither year nor Nrate and its interaction in both rotations had a statistically significant effect on two fertilizer-based indices, RE and AE, and the plant-based index IE (Table 5). Both indices were higher in CC (22.4 kg of grain kg^{-1} N added and 55.2%) than in CP (20.4 kg of grain kg^{-1} N added and 38.3%).

Table 5. Means and standard errors for fertilizer-based indices: agronomic efficiency (AE), recovery efficiency (RE), internal efficiency (IE), partial factor productivity (PFP), and partial nutrient balance (PNB) for wheat crops by year, N rate, and rotation system: continuous cropping (CC) and crop-pasture (CP).

		AE		RE		IE		PFP		PNB	
year	N rate	kg yield increase kg N applied^{-1}		kg N uptake increase ha^{-1} kg N applied ha^{-1}		kg yield increase kg N uptake increase ha^{-1}		kg grain yield kg N applied^{-1}		kg N removed kg N applied^{-1}	
		CC	CP	CC	CP	CC	CP	CC	CP	CC	CP
†2014	30	8.1 ± 8.0	13.5± 14.8	0.32 ± 0.2	0.40 ± 0.3	100.4 ± 68.8	109.7 ± 57.8	67.0± 2.4	60.6 ± 6.8	1.1 ± 0.1	1.0 ± 0.1
2015	30	37.3 ± 15.7^{a}	20.6 ± 16.7	0.75 ± 0.3	0.38 ± 0.2	46.0 ± 5.7	38.6 ± 16.3	98.2 ± 6.2^{a}	60.7 ± 12.6^{a}	1.4 ± 0.0^{a}	0.8 ± 0.2^{a}
	60	21.8 ± 6.8^{b}	32.6 ± 2.3	0.51 ± 0.1	0.64 ± 0.0	40.0 ± 7.3	52.7 ± 9.3	52.2 ± 3.4^{b}	52.6 ± 7.7^{a}	0.7 ± 0.1^{b}	0.7 ± 0.1^{ab}
	90	22.9 ± 10.0^{ab}	13.8 ± 2.3	0.47 ± 0.2	0.30 ± 0.1	42.0 ± 11.9	48.5 ± 4.9	43.2 ± 4.1^{b}	27.1 ± 3.4^{b}	0.6 ± 0.1^{b}	0.4 ± 0.1^{b}
2016	30	21.1 ± 4.8	15.0± 7.4	0.51 ± 0.2	0.30 ± 0.2	43.3 ± 3.7^{ab}	74.4 ±18.7	61.5 ± 3.2^{a}	46.9 ± 3.9	1.1 ± 0.1^{a}	1.0 ± 0.1^{a}
	60	23.0 ± 0.5	19.1 ± 3.3	0.47 ± 0.0	0.35 ± 0.0	49.2 ± 2.6^{a}	54.8 ± 7.3	43.2 ± 1.3^{b}	35.1 ± 4.2	0.8 ± 0.0^{b}	0.7 ± 0.1^{ab}
	90	20.2 ± 1.3	15.6 ± 0.4	0.60 ± 0.0	0.29 ± 0.0	33.6 ± 2.8^{b}	55.1 ± 5.0	33.7 ± 1.7^{b}	26.2 ± 1.3	0.7 ± 0.1^{b}	0.5 ± 0.0^{b}
Variation source		*p*-value									
Year		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0009	0.0730	n.s.	n.s.
Nrate		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	<0.0001	0.0085	<0.0001	0.0062
Year × Nrate		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.0207	n.s.	0.0273	n.s.
Replication (Year)		0.0076	n.s.	0.0245	n.s.	0.0042	n.s.	n.s.	n.s.	n.s.	n.s.

Different letters within a column indicate differences among N rates each year, which are significant at a 95% confidence level; ns means no significant difference. †File data from 2014 were not included in the ANOVA analysis.

The PFP analysis indicated that the main effects of year and Nrate were statistically significant in both rotation systems. However, the interaction effect of year × Nrate was statistically significant only in the CC rotation system. Regarding PNB, the year's effect was not statistically significant; however, the Nrate effect was significant in both rotation systems. Similar to PFP, the interaction effect was only significant in CC.

The average AE achieved across years, and N rate was 24.4 and 19.4 kg ka^{-1} in CC and CP, respectively. Furthermore, the AE values were significantly associated with RE ($r = 0.94$, $P < 0.0001$ and $r = 0.91$, $P < 0.0001$, respectively) but not with IE in both rotations.

2.4. Discussion

2.4.1. Year and N rate effects segregated by rotation on soil mineral N

The presence of *Cynodon dactylon* was likely responsible for the lowest concentration of NO_3–N observed in 2015 in CP at wheat seeding. Its residues probably reduced the soil's mineral N content by absorbing it and competing with other pasture species, particularly legumes, thereby influencing their endurance towards the end of the pasture cycle. Consequently, the C: N ratio of the pasture residues might have increased, leading to either N immobilization in the soil or limited soil N availability to plants due to slow residue breakdown (Dang et al., 2020). In 2015, NO_3–N concentrations at tillering were similar to those at seeding, whereas in 2014 and 2016, they were only a third of those at seeding. The 2015 results could be related to low rainfall during the early stages of crop growth, while those of 2014 and 2016 might be due to heavy July rain (135 and 163 mm, respectively), which was more than doubled the expected July rain based on the 30-year historical average. The N response on NO_3–N in CP in 2016 was less than in CC, even though the values were similar in 0N plots under both rotation systems. This finding suggests that a fraction of the N applied at wheat seeding was lost or immobilized in the soil in CP, leading to a lesser increase in soil N availability at tillering.

The noteworthy difference in the quantity and proportion of N forms (ammonium vs. nitrate) between systems observed after the wheat harvest can be attributed to the more favorable soil conditions for net mineralization at this point in the season in CP rotation (supplementary materials, Table S2). The decomposition process of the remaining pasture residues was in its final phase, likely resulting in a lower rate of N immobilization.

The increased NH_4–N to NO_3–N ratio in 2014 and 2016 aligned with the periods of heaviest rainfall during the wheat tillering seasons. On the other hand, these findings suggest that under CP rotation, where the litter input has a chemistry conducive to decomposition (low lignin content and low C/N, such as in forage legumes and rhizodeposition), it may have led to higher NH_4–N accumulation in the soil but reduced nitrification rates. (Sun et al., 2019; Walkup et al., 2020; Fontaine et al., 2023). Furthermore, soil compaction might uphold higher moisture soil levels, particularly during slower drying periods like winter, leading to N loss through denitrification (Dang et al., 2020; Shaheb et al., 2021). Regarding these changes in soil N dynamics, Booth et al. (2005) showed that nitrification emerged as the primary process for NH_4^+ transformation at low mineralization rates, with only minor changes observed at higher rates. Conversely, the rate of NH_4^+ immobilization showed a steady increase across the entire spectrum of mineralization rates. They suggested that nitrifiers effectively outcompete heterotrophic microorganisms for NH_4^+ when soil C content is low. Based on this, we hypothesize that the competitive capacity of nitrifiers for NH_4^+ could be higher in CC under soil conditions with a low lability pool C and with a higher C: N ratio under this system (Table S3). In contrast, heterotrophic microorganisms may have a higher competition capacity for NH_4^+ in soils with high-quality C inputs in the soil rhizosphere, such as CP soil, where the C source would drive N immobilization (Sun et al., 2019 ;Walkup et al., 2020).

In our study, under soil no-till conditions, we found no disparities in soil C and N concentration levels between cropping systems rotating with pastures and those focused on continuous annual cropping systems (Supplementary material, Table S3). These findings align with studies indicating that reduced tillage practices are crucial for preserving SOC (Dang et al., 2020; Page et al., 2020; Rubio et al., 2022).

Additionally, comparing our C data with that reported by Salvo et al. (2010), whose study was carried out in the same experimental site as this work, it becomes evident that merely combining no-till techniques with crop-pasture rotations may not result in higher SOC levels (Dang et al., 2020; Grant et al., 2020; Wang et al., 2023b). This limitation in C sequestration could be associated with the system's N balance, which is generally negative and similar between CC and CP when their cropping phase includes C4 species (Pravia et al., 2019; Dang et al., 2020).

2.4.2. Effects of year and N rate segregated by rotation on physical productivity and quality of wheat

2.4.2.1. Wheat grain yield and grain protein concentration

The WGY response observed in CP aligned with expectations, as crops following legume pastures showed reduced response to applied N, consistent with previous studies (Peoples et al., 2015; Allen et al., 2021; Herridge et al., 2022; Nurbekov et al., 2024). The maximum yield was achieved with a dose of 60 N. In contrast, the response observed in CC indicates that the optimal N rate would have been above the maximum dose applied (> 90N). However, the higher WGY under CC suggested that other growth factors, which were less favorable under CP rotation, contributed to better wheat growth under CC. Although in this study, we did not measure physical properties such as bulk density or soil macroporosity, the difference in performance between systems may have stemmed from increased soil compaction under CP rotation due to cattle trampling. Barreto et al. (2022) also found higher surface runoff under CP than CC, attributing this difference to the trampling effect. These authors noted that under the CP system, there would be higher risks of nutrient losses with runoff water. Additionally, there was no indication of the expected increase in soil residual N under CP through elevated soil NO_3–N concentrations in this rotation. As mentioned, this higher soil N contribution may occur later in the crop cycle (post-Z22 growth stage). Alternatively, N losses due to denitrification could increase, potentially exacerbated by soil compaction (Dang et al., 2020; Shaheb et al., 2021).

For both systems, the highest WGY was obtained in 2015, a year with more favorable climate conditions, alongside the highest levels of NO_3–N during tillering compared to the other evaluated seasons. Furthermore, in 2015, the wheat seeding occurred amidst dry soil conditions, along with a notable presence of *Cynodon dactylon* in the CP rotation, resulting in poor crop establishment (though not quantified) and uneven emergence, leading to a negative impact on WGY, compared to CC. This weed invades the N-enriched areas in pastures created by the death of legume plants during summer droughts, effectively competing with the most commonly used pasture species (Pañella et al., 2022). Additionally, its underground biomass binds soil aggregates into a dense structure, which, combined with surface compaction from grazing, would lead to poor soil quality (García-Préchac et al., 2004; Dang et al., 2020; Shaheb et al., 2021). In 2016, yields were lower than in 2015, possibly due to low NO_3–N levels during tillering caused by heavy rainfall in July (163 mm total, with a third falling two weeks after N application at tillering) or due to reduced water availability in the subsequent period, as precipitation from August to October was below the 30-year average. The effect of soil N availability on wheat yield was evident in 2015; even though the NO_3–N concentration during tillering was close to the critical range (12-14 mg kg^{-1}) (Fassana et al., 2022), there was a noticeable N response under CC system. As has noted by other works, N assimilation is generally more negatively affected than mineralization by soil drying (Compton and Boone, 2002). Furthermore, in drying soils, NO_3^- assimilation is impaired before nitrification, suggesting that the overall production rates of inorganic N may increase as soils become drier, which was observed in our study during wheat tillering stage in 2015.

Our results showed that wheat crops seeded after pasture termination yielded less than the wheat-followed soybean crop under CC. This suggests that the effect of the previous crop (soybean) on wheat yield was more relevant than the expected residual effect of the pasture phase under CP (Lollato et al., 2019b; Griffiths et al., 2022; Arnhold et al., 2023). The grain yield of wheat under CC would have depended more on soil N availability at tillering. In contrast, following a degraded pasture under CP, other factors would limit the wheat yield (Fig.3 A). Furthermore, under CP, higher standard errors were recorded in WGY and other measured variables among experimental units, potentially attributed to the trampling and nutrient distribution (Carvalho et al., 2018; Dubeux and Sollenberger, 2020).

The measured GPC values were within the range described by other authors (Ding et al., 2020), being higher under CP than CC, as expected. Additionally, WGY and GPC had a significant negative association in the CP system, confirming that they are usually negatively correlated (Ghimire et al., 2021; Giordano et al., 2023) (Fig. 3C).

2.4.3. Relationships between nitrate-N at tillering and grain yield and quality segregated by rotation

The relationship between WGY and GPC is closely linked to N availability. When N supply is low, adding N increases yield but does not change or decrease GPC, with medium N availability, both yield and protein can increase, whereas with high N availability, fertilization mainly affects grain protein concentration (Ma et al., 2019; Ghimire et al., 2021; Giordano et al., 2023; Wang et al., 2023a). This relationship suggests that the negative correlations between WGY and GPC occur when wheat growth is severely N-deficient or when the N supply is low or unsynchronized with the crop's N demand for its potential yield. For instance, in high-production years like 2015 with favorable weather conditions, even small increases in N availability led to significant yield increases (Table 3). At this point, GPC either did not increase or sometimes decreased with higher N rates (Table 3, Figure 3C) because the increase in N uptake by the crop was small relative to the increase in WGY. Additionally, the negative correlations between WGY and GPC in CP (Figure 3C) could be attributed to other factors limiting wheat yield (Fig. 3A). These include increased N immobilization by the pasture residue and the delayed release of this N beyond the critical uptake periods (heading and grain filling stages) of the following wheat crop. Poor soil physical conditions that hinder wheat performance, N uptake, and NUE might have also played a role. This latter factor is particularly plausible as it could reduce the plant's growth rate and N uptake capacity. Soil N availability for plants depends on the soil's ability to provide resources and the plant's sink strength, which enhances crop N demand and, consequently, the efficiency of absorbing these resources. (Lemaire et al., 2021; Giordano et al., 2023). The higher N response on WGY in 2015 compared with 2016 explains the lower GPC values observed in that year (Table 2). This difference is likely due to differences in the amount and distribution of rainfall (Fig. 2A). The wetter season in 2015, with rainfall 100 mm higher than in 2016, may have led to higher N dilution in the plant (Grahmann et al., 2013; Ghimire et al., 2021). Moreover, the differences in NO_3-N availability at wheat tillering between years (Table 2) accounted for the year effect on GPC; the greater the NO_3-Navailability, as seen in 2015, the higher WGY and, consequently, the more significant dilution of grain protein (Ghimire et al., 2021).

2.4.4. Effects of year and N rate segregated by rotation on NUE indices

The two fertilizer-based indices, RE and AE, were higher under CC than CP (Table 5) and within the typical N range for cereal crops harvested for grain (Ma et al., 2019; Hausherr Lüder et al., 2020; Hu et al., 2023; Li et al., 2023). The AE data remained stable across different N doses in 2016, marked by unfavorable weather conditions and reduced soil N availability during wheat tillering. Comparing AE values across cropping systems, the CP rotation exhibited greater variability among N treatments and generally lower values than CC. This variability in CP is likely due to factors other than temporary N deficiency, as a more significant N deficiency would have led to a greater wheat yield response to applied N. The inherent variability of integrated crop-livestock systems could also explain this fluctuation.

The PFP and PNB indices were higher under CC than CP, suggesting that fertilized wheat in CC, especially at low N rates, likely absorbed more native soil N than unfertilized crops. Consequently, this native soil N might have been mistakenly quantified as coming from the fertilizer, possibly leading to an overestimation of RE (Liang et al., 2017, 2023; Chen et al., 2024). Additionally, both indices decreased with increasing N rates, a trend observed in many studies (Gastal et al., 2015; Sieling and Kage, 2021; Hu et al., 2023; Li et al., 2023). In both rotations, PNB values were higher than 1 for wheat fertilized with low N levels, indicating a potential threat to soil fertility or productivity degradation (Pravia et al., 2019; Congreves et al., 2021). The highest values were seen in CC, where nutrient availability is typically low. Fontaine et al.

(2023) reported that soils with significant SOM accumulation, such as those in CP systems, often exhibit reduced N availability for plants. This is attributed to continuous C and nutrient sequestration, driven by high microbial N assimilation, which leads to a limited response to N fertilization because of the low nutrient demand from plants in this scenario (Cotrufo and Lavalle, 2022). Conversely, CC systems are expected to show lower microbial N immobilization in the soil and greater depletion of SOM, particularly in the MAOM-associated fraction (Cotrufo and Lavalle, 2022). This suggests that N immobilization would be higher in CP, which explains the lower AE in CP compared to CC.

The efficiency of the N recovered from the added fertilizer was significantly associated with the AE index, as was seen in another study (Hausherr Lüder et al., 2020). The higher AE in CC due to its higher RE can be explained by an expected lower soil N availability in this system. The results indicate that soil N availability was an important factor limiting wheat yield in both rotations, particularly in the CC. These findings align with Ernst et al. (2020), who, comparing systems at equivalent yield (using as target yield obtained in the system that yielded the least). They found that CC,-including C4 crops, required a slightly more N fertilizer than CP, both systems under no-till management, to achieve comparable grain yield because CC was more dependent on N fertilizer inputs. The lack of pasture legumes in CC led to a lower potentially mineralizable N, which could significantly reduce N uptake in unfertilized treatments by 20% on average (Ernst et al., 2020). In CP, however, the quadratic N response revealed that factors other than N could have limited the wheat performance of that system. The factor limiting the yield response in CP rotation likely disrupted the synchrony between the N demand of crop and the amounts of N provided by both N sources, fertilizer, and soil. In our study, these factors could not be identified through our data analysis.

2.4.5. Implications and limitations of the study

Based on the assumption that the total residue dry matter input and soil C were similar between rotations (Table S3), a finding also reported by Ernst et al. (Ernst et al., 2020) comparing similar rotation systems that this study, we propose the following hypothesis. The growth-limiting factor, which could explain the disparities between systems, is likely to be associated with factors other than nutrients and the previous crop effect. These factors could include adverse effects on soil physical properties caused by animal trampling. The discordant result in the CP system, which consistently showed lower WGY, despite the expected higher soil quality and higher soil productivity potential (Ernst et al., 2018; Rubio et al., 2022) compared to CC, could be explained by considering two reasons:

1. Residues quality from tcrop sequences. We compared wheat performance seeded after a degraded pasture due to grazing (invaded by *Cynodon dactylon* in 2015), which led to a low decomposition rate and potential N immobilization, versus wheat seeded after soybean, which provides a favorable environment for residue breakdown and N response. While incorporating perennial pastures into crop rotations may offer advantages in accumulating SOC, due to greater root and shoot allocation (Mazzilli et al., 2015; Pinto et al., 2021), the low-quality residue left in the soil at the final pasture phase negatively affected the wheat performance. This, in turn, affected the soil's physical conditions for the subsequent wheat crop, limiting its performance and the efficiency of N recovery from added fertilizer. However, this effect is likely temporary, as soil quality improvment would become evident with the sequence of crops that constitute the agricultural phase (Ernst et al., 2018). When comparing the annual cropping phase only, CC and CP represent a diversified and intensive cropping system (5 crops in 3 years), a relevant variable for mitigating soil quality depletion (Novelli et al., 2017; Allen et al., 2021; Rubio et al., 2022; Alvarez and Ernst, 2024). Consequently, the differences between CP and CC are minimized when crop productivity is evaluated across the entire system (Ernst et al., 2020).

2. Temporary productivity limitation in CP. The limiting factor for productivity in CP might be temporary, affecting only the first crop after pasture termination since the negative impacts of animal trampling typically affect only shallow soil depths. Additionally, the soil's physical properties, such as macroporosity, may recovered through natural processes (Bell et al., 2011; Dang et al., 2020). The response of wheat growing in compacted soil could be highly dependent on the season, with the influence

of the limiting factor and dissimilarities between rotations being more noticeable in high-production years with favorable weather, as observed in 2015.

Our results highlight potential areas for improvement, such as increasing crop diversity by introducing alternative crops like intermediate wheatgrass (*Thinopyrum intermedium* L.). This perennial grain crop is promising for temperate regions with mild winters (Locatelli Fagúndez, 2023). Its dense and fibrous root system can enhance soil aggregate stability, as was reported with other perennial species following an agricultural phase (García-Préchac et al., 2004); thus, integrating this perennial species into the rotation could help mitigate or reduce soil compaction damage. Lupine cultivation is another promising option for diversifying crops within agricultural systems and reducing N and P fertilizer use (Griffiths et al., 2022; Mori Alvez et al., 2024). For lupines, the potential impact of its roots on improving soil structure alongside other crops and forage pastures has been evaluated (Bodner et al., 2021; Griffiths et al., 2022). Legumes were more effective in stabilizing soil structure than non-legumes, with lupine and lucerne being especially effective (Bodner et al., 2021). The researchers also observed that roots might stabilize some soil fractions for particular plant/soil combinations that influence soil physical properties, such as soil structure through biopore channels, with this impact being more pronounced in species with coarse roots, such as legumes (Boldrin et al., 2022; Wang et al., 2023b; Giuliani et al., 2024). The integration and diversification of functional groups—such as annual winter legumes like lupine—may be a pivotal strategy to reverse soil deterioration processes (Hallama et al., 2019; Fontaine et al., 2023; Nurbekov et al., 2024). An additional adjustment can be to shorten the pasture phase by changing the existing crop arrangement. This shortening in the pasture phase intends to reach the end of its cycle with better aboveground and belowground biomass, a higher legume proportion in mixed pasture swards, and roots in active growth (Hamza and Anderson, 2005; Huang et al., 2020; Zhou et al., 2021; Crecetto et al., 2024), increasing system sustainability and resilience derived from increasing non-provisioning ecosystem services with long pasture phases, such as improved soil physical conditions and the legume persistence at pasture termination. In this context, and following the recommendation of Cerecetto et al. (2024), an effective strategy to maximize the benefits of pastures would be to establish crops with high nutritional demands, such as maize, immediately after a pasture with a high proportion of legumes.

In this study, we assessed only changes in soil N availability, WGY, N uptake, GPC, and the NUE as affected by the N response trials, inferring from these experiments performed consistencies or dissimilarities between cropping systems. However, this approach may miss other system-wide benefits or tradeoffs.

Due to the limitations of our field experiment, we propose an effective and practical method for a fair comparison of the potential of CP and CC systems. Additionally, this approach allows for direct inferences about the rotation effect, as this experimental design would include three replicates with the rotation factor, minimizing the pre-crop effect observed on wheat crops and isolating the year effect from the rotation effect. This method involves using data from the same long-term experiment, involving more than one crop in the same year of the evaluation, and associating them with the same pre-crop under both systems. For instance, in this approach, we could use winter crops as pre-crops or the fallow period as pre-conditions for summer crops used as test crops (whose crops offer clear advantages under no-till conditions). This proposal must be replicated for more than two years. It is also necessary to consider soil C and N levels and the quantity and quality of residues and correlate this with soil health indicators. Equally important is using the best available knowledge and technologies to reduce the impact of nutrient stress, pests, and weeds affecting crop establishment (e.g., uneven emergence) in both CP and CC systems.

2.5. Conclusions

Our three-year research study on Uruguayan soils under a long-term experiment spanning over 20 years revealed that wheat crops in the CC system achieved higher yields than those seeded as the first annual crop after the long perennial pasture phase in the CP system in a rainfed environment. Additionally, the greater N response in CC was likely due to reduced soil N availability, which, combined with healthier soil physical properties, supported improved productivity. This resulted in higher N uptake and better NUE indices than the CP system. In contrast, adverse soil conditions in the

CP rotation may have impeded the effective utilization of applied N in wheat production. Our findings suggested that under continuous no-till systems, the performance of the first crop seeded after a long perennial pasture phase (3.5 years) was negatively affected by factors such as animal trampling and the quality of crop residues returned to the soil. While such a system would support greater soil fertility and GPC values, it had a detrimental effect on WGY due to temporarily restricted soil physicochemical and biological properties. These restrictions limited the N response, consequently reducing wheat yield and NUE indices. Our hypothesis that integrating pastures into crop rotations would improve wheat yields (as the first crop after the pasture phase) was not supported. The expected advantages of enhanced soil quality and N availability were insufficient to counteract the negative impacts of cattle trampling and grazing.

However, increased N immobilization could enhance C and N sequestration in SOM. This N process could be encouraged by temporary soil compaction, creating more conducive soil conditions typically characterized by moisture and relatively low temperatures. For future research, our findings underscore the need to directly assess soil properties linked to physical degradation that affect water dynamics, such as infiltration and retention. It is also crucial to evaluate highly sensitive C and N indicators in response to changes in their balance, including the distribution between POM and MAOM and the potentially mineralizable N from these SOM fractions and soil C respiration. This assessment highly depends on factors like soil type, environmental conditions, and the quantity and quality of residues left in the soil. Therefore, future research should focus on characterizing all these aspects within the evaluated system.

Author Contributions

The authors confirm their contribution to the paper: Study conception and design: CM and CP. Data collection: CM. Analysis and interpretation of results: CM and CP. Draft manuscript preparation: CM and CP. Writing – review & editing: CM, PG, OE and CP. All authors contributed to the article and approved the submitted version.

Funding

This research received funds from IAEA within the Coordinated Research Project D12012 framework, entitled ‘Optimizing Soil, Water and Nutrient Use Efficiency in Integrated Cropping-Livestock Production Systems.’ The National Research of our Institution, the University of the Republic (UdelaR, Uruguay), funded the publication of this article. This work is part of the senior author's doctoral dissertation at the Doctoral Program in Agricultural Sciences, Agronomy College, UdelaR, Uruguay.

Acknowledgments

The authors thank Pablo Fontes, Andrei Assanelli, Nicola Di Muro, and Daniel Arana for their assistance throughout the field experiment and laboratory work. We also thank the EEMAC staff for their field assistance and collaboration during the entire period of this investigation work (2014-2017). Finally, we thank Dra. (Ing. Agr.) Amabelia del Pino for careful and extensive revision, guiding data analysis, and comprehensive feedback on the work.

Supplementary Material

Supplementary Materials: Table S1. ANOVA results (p-values) based on the segmented model by rotation system: continuous cropping (CC) and crop-pasture (CP), for the effects of year on nitrate-N at planting and tillering, grain yield (WGY), grain protein concentration (GPC) and N content in the straw of wheat crops cultivated in non-fertilized control plots; Table S2. Ammonium–N, Nitrate–N, and Mineral–N at planting of the succeeding summer crop corresponding to Soybean 1 as affected by experimental year (December 2015 and 2016), based on the segmented model by rotation system: continuous cropping (CC) and crop-pasture (CP). Values are means ± standard error; Table S3. Means and standard errors for soil organic carbon (SOC) and total N concentration in systems under NT as a function of depth in the three experimental years (2014-2016) in continuous cropping (CC) and crop-pasture (CP) rotation systems.

2.6. References

Allen, B. L., Lenssen, A. W., Sainju, U. M., Jabro, J. D., and Stevens, W. B. (2021). Nitrogen use in spring wheat affected by crop diversification, management, and tillage. *Agron. J.* 113, 2437–2449. doi: 10.1002/agj2.20686

Alvarez, S., and Ernst, O. (2024). Impact of cropping systems on soil quality. *Eur. J. Agron.* 158. doi: 10.1016/j.eja.2024.127197

Arnhold, J., Grunwald, D., Braun-Kiewnick, A., and Koch, H. J. (2023). Effect of crop rotational position and nitrogen supply on root development and yield formation of winter wheat. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1265994

Baethgen, W. E., Parton, W. J., Rubio, V., Kelly, R. H., and M. Lutz, S. (2021). Ecosystem dynamics of crop–pasture rotations in a fifty-year field experiment in southern South America: Century model and field results. *Soil Sci. Soc. Am. J.* 85, 423–437. doi: 10.1002/saj2.20204

Baiyeri, P. K., Foleng, H. N., Machebe, N. S., and Nwobodo, C. E. (2019). "Crop-livestock interaction for sustainable agriculture," in *Innovations in Sustainable Agriculture*, 557–582. doi: 10.1007/978-3-030-23169-9_18

Barbazán, M., Bautes, C., Beux, L., Bordoli, M., Cano, J. D., Ernst, O., et al. (2011). Fertilización potásica en cultivos de secano sin laboreo en Uruguay: rendimiento según análisis de suelos. *Agrociencia* 15, 93–99. doi: 10.31285/agro.15.597

Barreto, P., Ernst, O., Pérez Bidegain, M., and Perdomo, C. (2022). Effects of grazing, rotation, and tillage on surface runoff in a heavy textured Uruguayan soil. *Soil Sci. Soc. Am. J.* 86, 1096–1112. doi: 10.1002/saj2.20427

Bedoussac, L., and Justes, E. (2010). The efficiency of a durum wheat-winter pea intercrop to improve yield and wheat grain protein concentration depends on N availability during early growth. *Plant Soil* 330, 19–35. doi: 10.1007/s11104-009-0082-2

Bell, L. W., Kirkegaard, J. A., Swan, A., Hunt, J. R., Huth, N. I., and Fettell, N. A. (2011). Impacts of soil damage by grazing livestock on crop productivity. *Soil Tillage Res.* 113, 19–29. doi: 10.1016/j.still.2011.02.003

Bodner, G., Mentler, A., and Keiblinger, K. (2021). “Plant Roots for Sustainable Soil Structure Management in Cropping Systems,” in *The Root Systems in Sustainable Agricultural Intensification*, 45–90. doi: 10.1002/9781119525417.ch3

Boldrin, D., Knappett, J. A., Leung, A. K., Brown, J. L., Loades, K. W., and Bengough, A. G. (2022). Modifying soil properties with herbaceous plants for natural flood risk-reduction. *Ecol. Eng.* 180. doi: 10.1016/j.ecoleng.2022.106668

Booth, M. S., Stark, J. M., and Rastetter, E. (2005). Controls on nitrogen cycling in terrestrial ecosystems: A synthetic analysis of literature data. *Ecol. Monogr.* 75, 139–157. doi: 10.1890/04-0988

Carvalho, P. C. de F., Peterson, C. A., Nunes, P. A. de A., Martins, A. P., Filho, W. de S., Bertolazi, V. T., et al. (2018). Animal production and soil characteristics from integrated crop-livestock systems: Toward sustainable intensification. *J. Anim. Sci.* 96, 3513–3525. doi: 10.1093/jas/sky085

Cassman, K. G., and Grassini, P. (2020). A global perspective on sustainable intensification research. *Nat. Sustain.* 3, 262–268. doi: 10.1038/s41893-020-0507-8

Chen, S., Guo, J., Guo, R., Huang, B., Huang, J., Wang, M., et al. (2024). Deciphering the active bacteria involving glucose-triggered priming effect in soils with gradient N inputs. *Soil Biol. Biochem.* 199. doi: 10.1016/j.soilbio.2024.109612

Colombi, T., and Keller, T. (2019). Developing strategies to recover crop productivity after soil compaction—A plant eco-physiological perspective. *Soil Tillage Res.* 191, 156–161. doi: 10.1016/j.still.2019.04.008

Compton, J. E., and Boone, R. D. (2002). Soil nitrogen transformations and the role of light fraction organic matter in forest soils. *Soil Biol. Biochem.* 34, 933–943. doi: 10.1016/S0038-0717(02)00025-1

Congreves, K. A., Otchere, O., Ferland, D., Farzadfar, S., Williams, S., and Arcand, M. M. (2021). Nitrogen Use Efficiency Definitions of Today and Tomorrow. *Front. Plant Sci.* 12. doi: 10.3389/fpls.2021.637108

Cotrufo, M. F., and Lavallee, J. M. (2022). Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. Adv. Agron. 172, 1–66. doi: 10.1016/bs.agron.2021.11.002

Dang, Y. P., Dalal, R. C., and Menzies, N. W. (2020). *No-till Farming Systems for Sustainable Agriculture: Challenges and Opportunities*., Yash P. Da. Springer. doi: 10.1007/978-3-030-46409-7

Ding, J., Li, F., Le, T., Wu, P., Zhu, M., Li, C., et al. (2020). Nitrogen management strategies of tillage and no-tillage wheat following rice in the yangtze river basin, China: Grain yield, grain protein, nitrogen efficiency, and economics. *Agronomy* 10. doi: 10.3390/agronomy10020155

Dong, F., and Zeng, W. (2024). Effects of Fall and Winter Cover Crops on Weed Suppression in the United States: A Meta-Analysis. *Sustain.* 16. doi: 10.3390/su16083192

Drewry, J. J., Cameron, K. C., and Buchan, G. D. (2008). Pasture yield and soil physical property responses to soil compaction from treading and grazing - A review. *Aust. J. Soil Res.* 46, 237–256. doi: 10.1071/SR07125

Dubeux, J. C. B., and Sollenberger, L. E. (2020). “Nutrient cycling in grazed pastures,” in *Management Strategies for Sustainable Cattle Production in Southern Pastures*, 59–75. doi: 10.1016/B978-0-12-814474-9.00004-9

Duru, M., Therond, O., Martin, G., Martin-Clouaire, R., Magne, M. A., Justes, E., et al. (2015). How to implement biodiversity-based agriculture to enhance ecosystem services: a review. *Agron. Sustain. Dev.* 35, 1259–1281. doi: 10.1007/s13593-015-0306-1

Ernst, O. R., Dogliotti, S., Cadenazzi, M., and Kemanian, A. R. (2018). Shifting crop-pasture rotations to no-till annual cropping reduces soil quality and wheat yield. *F. Crop. Res.* 217, 180–187. doi: 10.1016/j.fcr.2017.11.014

Ernst, O. R., Kemanian, A. R., Mazzilli, S., Siri-Prieto, G., and Dogliotti, S. (2020). The dos and don’ts of no-till continuous cropping: Evidence from wheat yield and nitrogen use efficiency. *F. Crop. Res.* 257. doi: 10.1016/j.fcr.2020.107934

FAO (2011). Save and grow A policymaker's guide to the sustainable intensification of smallholder crop production FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS Rome.

Fassana, C. N., Hoffman, E. M., Berger, A., and Ernst, O. (2022). Nitrogen nutrition index at GS 3.3 is an effective tool to adjust nitrogen required to reach attainable wheat yield. *Agrociencia Uruguay* 26. doi: 10.31285/AGRO.26.924

Fontaine, S., Abbadie, L., Aubert, M., Barot, S., Bloor, J. M. G., Derrien, D., et al. (2023). Plant–soil synchrony in nutrient cycles: Learning from ecosystems to design sustainable agrosystems. *Glob. Chang. Biol.* 30. doi: 10.1111/gcb.17034

Franzluebbers, A. J. (2013). Pursuing robust agroecosystem functioning through effective soil organic carbon management. *Carbon Manag.* 4, 43–56. doi: 10.4155/cmt.12.78

Franzluebbers, A. J., and Stuedemann, J. A. (2014). Crop and cattle production responses to tillage and cover crop management in an integrated crop-livestock system in the southeastern USA. *Eur. J. Agron.* 57, 62–70. doi: 10.1016/j.eja.2013.05.009

García-Préchac, F., Ernst, O., Siri-Prieto, G., and Terra, J. A. (2004). Integrating no-till into crop-pasture rotations in Uruguay. *Soil Tillage Res.* 77, 1–13. doi: 10.1016/j.still.2003.12.002

Gastal, F., Lemaire, G., Durand, J. L., and Louarn, G. (2015). "Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency," in *Crop Physiology: Applications for Genetic Improvement and Agronomy: Second Edition*, 161–206. doi: 10.1016/B978-0-12-417104-6.00008-X

Ghimire, D., Das, S., Mueller, N. D., Creech, C. F., Santra, D., Baenziger, P. S., et al. (2021). Effects of cultivars and nitrogen management on wheat grain yield and protein. *Agron. J.* 113, 4348–4368. doi: 10.1002/agj2.20836

Giordano, N., Sadras, V. O., and Lollato, R. P. (2023). Late-season nitrogen application increases grain protein concentration and is neutral for yield in wheat. A global meta-analysis. *F. Crop. Res.* 290. doi: 10.1016/j.fcr.2022.108740

Giuliani, L. M., Hallett, P. D., and Loades, K. W. (2024). Effects of soil structure complexity to root growth of plants with contrasting root architecture. *Soil Tillage Res.* 238. doi: 10.1016/j.still.2024.106023

Giunta, F., Mefleh, M., Pruneddu, G., and Motzo, R. (2021). Role of nitrogen uptake and grain number on the determination of grain nitrogen content in old durum wheat cultivars. *Agronomy* 11. doi: 10.3390/agronomy11010042

Grahmann, K., Rubio Dellepiane, V., Terra, J. A., and Quincke, J. A. (2020). Long-term observations in contrasting crop-pasture rotations over half a century: Statistical analysis of chemical soil properties and implications for soil sampling frequency. *Agric. Ecosyst. Environ.* 287. doi: 10.1016/j.agee.2019.106710

Grahmann, K., Verhulst, N., Buerkert, A., Ortiz-Monasterio, I., and Govaerts, B. (2013). Nitrogen use efficiency and optimization of nitrogen fertilization in conservation agriculture. *CAB Rev. Perspect. Agric. Vet. Sci. Nutr. Nat. Resour.* 8, 1–19. doi: 10.1079/PAVSNNR20138053

Grant, R. F., Dyck, M., and Puurveen, D. (2020). Nitrogen and phosphorus control carbon sequestration in agricultural ecosystems: Modelling carbon, nitrogen, and phosphorus balances at the breton plots with ecosys under historical and future climates1. *Can. J. Soil Sci.* 100, 408–429. doi: 10.1139/cjss-2019-0132

Griffiths, M., Delory, B. M., Jawahir, V., Wong, K. M., Bagnall, G. C., Dowd, T. G., et al. (2022). Optimisation of root traits to provide enhanced ecosystem services in agricultural systems: A focus on cover crops. *Plant Cell Environ.* 45, 751–770. doi: 10.1111/pce.14247

Habbib, H., Hirel, B., Verzeaux, J., Roger, D., Lacoux, J., Lea, P., et al. (2017). Investigating the combined effect of tillage, nitrogen fertilization and cover crops on nitrogen use efficiency in winter wheat. *Agronomy* 7. doi: 10.3390/agronomy7040066

Hallama, M., Pekrun, C., Lambers, H., and Kandeler, E. (2019). Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant Soil* 434, 7–45. doi: 10.1007/s11104-018-3810-7

Hamza, M. A., and Anderson, W. K. (2005). Soil compaction in cropping systems: A review of the nature, causes and possible solutions. *Soil Tillage Res.* 82, 121–145. doi: 10.1016/j.still.2004.08.009

Hatfield, J. L., and Beres, B. L. (2019). Yield Gaps in Wheat: Path to Enhancing Productivity. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.01603

Hausherr Lüder, R. M., Qin, R., Richner, W., Stamp, P., Streit, B., Herrera, J. M., et al. (2020). Small-scale variation in nitrogen use efficiency parameters in winter wheat as affected by N fertilization and tillage intensity. *Sustain.* 12. doi: 10.3390/su12093621

Herridge, D. F., Giller, K. E., Jensen, E. S., and Peoples, M. B. (2022). Quantifying country-to-global scale nitrogen fixation for grain legumes II. Coefficients, templates and estimates for soybean, groundnut and pulses. *Plant Soil* 474, 1–15. doi: 10.1007/s11104-021-05166-7

Hochman, Z., and Horan, H. (2018). Causes of wheat yield gaps and opportunities to advance the water-limited yield frontier in Australia. *F. Crop. Res.* 228, 20–30. doi: 10.1016/j.fcr.2018.08.023

Hu, S., Qiao, B., Yang, Y., Rees, R. M., Huang, W., Zou, J., et al. (2023). Optimizing nitrogen rates for synergistically achieving high yield and high nitrogen use efficiency with low environmental risks in wheat production – Evidences from a long-term experiment in the North China Plain. *Eur. J. Agron.* 142. doi: 10.1016/j.eja.2022.126681

Huang, N., Athmann, M., and Han, E. (2020). Biopore-induced deep root traits of two winter crops. *Agric.* 10, 1–16. doi: 10.3390/agriculture10120634

Lemaire, G., Tang, L., Bélanger, G., Zhu, Y., and Jeuffroy, M. H. (2021). Forward new paradigms for crop mineral nutrition and fertilization towards sustainable agriculture. *Eur. J. Agron.* 125. doi: 10.1016/j.eja.2021.126248

Li, B., Ma, X., Zhao, B., Wang, L., and Ata-Ul-Karim, S. T. (2023). In-season assessment of agronomic nitrogen use efficiency and its components in winter wheat using critical nitrogen dilution curve. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1128799

Liang, X., Yuan, J., Yang, E., and Meng, J. (2017). Responses of soil organic carbon decomposition and microbial community to the addition of plant residues with different C:N ratio. *Eur. J. Soil Biol.* 82, 50–55. doi: 10.1016/j.ejsobi.2017.08.005

Liang, Z., Rasmussen, J., Poeplau, C., and Elsgaard, L. (2023). Priming effects decrease with the quantity of cover crop residues – Potential implications for soil carbon sequestration. *Soil Biol. Biochem.* 184. doi: 10.1016/j.soilbio.2023.109110

Liu, J., Wang, Y., Li, Y., Peñuelas, J., Zhao, Y., Sardans, J., et al. (2023). Soil ecological stoichiometry synchronously regulates stream nitrogen and phosphorus concentrations and ratios. *Catena* 231. doi: 10.1016/j.catena.2023.107357

Locatelli Fagúndez, A. (2023). Genetic and phenotypic characterization of Tinopiro (Thinopyrum intermedium) as a dual-purpose perennial crop in the Southern Cone: Doctoral thesis abstract. *Agrociencia Uruguay* 27, e1463. doi: 10.31285/agro.27.1463

Lollato, R. P., Diaz, D. A. R., Dewolf, E., Knapp, M., Peterson, D. E., and Fritz, A. K. (2019a). Agronomic practices for reducing wheat yield gaps: A quantitative appraisal of progressive producers. *Crop Sci.* 59, 333–350. doi: 10.2135/cropsci2018.04.0249

Lollato, R. P., Figueiredo, B. M., Dhillon, J. S., Arnall, D. B., and Raun, W. R. (2019b). Wheat grain yield and grain-nitrogen relationships as affected by N, P, and K fertilization: A synthesis of long-term experiments. *F. Crop. Res.* 236, 42–57. doi: 10.1016/j.fcr.2019.03.005

Longepierre, M., Feola Conz, R., Barthel, M., Bru, D., Philippot, L., Six, J., et al. (2022). Mixed Effects of Soil Compaction on the Nitrogen Cycle Under Pea and Wheat. *Front. Microbiol.* 12. doi: 10.3389/fmicb.2021.822487

Lussich Rachetti, F. (2020). Variabilidad de la fijación biológica de nitrógeno de leguminosas forrajeras en Uruguay : posibles causas y consecuencias nutricionales. 1–85.

Ma, G., Liu, W., Li, S., Zhang, P., Wang, C., Lu, H., et al. (2019). Determining the optimal N input to improve grain yield and quality in winter wheat with reduced apparent N loss in the north China plain. *Front. Plant Sci.* 10. doi: 10.3389/fpls.2019.00181

Mălinaş, A., Vidican, R., Rotar, I., Mălinaş, C., Moldovan, C. M., and Proorocu, M. (2022). Current Status and Future Prospective for Nitrogen Use Efficiency in Wheat (Triticum aestivum L.). *Plants* 11. doi: 10.3390/plants11020217

Mazzilli, S. R., Kemanian, A. R., Ernst, O. R., Jackson, R. B., and Piñeiro, G. (2015). Greater humification of belowground than aboveground biomass carbon into particulate soil organic matter in no-till corn and soybean crops. *Soil Biol. Biochem.* 85, 22–30. doi: 10.1016/j.soilbio.2015.02.014

Mori Alvez, C., Perdomo Varela, C., González Barrios, P., Bentos Guimaraes, A., and del Pino Machado, A. (2024). Lupine Cultivation Affects Soil’s P Availability and Nutrient Uptake in Four Contrasting Soils. *Agronomy* 14. doi: 10.3390/agronomy14020389

Mosier, S., Apfelbaum, S., Byck, P., Calderon, F., Teague, R., Thompson, R., et al. (2021). Adaptive multi-paddock grazing enhances soil carbon and nitrogen stocks and stabilization through mineral association in southeastern U.S. grazing lands. *J. Environ. Manage.* 288. doi: 10.1016/j.jenvman.2021.112409

Mulvaney, R. L. (2018). “Nitrogen-inorganic forms,” in *Methods of Soil Analysis, Part 3: Chemical Methods*, 1123–1184. doi: 10.2136/sssabookser5.3.c38

Novelli, L. E., Caviglia, O. P., and Piñeiro, G. (2017). Increased cropping intensity improves crop residue inputs to the soil and aggregate-associated soil organic carbon stocks. *Soil Tillage Res.* 165, 128–136. doi: 10.1016/j.still.2016.08.008

Nurbekov, A., Kosimov, M., Islamov, S., Khaitov, B., Qodirova, D., Yuldasheva, Z., et al. (2024). No-till, crop residue management and winter wheat-based crop rotation strategies under rainfed environment. *Front. Agron.* 6. doi: 10.3389/fagro.2024.1453976

Page, K. L., Dang, Y. P., and Dalal, R. C. (2020). The Ability of Conservation Agriculture to Conserve Soil Organic Carbon and the Subsequent Impact on Soil

Physical, Chemical, and Biological Properties and Yield. *Front. Sustain. Food Syst.* 4. doi: 10.3389/fsufs.2020.00031

Pañella, P. G., Guido, A., Jaurena, M., Cardozo, G., and Lezama, F. (2022). Fertilization and overseeding legumes on native grasslands leads to a hardly reversible degraded state. *Appl. Veg. Sci.* 25. doi: 10.1111/avsc.12693

Peoples, M. B., Hauggaard-Nielsen, H., and Jensen, E. S. (2015). "The potential environmental benefits and risks derived from legumes in rotations," in *Nitrogen Fixation in Crop Production*, (wiley), 349–385. doi: 10.2134/agronmonogr52.c13

Peterson, C. A., Deiss, L., and Gaudin, A. C. M. (2020). Commercial integrated crop-livestock systems achieve comparable crop yields to specialized production systems: A meta-analysis. *PLoS One* 15. doi: 10.1371/journal.pone.0231840

Pinto, P., Rubio, G., Gutiérrez, F., Sawchik, J., Arana, S., and Piñeiro, G. (2021). Variable root:shoot ratios and plant nitrogen concentrations discourage using just aboveground biomass to select legume service crops. *Plant Soil* 463, 347–358. doi: 10.1007/s11104-021-04916-x

Pittelkow, C. M., Liang, X., Linquist, B. A., Van Groenigen, L. J., Lee, J., Lundy, M. E., et al. (2015a). Productivity limits and potentials of the principles of conservation agriculture. *Nature* 517, 365–368. doi: 10.1038/nature13809

Pittelkow, C. M., Linquist, B. A., Lundy, M. E., Liang, X., van Groenigen, K. J., Lee, J., et al. (2015b). When does no-till yield more? A global meta-analysis. *F. Crop. Res.* 183, 156–168. doi: 10.1016/j.fcr.2015.07.020

Pravia, M. V., Kemanian, A. R., Terra, J. A., Shi, Y., Macedo, I., and Goslee, S. (2019). Soil carbon saturation, productivity, and carbon and nitrogen cycling in crop-pasture rotations. *Agric. Syst.* 171, 13–22. doi: 10.1016/j.agsy.2018.11.001

Quemada, M., and Lassaletta, L. (2024). Fertilizer dependency: a new indicator for assessing the sustainability of agrosystems beyond nitrogen use efficiency. *Agron. Sustain. Dev.* 44. doi: 10.1007/s13593-024-00978-0

Rabuffetti, A. (2017). La fertilidad del suelo y su manejo. *Montevideo Hemisferio Sur*.

Rayment, G. E., and Lyons, D. J. (2011). *Soil_Chemical_Methods*. CSIRO publishing,.

Rhine, E. D., Mulvaney, R. L., Pratt, E. J., and Sims, G. K. (1998). Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. *Soil Sci. Soc. Am. J.* 62, 473–480. doi: 10.2136/sssaj1998.03615995006200020026x

Romano, I., Bodenhausen, N., Basch, G., Soares, M., Faist, H., Trognitz, F., et al. (2023). Impact of conservation tillage on wheat performance and its microbiome. *Front. Plant Sci.* 14. doi: 10.3389/fpls.2023.1211758

Rovira, P., Ayala, W., Terra, J., García-Préchac, F., Harris, P., Lee, M. R. F., et al. (2020). The 'Palo a Pique' long-term research platform: First 25 years of a crop–livestock experiment in Uruguay. *Agronomy* 10. doi: 10.3390/agronomy10030441

Rubio, V., Diaz-Rossello, R., Quincke, J. A., and van Es, H. M. (2021a). Quantifying soil organic carbon's critical role in cereal productivity losses under annualized crop rotations. *Agric. Ecosyst. Environ.* 321. doi: 10.1016/j.agee.2021.107607

Rubio, V., Núñez, A., Berger, A., and van Es, H. (2025). Biomass inputs drive agronomic management impacts on soil health. *Agric. Ecosyst. Environ.* 378. doi: 10.1016/j.agee.2024.109316

Rubio, V., Quincke, A., and Ernst, O. (2021b). Deep tillage and nitrogen do not remediate cumulative soil deterioration effects of continuous cropping. *Agron. J.* 113, 5584–5596. doi: 10.1002/agj2.20927

Rubio, V., Sawchik, J., and van Es, H. (2022). Soil health benefits from sequence intensification, fertilization, and no-tillage in annual cropping systems. *Soil Secur.* 9. doi: 10.1016/j.soisec.2022.100074

Salvo, L., Hernández, J., and Ernst, O. (2010). Distribution of soil organic carbon in different size fractions, under pasture and crop rotations with conventional tillage and no-till systems. *Soil Tillage Res.* 109, 116–122. doi: 10.1016/j.still.2010.05.008

Shaheb, M. R., Venkatesh, R., and Shearer, S. A. (2021). A Review on the Effect of Soil Compaction and its Management for Sustainable Crop Production. *J. Biosyst. Eng.* 46, 417–439. doi: 10.1007/s42853-021-00117-7

Sieling, K., and Kage, H. (2021). Apparent fertilizer N recovery and the relationship between grain yield and grain protein concentration of different winter wheat varieties in a long-term field trial. *Eur. J. Agron.* 124. doi: 10.1016/j.eja.2021.126246

Stanley, P. L., Wilson, C., Patterson, E., Machmuller, M., and Cotrufo, M. F. (2024). Ruminating on soil carbon: Applying current understanding to inform grazing management. *Glob. Chang. Biol.* 30. doi: 10.1111/gcb.17223

Sun, L., Xia, Z., Sang, C., Wang, X., Peng, B., Wang, C., et al. (2019). Soil resource status affects the responses of nitrogen processes to changes in temperature and moisture. *Biol. Fertil. Soils* 55, 629–641. doi: 10.1007/s00374-019-01379-2

Taylor, K. M., Nelsen, T. S., Scow, K. M., and Lundy, M. E. (2024). No-till annual wheat increases plant productivity, soil microbial biomass, and soil carbon stabilization relative to intermediate wheatgrass in a Mediterranean climate. *Soil Tillage Res.* 235. doi: 10.1016/j.still.2023.105874

Teague, R., and Kreuter, U. (2020). Managing Grazing to Restore Soil Health, Ecosystem Function, and Ecosystem Services. *Front. Sustain. Food Syst.* 4. doi: 10.3389/fsufs.2020.534187

Walkup, J., Freedman, Z., Kotcon, J., and Morrissey, E. M. (2020). Pasture in crop rotations influences microbial biodiversity and function reducing the potential for nitrogen loss from compost. *Agric. Ecosyst. Environ.* 304. doi: 10.1016/j.agee.2020.107122

Wang, Y., Peng, Y., Lin, J., Wang, L., Jia, Z., and Zhang, R. (2023a). Optimal nitrogen management to achieve high wheat grain yield, grain protein content, and water productivity: A meta-analysis. *Agric. Water Manag.* 290. doi: 10.1016/j.agwat.2023.108587

Wang, Y., Wu, P., Qiao, Y., Li, Y., Liu, S., Gao, C., et al. (2023b). The potential for soil C sequestration and N fixation under different planting patterns depends on the carbon and nitrogen content and stability of soil aggregates. *Sci. Total Environ.* 897. doi: 10.1016/j.scitotenv.2023.165430

Yin, X., Song, Z., Shi, S., Bai, Z., Jiang, Y., Zheng, A., et al. (2024). Developments and prospects of multiple cropping in China. *Farming Syst.* 2. doi: 10.1016/j.farsys.2024.100083

Zhou, H., Whalley, W. R., Hawkesford, M. J., Ashton, R. W., Atkinson, B., Atkinson, J. A., et al. (2021). The interaction between wheat roots and soil pores in structured field soil. *J. Exp. Bot.* 72, 747–756. doi: 10.1093/jxb/eraa475

Supplementary Material

Table S1. ANOVA results (p-values) based on the segmented model by rotation system: continuous cropping (CC) and crop-pasture (CP), for the effects of year on nitrate-N at planting and tillering, grain yield (WGY), grain protein concentration (GPC) and N content in the straw of wheat crops cultivated in non-fertilized control plots.

Variables	Rotation system	
	CC	CP
Nitrate-N at planting	n.s.	0.0243
Nitrate-N at tillering	0.0196	< 0.0001
WGY	n.s.	n.s.
GPC	0.0001	<0.0001
N content in straw	n.s.	n.s.

Significant effects at $p < 0.05$. n.s: means no significant

Table S2. Ammonium–N, Nitrate–N, and Mineral–N at planting of the succeeding summer crop corresponding to Soybean 1 as affected by experimental year (December 2015 and 2016), based on the segmented model by rotation system: continuous cropping (CC) and crop-pasture (CP). Values are means ± standard error.

Rotation system	year	Ammonium–N	Nitrate–N	Mineral–N
		mg kg^{-1}		
CC	2015	11.5 ± 0.9	8.0 ± 0.1	19.5 ± 0.9
	2016	12.3 ± 1.2	7.1 ± 0.8	19.4 ± 1.0
	p-value	n.s	n.s	n.s
CP	2015	15.2 ± 1.7	8.7 ± 2.1	23.9 ± 3.8
	2016	16.0 ± 1.0	9.1 ± 1.0	25.1 ± 1.5
	p-value	n.s	n.s	n.s

Significant at a p-level of 0.05.; n.s means no significant

Table S3. Means and standard errors for soil organic carbon (SOC) and total N concentration in systems under NT as a function of depth in the three experimental years (2014-2016) in continuous cropping (CC) and crop-pasture (CP) rotation systems.

Year	Rotation system	C	N	C	N	C	N
		Soil depth					
		0-5		5-10		10-15	
		g kg^{-1}					
2014	CC	21.8±0.55	1.8±0.13	19.6±0.47	1.7±0.04	16.5±0.74	1.4±0.03
2015		25.4±0.52	2.2±0.06	16.7±0.63	1.3±0.05	18.6±0.27	1.5±0.03
2016		25.6±0.90	2.1±0.07	18.2±0.31	1.5±0.04	17.5±0.42	1.4±0.04
2014	CP	23.4±1.07	2.0±0.12	20.0±1.03	1.6±0.07	21.9±0.90	1.7±0.11
2015		24.8±1.59	2.2±0.19	16.9±0.65	1.4±0.04	17.7±0.45	1.5±0.05
2016		22.8±0.68	2.0±0.07	14.5±0.39	1.2±0.03	16.2±0.62	1.4±0.05

3. Nitrogen Residual Effect of Winter Cover Crops on Maize in Uruguay: Conventional and Isotopic Evaluation

This article belongs to the Special Issue Benefits and Challenges of Cover Crops in Agricultural Systems

El estudio se llevó a cabo en campos agrícolas en dos zonas agroecológicas de Uruguay, durante dos ciclos agrícolas, lo que permitió analizar el efecto residual de los cultivos de cobertura (CC) en la producción de maíz y la eficiencia de uso del nitrógeno (NUE) bajo diferentes condiciones. Se exploró el impacto de cuatro cultivos de cobertura (CC) de invierno en la dinámica del N en el suelo, el rendimiento del maíz subsiguiente y la eficiencia en el uso del N (EUN). Los cultivos evaluados fueron dos leguminosas (*Lupinus angustifolius* L. y *Trifolium alexandrinum* L.), una gramínea (*Avena strigos*a L.) y un cultivo mixto (lupino-avena), junto con un tratamiento control sin CC.

Principales resultados:

El estudio mostró que la producción de biomasa de los cultivos de cobertura varió según el sitio, con mayor rendimiento de la avena en monocultivo en el sitio 1 y una mayor productividad de la mezcla avena-lupino en el sitio 2. Además, la cantidad y composición de los residuos de los cultivos de cobertura afectaron la dinámica del nitrógeno en el suelo, influyendo en la eficiencia de recuperación del N y en el rendimiento del maíz, con los mayores valores de apNREFert asociados a la avena en monocultivo en el sitio 1 y a la mezcla lupino-avena en el sitio 2.

El rendimiento del maíz se relacionó con la cantidad y calidad de la biomasa residual de los CC. La relación C:N de los residuos tuvo un efecto importante en la eficiencia de uso del fertilizante N, lo que contribuye a mitigar las pérdidas potenciales de N.

La avena destacó en el control de malezas, el trébol alejandrino sobresalió en su capacidad de aporte de N y las mezclas de CC mostraron un buen desempeño en el sitio 2. El CC mezcla de avena-lupino, demostró aportar biomasa rica en C para la formación de materia orgánica del suelo (SOM) y un suministro de N comparable al trébol alejandrino, pero con mayor capacidad para reducir pérdidas de N gracias a la relación C:N más alta proporcionada por la avena.

En el sitio 1 (suelos de fertilidad media a baja y precipitaciones superiores al promedio): Los mejores resultados de rendimiento, eficiencia de uso del N y menor riesgo de pérdidas de N se lograron con maíz cultivado sobre trébol alejandrino sin fertilización o sobre avena fertilizada, que además resultó excelente para el control de malezas. En el sitio 2 (suelos más fértiles y precipitaciones por debajo del promedio): El maíz presentó mayor eficiencia de uso del N cuando se cultivó sobre lupino en monocultivo o en mezcla con avena, que además en este CC aportó mayor cantidad de C.

Conclusión: El impacto del cultivo antecesor sobre el maíz estuvo determinado por la cantidad y calidad de la biomasa residual de los CC, las características inherentes del suelo o de manejo en cada sitio y las condiciones ambientales durante el período experimental. Esto resalta la importancia de adaptar la elección de los CC a las condiciones locales para maximizar los beneficios de esta práctica en los sistemas agrícolas.

agriculture

Article

Nitrogen Residual Effect of Winter Cover Crops on Maize in Uruguay: Conventional and Isotopic Evaluation

Cristina Mori Alvez *, Carlos Perdomo Varela and Amabelia del Pino Machado

Soil and Water Department, Agronomy College, University of the Republic,
Av. Garzon 780, Montevideo CP 12900, Uruguay; chperdom@fagro.edu.uy (C.P.V.);
amabelia@fagro.edu.uy (A.d.P.M.)
* Correspondence: cristinamori@fagro.edu.uy

Abstract: This study aimed to evaluate the nitrogen (N) residual effects of winter cover crops (CCs) on soil N availability and corn (*Zea mays* L.) performance over two growing seasons and at two sites in Uruguay. Both conventional and isotopic methods were used to assess the N residual effects of two legume monocultures, a legume–grass mixture, an oat monoculture, and a control without CCs. The experimental design was a randomized block with split plots, where CCs were applied to main plots and N rates (0 and 100 kg ha^{-1}) to subplots. An isotopic trial with ^{15}N was included to measure fertilizer N use efficiency (NUE). Results varied between sites: at Site 1, legume monocultures enhanced soil N availability and, along with N rate, significantly increased corn yield and N uptake. At Site 2, only the N rate affected these variables. Site 1 had a low crop ^{15}N recovery, averaging 9.5% due to weeds and heavy rainfall, while Site 2 showed higher recovery, notably when corn succeeded lupine (35%) and mixture CCs (40%). The soil's top layer and corn grain showed the highest ^{15}N concentration. The study suggests that specific CC combinations tailored to site conditions may optimize corn yield and NUE.

Keywords: legume cover crops; nitrogen use efficiency; residue quality; N residuality; sustainable agriculture

3.1. Introduction

Rainfed agricultural systems have experienced significant shifts in Uruguay since the early 2000s [1,2]. At the time, introducing new actors in the sector led to a simplified approach to system management, primarily marked by cropping sequences such as wheat–soybean and a reduction in perennial pastures inclusion into agricultural rotations. This shift extended the annual cropping phase and facilitated the expansion of farming activities into soils less suited for agriculture [1,3]. However, these changes have gradually negatively impacted soil nitrogen (N) balance and carbon (C) sequestration, affecting soil quality and the system's sustainability [3–5]. Simulation results for three agricultural production systems indicated that continuous cropping leads to low and negative partial N balances, with values ranging from −40 to −30 kg N ha^{-1} [5]. Since 2015, livestock and crop production have increasingly been managed as part of a unified farming system, fostering greater integration and interconnection [1]. Such current management has reintroduced pasture phases and expanded the presence of double cropping to enhance crop diversity and utilization efficiency of production factors while protecting natural resources [6–8]. This shift has promoted an eco-sustainable approach, driven mainly by implementing Uruguay's legal framework (Ley 15.239, Decreto 333/04 and Ley 18.564) for regulating land use and soil management [9], which has played a crucial role in this transformation. In this context, the sequence and type of crops in rotation, along with the amount of remaining residue (both aboveground and belowground), are critical factors in maintaining the balance of C [10], N [11], and other soil nutrients. Including CCs, also known as "service crops," in crop rotations is essential for sustainable agriculture, as they enhance soil health and contribute to a more balanced and resilient agricultural system. Due to their role in erosion prevention, this practice also aligns with Uruguay's legal framework, which requires the submission of Soil Use and Conservation Plans (Ley

15.239, Decreto 333/04 and Ley 18.564). Cover crops are essential for controlling water erosion, improving the soil's physical properties, and conserving and recycling nutrients [12,13]. Therefore, it is crucial to understand and assess different CC options to select those most suitable for specific soil and climate conditions, offering advantages within a given rotation scheme [14,15]. This technological measure requires knowledge of the agronomic performance of various species across different soils, years, and production systems to identify those that provide greater productive, economic, and environmental benefits [16–18]. The benefits of using legumes as CCs include N inputs through biological N fixation (BNF), improved soil quality and fertility, increased crop productivity, C sequestration, and protein-rich grain and forage for animal feed. The role of CCs in mitigating N losses is particularly important in today's agricultural systems, where intensification demands more nutrients, increasing fertilizer use, and raising the risk of nitrate-N contamination in surface and groundwater [19,20]. This agronomic practice can help mitigate N losses, increase soil N reserves, and improve nutrient use efficiency [16,19–21].

The N recovery efficiency from crop residues depends on the synchronization between N supply, fertilizer management, and the crop's demand for the nutrient. According to several studies [11,22–25], the recovery efficiencies of N fixed by legume cover crops were relatively inconsistent, ranging from 3% to 56%. Nitrogen recovery efficiency (NRE) can be estimated using conventional or isotopic trial data. In conventional trials, apparent NRE (apNRE) is calculated. In isotopic trials, ^{15}NRE is directly measured by the ratio of ^{15}N absorbed by the crop to the amount of N applied. The term "apparent" is used because the N recovery is indirectly estimated through plant analysis. This method assumes that the soil supplies the same amount of N to both treatments, with and without residue, attributing the difference in N absorption between the two to the N derived from the residue [11].

Several legume cover crop species and their subsequent use as green manure has been evaluated in Uruguay under both tilled and no-till systems [11,26,27]. However, more research on using CCs in Uruguayan agricultural production is needed, emphasizing the importance of developing soil and nutrient management strategies that enhance sustainability and reduce environmental pollution. In this context, CCs present a viable option to minimize the degenerative effects caused by continuous no-till farming and soybean monoculture [28]. Understanding and quantifying the effects of CCs is especially important now, as fertilizer use significantly impacts production systems' profitability and poses environmental risks, a growing concern for society.

Our study was driven by the practical implications of understanding how CCs can influence soil fertility for the subsequent cash crop. The N provided by CC biomass can be a valuable resource for the next crop in the short term and, in the long term, can contribute to increasing soil N content and reducing fertilization costs. However, the soil N availability is not straightforward, as it depends on residual N (mineral N already present in the soil) and mineralized N (released from soil organic matter and previous crop residues). The dynamics of CC decomposition, influenced by environmental and management factors, make it challenging to consistently and accurately predict the amount of N available and when it will be accessible to the following crop. The studied winter CCs included a grain legume, blue lupine (*Lupinus angustifolius* L.), a forage legume, berseem clover (*Trifolium alexandrinum* L.), a grass, black oats (*Avena strigosa* L.), and blue lupine grown in mixture with black oats (L-O mixture). The objectives of this study were (i) to assess the effect of those CCs on N availability derived from the mineralization of their residues by measuring changes in soil mineral N; (ii) to evaluate the impact of CCs as predecessor crops on the performance of a maize crop measuring grain yield, N uptake, and the relative response in plant N uptake to N fertilization, and estimating N recovery in the crop using a conventional method; and (iii) to analyze how the residue quality (C:N ratio, biochemical composition) of the preceding crop affects N use efficiency (NUE) by measuring N recovery in the crop and soil from fertilizer applied at sowing and other stages (V6 and V10) of the corn crop using isotopic methods.

3.2. Materials and Methods

3.2.1. Experimental Sites Description

The experiments for this study were conducted in farmers' fields. The first site was established in Libertad, San José Department, at 34°36′27.5″ S, 56°32′57.4″ W

(Site 1) during the 2018/2019 growing season. The second site was set up in Ombúes de Lavalle, Colonia Department, at 33°58′56.9″ S, 57°50′57.1″ W (Site 2) during the 2019/2020 season. According to the local soil classification, soil in both sites was classified as Li1 (Libertad) unit. The parent material in Site 1 was silty clay sediments, while for Site 2, it was a combination of silty clay sediments and crystalline rock. According to the USDA International Soil Taxonomy, the soils at Site 1 were classified as Typic Argiaquolls and Site 2 as Typic Argiudolls [29]. Both suborders belong to the Mollisol order and share characteristics such as an argillic horizon (Bt horizon) with clay accumulation and a surface layer rich in organic matter (OM). However, they differ in their moisture regimes, which influence their physical and chemical properties: Argiudolls have a udic moisture regime, while Argiaquolls have an aquic regime [29,30].

In the top 20 cm, Site 1 soil had lower total organic C (TOC) content, exchangeable bases, and pH than Site 2 (Table 1). The soil texture in both studied layers (the top 7 cm and from 7 to 15 cm) was heavier at Site 2, which also had a higher C content (Table 2) and lower bulk density (1.2 vs. 1.4 Mg m^3).

Table 1. Chemical characterization of the soils (at 0–20 cm soil depth) at each experimental site (Site 1—season 2018–2019 and Site 2—season 2019–2020).

Site	P Bray N°1	Ca	Mg	K	Na	pH
	mg kg^{-1}	cmol$_c$kg^{-1}				
1	18.0	5.9	1.9	0.5	0.4	4.7
2	9.2	16.0	4.7	1.0	0.2	5.6

Table 2. Total organic C and N content in the soil and texture in 0–7 and 7–15 cm soil depth layers at each experimental site (Site 1—season 2018–2019 and Site 2—season 2019–2020).

Site	Soil Depth	TOC	TN	Sand	Clay	Silt	Texture
	cm	%					
1	0–7	3.0	0.3	8.3	35.5	56.1	Silty clay loam
1	7–15	1.7	0.2	7.6	38.8	53.6	Silty clay loam

2	0–7	3.3	0.3	8.8	53.6	37.6	Clayey
2	7–15	2.2	0.2	8.0	55.0	37.0	Clayey

Meteorological data, including rainfall and air temperature, were collected on-site by a Meter-Group weather station, which recorded hourly data. This station was installed during Phase 2 of the experiment, during the growth period of the corn crop at both sites.

3.2.2. Treatments and Experimental Design

The experiment was conducted on soybean crop stubble at both sites. The activities at each site were carried out in two phases (Supplementary Materials, Table S1).

In Phase 1, CCs were established, and the biomass production and BNF rates of the legume cover crops were estimated. The species sown at both sites included two legume CCs—*Lupinus angustifolius* L. and *Trifolium alexandrinum* L.—a non-legume species as a reference (*Avena strigosa* L.), and L-O mixture culture in a 60:40 ratio in Site 1 and 70:30 ratio in Site 2. The experimental design for this phase followed a randomized complete block design with three replications. Each experimental unit covered an area of 380 m^2 (10 m wide × 38 m long), resulting in a total area of 0.57 ha across 15 plots.

The CCs were sown using a seed-drill Semeato brand model SHM 17-13-Germany (with 13 rows and 17 cm row spacing). The sowing density for each species is presented in Supplementary materials, Table S1. The legumes were inoculated with commercially recommended inoculants specific to each species, following the manufacturer’s instructions (NITRASEC, Lage y Cía S.A., Uruguay). Since no particular inoculant was available for lupine, *Bradyrhizobium* sp. strains U-612 + U-620, typically recommended for *Ornithopus compressus*, were used. After soil sampling simultaneously with sowing, base fertilization was applied across the entire experimental area with non-limiting doses of phosphorus (P) and potassium (K).

The CCs at Site 1 were sown earlier, within the optimal sowing window, compared to Site 2. This difference in sowing dates resulted in a total growth cycle of

164 days at Site 1, while Site 2 had a shorter cycle of 119 days, giving Site 1 an additional 45 days for biomass production.

Phase 2 began with the corn sowing in the same experimental units where the winter CCs had been grown 60 days after terminating them with herbicide at Site 1 and 58 days after herbicide application at Site 2. Corn was sown using no-till planting with a mechanical plate planter at a depth of 3.5 cm, with 0.7 m row spacing and 0.2 m between plants, aiming for a density of 65,000–70,000 plants ha^{-1} [31].

At this phase, the main plots were divided to introduce an additional study factor: N rate. As a result, the experimental design for this phase followed a randomized complete block design with a split-plot arrangement and three replications. The main plots (33 m × 21 m) were randomly assigned one of five "preceding crop" treatments: (1) lupine, (2) lupine grown in mixture with black oat, (3) black oat, (4) berseem clover, and (5) no CC (control). The subplots (6.6 m ×10.5 m) were assigned the "N rate" factor for maize cultivation with two levels: (1) 0 kg N ha^{-1} and (2) 100 kg N ha^{-1}. The N rate was applied to the maize crop in two stages: one-third at the time of seeding and the remaining two-thirds when the maize reached the phenological stage, equivalent to V6 at Site 1 and V10 at Site 2, according to the Ritchie and Hanway growth scale (see Supplementary Materials, Table S2) [32].

Alongside the conventional experiment, an isotopic trial was carried out to evaluate the fertilizer's NUE, measuring the recovery ^{15}N in both the plant and soil. Isotopic microplots were carefully established within each subplot of the conventional trial, with dimensions of 2.25 m^2 (1.5 m wide and 1.5 m long). Each microplot contained three rows, each with seven plants. Urea enriched with ^{15}N at 6% and 3% atom excess was applied at the same phenological stages of maize and using the same N rate as in the fertilized treatment of the conventional trial but split across two applications in the corn growth cycle, with only one application using labeled urea (Supplementary Materials, Table S2). The labeled urea was dissolved in distilled water (300 mL per microplot) and sprayed to the soil to ensure uniform ^{15}N distribution. This isotopic trial allowed us to independently evaluate NUE from the fertilizer at each application time without interacting with the N rate used [33]. The experimental design followed the same structure as the conventional experiment, with three replications.

3.2.3. Soil and Plant Sampling

Soil sampling was conducted at both experimental sites at the start of the experiment in Phase 1 to characterize the soils. Composite soil samples were obtained from each plot at 0 to 20 cm depth to assess mineral N concentration. Additionally, samples were collected from the 0–7 cm and 7–15 cm layers to evaluate physical properties such as texture and bulk density.

In Phase 1, soil sampling was performed at three points in time: sowing, harvest, and post-harvest of the CCs to evaluate soil N concentration. Additionally, three sampling times were implemented for soil water content determination using the gravimetric method [34], recording the difference between the weight of soil at field moisture and the weight after drying at 105 °C in an oven for 48 h until a constant weight (Equation (1)).

$$\text{Soil water content (g water g dry soil}^{-1}\text{)} = \frac{\text{soil wet weight } - \text{soil dry weight}}{\text{soil dry weight}} \qquad 1)$$

In Phase 2, soil sampling was conducted at two points in time: corn sowing at both sites and at the V6 in Site 1 and V10 stages of the corn in Site 2, to assess nitrate (NO_3-N) and ammonium (NH_4-N) concentrations. Fifteen subsamples were collected from each plot using a sharpened stainless-steel probe with a diameter of 2 cm. In Phase 2, soil sampling within the isotopic microplots, conducted after the corn harvest, was carefully carried out to avoid cross-contamination of ^{15}N between soil layers. The sampling was performed in two steps: first, the top 15 cm of the soil profile was collected and divided into three intervals (0–5, 5–10, and 10–15 cm) to quantify the remaining ^{15}N from fertilization (sowing and V6 stage). Next, a second sampling was obtained from the same hole, collecting the subsequent 15–30 cm soil depth layer. Each layer was processed and analyzed separately.

The first plant sampling was conducted during Phase 1, the day before the CC termination, defined by the lupine reaching the beginning of grain filling. The area harvested for each CC's aboveground biomass was 4.6 m^2 (1.15 m ×× 4 m), and the fresh weight of each sample was recorded in the field. Simultaneously, subsamples were taken to the laboratory to estimate the dry matter of each CC and to perform the relevant chemical analyses for this study.

In Phase 2, at physiological maturity and during the harvest of the corn from the conventional experiment, the corn grain yield was calculated based on the ears harvested from the two central rows of 8 m long. Another plant sampling was conducted at physiological maturity, cutting three rows of 1 m each at ground level. Each sample was separated into different components: stems, leaves, cobs, and grain. The grain from the ears was threshed using experimental equipment, and its moisture content was measured with a Model MC2000 moisture meter (OHAUS Europe GmbH, Switzerland). The grain yield was then corrected to a baseline moisture level of 14.5%. The total aboveground biomass yield per hectare was calculated based on the number of plants harvested and the planting spacing. Additionally, during Phase 2, corn plants were sampled at physiological maturity from the isotopic plots. Three corn plants were harvested from the central row of each microplot and separated into three components: stems–leaves, cobs, and grain, for independent processing and analysis. The first two components were referred to as stubble to distinguish them from the harvested product, which is the grain component.

3.2.4. Sample Processing and Analytical Determinations

3.2.4.1. Soil Samples from Conventional and Isotopic Experiments

Soil samples were dried in a forced-air oven at 40 °C for at least 48 h and were ground through a 2 mm sieve after removing any visible plant residue. The following properties were determined to characterize the soils of each experimental site: OM, pH, available phosphorus (PBray1), mineral N (nitrate and ammonium), exchangeable cations (Ca, Mg, K, and Na), and granulometric composition. The OM content was determined by the Walkley–Black method [35], while exchangeable cations Ca and Mg were determined by atomic absorption, and K and Na by flame spectrophotometry, after extraction with 1 M ammonium acetate at pH 7 [36]. Soil pH was measured in water (1:2.5 soil/water ratio) with Orion Research 701 pH electrode (Massachusetts, USA). Available P content was measured by Bray extraction method No. 1 [37], the most used technique in Uruguay. The Griess–Ilosvany method [38] was used to determine the concentration of NO_3-N after extraction with 2 M KCl, while the concentration of N-NH_4 was determined in the same extract using a colorimetric method based on the Berthelot reaction [39]. The soil granulometric composition was analyzed by the hydrometer method according to Gee and Bauder [40]. Each soil's water retention curve was determined in the 0–15 cm layer, for which the water potential at different soil water contents was measured with a WP4-C Dew Point Potentiometer (Decagon Brand, WA, USA).

Soil samples collected from the isotopic microplots were processed differently in the laboratory. For each sample, the recorded volume and fresh and dry weight at 105 °C were used to estimate the bulk density at each sampling depth and the equivalent N mass, allowing for the expression of ^{15}N concentrations in each soil layer in kg N ha^{-1}. Bulk density data were obtained by dividing the total dry weight of each soil layer by the total volume (the volume of each soil sample at the specified depth multiplied by the number of subsamples in the composite sample). The soil grinding for isotopic analysis was carried out in two steps: first, a coarse grinding using a blade mill, followed by finer grinding in a rotary mill (SampleTek Model 200 Vial Rotator, Lincoln, NE, USA) to produce a fine powder with a consistency similar to talcum powder, which is necessary for ^{15}N analysis via mass spectrometry. The concentrations of total C and N and the ^{15}N/^{14}N ratios of the soil samples from each layer in the isotopic plots were determined using an elemental analyzer (Flash EA 112) connected to an isotope ratio mass spectrometer (DeltaPLUS, Finnigan MAT, Bremen, Germany).

The ^{15}N recovery at each soil depth layer and the total for the combined layers (Ndff 0–30 cm) were determined using isotopic data. To achieve this, estimating the fraction of N derived from the fertilizer (Ndff) and the total N content in each soil layer was necessary. For example, the calculation for the 0–5 cm layer was performed as follows [41]:

$$\mathrm{Ndff}(\%) = \frac{\%\mathrm{at.\,exc}^{15}\mathrm{Nsoil\ sample}_{0-5\mathrm{cm}}}{\%\mathrm{at.\,exc}^{15}\mathrm{Nfertilizer}} \times 100 \qquad 2)$$

$$N_{0-5cm}(kgha^{-1}) = \frac{(\text{soil mass}_{0-5cm}) \times N_{0-5cm}(\%)}{100} \quad 3)$$

$$Ndff\ (kgha^{-1}) = \frac{N_{0-5}(kgha^{-1}) \times Ndff_{0-5cm}(\%)}{100} \quad 4)$$

3.2.5. Plant Samples from Conventional and Isotopic Experiments

All samples of maize plant components (grain, leaf + stem, and cobs) were dried in a forced air oven at 65 °C until a constant weight. Then, the samples were ground in a mill with fixed and mobile blades (Model MA-580, Marconi Equipments, Piracicaba, SP, Brazil)) until being passed through a 0.5 mm mesh. The samples analyzed by mass spectrometry were newly ground in a rotary mill (SampleTek Model 200 Vial Rotator, Lincoln, NE, USA)) until achieving the granulometry required for the analyses of C and total N concentration and $^{15}N/^{14}N$ composition of the plant samples (at the natural abundance level and the enriched level).

The Kjeldahl method determined the N content in all plant and grain samples from the conventional experiment. The total amount of N absorbed was defined as the product of the accumulated dry matter (DM) expressed in kg ha^{-1} and the DM's N concentration expressed in percentage (%N), as shown in Equation (5) [41].

$$\text{Total N Uptake}\ \ (\text{kg ha}^{-1}) = \frac{\text{DM (kg ha}-^{1}) \times\ N(\%)}{100} \quad (5)$$

The relative response of N absorption to fertilization according to N supply (Rs) was estimated as the difference between 1 minus the ratio between soil N supply and crop N demand using the following equation [42]:

$$Rs = \left[1 - \frac{(\text{ Total N Uptake}_{T0N})}{(\text{Total N Uptake}_{T100N})}\right] \quad (6)$$

Total N Uptake$_{T0N}$ is the N absorbed in the control treatment and constitutes a measure of the soil N supply, and the total N uptake$_{T100N}$ is an estimate of the N demand of the crop in the treatment with N addition. The values close to 1 indicate a greater demand for fertilizer N (sites with N response), while values close to 0 represent flat response curves, that is, cases without response to N (when the soil N supply satisfies the crop's demand).

The apparent N recovery efficiency derived from CC residues (apNRE$_{CC}$) was estimated from the 0 N treatments as the difference in N uptake between corn sown on CC residues and without CCs (control) [11].

$$\mathrm{apNRE_{CC}(\%)} = \frac{\text{N Uptake on CC} - \text{NUptake on Control}}{\text{N at CC termination}} \times 100 \tag{7}$$

Furthermore, the apparent N recovery efficiency derived from fertilizer (apNRE$_{Fert}$) was estimated from the 0 N and 100 N treatments as the difference in N uptake between corn sown on fertilized CC and unfertilized CC [11].

$$\mathrm{apNRE_{Fert}(\%)} = \frac{\text{NUptake on } \mathrm{CC_{100N}} - \text{NUptake on } \mathrm{CC_{0N}}}{\text{Nrate fertilizer}} \times 100 \tag{8}$$

The estimation of apNRE$_{CC}$ (Equation (7)) and apNRE$_{Fert}$ (Equation (8)) assumes that the rates of N mineralization in the soil are similar in plots with and without the contribution of CC or fertilizer, respectively.

Using the natural abundance method, the ^{15}N isotopic composition of the CC was used to estimate BNF for berseem clover and lupine in monoculture and L-O mixture culture [43]. Oats served as the reference crop. Another important parameter for estimating BNF is the B value, which was determined using the same legume variety and rhizobium strain as in the field experiment but grown in an N-free medium under greenhouse conditions. The estimated B values were −0.8‰ for lupine and −0.5‰ for berseem clover.

$$\mathrm{BNF(\%)} = \frac{\delta^{15}\text{N reference} - \delta^{15}\text{N legume}}{\delta^{15}\text{N reference} - \text{B value}} \times 100 \tag{9}$$

From the data of kg DM ha^{-1}, the sample's N concentration, and the ^{15}N enrichment(% atom ^{15}N excess, the fraction of Ndff in each plant component (grain, stem + leaves, and cob) at each fertilization time (Supplementary Materials, Table S2) with ^{15}N was determined [41,44].

$$\mathrm{Ndff}(\%) = \frac{\%\mathrm{atom}^{15}\mathrm{Nexc\ foliar\ sample}}{\%\mathrm{atom}^{15}\mathrm{N\ exc\ fertilizer}} \times 100 \quad (10)$$

The amount of Ndff in each plant component was estimated using Equations (5) and (10) [41].

$$\mathbf{Ndff}(\mathbf{kg\ ha^{-1}}) = \frac{\mathbf{N\ uptake} \times \mathbf{Ndff}(\%)}{\mathbf{100}} \quad (11)$$

Then, based on the kg ha^{-1} of Ndff in the plant components, the crop's N recovery efficiency ($^{15}NRE_{crop}$) was calculated [41], expressed as a percentage of the ^{15}N fertilizer added. On the other hand, N recycling was calculated as the sum of Nddf remaining in the crop's residue and the soil at harvest, and the unaccounted-for N was estimated as the difference between the total N applied minus the N exported by the grain and recycled N (N remaining in the soil plus N in wheat stubble).

$$\mathbf{NRE}(\%) = \frac{\mathbf{Ndff}(\mathbf{kgha^{-1}})}{\mathbf{N\ rate}(\mathbf{kgha^{-1}})} \times \mathbf{100} \quad (12)$$

The weighted average ^{15}NRE of the crop was estimated based on the amount of N and the ^{15}N enrichment used at each N fertilization time (Supplementary Materials, Table S2). The amount of Ndff in the crop fertilized at each fertilization time was estimated, and then the sum of these amounts was divided by the total N rate applied (100 kg N ha^{-1}).

The biochemical composition of CC (non-fibrous carbohydrates, cellulose, and lignin, expressed as a percentage of total C) was determined at the Agricultural and Environmental Service Laboratory of the University of Georgia, USA, using near-infrared reflectance spectroscopy (NIRS) equipment.

3.2.6. Statistical Analysis

The results were analyzed using ANOVA with the MIXED procedure in the SAS statistical program (SAS ® Studio on Demand for Academics Cary, NC, USA). The predecessor crops (cover crops), N treatments, and their interactions were treated as fixed effects in the statistical model, while the block × species interaction was considered a random effect. To assess mineral N dynamics over time in the non-fertilized treatments, the model included predecessors, sampling time, and their interaction as fixed effects, with the block effect (plot) treated as random. A Tukey test with a 95% confidence level was employed to determine the statistical significance of the fixed effects within each experimental site (and year effect). The Shapiro–Wilk and Levene tests were conducted to check for data normality and the homogeneity of variance assumptions. The variables analyzed from the conventional experiments included mineral N concentration at sowing and the V6 stage, yield, nitrogen concentration, absorbed N in grain, and the relative response of N absorption to fertilization. In the isotopic experiment, the study focused on variables related to the recovery ^{15}N in both plant and soil. Measurements from the WP4-C Dewpoint Potentiometer were plotted, and nonlinear regression analysis of water potential (WP in MPa) and gravimetric water content (GWC in g H_2O g oven dry soil^{-1}) was performed using SigmaPlot v. 14.0 (Systat Software).

3.3. Results

3.3.1. Weather Conditions during the Study Period

The total precipitation from sowing to physiological maturity of the corn was 517 mm at Site 1 and 324 mm at Site 2. Meanwhile, the average temperatures for the same period were 21.3 °C at Site 1 and 21.8 °C at Site 2. From chemical fallow to corn sowing, accumulated rainfall was 85 mm at Site 1 and 60 mm at Site 2, with average temperatures of 17.8 °C and 21.7 °C, respectively. At the time of corn sowing, there were no differences in soil water content between cover crop treatments (Supplementary Materials, Table S3).

Based on the weather data in Figure 1, the water supply for the summer crop at Site 2 was deficient, as the monthly rainfall throughout the corn cycle was consistently below the 30-year average. The most significant deviation from the historical rainfall pattern occurred between December and March at Site 2. In contrast, at Site 1, rainfall was lower than the historical average in December but exceeded the average during the rest of the crop cycle, including the critical growth period.

Regarding temperature records, the monthly average temperature during the crop's critical period, defined as the 15 days before and after female flowering [45], was 21.6 °C at Site 1 and 21.9 °C at Site 2. These temperatures were similar to the historical averages for the same growth period (Figure 1).

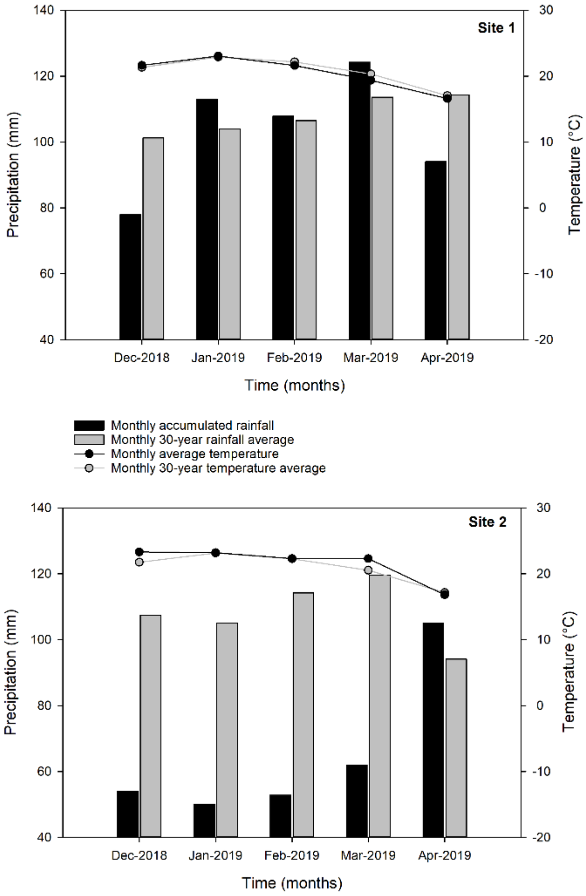


Figure 1. Monthly average temperature and rainfall in 2018–2019 (Site 1) and 2019/2020 (Site 2) and the historical average (30 years including the experimental period) temperature and rainfall.

3.3.2. Phase 1: Aboveground Biomass Yield and Chemical and Biochemical Traits of CC

Regarding biochemical composition of CC, aerial biomass of legumes had a higher concentration of non-fibrous carbohydrates and a lower concentration of cellulose than grasses, whether grown in monoculture or mixture (Table 3). In this dataset, there was a strong linear correlation between the soluble carbohydrates concentration and total N concentration in the residues (r = 0.9, $p < 0.0001$), which was influenced by the species and the maturity stage at which the crop residues were harvested. No clear association was observed between lignin concentration and specific species; however, a trend showed higher lignin levels in the CCs from Site 2.

At Site 1, the biomass yield was the highest for oats grown in monoculture, while at Site 2, the L-O mixture yielded more (Table 3). Overall, CCs produced more at Site 1, except for the mixture culture, where Site 2 produced a greater biomass. Regarding the legumes evaluated, the yield of berseem clover was comparable at both sites. However, the yield of lupine monoculture was significantly higher at Site 1, quadrupling the yield reached at Site 2.

The results concerning legumes' BNF rates were similar across sites. At Site 2, a notable increase in N fixation by lupine was observed in the mixed culture compared to the monoculture, while no differences were found at Site 1 (Table 3). Coinciding with the higher biomass yield, the highest AG N yield of legumes in monoculture was produced in Site 1 because the CC was sown earlier than in Site 2, giving Site 1 further time for both biomass production and N fixation.

Table 3. Initial chemical composition of the predecessor crop from Site 1 (cropping season 2018–2019) and Site 2 (cropping season 2019–2020). Values are replicate averages for each site–predecessor crop.

Site	**Predecessor Crop**	**AGBiomass**	**C**	**N**	**C:N**	**CARB**	**CELL**	**LIG**	**BNF**	**AG N Yield**	**AG N Yield Fixed**
		kg ha^{-1}	**%**			**% of Total C**			**%**	**kg ha^{-1}**	
1	Berseem clover	2444.4	42.2	3.0	14.2	54.5	38.9	6.6	82.8	72.9	64.1
	Lupine	4674.5	42.0	2.4	17.9	59.6	34.2	6.2	80.8	110.0	60.1
	L-O mixture	3253.0	44.3	1.1	40.2	49.8	45.1	5.1	80.4	35.8	1.4
	Oat	4849.9	40.4	1.0	40.6	31.4	61.7	6.9	n.c	48.3	n.c

2	Berseem clover	2183.8	40.5	2.7	15.2	55.8	37.5	6.7	79.3	59.8	47.4
	Lupine	1134.2	41.0	2.5	16.4	58.3	33.5	8.2	76.3	28.4	21.6
	L-Omixture	3619.7	41.2	1.8	28.2	44.4	48.9	6.7	93.8	65.1	13.5
	Oat	2621.4	41.9	1.0	40.2	35.8	56.4	7.8	n.c	27.4	n.c

AG, aboveground; C, carbon; N, nitrogen; CARB, soluble carbohydrates; CELL, cellulose + hemicellulose; LIG, lignin; BNF, biological nitrogen fixation. n.c: means not corresponding.

3.3.3. Phase 2: Conventional Experiment

3.3.3.1. Soil Mineral N Dynamics after CC Termination

Although the fallow period was similar at both sites (Table S2), the predecessor crop effect on the soil N availability at corn sowing at Site 1 was statistically significant (*p* = 0.0006). In contrast, Site 2 showed no differences among CCs (Table 4). At Site 1, soil mineral N availability varied among CCs, with higher concentrations of NO_3 -N associated with legume monocultures like lupine and berseem clover. The other treatments (oats in monoculture and L-O mixture) had similar N concentrations to the control (with no CC). At Site 2, while no statistically significant effect of the predecessor crops was observed, the control treatment tended to have higher N availability than CCs. Ammonium (N-NH_4) largely accounted for a smaller proportion of the available mineral N in both sites.

Since the interaction between the predecessor crop and sampling time was not significant (Figure 2), the average mineral N concentration across all points in time was analyzed. For this variable, the ranking of the predecessor crops from highest to lowest concentration for Site 1 was berseem clover, lupine, control, L-O mixture, and oats. In contrast, for Site 2, the ranking was lupine, control, berseem clover, L-O mixture, and oats (Figure 2).

Table 4. Soil nitrate, ammonium, and mineral N concentration in the soil (at 0–20 cm soil depth) at corn sowing according to predecessor crop at Site 1 (cropping season 2018–2019) and Site 2 (cropping season 2019–2020). Values are replicate averages for each site–predecessor crop ± standard error.

Site	**Predecessor Crop**	**NO_3-N**	**NH_4-N**	**Mineral N**
			mg kg^{-1}	
1	Berseem clover	14.2 ± 1.5 ^{a}	5.1 ± 0.4	19.3 ± 1.9 ^{ab}
	Oat	6.3 ± 1.0 ^{b}	7.9 ± 0.3	14.2 ± 1.0 ^{c}
	Lupine	14.8 ± 0.9 ^{a}	7.4 ± 1.2	22.2 ± 1.9 ^{a}
	L-O mixture	8.3 ± 0.1 ^{b}	5.8 ± 0.6	14.1 ± 0.6 ^{c}
	No CC (Control)	7.9 ± 0.6 ^{b}	8.1 ± 0.9	16.1 ± 1.4 ^{bc}
Significance of treatment effect			*p*-value	
Predecessor crop		0.0006	n.s	0.0187
2	Berseem clover	8.5 ± 1.2	6.9 ± 0.1	15.4 ± 1.3
	Oat	8.2 ± 0.9	7.4 ± 1.1	15.6 ± 1.8
	Lupine	9.4 ± 0.5	7.6 ± 0.5	17.0 ± 0.7
	L-O mixture	8.7 ± 0.8	8.9 ± 1.5	17.6 ± 2.3
	No CC (Control)	11.6 ± 0.2	7.4 ± 0.0	19.0 ± 0.2
Significance of treatment effect			*p*-value	
Predecessor crop		n.s	n.s	n.s

Different letters within a column indicate differences between the predecessor crop within each site, being significant at a *p*-level of 0.05; n.s: means no significant difference.

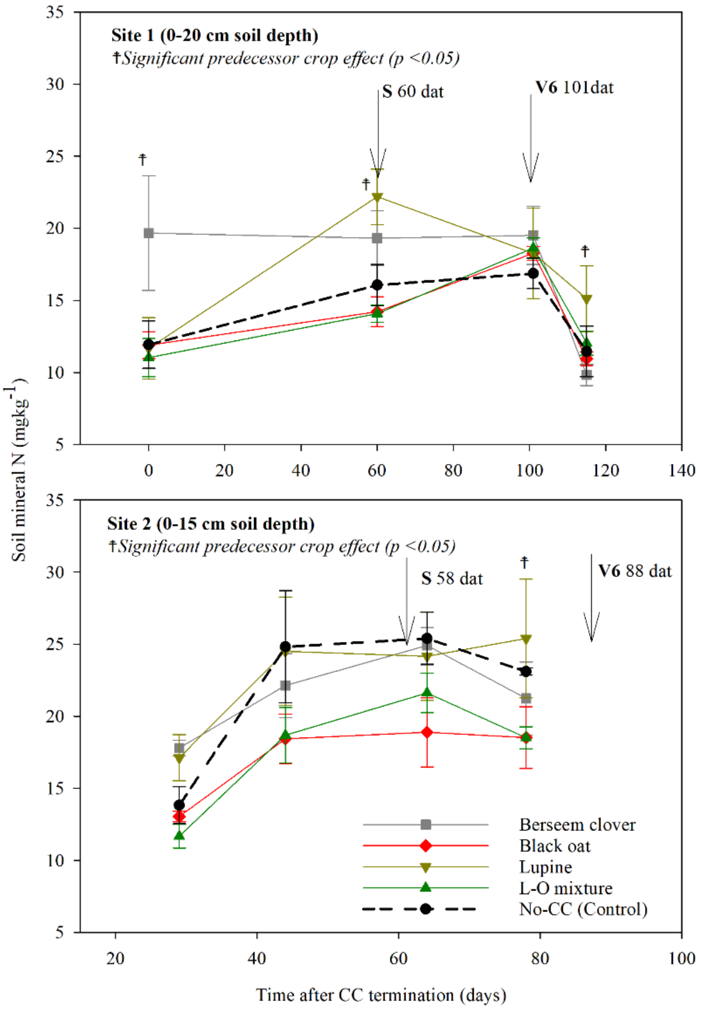


Figure 2. Soil mineral nitrogen concentration (Ammonium-N + Nitrate-N) by preceding crop in 0 N treatments at two soil depths and sites, during fallow and early corn growth stages (sowing to V6). The vertical bars indicate the standard error.

The predecessor crop significantly affected N availability at V6 and V10 stages in Site 2 but not in Site 1 (Table 5), dissimilar to what was observed at corn sowing (Table 4). When comparing soil NO_3-N concentration at sowing and V6 or V10 in the non-fertilized treatment and without a CC, it was found that the concentration remained stable at Site 1 but decreased at Site 2 (Tables 4 and 5). At Site 1, none of the evaluated effects on soil mineral N were significant. However, at Site 2, there was a significant interaction between the N rate and the predecessor crop.

Table 5. Soil nitrate, ammonium, and mineral N concentration (at 0–20 cm soil depth) in the stages between six leaves (Site 1) and ten leaves (Site 2) according to the N rate and predecessor crop at Site 1 (cropping season 2018–2019) and Site 2 (cropping season 2019–2020). Values are replicate averages for each site–N rate–predecessor crop ± standard error.

Site	**N Rate**	**Predecessor Crop**	**NO_3-N**	**NH_4-N**	**Mineral N**
			mg kg^{-1}		
1	0	Berseem clover	10.3 ± 0.7	9.1 ± 1.4	19.5 ± 2.0
		Oat	8.6 ± 0.7	9.7 ± 0.7	18.3 ± 0.5
		Lupine	8.5 ± 3.0	9.7 ± 0.7	18.3 ± 3.2
		L-O mixture	9.0 ± 2.0	9.7 ± 2.3	18.6 ± 0.7
		No CC (control)	7.3 ± 0.5	9.6 ± 1.4	16.9 ± 1.1
	100	Berseem clover	12.6 ± 3.5	8.4 ± 0.6	20.9 ± 3.1
		Oat	11.9 ± 2.5	8.8 ± 0.6	20.8 ± 2.4
		Lupine	12.6 ± 1.9	8.3 ± 0.6	20.9 ± 2.4
		L-O mixture	7.9 ± 1.3	8.6 ± 0.1	16.5 ± 1.2
		No CC (control)	10.2 ± 0.3	8.4 ± 0.3	18.6 ± 0.2
Significance of treatment effect				*p*-value	
		Nrate	n.s	n.s	n.s
		Predecessor crop	n.s	n.s	n.s
		Nrate× Predecessor crop	n.s	n.s	n.s
2	0	Berseem clover	4.4 ± 0.9 ^{abB}	8.8 ± 1.0	13.2 ± 1.6 ^{B}
		Oat	2.6 ± 0.6 ^{b}	9.5 ± 1.0	12.1 ± 1.0 ^{A}
		Lupine	4.4 ± 1 ^{abB}	9.5 ± 0.6	13.8 ± 1.5 ^{B}
		L-O mixture	3.2 ± 0.7 ^{b}	8.1 ± 1.0	11.2 ± 0.6

	No CC (control)	6.5 ± 2.0 ^{a}	8.7 ± 1.0	15.3 ± 1.3
100	Berseem clover	11.6 ±2.1^{aA}	10.7 ± 1.5	22.3 ± 2.3 ^{aA}
	Oat	1.9 ± 0.5 ^{c}	7.5 ± 0.6	9.4 ± 0.6 ^{cB}
	Lupine	11.0 ± 0.7 ^{aA}	10.3 ± 2.2	21.3 ± 1.8 ^{aA}
	L-O mixture	3.9 ± 0.7 ^{bc}	7.6 ± 0.8	11.5 ± 0.1 ^{bc}
	No CC (control)	8.4 ± 1.8 ^{b}	6.8 ± 0.2	15.2 ± 2.0 ^{b}
Significance of treatment effect			*p*-value	
	Nrate	<0.0001	n.s	0.0002
	Predecessor crop	0.0036	n.s	0.0113
	Nrate× Predecessor crop	0.0014	n.s	<0.0011

Different lowercase letters within a column indicate differences between predecessor crops within each site and each N rate, and different capital letters within a column indicate differences between N rates within each site and predecessor crop, being significant at a *p*-level of 0.05; n.s: means no significant difference.

3.3.4. Grain Yield and Plant N Uptake by Maize Cropping

The ANOVA results for the variables measured in corn showed that the main effects (N rate and predecessor crop) were significant for grain yield and grain N content at Site 1. At Site 2, only the N rate significantly affected those variables and grain N concentration (Table 6). At Site 1, the interaction between the N rate and predecessor crop was also significant for grain yield and whole plant N content. Additionally, for the relative response in whole plant N content (N absorbed in grain plus N absorbed in the remaining aerial biomass), the predecessor crop effect was significant only at Site 1.

In Site 1, the highest grain yield and N uptake were recorded in the fertilized corn plots without CCs. In contrast, among the 0 N plots, the highest yield was observed in the berseem clover treatment. At Site 2, the response was similar across

CCs, making the effect of the predecessor crop non-significant (Table 6). At both sites, the response to N fertilization in grain yield was significant for oats and lupine as predecessor crops. In Site 1, the N absorbed in grain was high for those CCs, while in Site 2, it was significant for all CCs treatments, as grain N concentration increased significantly in all fertilized treatments. However, when whole plant N content was analyzed, the response to N fertilization was significant across all treatments, except for berseem clover in Site 1. In contrast, in Site 2, the response to applied N in this variable did not differ between CCs, like the response observed for grain N content (Table 6).

Another noteworthy finding was that in the 0 N treatments, grain yield with no CC in Site 1 was higher and statistically different from that of the lupine and oat monocultures. However, it was not significantly different from the yields with berseem clover and the mixture culture, although the difference with the mixture was nearly 2000 kg ha^{-1} (Table 6). In contrast, in Site 2, the grain yield without a CC did not show significant differences compared to any evaluated CC.

Table 6. Grain yield, grain N concentration, grain N content, plant N content, and relative N response in plant N content by the maize crop, according to predecessor crop, and N rate at Site 1 (cropping season 2018–2019) and Site 2 (cropping season 2019–2020) (Site 1—season 2018–2019 and Site 2—season 2019–2020). Values are replicate averages for each site–predecessor crop ± standard error.

Site	N Rate	Predecessor Crop	Grain Yield	Grain N Concentration	Grain N Content	Whole Plant N Content †	Relative N Response in Whole Plant N Content (Rs)
			kg ha^{-1}	g kg^{-1} Dry Weight	kg ha^{-1}		
1	0	Berseem clover	6439 ± 526 ^{a}	10.3 ± 0.9	65.3 ± 1.1 ^{a}	99.9 ± 8.2 ^{a}	
		Oat	2785 ± 232 ^{cB}	9.9 ± 0.3	27.5 ± 2.3 ^{cB}	53.9 ± 3.6 ^{bB}	
		Lupine	2437 ± 846 ^{cB}	9.4 ± 0.6	23.8 ± 9.5 ^{cB}	62.2 ± 14.0 ^{bB}	
		Mixture	3744 ± 135 ^{bc}	9.4 ± 0.6	35.9 ± 2.1 ^{bc}	66.9 ± 3.8 ^{bB}	
		No CC (control)	5542 ± 546 ^{ab}	9.4 ± 0.5	52.5 ± 7.4 ^{ab}	82.4 ± 13.7 ^{abB}	
	100	Berseem clover	5582 ±762 ^{ab}	10.5 ± 0.2	58.3 ± 7.6 ^{ab}	93.6 ± 11.4	−0.08 ± 0.0 ^{b}
		Oat	5870 ±558 ^{abA}	10.4 ± 0.7	61.4 ± 8.9 ^{abA}	96.9 ± 13.1 ^{A}	0.43 ± 0.1 ^{a}
		Lupine	4882 ± 629 ^{bA}	10.4 ± 1.2	49.9 ± 7.2 ^{bA}	82.7 ± 8.5 ^{A}	0.26 ± 0.1 ^{a}
		Mixture	5124 ± 374 ^{ab}	9.6 ± 0.5	49.2 ± 5.0 ^{b}	80.4 ± 10.3 ^{A}	0.25 ± 0.0 ^{a}
		No CC (control)	7148 ± 1040 ^{a}	10.2 ± 0.5	73.8 ± 13.7 ^{a}	101.2 ± 14.0 ^{A}	0.19 ± 0.0 ^{a}
Significance of treatment effect					*p*-value		

		Nrate	0.0017	n.s	0.0028	0.0004	0.0197
		Predecessor crop	0.0313	n.s	0.0399	n.s.	
		Nrate× Predecessor crop	0.0457	n.s	n.s	0.0197	
2	0	Berseem clover	7267 ±676	11.3 ± 0.9 ^{B}	81.3 ± 4.2 ^{B}	149.3 ± 12.5 ^{B}	
		Oat	6574 ± 405 ^{B}	10.3 ± 1.0 ^{B}	68.0 ± 9.9 ^{B}	124.6 ± 11.7 ^{B}	
		Lupine	7866 ± 745 ^{B}	10.2 ± 0.3 ^{B}	80.1 ± 6.8 ^{B}	131.1 ± 7.1 ^{B}	
		Mixture	8056 ± 977	10.4 ± 0.2 ^{B}	84.2 ± 11.2 ^{B}	125.8 ± 16.2 ^{B}	
		No CC (control)	7121 ± 570	11.7 ± 0.5 ^{B}	82.7 ± 3.8 ^{B}	136.7 ± 10.4 ^{B}	
	100	Berseem clover	8707 ± 1275	16.3 ± 2.2 ^{A}	136.9 ± 8.6 ^{A}	214.3 ± 12.4 ^{A}	0.30 ± 0.1
		Oat	8969 ± 815 ^{A}	13.8 ± 0.4 ^{A}	122.6 ± 8.2 ^{A}	181.9 ± 18.0 ^{A}	0.30 ± 0.1
		Lupine	10,116 ± 952 ^{A}	13.8 ± 1.3 ^{A}	140.7 ± 24.4 ^{A}	203.1 ± 29.7 ^{A}	0.26 ± 0.1
		Mixture	9583 ± 663	13.6 ± 0.3 ^{A}	129.8 ± 7.1 ^{A}	197.1 ± 11.0 ^{A}	0.32 ± 0.1
		No CC (control)	7999 ± 610	15.0 ± 1.0 ^{A}	120.6 ± 10.0 ^{A}	186.1 ± 6.4 ^{A}	0.37 ± 0.1
Significance of treatment effect					*p*-value		
		Nrate	0.0022	<0.0001	<0.0001	<0.0001	n.s
		Predecessor crop	n.s	n.s	n.s	n.s	
		Nrate× Predecessor crop	n.s	n.s	n.s	n.s	

Different lowercase letters within a column indicate differences between predecessor crops within each site and each N rate, and different capital letters within a column indicate differences between N rates within each site and predecessor crop, being significant at a *p*-level of 0.05; n.s: means no significant difference. † Whole plant N content: grain N content + N content in the aboveground remanent biomass.

3.3.5. Apparent Nitrogen Recovery Efficiency from CCs and Fertilizer

The average apNRE_{CC} was negative and similar at both sites, with values of −13% at Site 1 and −11% at Site 2. However, excluding oats, which always had negative values, the average apNRE_{CC} was 0 and 3% in Site 1 and 2, respectively. In contrast, $\text{apNRE}_{\text{Fert}}$ was consistently positive and varied between sites, reaching an average of 20% at Site 1 and 66% at Site 2. As shown in Figure 3, $\text{apNRE}_{\text{Fert}}$ exhibited more significant variability among CCs at Site 1 than Site 2, which was also reflected in the Rs values, with a significant effect from the predecessor crop (Table 6). At Site 1, a considerable negative correlation was found between apNRE_{CC} and $\text{apNRE}_{\text{Fert}}$, suggesting that when corn received an adequate N supply from the CC (such as berseem clover), there was no significant response to N fertilization. In contrast, a positive response to fertilization was observed when the CC immobilized N, as seen with oats. Therefore, there was a positive correlation between $\text{apNRE}_{\text{Fert}}$ and the C:N ratio of the CC.

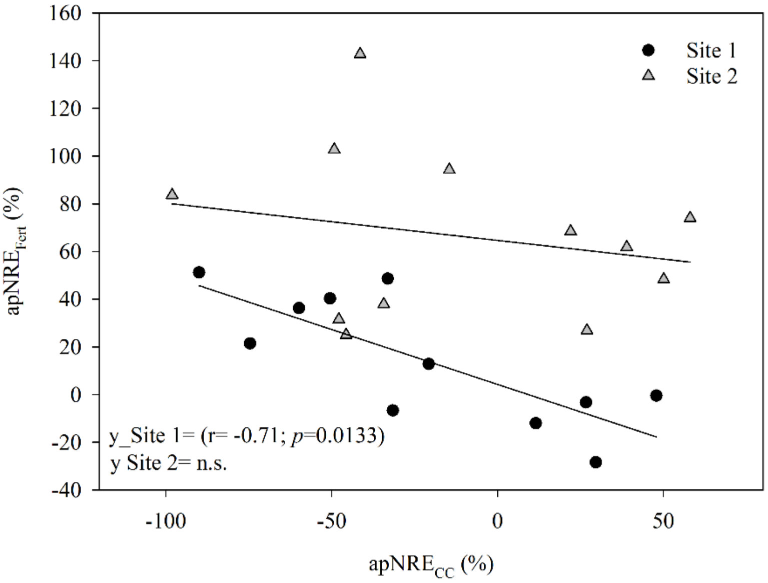


Figure 3. Relationship between the apparent N recovery efficiency derived from cover crops (apNRE_{CC}) and fertilizer ($\text{apNRE}_{\text{Fert}}$) at each experimental site.

3.3.6. Isotopic Experiment

3.3.6.1. Plant ^{15}N Recovery in the Maize Cropping

The ^{15}N recovery of the entire plant resulted from the ^{15}N recovery of each corn plant component (Table 7), confirming the differences observed in the conventional experiment. The crop's ^{15}NRE was significantly lower at Site 1, likely due to competition for N between the crop and weeds (Table 7). At Site 2, only the estimator of ^{15}NRE of corn grown on berseem clover stubble did not differ between the timing of ^{15}N labeling (at sowing and V10 stages), whereas, in the other treatments, ^{15}NRE was significantly higher in V10 than sowing time. At Site 1, the differences in ^{15}NRE between ^{15}N application timings were significant, especially in the berseem clover and mixture CC treatments.

Regarding the predecessor crop effect, in Site 1, the ^{15}NRE of N fertilizer applied at sowing was similar across all predecessors. However, at the V6 stage, legume CCs or the mixture of lupine and oats showed higher ^{15}NRE. At Site 2, the interaction between ^{15}N application timing and predecessor crop significantly affected ^{15}NRE. At sowing, ^{15}NRE was higher in the berseem clover treatment and the control, while at the V10 stage, lupine monoculture and lupine–oat mixture treatments had the highest ^{15}NRE. Although no significant differences in mineral N availability were found between treatments at Site 1 at the V6 stage, the soil N concentration was considerably lower at sowing with oats grown in monoculture or mixture (Table 4).

Table 7. Plant ^{15}N recovery (en % y kg ha^{-1}) from N fertilizer applied (100 kg ha^{-1}) at two ^{15}N application timings (sowing and vegetative stages), and four predecessor crop and one control treatment (No CC) at Site 1 (cropping season 2018–2019) and Site 2 (cropping season 2019–2020). Values are replicate averages for each site–N rate–predecessor crop ± standard error.

Site	**^{15}N Application Timing**	**Predecessor Crop**	**^{15}NRE $_{crop}$**	**Ndff Grain**	**Ndff Grain**	**Ndff Stubble †**	**Nddf Stubble**
			(%)		**kg ha^{-1}**	**%**	**kg ha^{-1}**
1	Sowing	Berseem clover	7.4 ± 2.6 ^{B}	2.3 ± 0.4 ^{B}	1.4 ± 0.5 ^{B}	1.2 ± 0.2 ^{B}	0.4 ± 0.1 ^{B}
		Oat	3.9 ± 1	0.8 ± 0.2	0.7 ± 0.2	0.5 ± 0.1 ^{B}	0.2 ± 0.1 ^{B}
		Lupine	7.2 ± 2.0	2.4 ± 0.5 ^{B}	1.1 ± 0.3 ^{B}	1.5 ± 0.3	0.6 ± 0.2 ^{B}
		L-O mixture	3.1 ± 0.1 ^{B}	1.1 ± 0.1 ^{B}	0.6 ± 0.1 ^{B}	0.6 ± 0.0 ^{B}	0.2 ± 0.0 ^{B}
		No CC (control)	7.3 ± 0.8	2.2 ± 0.2	1.5 ± 0.3 ^{B}	0.9 ± 0.2	0.3 ± 0.0
	V6 stage	Berseem clover	17.5 ± 2.0^{aA}	12.3 ± 3.0^{aA}	6.2 ± 0.7 ^{aA}	6.7 ± 1.8 ^{A}	2.0 ± 0.4 ^{A}
		Oat	7.0 ± 2.1^{c}	4.6 ± 1.5^{c}	2.8 ± 1.0^{b}	6.7 ± 1.8 ^{A}	1.9 ± 0.2 ^{A}
		Lupine	11.4 ± 2.4^{bc}	9.2 ± 2.5^{abA}	4.2 ± 1.1^{bA}	4.3 ± 1.1	1.5 ± 0.3 ^{A}
		L-O mixture	12.6 ±2.2^{abA}	9.9 ± 2.6^{abA}	4.5 ± 0.8^{abA}	5.0 ± 1.3 ^{A}	1.5 ± 0.5 ^{A}
		No CC (control)	8.9 ± 1.2^{c}	5.4 ± 1.2^{bc}	4.0 ± 0.9^{bA}	3.0 ± 0.6	0.9 ± 0.1
Significance of treatment effect					*p*-value		
^{15}N application timing			0.0016	0.0001	<0.0001	0.0001	<0.0001

Predecessor crop			0.0201	n.s	n.s	n.s	n.s
^{15}N appl. timing × Predecessor crop			n.s	n.s	n.s	n.s	n.s
2	Sowing	Berseem clover	34.4 ± 1.7^{a}	8.6 ± 0.6^{B}	7.4 ± 1.3^{aB}	8.8 ± 1.0	4.0 ± 0.8
		Oat	19.6 ± 2.5^{bB}	5.0 ± 0.2^{B}	3.6 ± 0.5^{bB}	8.5 ± 1.1	2.7 ± 0.4
		Lupine	21.4 ± 0.9^{bB}	5.1 ± 0.4^{B}	4.8 ± 0.2^{bB}	6.5 ± 0.1^{B}	2.3 ± 0.1^{B}
		L-O mixture	22.8 ± 3.7^{bB}	6.2 ± 0.7^{B}	5.0 ± 0.9^{bB}	7.9 ± 0.8^{B}	2.5 ± 0.4^{B}
		No CC (control)	30.7 ± 4.4^{aB}	7.6 ± 1.6^{B}	6.0 ± 1.1^{abB}	10.5 ± 0.6	4.1 ± 0.5
	V10 stage	Berseem clover	32.4 ± 2.0^{b}	19.2 ± 3.5^{cA}	16.2 ± 1.3^{bA}	11.6 ± 2.2^{b}	5.2 ± 1.1^{ab}
		Oat	30.4 ± 2.5^{bA}	21.5 ± 4.1^{bcA}	16.2 ± 1.8^{bA}	11.7 ± 1.6^{b}	3.9 ± 0.5^{b}
		Lupine	50.9 ± 3.0^{aA}	25.6 ± 2.4^{abA}	28.0 ± 2.1^{aA}	14.5 ± 1.7^{abA}	5.6 ± 0.7^{abA}
		L-O mixture	48.0 ± 2.3^{aA}	30.9 ± 0.8^{aA}	25.4 ± 0.8^{aA}	18.3 ± 1.5^{aA}	6.3 ± 0.9^{aA}
		No CC (control)	37.2 ± 1.4^{bA}	24.3 ± 0.1^{bcA}	19.4 ± 0.3^{bA}	14.3 ± 1.3^{ab}	5.2 ± 0.9^{ab}
Significance of treatment effect					*p*-value		
^{15}N application timing			<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
Predecessor crop			0.0444	n.s	0.0009	n.s	n.s
^{15}N appl. timing × Predecessor crop			<0.0001	0.0284	0.0002	0.0091	0.0214

Different lowercase letters within a column indicate differences between predecessor crops within each site and each ^{15}N application timing, and different capital letters within a column indicate differences between ^{15}N application timing within each site and each predecessor crop being significant at a level $p < 0.05$. n.s: not significant. [†] %Ndff stubble was estimated as the weighted sum of the N absorbed and the %at.exc. ^{15}N for each plant component (stem + leaves + cob).

3.3.7. Soil 15N Recovery After Corn Harvest

At corn harvest, ^{15}N recovery was measured across different soil layers (0–5, 5–10, 10–15, and 15–30 cm, Supplementary Materials, Figure S3) and evaluated as the total sum of Ndff from all layers, as well as specifically the top soil layer (Table 8). The highest proportion of ^{15}N in the soil profile at both sites was found in the top 0–5 cm soil layer. Consequently, the association between these data and the Ndff (0–30 cm) was strong and significant (Supplementary Materials, Table S4). However, the CC biomass or its quality did not explain it but rather the soil C content and the increased rhizospheric and microbial activity in that top soil layer. It was also observed that residual ^{15}N in the soil was highly related to the fertilizer N rate (Table 8).

The aboveground CC biomass did not explain the ^{15}N recovery in the corn crop at either site (Supplementary Materials, Table S4); however, the C:N ratio did, as previously mentioned (Figure 4). Additionally, the amount of unaccounted-for N showed a significant negative association with crop N recovery (Supplementary Materials, Table S4). In other words, the higher the ^{15}NRE of the crop (Figure 3, Table 7), the lower the proportion of unaccounted-for N (or N losses from the system) (Table 8).

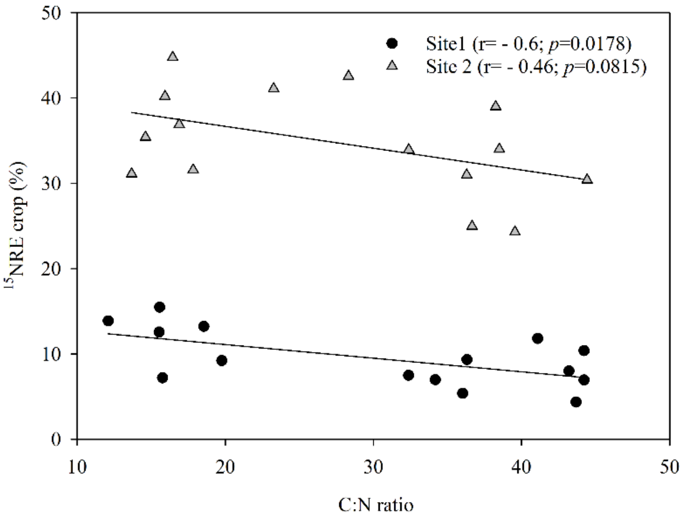


Figure 4. Relationship between the weighted N recovery efficiency of the corn crop (^{15}NRE$_{\text{crop}}$) and the C:N ratio of cover crops at each experimental site.

Table 8. Soil ^{15}N recovery (en % y kgha^{-1}) at 0–30 cm and en 0–5 cm depth layer, and unaccounted-for N from N fertilizer applied (100 kg ha^{-1}) at two ^{15}N application timings (sowing and vegetative stages) and four predecessor crops and one control treatment (no CC) at Site 1 (cropping season 2018–2019) and Site 2 (cropping season 2019–2020). Values are replicate averages for each site–N rate–predecessor crop ± standard error.

Site	**^{15}N Application Timing**	**Predecessor Crop**	**Ndff-0–30 cm Soil Depth**	**Ndff-0–5 cm Soil Depth**	**Unaccounted-for N**
			kg ha^{-1}		
1	Sowing	Berseem clover	2.9 ± 0.3 ^{B}	1.2 ± 0.1	27.6 ± 1.0 ^{B}
		Oat	3.0 ± 0.3	1.4 ± 0.2	28.7 ± 0.4 ^{B}
		Lupine	4.2 ± 1.1	2.5 ± 0.9	26.4 ± 0.4 ^{B}
		L-O mixture	3.2 ± 0.2 ^{B}	1.5 ± 0.2 ^{B}	28.8 ± 0.2 ^{B}
		No CC (control)	2.9 ± 0.2 ^{B}	1.5 ± 0.2	27.7 ± 0.1 ^{B}
	V6 stage	Berseem clover	9.7 ± 2.8 ^{A}	5.3 ± 1.8	44.8 ± 4.1 ^{A}
		Oat	6.2 ± 0.5	2.4 ± 0.2	55.2 ± 1.7 ^{A}
		Lupine	8.2 ± 1.2	5.3 ± 1.4	50.3 ± 1.7 ^{A}
		L-O mixture	11.1 ± 4.4 ^{A}	6.6 ± 3.7 ^{A}	46.6 ± 5.7 ^{A}

		No CC (control)	9.7 ± 3.1 ^{A}	5.8 ± 3.0	50.4 ± 2.9 ^{A}
Significance of treatment effect				*p*-value	
^{15}N application timing			0.0008	0.0055	<0.0001
Predecessor crop			n.s	n.s	n.s
^{15}N appl. timing × Predecessor crop			n.s	n.s	n.s
2	Sowing	Berseem clover	12.0 ± 2.5 ^{B}	5.6 ± 1.8	9.7 ± 3.0
		Oat	9.7 ± 0.9 ^{B}	4.9 ± 1.1	16.8 ± 1.7
		Lupine	11.4 ± 1.1 ^{B}	5.2 ± 1.2	14.5 ± 1.4
		L-O mixture	12.0 ± 3.2 ^{B}	6.8 ± 2.5	13.5 ± 4.4
		No CC (control)	8.5 ± 1.1 ^{B}	3.6 ± 0.9 ^{B}	14.4 ± 1.9
	V10 stage	Berseem clover	25.2 ± 4.7 ^{A}	8.7 ± 0.7	19.5 ± 5.2 ^{ab}
		Oat	22.7 ± 2.2 ^{A}	9.7 ± 2.0	23.2 ± 3.1 ^{a}
		Lupine	25.7 ± 3.6 ^{A}	8.7 ± 1.1	6.7 ± 5.0 ^{b}
		L-O mixture	23.7 ± 1.8 ^{A}	9.3 ± 1.1	10.7 ± 2.9 ^{ab}
		No CC (control)	19.8 ± 3.4 ^{A}	8.9 ± 2.2 ^{A}	21.7 ± 3.1 ^{ab}
Significance of treatment effect				*p*-value	
^{15}N application timing			<0.0001	0.0043	n.s
Predecessor crop			n.s	n.s	n.s
^{15}N appl. timing × Predecessor crop			n.s	n.s	n.s

Different lowercase letters within a column indicate differences between predecessor crops within each site and each ^{15}N application timing, and different capital letters within a column indicate differences between ^{15}N application timing within each site and each predecessor crop being significant at a level $p < 0.05$. Ndff, nitrogen derived from fertilizer; n.s, not significant.

3.4. Discussion

3.4.1. Soil Mineral N Dynamic as Affected by Chemical and Biochemical Traits of CCs

The effectiveness of CCs in delivering various agronomical and environmental services depends on the chemical and biochemical traits of CCs, which control N mineralization and the duration of the stubble's soil coverage. During decomposition, the rate at which microorganisms assimilate C depends on both plant material quantity and quality and soil microorganisms' carbon use efficiency [46]. Carbon that is not assimilated is released as CO_2. The gross N mineralization process is linked to C assimilation and the microbial community's C:N ratio. When N is present over microbial requirements, net N mineralization occurs, converting organic N into ammonium. However, if N is lacking, inorganic N is immobilized to maintain the microbial biomass [47]. In our study, the aboveground biomass of legume CCs exhibited higher levels of soluble carbohydrates and lower cellulose content than oats, whether grown in monoculture or mixture with oats. These chemical and biochemical traits of the CC legumes, together with high N content, may lead to net N mineralization in the soil, whereas for CC grasses, net immobilization occurs, decreasing soil N availability. These expected changes in soil N availability are in agreement with the findings obtained in our study. The soil mineral N results in Table 4 indicate that the predecessor crop effect was primarily driven by the amount of biomass produced; the higher the biomass production at Site 1, the more pronounced the differences compared to the no-CC treatment. In addition, the C:N ratio as an indicator of the residue quality also played a role. Crops like oats and the mixture treatment supply less N, lowering soil nitrate availability [48,49]. The significant difference in soil mineral N between these two treatments in Site 1 (Table 4) may be attributed to the differing proportions of oats and lupine seeded at each site. In Site 1, the mixture's composition was similar to that of the oat monoculture treatment, prompting a decision to increase the proportion of lupine in the mix during the following cropping season, achieved in Site 2. This adjustment aimed to perform a CC with a more significant presence of lupine, which resulted in a higher N biomass concentration and a lower C:N ratio in Site 2, increasing the difference in AG N yield between CC's oat and mixture, and consequently, the N supply for the succeeding crop (Table 3). We suggest this occurred because heterotrophic bacteria (microbial net immobilization of ammonium) out-compete nitrifiers for the available ammonium, meaning that nitrification requires a low C:N ratio to proceed effectively, i.e., when C availability is low [50]. In addition, the lupine yield and AG N yield were higher at Site 1, about quadrupling them concerning Site 2. This difference can mainly be attributed to the crops' growth period, which was 45 days longer at Site 1 because the sowing date was two months earlier than at Site 2.

The more significant impact of the predecessor crop at Site 1 compared to Site 2 could not be attributed to climatic conditions during the fallow period because the minor differences in rainfall (25 mm higher in Site 1) and temperature (3.9 °C lower in Site 1) were unlikely to account for the higher mineral N levels at Site 1. Soil texture could also influence N mineralization given the lower clay content and a reduced soil water capacity retention of Site 1 (Supplementary Materials, Figure S1), which may have led to more favorable conditions for the N mineralization from the CC stubble [15]. Since the interaction between the predecessor crop and sampling time was not significant (Figure 2), the average mineral N concentration across all points in time was analyzed. For this variable, the ranking of the predecessor crops from highest to lowest concentration for Site 1 was berseem clover, lupine, no CC, L-O mixture, and oats. In contrast, for Site 2, the ranking was lupine, no CC, berseem clover, L-O mixture, and oats (Figure 2). These results suggest that the CC effect on soil mineral N was similar between sites, with no significant differences between the legume in monoculture (berseem clover and lupine) and the treatment without a CC, except in Site 1, where the soil mineral N concentration with berseem clover was higher than control. (Figure 2). Although oats in monoculture or mixture had the lowest soil mineral N concentration. These CCs did not differ from the control treatment, except in Site 2, where the soil mineral N concentration with oats was significantly lower than in the treatment without a CC. This lower N availability with oats is expected, as it presented a high C:N ratio, which generally leads to increased rates of N immobilization [48].

The lower NO_3-N concentration at Site 2 obtained at the V10 stage can be partially explained by the more advanced corn physiological stage compared to Site 1. At the V6 stage, spike differentiation and stem elongation begin, while at V10, the spike determines the number of rows and grains per row. At the V10 stage, the corn plant starts rapidly accumulating nutrients and biomass, continuing into the reproductive stage [51]. At Site 1, these changes coincided with a humid period, with rainfall close to the historical average and no water excess. In contrast, Site 2 experienced an exceptionally dry cycle compared to the historical average (Figure 1), explaining the lower NO_3-N values due to unfavorable conditions for nitrification. In treatments involving legumes, such as lupine monoculture and berseem clover, soil NO_3^--N concentrations at the V10 stage were higher in fertilized plots than in unfertilized ones, suggesting that there was residuality of the N fertilizer applied at sowing corn (Table 5), which remained available for plant uptake. In contrast, in other treatments, mainly with oats, NO_3^--N concentrations of the fertilized plots were similar or even lower. Additionally, although no significant differences in mineral N availability were found between unfertilized treatments at Site 2, the control treatment tended to have higher N availability, implying that CC may help to retain N in the soil, potentially reducing N losses.

While no distinct relationship between CC's lignin concentration and particular species was identified, there was a tendency for CCs at Site 2 to have higher lignin levels (Table 3). This difference in lignin concentration between sites could be attributed to dissimilar climatic conditions and soil nutrient availability, particularly potassium (K) and phosphorus (P), which may have promoted more significant lignin biosynthesis. Liu et al. [52] highlighted that lignin metabolism is actively involved in responding to various environmental stresses (both abiotic and biotic) and can be influenced by management factors such as nutrient availability and plant density. Regarding the effect of climate, Site 1 experienced higher accumulated rainfall and average temperatures during the CC growth cycle, with 548 mm compared to 383 mm and 13.1 °C versus 12.8 °C, respectively. Numerous studies indicate that lignin biosynthesis tends to increase under drought stress. This enhanced accumulation of lignin, which serves as an adaptation mechanism to drought or high salinity, has also been observed in Leucaena, sweet potato (Ipomoea batatas), and soybeans [52]. Although scarce differences were found in SOM (5.2% at Site 1 and 5.7% at Site 2) and nutrient availability (Tables 1 and 2), soil characterization revealed a lower concentration of exchangeable bases, particularly K, at Site 1. Research has shown that low K levels can lead to unregulated absorption of ammonium and inhibit K absorption, potentially resulting in decreased lignin biosynthesis [53]. Thus, the drier climatic conditions and greater K availability at Site 2 would account for the higher lignin content in the CCs. This biochemical fraction of the total C was linked to the water potential (ψ) of CC residues on the soil surface [54]. The ψ of cover crop residues is crucial for microbial survival and activity and, consequently, has a strong influence over crop decomposition and subsequent release of N in no-till systems [54]. That research demonstrated that the parameters “a” and “b” in a model describing water potential ($\psi = a\,\theta_g^{-b}$) in CC residues are influenced by lignin content. This study found that when lignin concentrations increase, the “a” parameter tends to increase (becoming less negative), indicating a decrease in the water retention capacity of the residue. Conversely, a decrease in the “b” parameter (becoming more negative) suggests a reduction in the water retention rate, meaning the residue dries out more quickly and loses moisture faster. Together, these changes imply that residues with higher lignin content may have a diminished ability to retain water, which could negatively impact microbial activity, decomposition rates, and nutrient cycling in the soil. In our study, the higher lignin content of oats may have contributed to the observed lower N release to the maize crop (Tables 4 and 5).

The results for legumes’ BNF rates were consistent across sites. At Site 2, lupine in mixture culture increased N fixation compared to monoculture. At the same time, no differences were observed at Site 1 (Table 4). Previous studies have indicated that the proportion of N derived from BNF increases when legumes are sown alongside non-legumes like oats. This increment in N fixation occurs because grasses are generally more efficient at competing for available soil N than legumes [55,56]. However, the ability of legumes to absorb inorganic N during their growth cycle may also explain the variability in their N fixation response to soil inorganic N levels [55,57,58]. In a field experiment, Guinet et al. [40] classified ten legume species based on their efficiency in inorganic N uptake. They found that species such as broad bean, lupine, fenugreek, and pea exhibited efficiencies of less than 0.5. In contrast, chickpea, bean, vetch, lentil, pea, and soybean demonstrated efficiencies equal to or greater than 0.5. These findings suggest that, for the lupine genus, N fixation is minimally impacted by the availability of soil inorganic N compared to other legume species.

3.4.2. Cover Crops Effects on Succeeding Maize Crop

The substantial differences in CC biomass production and N content (Table 3) within each site suggest that the predecessor crop was an essential source of N for the succeeding corn, particularly in the case of berseem clover, but this was not true for lupine or oats. Moreover, the higher CC’s stubble biomass production at Site 1 likely promoted N immobilization, explaining the lower corn N uptake in treatments with CC (except for berseem clover) than those without CC. However, the ANOVA did not show significant differences between oats, lupine, and mixtures (Table 6). Additionally, the pedoclimatic conditions at Site 1, characterized by coarser-textured soils and a cropping season with rainfall levels higher than the historical average, could have led to some N losses, mainly through leaching and denitrification in areas prone to waterlogging within the experimental site. Moreover, weed control at this site was ineffective, and probably, part of the available N was absorbed by weeds. Although CCs generally suppress weeds, grasses like oats are more effective in early-season weed control than legumes because they rapidly establish roots and emerge [59,60]. In our study, the highest incidence of weeds was observed in Site 1 plots with berseem clover, where weed biomass was 3 to 8 times higher than in the plots with oats. This result confirms the more remarkable ability of CC grasses to reduce weed incidence.

Based on the model for corn N fertilization in Uruguay proposed by Perdomo and Cardellino [61], which predicts response to N fertilization when N-NO_3^- concentrations are below 20 mg kg^{-1} in the top 20 cm of soil at sowing, all treatments at both sites would meet the criteria for a positive N response of corn. This positive N response was particularly evident for lupine and oats in monoculture and mixture culture at Site 1 and in all treatments in Site 2. The significant response in N uptake in both grain and whole plant in Site 2 may be attributed to the corn phenological stage when N was applied at the V10 stage, a period of high N demand. On the other hand, in Site 1, it was used earlier, at the V6 stage. Applying N at that later stage allowed for better synchronization of N supply with the crop's peak demand, improving the rate of N uptake by the corn [62].

Interestingly, in the 0 N treatments, the grain yield without a CC at Site 1 was significantly higher than that of lupine and oat monocultures but not substantially different from that of berseem clover. At Site 2, grain yield without a CC showed no significant differences compared to any evaluated CC. This suggests that a 100 kg N ha^{-1} fertilizer application was needed in the treatment without CC to match the grain yield of unfertilized CC treatments. At Site 1, only berseem clover achieved this yield, while at Site 2, no significant predecessor crop effect was observed, where the yield with 100 kg N ha^{-1} and no CC matched that of any unfertilized CC treatment. Maize grain yield and whole N plant content tend to be higher on legumes CC than oats (Table 6), which could be attributed to increased soil N supply, most likely due to the greater quantity of N-rich residues returned by these CC (Table 3). These results agree with Alvarez et al. [63], in a meta-analysis of 67 experiments across the Pampas region, reported that maize yield generally decreased by 8% following non-legume CC; however, it increased by 7% after legume CCs compared to fallow. In addition, they concluded that adopting legume CCs in the Pampas region is particularly recommended for optimizing corn production.

3.4.3. Cover Crops Effects on Nitrogen Recovery Indices from Conventional and Isotopic Experiments

Except for oats, which consistently showed negative values, the average $apNRE_{CC}$ was roughly zero at both sites. This low value of $apNRE_{CC}$ indicates that, in most cases, N derived from the other predecessor crops has contributed minimally as an N source for maize. Apparent NRE_{CC} was positively associated with N content of the CC biomass or low C:N ratio, while the opposite association with apparent NRE_{Fert} was observed due to the negative association between $apNRE_{CC}$ and $apNRE_{Fert}$, but this was only observed at Site 1 (Figure 3). This finding indicates that a higher residue biomass of CCs, and consequently higher C contribution, was linked to more efficient use of fertilizer N, highlighting the crucial role of soil C inputs in enhancing crop productivity, especially in cropping seasons with a high risk of N losses, such as in Site 1, which experienced above-average rainfall and presented low soil water retention capacity (Supplementary Materials, Figure S1). At Site 2, the variation in apNRE_{CC} did not account for the variation in $\text{apNRE}_{\text{Fert}}$, as the RS was similar across predecessor crops (Table 6). The lack of a significant predecessor crop effect may have been partly due to the drought during much of the corn growth cycle, which hindered residue decomposition. Lower CC biomass production at Site 2 could have also contributed to the reduced N input from CC residues.

Regarding the ^{15}N application timing, as expected, ^{15}NRE was higher when the N supply coincided with the periods of highest N demand by the crop (V6 and V10), which are stages of rapid growth and increased N uptake. The weighted $^{15}\text{NRE}_{\text{crop}}$ was linked to residue quality. At Site 1, the highest $^{15}\text{NRE}_{\text{crop}}$ was achieved with berseem clover, which had the lowest C:N ratio. At Site 2, the highest $^{15}\text{NRE}_{\text{crop}}$ was obtained with lupine grown in monoculture or mixture, which also had favorable C:N ratios for net N mineralization in the soil (Supplementary Materials, Figure S2). However, at Site 1, weeds may have also absorbed a significant portion of the N mineralized from berseem clover.

An unexpected outcome was the low recovery of ^{15}N in both the crop and soil in the oat treatments, observed at both sites and for both N application times. Additionally, at Site 1, even though this treatment resulted in the lowest weed invasion, it also had the lowest ^{15}N recovery (Tables 7 and 8). These results may be linked to the biochemical characteristics of the oat CC residue, such as its high lignin content, which slowed down decomposition and affected N cycling. Despite no significant effect of the preceding crop (Table 8), maize following oats had greater ^{15}N fertilizer losses (unaccounted-for N) than other CCs. The ^{15}N losses ranged from 40 to 84 kg N ha^{-1} at Sites 2 and 1, respectively, accounting for 40–80% of the applied N. We propose that this high proportion of unaccounted-for N could be due to two factors: 1. the low water retention of oat residues results in increased N losses (through leaching, denitrification, or volatilization), mainly because the labeled N was applied in liquid form. Moreover, this effect would be more pronounced at Site 1 due to edaphic factors such as the soil's lower cation exchange capacity and clay content. 2. the biochemical characteristics of oat stubble likely caused the added ^{15}N urea fertilizer to be diluted by native soil N more significantly than with other CCs [41,64]. The dilution effect observed with oats aligns with the findings of Schmatz et al. [65], who, through measuring soil N dynamic with labeled residues, reported a significant decrease in ^{15}N enrichment for wheat residue (which had a lower initial soluble C fraction). These authors suggested that some of the N measured in the remaining residue came from unlabelled soil N, which was assimilated by decomposers on the residue particles. In contrast, this effect was much less pronounced or absent in legume residues (*Vicia sativa* L., and *Pisum sativum* L.), where the labeled N was sufficiently available to meet microbial needs throughout the incubation period. The low soil ^{15}N recovery in oats treatments could also be attributed to the effects of quality and quantity of oat stubble on the intensity and direction of the priming effect, i.e., the change in the rate bulk SOC mineralization induced by the input of fresh organic residues. In this regard, studies by Liang et al. [66,67] have shown that CC residues with low soluble C fraction and high productivity, such as oat residues, are not associated with positive priming, meaning that the residue's decomposers microorganisms would be more dependent on N fertilizer, which would lead to have higher soil ^{15}N dilution.

Including CCs in both sites had significant practical implications: it improved maize's ^{15}NUE from N fertilizer, but specifically with berseem clover and mixture in Site 1 and with lupine in monoculture and mixture with oats in Site 2 (Table 7). The predecessor crop effect on soil microbial activity may explain the higher plant ^{15}N recovery [66]. Although this effect was statistically not significant on residual ^{15}N remaining in the soil, this variable had higher values in plots with CC compared with treatments without CCs (Table 8).

A negative correlation was observed between the C:N ratio of the CC residues and the weighted ^{15}NRE crop (Figure 4), suggesting that NUE improves with higher residue quality. This negative relationship arises because, with a high C:N ratio—such as in the case of oats—microorganisms likely immobilize some of the ^{15}N from the fertilizer to decompose the stubble, leading to lower recovery of the applied ^{15}N [68,69]. The strong negative association between the C:N ratio of CC residues and the weighted $^{15}NRE_{Crop}$ at Site 1 is intriguing, as one would expect a higher C:N ratio to increase reliance on fertilizer N due to limited N release from the stubble, resulting in higher $^{15}NRE_{crop}$. The lower N availability at Site 1 could explain the higher relative total N uptake by corn grown after oats, the highest among the CCs (Table 7). Therefore, the weighted $^{15}NRE_{crop}$ reflects the impact of CCs (especially its N biomass content) on the fate of ^{15}N more than $apNRE_{Fert}$, which showed a positive association with the C:N ratio of the CC. At Site 1, where the predecessor crop had a significant effect, $apNRE_{Fert}$ was higher in corn grown after oats and lower after berseem clover, opposite to the ^{15}NRE estimated by the isotopic method (Table 7). At Site 2, although the predecessor crop effect was not significant (Table 6), the highest $apNRE_{Fert}$ was found in corn grown on the mixture treatment, aligning with the ^{15}NRE results estimated by the isotopic method (Table 7). This outcome at Site 2 highlights the potential benefits of using a mixed CC; the more favorable C:N ratio in this mixed culture positioned it as one of the best treatments, achieving the highest yield and NRE (Tables 6 and 7). This success resulted from a more balanced proportion of species in the mixture, maximizing the advantages of each CC's species growing in mixture cultures [49,70–72]. These results agree with those obtained by Moreno-Cadena et al. [72], who assessed the performance of cereal rye and crimson clover mixture under varying soil N levels and sampling times, comparing it to monocultures. That study showed that the mixture offered advantages in maximizing CC performance and ecosystem services under different soil N conditions and termination times. In our study, the highest weighted $^{15}NRE_{crop}$ was observed with berseem clover at Site 1 and with lupine grown in monoculture or in mixed culture at Site 2 (Supplementary Materials, Figure S2), which coincides with the highest grain yields achieved in the corresponding site under fertilized treatments (Table 6).

It is also important to note that both conventional and isotopic methods may have evaluation errors, mainly due to the so-called "priming effect" or "interaction with added N" [65,73,74]. This phenomenon is linked to changes in SOM mineralization and potential interactions between the N from the mineralization of native organic N in the soil and the N added as fertilizer or from residues. In addition, this phenomenon could explain the discrepancies between findings derived from conventional and isotopic data, such as the negative association between the C:N ratio of CC residues and the weighted ^{15}NREcrop at Site 1 or the low plant and soil ^{15}N recovery in plots with oats stubble as was mentioned above.

Of the factors evaluated, only the timing of ^{15}N application significantly affected soil ^{15}N recovery. The findings suggest that weeds likely absorbed a substantial amount of the N applied to maize at Site 1. On average, 84% and 75% of the applied N at sowing and V6 or V10 stages were unaccounted for at Site 1, compared to 42% and 25% at Site 2. The high N losses at Site 1 may also be attributed to pedoclimatic conditions, such as higher rainfall and poorer soil structure and natural fertility than at Site 2.

3.5. Conclusions

The study was set up in two distinct agroecological zones and conducted during two cropping seasons, which allowed us to examine the CC residual effect on maize production and NUE under different conditions. At Site 1, oats in monoculture produced more biomass than at Site 2, while the oats grown in L-O mixture with lupine were more productive at Site 2. The biomass yield and the biochemical composition of the CCs also varied the soil mineral N dynamics, influencing the NRE and corn yield. At Site 1, the highest apNRE$_{\text{Fert}}$ value was associated with oats, while in Site 2, this was with lupine in L-O mixture culture. Differences in residue quality, such as C:N ratio, also impacted the fertilizer's N use efficiency. Overall, corn yield was related to both the quantity and quality of the CC biomass, with oats excelling in weed control, berseem clover in its N supply capacity, and the mixed CCs performing well at Site 2. This CC mixture would provide enhanced benefits by contributing C biomass to the formation of SOM and supplying N comparable to berseem clover but with a more remarkable ability to mitigate potential N losses due to the higher C:N ratio provided by oats in the L-O mixture culture.

The study concluded that at Site 1, which had medium- to low-fertility soils and above-average rainfall, the best grain yield, N use efficiency, and lowest risk of N losses were associated with maize cultivated on berseem clover without fertilization or oats fertilized, providing this last one CC with superior weed control. Corn performed best at Site 2, where soils were more fertile than at Site 1, and rainfall was below the historical average, having greater N use efficiency when grown over CCs of lupine grown in monoculture or mixture with oats, with the latter providing a more substantial contribution of C. Thus, the impact of the predecessor crop on corn was linked to the quantity and quality of the CC residual biomass, the inherent soil characteristics of each site, and environmental conditions during each experimental period.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: Each experimental site's soil moisture curves (0–15 cm depth) (Site 1 and 2). The filled circles (Site 1) and triangles (Site 2) correspond to the data used for model fitting (the nonlinear regression functions). At the same time, the unfilled symbols indicate the data used for model validation represented by the equation $\psi = a\ \theta_g^{-b}$; Figure S2: The weighted N recovery efficiency of the maize (^{15}NREcrop) according to site and predecessor cover crop. Different letters indicate significant differences between predecessor crops within each site at a *p*-level of < 0.05; Figure S3: Soil ^{15}N recovery at the four depth layers (0–5, 5–10, 10–15, and 15–30 cm) from N fertilizer applied (100 kg ha^{-1}) at two ^{15}N application timings (sowing and vegetative stages), and four predecessor crops and one control treatment (no CC) at Site 1 (panels a–e) and Site 2 (panels f–j); Table S1: Overview of activities in the experiments installed at Sites 1 and 2, organized into two phases: 1—Cover crop establishment and 2—Maize establishment (including conventional and isotopic experiments); Table S2: Treatments evaluated in the isotopic experiment; Table S3: Soil gravimetric moisture at 0–15 cm depth layer according to each sampling time and predecessor crop at Site 1 (2018–2019) and Site 2 (2019–2020). Values are replicate averages for each site–time–predecessor crop ± standard error; Table S4: Pearson correlation coefficients (r) within each experimental site (1 and 2) and ^{15}N application timing (sowing and vegetative stage) between variables related to plant and soil ^{15}N recovery and AG CC biomass.

Author Contributions: Conceptualization, C.M.A. and C.P.V.; methodology, C.M.A.; formal analysis, C.M.A. and C.P.V.; investigation, C.M.A.; resources, C.M.A.; data curation, C.M.A., C.P.V. and A.d.P.M.; writing—original draft preparation, C.M.A. and A.d.P.M.; writing—review and editing, C.M.A., C.P.V. and A.d.P.M.; visualization, C.M.A.; supervision, A.d.P.M.; project administration, C.M.A.; funding acquisition, C.M.A., C.P.V. and A.d.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research and Innovation Agency of Uruguay, Funds: María Viñas, grant number FMV 135487; University of Republic of Uruguay Funds: Fellowship CAP grants; and the APC was funded by the National Research of our Institution, the University of the Republic (UdelaR, Uruguay).

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors thank the farmers for allowing us to set up experiments and sample their fields. Leticia Martínez helped with soil characterization and classification of the soil. Lucas Sosa, Nicola Di Muro, Bruno Romano, Andrea Bentos Guimaraes, and Ana Elhordoy helped us with their assistance throughout the field experiments and laboratory work. We also thank Juan P. Viera for their technical support during the entire period of this investigation work (2018–2020), and Jorge Foderé (FADISOL S.A Uruguayan Company) for their field assistance and collaboration in materials used for experiments (fertilizers, herbicides, and seeds).

Conflicts of Interest: The authors declare no conflicts of interest.

3.6. References

1. Arbeletche, P. Agribusiness in Uruguay: Evolution and Its Changeant Estrategies in the 20th Century. *Rev. Iberoam. Vitic. Agroind. Rural.* **2020**, *7*, 109–129. https://doi.org/10.35588/rivar.v7i19.4355.

2. Vassallo, M. Dinámica y Competencia Intrasectorial En La Agricultura Uruguaya: Los Cambios En La Última Década. *Agrociencia Uruguay.* **2013**, *17*, 170–179. https://doi.org/10.31285/agro.17.488.

3. Ernst, O.R.; Dogliotti, S.; Cadenazzi, M.; Kemanian, A.R. Shifting Crop-Pasture Rotations to No-Till Annual Cropping Reduces Soil Quality and Wheat Yield. *Field Crop. Res.* **2018**, *217*, 180–187. https://doi.org/10.1016/j.fcr.2017.11.014.

4. Rubio, V.; Quincke, A.; Ernst, O. Deep Tillage and Nitrogen Do Not Remediate Cumulative Soil Deterioration Effects of Continuous Cropping. *Agron. J.* **2021**, *113*, 5584–5596. https://doi.org/10.1002/agj2.20927.

5. Pravia, M.V.; Kemanian, A.R.; Terra, J.A.; Shi, Y.; Macedo, I.; Goslee, S. Soil Carbon Saturation, Productivity, and Carbon and Nitrogen Cycling in Crop-Pasture Rotations. *Agric. Syst.* **2019**, *171*, 13–22. https://doi.org/10.1016/j.agsy.2018.11.001.

6. Xie, H.; Huang, Y.; Chen, Q.; Zhang, Y.; Wu, Q. Prospects for Agricultural Sustainable Intensification: A Review of Research. *Land* **2019**, *8*, 157. https://doi.org/10.3390/land8110157.

7. Rubio, V.; Núñez, A.; Berger, A.; van Es, H. Biomass Inputs Drive Agronomic Management Impacts on Soil Health. *Agric. Ecosyst. Environ.* **2024**, *378*, 109316. https://doi.org/10.1016/j.agee.2024.109316.

8. Mazzilli, S.R.; Ernst, O.R. Rapeseed-to-Wheat Yield Ratio in Different Production Environments and Effects on Subsequent Summer Crops Yields. *Agrosyst. Geosci. Environ.* **2019**, *2*, 1–7.

9. Rossini, G.F.I. Soil and Subsoil Conservation from an Environmental Perspective. *Rev. Derecho* **2022**, *21*, 41–52. https://doi.org/10.47274/DERUM/42.3.

10. Mazzilli, S.R.; Kemanian, A.R.; Ernst, O.R.; Jackson, R.B.; Piñeiro, G. Greater Humification of Belowground than Aboveground Biomass Carbon into Particulate Soil Organic Matter in No-Till Corn and Soybean Crops. *Soil Biol. Biochem.* **2015**, *85*, 22–30. https://doi.org/10.1016/j.soilbio.2015.02.014.

11. Ernst, O. Efecto De Una Leguminosa Invernal Como Cultivo de Cobertura Sobre Rendimiento En Grano y Respuesta a Nitrógeno DE Maíz Sembrado Sin Laboreo. *Agrociencia Uruguay.* **2006**, *10*, 25–35. https://doi.org/10.31285/agro.10.937.

12. Constantin, J.; Mary, B.; Laurent, F.; Aubrion, G.; Fontaine, A.; Kerveillant, P.; Beaudoin, N. Effects of Catch Crops, No Till and Reduced Nitrogen Fertilization on Nitrogen Leaching and Balance in Three Long-Term Experiments. *Agric. Ecosyst. Environ.* **2010**, *135*, 268–278. https://doi.org/10.1016/j.agee.2009.10.005.

13. Prieto, G.S.; Ernst, O. Effect of Legume or Grass Cover Crops and Nitrogen Application Rate on Soil Properties and Corn Productivity. *Agrociencia Uruguay.* **2012**, *16*, 294–301. https://doi.org/10.31285/agro.16.686.

14. Kaye, J.P.; Quemada, M. Using Cover Crops to Mitigate and Adapt to Climate Change. A Review. *Agron. Sustain. Dev.* **2017**, *37*, 4. https://doi.org/10.1007/s13593-016-0410-x.

15. Iqbal, A.; Aslam, S.; Alavoine, G.; Benoit, P.; Garnier, P.; Recous, S. Rain Regime and Soil Type Affect the C and N Dynamics in Soil Columns that are Covered with Mixed-Species Mulches. *Plant Soil* **2015**, *393*, 319–334. https://doi.org/10.1007/s11104-015-2501-x.

16. Meisinger, J.J.; Randall, G.W. Estimating Nitrogen Budgets for Soil-Crop Systems. *Manag. Nitrogen Groundw. Qual. Farm Profitab.* **2015**, *1*, 85–124. https://doi.org/10.2136/1991.managingnitrogen.c5.

17. Moukanni, N.; Brewer, K.M.; Gaudin, A.C.M.; O'Geen, A.T. Optimizing Carbon Sequestration Through Cover Cropping in Mediterranean Agroecosystems: Synthesis of Mechanisms and Implications for Management. *Front. Agron.* **2022**, *4*, 844166. https://doi.org/10.3389/fagro.2022.844166.

18. Abdalla, M.; Hastings, A.; Cheng, K.; Yue, Q.; Chadwick, D.; Espenberg, M.; Truu, J.; Rees, R.M.; Smith, P. A Critical Review of the Impacts of Cover Crops on Nitrogen Leaching, Net Greenhouse Gas Balance and Crop Productivity. *Glob. Chang. Biol.* **2019**, *25*, 2530–2543. https://doi.org/10.1111/gcb.14644.

19. Zhang, Z.; Wang, J.; Huang, W.; Chen, J.; Wu, F.; Jia, Y.; Han, Y.; Wang, G.; Feng, L.; Li, X.; et al. Cover Crops and N Fertilization Affect Soil Ammonia Volatilization and N2O Emission by Regulating the Soil Labile Carbon and Nitrogen Fractions. *Agric. Ecosyst. Environ.* **2022**, *340*, 108188. https://doi.org/10.1016/j.agee.2022.108188.

20. Johnson, F.E.; Roth, R.T.; Ruffatti, M.D.; Armstrong, S.D. Cover Crop Impacts on Nitrogen Losses and Environmental Damage Cost. *Agric. Ecosyst. Environ.* **2024**, *363*, 108859. https://doi.org/10.1016/j.agee.2023.108859.

21. Oberson, A.; Jarosch, K.A.; Frossard, E.; Hammelehle, A.; Fliessbach, A.; Mäder, P.; Mayer, J. Higher than Expected: Nitrogen Flows, Budgets, and Use Efficiencies Over 35 Years of Organic and Conventional Cropping. *Agric. Ecosyst. Environ.* **2024**, *362*, 108802. https://doi.org/10.1016/j.agee.2023.108802.

22. Landriscini, M.R.; Galantini, J.A.; Martínez, J.M. Eficiencia de Recuperación de Nitrógeno Por Los Métodos de la Diferencia y de la Dilución Isotópica. *Cienc. Suelo* **2016**, *34*, 155–162.

23. Ovalle, C.; del Pozo, A.; Peoples, M.B.; Lavín, A. Estimating the Contribution of Nitrogen From Legume Cover Crops to the Nitrogen Nutrition of Grapevines Using a 15N Dilution Technique. *Plant Soil* **2010**, *334*, 247–259. https://doi.org/10.1007/s11104-010-0379-1.

24. Espinoza, S.; Ovalle, C.; Zagal, E.; Matus, I.; Tay, J.; Peoples, M.B.; Del Pozo, A. Contribution of Legumes to Wheat Productivity in Mediterranean Environments of Central Chile. *Field Crop. Res.* **2012**, *133*, 150–159. https://doi.org/10.1016/j.fcr.2012.03.006.

25. Wang, J.; Sainju, U.M.; Zhang, S. A Mixture of Summer Legume and Nonlegume Cover Crops Enhances Winter Wheat Yield, Nitrogen Uptake, and Nitrogen Balance. *Nitrogen* **2024**, *5*, 871–890. https://doi.org/10.3390/nitrogen5040056.

26. Sawchik, J.; Pérez-Bidegain, M.; García, C. Impact of Winter Cover Crops on Soil Properties Under Soybean Cropping Systems. *Agrociencia Urug.* **2012**, *16*, 288–293. https://doi.org/10.31285/agro.16.685.

27. Sawchik, J.; Siri, G.; Ayala, W.; Barrios, E.; Bustamante, M.; Ceriani, M.; Gutierrez, F.; Mosqueira, J.; Otaño, C.; Perez, M.; et al. El Sistema Agrícola Bajo Amenaza: ¿Qué Aportanlos Cultivos de Cobertura y/o Laspasturas Cortas? *IV Simp. Nac. Agric.* **2015**, *4*, 149–168.

28. Alvarez, S.; Ernst, O. Impact of Cropping Systems on Soil Quality. *Eur. J. Agron.* **2024**, *158*, 127197. https://doi.org/10.1016/j.eja.2024.127197.

29. Durán, A.; Califra, A.; Molfino, J.H.; Lynn, W. *Keys to Soil Taxonomy for Uruguay*; USDA, Natural Resources Conservation Service (NRCS): Washington, DC, USA, 2006; p. 77.

30. Durán, A.; Fernando, G.P. *Suelos Del Uruguay, Origen, Clasificación, Manejo Y Conservación*; Southern Hemisphere: Montevideo, Uruguay, 2007; Volume 2, ISBN 978-9974-674-02-8.

31. Assefa, Y.; Carter, P.; Hinds, M.; Bhalla, G.; Schon, R.; Jeschke, M.; Paszkiewicz, S.; Smith, S.; Ciampitti, I.A. Analysis of Long Term Study Indicates Both Agronomic Optimal Plant Density and Increase Maize Yield per Plant Contributed to Yield Gain. *Sci. Rep.* **2018**, *8*, 1–11. https://doi.org/10.1038/s41598-018-23362-x.

32. Ritchie, S.W.; Hanway, J.J. *How a Corn Plant Develops*; Iowa State University of Science and Technology: Ames, IA, USA, 1993; pp. 1–25.

33. IAEA (1990) Use of nuclear techniques in studies of soil-plant relationships. Training course series no 2 (Hardarson G, ed). International Atomic Energy Agency, Vienna, Austria, 223 pp .

34. Rayment, G.E.; Lyons, D.J. *Soil Chemical Methods*; CSIRO Publishing: Clayton, Australia, 2011; ISBN 978-0-643-06768-4.

35. Nelson, D.W.; Sommers, L.E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis*; Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Soltanpour, P.N., Tabatabai, M.A., Johnston, C.T., Sumner, M.E., Eds.; SSSA: Madison, WI, USA, 1996.

36. Thomas, G.W. Exchangeable Cations. In *Methods of Soil Analysis*; Page, A., Ed.; SSSA: Madison, WI, USA, 1983; pp. 159–165.

37. Lajtha, K.; Driscoll, C.T.; Jarrell, W.M.; Elliott, E.T. Soil phosphorus: Characterization and total element analysis. In *Standard Soil Methods for Long Term Ecological Research*; Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P., Eds.; Oxford University Press: Oxford, UK, 1999; pp. 115–143, ISBN 0-19-512083-3.

38. Mulvaney, R.L. Nitrogen-Inorganic Forms. In *Methods of Soil Analysis, Part 3: Chemical Methods*; Wiley: New Jersey, NJ, USA, 2018; pp. 1123–1184, ISBN 9780891188667.

39. Rhine, E.D.; Mulvaney, R.L.; Pratt, E.J.; Sims, G.K. Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. *Soil Sci. Soc. Am. J.* **1998**, *62*, 473–480. https://doi.org/10.2136/sssaj1998.03615995006200020026x.

40. Gee, G.W.; Bauder, J.W. Particle Size Analysis by Hydrometer: A Simplified Method for Routine Textural Analysis and a Sensitivity Test of Measurement Parameters. *Soil Sci. Soc. Am. J.* **1979**, *43*, 1004–1007. https://doi.org/10.2136/sssaj1979.03615995004300050038x.

41. Van Cleemput, O.; Zapata, F.; Vanlauwe, B. Guidelines on Nitrogen Management in Agricultural Systems. In *Guidelines on Nitrogen Management in Agricultural System*; Soil Science Unit: Seibersdorf, Austria, 2008; pp. 19–125.

42. Tamagno, S.; Eagle, A.J.; McLellan, E.L.; van Kessel, C.; Linquist, B.A.; Ladha, J.K.; Pittelkow, C.M. Quantifying N leaching losses as a function of N balance: A Path to Sustainable Food Supply Chains. *Agric. Ecosyst. Environ.* **2022**, *324*, 107714. https://doi.org/10.1016/j.agee.2021.107714.

43. Shearer, G.; Kohl, D.H. N2-Fixation in Field Settings: Estimations Based on Natural 15N Abundance. *Aust. J. Plant Physiol.* **1987**, *13*, 699–756. https://doi.org/10.1071/pp9860699.

44. Hardarson, G.; Danso, S.K.A. Methods for Measuring Biological Nitrogen Fixation in Grain Legumes. *Plant Soil* **1993**, *152*, 19–23. https://doi.org/10.1007/BF00016330.

45. Giménez, L. Producción de Maíz Con Estrés Hídrico Provocado En Diferentes Etapas de Desarrollo. *Agrociencia* **2012**, *16*, 92–102. https://doi.org/10.31285/agro.17.544.

46. Soares, M.; Rousk, J. Microbial Growth and Carbon Use Efficiency in Soil: Links to Fungal-Bacterial Dominance, SOC-Quality and Stoichiometry. *Soil Biol. Biochem.* **2019**, *131*, 195–205. https://doi.org/10.1016/j.soilbio.2019.01.010.

47. Cabrera, M.L.; Kissel, D.E.; Vigil, M.F. Nitrogen Mineralization from Organic Residues. *J. Environ. Qual.* **2005**, *34*, 75–79. https://doi.org/10.2134/jeq2005.0075.

48. Chaves, B.; Redin, M.; Giacomini, S.J.; Schmatz, R.; Léonard, J.; Ferchaud, F.; Recous, S. The Combination of Residue Quality, Residue Placement AND Soil Mineral N Content Drives C and N Dynamics by Modifying N Availability to Microbial Decomposers. *Soil Biol. Biochem.* **2021**, *163*, 108434. https://doi.org/10.1016/j.soilbio.2021.108434.

49. Finney, D.M.; White, C.M.; Kaye, J.P. Biomass Production and Carbon/Nitrogen Ratio Influence Ecosystem Services from Cover Crop Mixtures. *Agron. J.* **2016**, *108*, 39–52. https://doi.org/10.2134/agronj15.0182.

50. Sun, L.; Xia, Z.; Sang, C.; Wang, X.; Peng, B.; Wang, C.; Zhang, J.; Müller, C.; Bai, E. Soil Resource Status Affects the Responses of Nitrogen Processes to Changes in Temperature and Moisture. *Biol. Fertil. Soils* **2019**, *55*, 629–641. https://doi.org/10.1007/s00374-019-01379-2.

51. Çakir, R. Effect of Water Stress at Different Development Stages on Vegetative and Reproductive Growth of Corn. *Field Crop. Res.* **2004**, *89*, 1–16. https://doi.org/10.1016/j.fcr.2004.01.005.

52. Liu, Q.; Luo, L.; Zheng, L. Lignins: Biosynthesis and Biological Functions in Plants. *Int. J. Mol. Sci.* **2018**, *19*, 335. https://doi.org/10.3390/ijms19020335.

53. Kong, L.; Sun, M.; Wang, F.; Liu, J.; Feng, B.; Si, J.; Zhang, B.; Li, S.; Li, H. Effects of High Nh+4 on k+ Uptake, Culm Mechanical Strength and Grain

Filling IN Wheat. *Front. Plant Sci.* **2014**, *5*, 703. https://doi.org/10.3389/fpls.2014.00703.

54. Dann, C.E.; Cabrera, M.L.; Thapa, R.; Mirsky, S.; Tully, K.; Reberg-Horton, C.; Hitchcock, R.; Morari, F. Modeling Water Potential of Cover Crop Residues on the Soil Surface. *Ecol. Model.* **2021**, *459*, 109708. https://doi.org/10.1016/j.ecolmodel.2021.109708.

55. Guinet, M.; Nicolardot, B.; Revellin, C.; Durey, V.; Carlsson, G.; Voisin, A.S. Comparative Effect of Inorganic N on Plant Growth and N2 Fixation of Ten Legume Crops: Towards a Better Understanding of the Differential Response Among Species. *Plant Soil* **2018**, *432*, 207–227. https://doi.org/10.1007/s11104-018-3788-1.

56. Schipanski, M.E.; Drinkwater, L.E. Nitrogen Fixation in Annual and Perennial Legume-Grass Mixtures Across a Fertility Gradient. *Plant Soil* **2012**, *357*, 147–159. https://doi.org/10.1007/s11104-012-1137-3.

57. Evans, J.; O’Connor, G.E.; Turner, G.L.; Bergersen, F.J. Influence of Mineral Nitrogen on Nitrogen Fixation by Lupin (Lupinus angustifolius) as Assessed by 15N Isotope Dilution Methods. *Field Crop. Res.* **1987**, *17*, 109–120. https://doi.org/10.1016/0378-4290(87)90086-4.

58. Liu, Y.; Wu, L.; Baddeley, J.A.; Watson, C.A. Models of Biological Nitrogen Fixation of Legumes. *Sustain. Agric.* **2009**, *2*, 883–905. https://doi.org/10.1007/978-94-007-0394-0_39.

59. Williams, A.; Wells, M.S.; Dickey, D.A.; Hu, S.; Maul, J.; Raskin, D.T.; Reberg-Horton, S.C.; Mirsky, S.B. Establishing the Relationship of Soil Nitrogen Immobilization to Cereal Rye Residues in a Mulched System. *Plant Soil* **2018**, *426*, 95–107. https://doi.org/10.1007/s11104-018-3566-0.

60. Koudahe, K.; Allen, S.C.; Djaman, K. Critical Review of the Impact of Cover Crops on Soil Properties. *Int. Soil Water Conserv. Res.* **2022**, *10*, 343–354. https://doi.org/10.1016/j.iswcr.2022.03.003.

61. Perdomo, C.; Cardellino, G. Respuesta de Maíz a Fertilizaciones Definidas con Diferentes Criterios de Recomendación. *Agrociencia* **2006**, *10*, 63–79. https://doi.org/10.31285/agro.10.942.

62. De Oliveira, S.M.; De Almeida, R.E.M.; Ciampitti, I.A.; Junior, C.P.; Lago, B.C.; Trivelin, P.C.O.; Favarin, J.L. Understanding N Timing in Corn Yield and Fertilizer N Recovery: An Insight from an Isotopic Labeled-N Determination. *PLoS ONE* **2018**, *13*, e0192776. https://doi.org/10.1371/journal.pone.0192776.

63. Alvarez, R.; Steinbach, H.S.; De Paepe, J.L. Cover Crop Effects on Soils and Subsequent Crops in the Pampas: A Meta-Analysis. *Soil Tillage Res.* **2017**, *170*, 53–65. https://doi.org/10.1016/j.still.2017.03.005.

64. Fillery, I.R.P.; Recous, S. Use of Enriched ^{15}N Sources to Study Soil N Transformations. *Curr. Plant Sci. Biotechnol. Agric.* **2001**, *9*, 167–194. https://doi.org/10.1007/978-94-015-9841-5_9.

65. Schmatz, R.; Recous, S.; Aita, C.; Tahir, M.M.; Schu, A.L.; Chaves, B.; Giacomini, S.J. Crop Residue Quality and Soil Type Influence the Priming Effect but Not the Fate of Crop Residue C. *Plant Soil* **2017**, *414*, 229–245. https://doi.org/10.1007/s11104-016-3120-x.

66. Liang, X.; Yuan, J.; Yang, E.; Meng, J. Responses of Soil Organic Carbon Decomposition and Microbial Community to the Addition of Plant Residues with Different C:N Ratio. *Eur. J. Soil Biol.* **2017**, *82*, 50–55. https://doi.org/10.1016/j.ejsobi.2017.08.005.

67. Liang, Z.; Rasmussen, J.; Poeplau, C.; Elsgaard, L. Priming Effects Decrease with the Quantity of Cover Crop Residues-Potential Implications for Soil Carbon Sequestration. *Soil Biol. Biochem.* **2023**, *184*, 109110. https://doi.org/10.1016/j.soilbio.2023.109110.

68. Baijukya, F.P.; De Ridder, N.; Giller, K.E. Nitrogen Release from Decomposing Residues of Leguminous Cover Crops and Their Effect on Maize Yield on Depleted Soils of Bukoba District, Tanzania. *Plant Soil* **2006**, *279*, 77–93. https://doi.org/10.1007/s11104-005-2504-0.

69. Asagi, N.; Ueno, H. Nitrogen Dynamics in Paddy Soil Applied with Various ^{15}N-labelled Green Manures. *Plant Soil* **2009**, *322*, 251–262. https://doi.org/10.1007/s11104-009-9913-4.

70. Dabney, S.M.; Delgado, J.A.; Collins, F.; Meisinger, J.J.; Schomberg, H.H.; Liebig, M.A; Kaspar, T.; Mitchell, J. Using Cover Crops and Cropping

Systems for Nitrogen Management. In *Advances in Nitrogen Management*; Soil and Water Conservation Society: Ankeny, SW, USA, 2010; pp. 230–281.

71. Chapagain, T.; Lee, E.A.; Raizada, M.N. The Potential of Multi-Species Mixtures to Diversify Cover Crop Benefits. *Sustainability* **2020**, *12*, 2058. https://doi.org/10.3390/su12052058.

72. Moreno-Cadena, P.; Salmeron, M.; Canisares, L.P.; Poffenbarger, H.J. Productivity Benefits of Cereal-Legume Cover Crop Mixtures Under Variable Soil Nitrogen and Termination Times. *Eur. J. Agron.* **2024**, *155*, 127114. https://doi.org/10.1016/j.eja.2024.127114.

73. Jenkinson, D.S.; Fox, R.H.; Rayner, J.H. Interactions Between Fertilizer Nitrogen and Soil Nitrogen—The so-Called 'Priming' Effect. *J. Soil Sci.* **1985**, *36*, 425–444. https://doi.org/10.1111/j.1365-2389.1985.tb00348.x.

74. Kuzyakov, Y.; Friedel, J.K.; Stahr, K. Review of Mechanisms and Quantification of Priming Effects. *Soil Biol. Biochem.* **2000**, *32*, 1485–1498. https://doi.org/10.1016/S0038-0717(00)00084-5.

Supplementary Material

Nitrogen Residual Effect of Winter Cover Crops on Maize in Uruguay: Conventional and Isotopic Evaluation

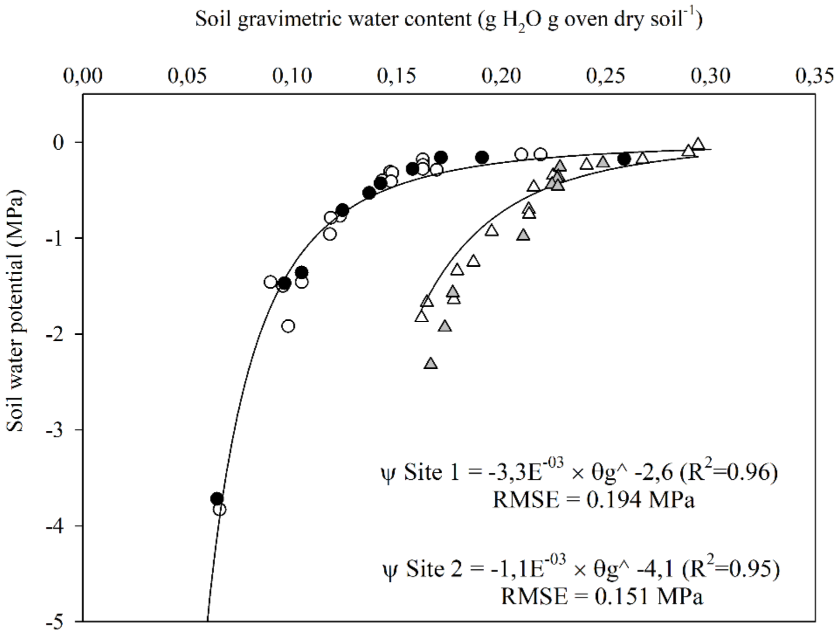


Figure S1. Each experimental site's soil moisture curves (0-15 cm depth) (Site 1 and 2). The filled circles (Site 1) and triangles (Site 2) correspond to the data used for model fitting (the nonlinear regression functions). At the same time, the unfilled symbols indicate the data used for model validation represented by the equation $\psi = a\ \theta_g^{-b}$.

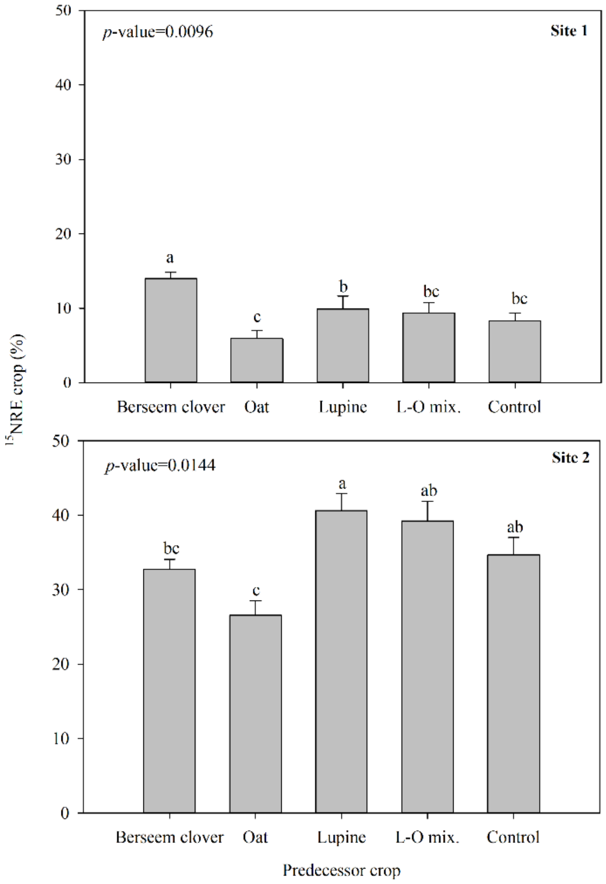


Figure S2. The weighted N recovery efficiency of the maize (^{15}NREcrop) according to site and predecessor cover crop. Different letters indicate significant differences between predecessor crops within each site at a p-level of < 0.05.

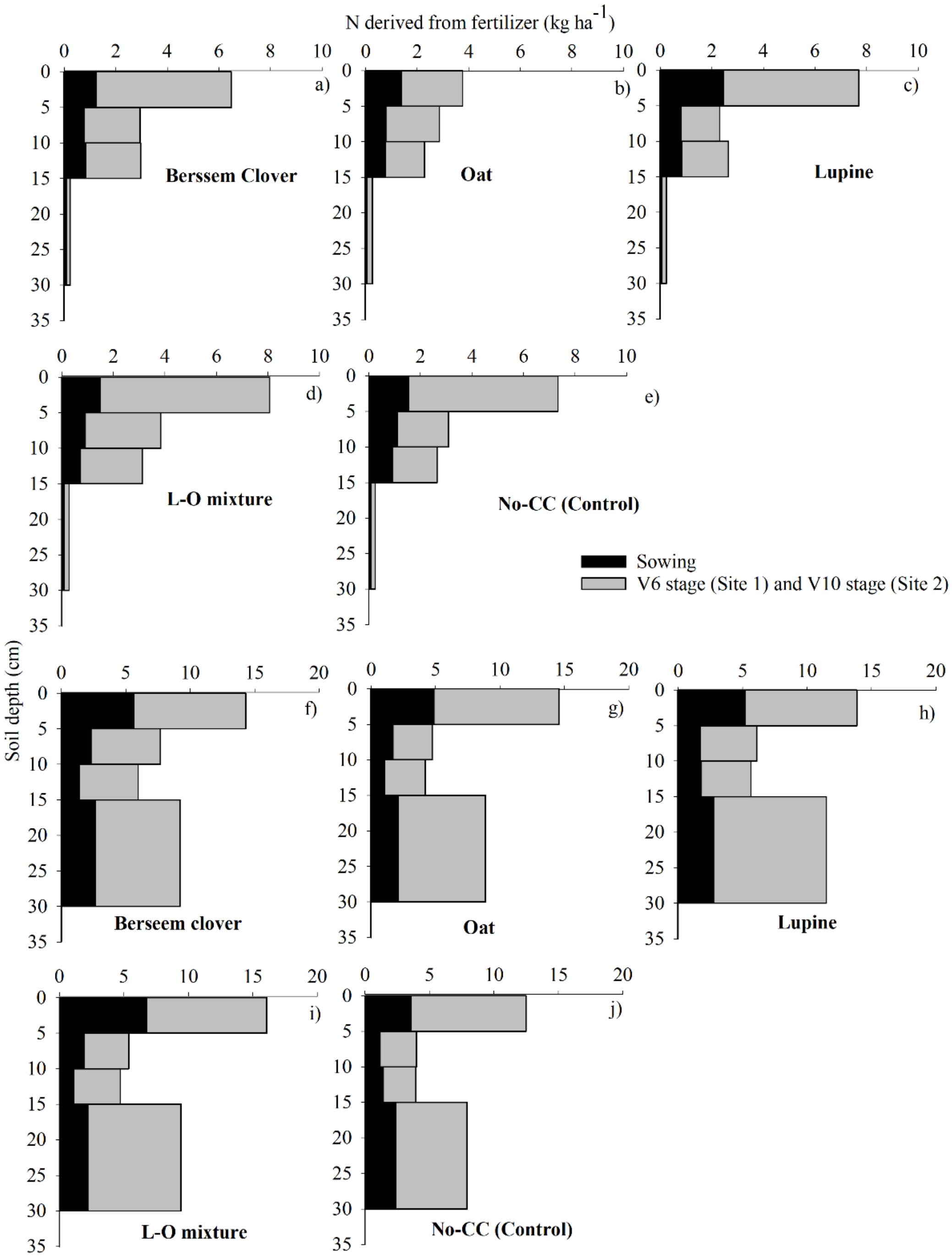


Figure S3. Soil ^{15}N recovery at the four depth layers (0-5, 5-10, 10-15, and 15-30 cm) from N fertilizer applied (100 kg ha^{-1}) at two ^{15}N application timings (sowing and vegetative stages), and four predecessor crops and one control treatment (No-CC) at Site 1 (panels a-e) and Site 2 (panels f-j).

Table S1. Overview of activities in the experiments installed at Sites 1 and 2, organized into two phases: 1- Cover crop establishment and 2- Maize establishment(including conventional and isotopic experiments).

	Location	San José, Libertad (Site 1)	Colonia, Ombúes de Lavalle (Site 2)
Cropping season Experimental phase	Activity	2018-2019	2019-2020
Cover crop (CC) establishment	Sowing date of CC	26-04-2018	9-07-2019
	Basal Fertilization	60 kg ha^{-1} K_2O (KCl) y 60 kg ha^{-1} P_2O_5 (triple superphosphate)	
	CC sowing density (kg ha^{-1})	Berseem clover: 15 Lupine:200 Oat:80 L-O Mixture: 160-180 y 65-60, first and second cropping season, respectively.	
	CC Termination date (herbicide)	10-10-2018	5-11-2019
Maize establishment: conventional experiment	Maize sowing date	10-12-2018	2-01-2020
	V6 stage	21-01-2019	2-02-2020
	Physiological maturity	23-04-2019	4-05-2020
	Vegetal material	Hybrid Dekalb 7 210	Hybrid SYN840 Viptera3

	Sowing density (plants ha^{-1})	65000-70000
	N Treatments (kgha^{-1})	0, 100 applied 1/3 at sowing, and 2/3 at V6 stage in 2019, and in 2020 N fertilizer was applied at V10 stage of maize.
Maize: isotopic experiment	Microplots† ^{15}N (1.5 m ×1.5 m)	Labeled urea was applied using an N rate of 100 kg ha^{-1}, split at two points in time: 1. First one-third at sowing, and 2. Two third at the V6 (Site 1) and V10 stages (Site 2) of maize, as in the conventional experiment).

†Microplots were adjacent to the 100N plots of the conventional experiment. In addition, all other tasks (sowing date, basal fertilization, N fertilization times, among others) were the same as those of the conventional experiment.

Table S2. Treatments evaluated in the isotopic experiment.

Treatments	Total N rate	N rate-at maize sowing	N rate-at V6 stage
		kg ha^{-1}	
1-	100	33⋆	66
2-	100	33	66⋆

⋆: ^{15}N Labeled urea (at sowing- 6 %at. ^{15}Nexc. and V6 stage- 3 % at. ^{15}Nexc.)

Table S3. Soil gravimetric moisture at 0-15 cm depth layer according to each sampling time and predecessor crop at Site 1(2018-2019) and Site 2 (2019-2020). Values are replicate averages for each site- time-predecessor crop ± standard error.

Site	Time after CC termination	Predecessor crop	Soil gravimetric moisture
	Days		g water g dry soil^{-1}

1	38	Berseem clover	0.30±0.02
		Oat	0.32±0.01
		Lupine	0.31±0.02
		L-O mixture	0.31±0.00
	57	Berseem clover	0.29±0.02
		Oat	0.30±0.01
		Lupine	0.27±0.02
		L-O mixture	0.30±0.01
	71	Berseem clover	0.32±0.02
		Oat	0.33±0.00
		Lupine	0.30±0.02
		L-O mixture	0.32±0.01
Significance of treatment effect			*p-value*
		Time	n.s
		Predecessor crop	n.s
		Time× Predecessor crop	n.s
2	45	Berseem clover	0.22±0.02^{B}
		Oat	0.27±0.03
		Lupine	0.27±0.02
		L-O mixture	0.24±0.01^{B}
	65	Berseem clover	0.28±0.01^{A}
		Oat	0.28±0.02
		Lupine	0.27±0.01
		L-O mixture	0.29±0.02^{A}
	79	Berseem clover	0.28±0.01^{A}
		Oat	0.30±0.03
		Lupine	0.28±0.01
		L-O mixture	0.28±0.02^{A}
Significance of treatment effect			*p-value*

Time	0.0005
Predecessor crop	n.s
Time× Predecessor crop	n.s

Different capital letters within a column indicate differences between sampling times within each site and predecessor crop, being significant at a *p*-level of 0.05; ns: means no significant difference

Table S4. Pearson correlation coefficients (r) within each experimental site (1 and 2) and ^{15}N application timing (Sowing and Vegetative stage) between variables related to plant and soil ^{15}N recovery and AG CC biomass.

Site	^{15}N application timing	Variable(1)	Variable(2)	Pearson (r)	*p*-value
1	Sowing	AG CC biomass	Ndff crop (corn)	0.09	n.s
		Ndff crop (corn)	N unaccounted-for	-0.65	0.0089
		Ndff (0-30 cm soil depth)	Ndff (0-5 cm soil depth)	0.96	0.0000
		Ndff (0-30 cm soil depth)	N unaccounted-for	-0.55	0.0334
		Ndff (0-5 cm soil depth)	N unaccounted-for	-0.56	0.0305
	V6 stage	AG CC biomass	Ndff crop (corn)	-0.06	n.s
		Ndff crop (corn)	N unaccounted-for	-0.78	0.0007

		Ndff (0-30 cm soil depth)	Ndff (0-5 cm soil depth)	0.98	0.0000
		Ndff (0-30 cm soil depth)	N unaccounted-for	-0.89	0.0000
		Ndff (0-5 cm soil depth)	N unaccounted-for	-0.86	0.0000
2	Sowing	AG CC biomass	Ndff crop (corn)	0.36	n.s
		Ndff crop (corn)	N unaccounted-for	-0.75	0.0012
		Ndff (0-30 cm soil depth)	Ndff (0-5 cm soil depth)	0.94	0.0000
		Ndff (0-30 cm soil depth)	N unaccounted-for	-0.87	0.0000
		Ndff (0-5 cm soil depth)	N unaccounted-for	-0.79	0.0004
	V10 stage	AG CC biomass	Ndff crop (corn)	-0.61	0.0157
		Ndff crop (corn)	N unaccounted-for	-0.81	0.0002
		Ndff (0-30 cm soil depth)	Ndff (0-5 cm soil depth)	0.37	n.s
		Ndff (0-30 cm soil depth)	N unaccounted-for	-0.75	0.0013
		Ndff (0-5 cm soil depth)	N unaccounted-for	-0.14	n.s

AG CC biomass, aboveground cover crop biomass; Nddf, Nitrogen derived from fertilizer; n.s : means not significant.

4. Lupine Cultivation Affects Soil’s P Availability and Nutrient Uptake in Four Contrasting Soils

(This article belongs to the Special Issue Legumes in Sustainable Cropping Systems)

Este estudio se centró en evaluar el impacto de dos especies de lupino (*L. albus* y *L. angustifolius*) en la movilización de P en el suelo y su asociación con la acidificación de este a través de la comparación de la respuesta en la productividad de biomasa aérea y en la absorción de nutrientes (P, N, cationes básicos, Mn y Fe) con la avena (*Avena strigosa*) en cuatro tipos de suelo contrastantes (en términos de disponibilidad de P, acidez, fertilidad y textura). Los objetivos fueron evaluar los cambios en la disponibilidad de P en el suelo y su relación con la acidificación, además de determinar qué especie de lupino tiene una mayor capacidad de solubilización de P y qué combinación suelo-lupino genera el efecto más eficiente de movilización de P. La capacidad de solubilización de P se evaluó mediante las variaciones en la disponibilidad de P (PBray1) en cuatro momentos específicos mediante la comparación de suelos con lupino frente a suelos con avena y sus valores iniciales.

Esta investigación contribuye al entendimiento de cómo las especies vegetales y el tipo de suelo influye en la movilización de P y la absorción de nutrientes, además de permitir explorar las interacciones entre especies y suelos. También demuestra que las plantas tienen un impacto significativo en la disponibilidad de P en la rizósfera. El lupino presenta menores requerimientos de P en comparación con otros cultivos, como la canola o el trigo, lo que permite reducir la dependencia de fertilizantes fosfatados y disminuir el riesgo de pérdidas de P y potencial contaminación de aguas superficiales. El conocimiento generado sobre estas dos especies de lupino constituye un aspecto clave para diversificar los sistemas agrícolas mediante nuevos tipos funcionales de cultivos, más allá del trigo y la cebada. Esta estrategia prometedora está alineada con las políticas gubernamentales de Uruguay que abordan el deterioro del suelo por erosión hídrica y la contaminación del agua causada por la escorrentía de fertilizantes.

Los resultados de este estudio podrían ser de gran utilidad para la selección de leguminosas destinadas al cultivo en áreas con disponibilidad variable de P en el suelo, lo cual puede contribuir a reducir la dependencia de la fertilización P. En el ámbito agronómico, resulta crucial abordar los cambios en el contenido mineral de P en el suelo, ya que estos no solo disminuyen la necesidad de fertilizantes minerales, sino que también mitigan su impacto negativo sobre el medioambiente. Las modificaciones en el contenido de P mineral pueden beneficiar tanto al cultivo principal como a los cultivos subsiguientes, ya que se genera una serie de beneficios que se extienden a lo largo del tiempo. Es fundamental evaluar estos cambios en distintos tipos de suelo, dado que existen interrelaciones significativas entre los niveles de nutrientes y otros parámetros edáficos que pueden influir en la eficacia de los procesos de movilización de P.

En comparación con los suelos que contenían avena, al momento de la cosecha, los suelos con lupino mostraron un aumento significativo en las concentraciones de P-Bray1, con un incremento promedio máximo de aproximadamente 5,3 mg kg^{-1}, registrado con *Lupinus albus* en los sitios 1 y 2, los cuales presentaban mayores contenidos de MO que los otros dos sitios estudiados. La acidificación del suelo inducida por el lupino no explicó completamente este aumento de P. La avena mostró el mayor incremento en peso seco de brotes en respuesta a la disponibilidad de P en el suelo, mientras que el lupino fue el cultivo menos afectado por esta disponibilidad. No obstante, *L. albus* presentó una absorción de nutrientes similar o incluso mayor que la avena en todos los suelos analizados. En cuanto a las concentraciones de manganeso (Mn), estas fueron elevadas en la biomasa aérea de ambas especies de lupino. Sin embargo, dentro de cada especie de lupino, los niveles de acumulación de Mn variaron según el tipo de suelo y su grado de acidez. *Lupinus albus* demostró una notable capacidad para movilizar P no lábil en suelos de textura ligera con alto contenido de MO, ya que alcanzó una acumulación de P en su biomasa comparable o superior a la de la avena, al mismo tiempo que aportó N al suelo mediante la FBN. Esto posiciona a *L. albus* como un cultivo adecuado para diversificar los sistemas de rotación agrícola en Uruguay, al mejorar la sostenibilidad y la salud del suelo.

Comparar estos resultados con los obtenidos en campo con las mismas especies sería necesario para determinar la robustez de los patrones identificados en la disponibilidad de P en el suelo debido a la presencia de lupino y analizar estos patrones desde la perspectiva de su estabilidad a largo plazo. Esto se debe a que la interacción y la retroalimentación con otros factores suelo-planta y procesos de actividad microbiana podrían dificultar el aumento en la disponibilidad de P en el suelo facilitado por las especies de lupino. Por lo tanto, es esencial realizar investigaciones en campo durante al menos dos temporadas de cultivo para verificar si los impactos beneficiosos del lupino se mantienen con la misma magnitud a lo largo del tiempo y si estas variaciones contribuyen a mejorar la salud del suelo y la producción de cultivos.

agronomy

Article

Lupine Cultivation Affects Soil's P Availability and Nutrient Uptake in Four Contrasting Soils

Cristina Mori Alvez [1,*], Carlos Perdomo Varela [1,†], Pablo González Barrios [2], Andrea Bentos Guimaraes [1] and Amabelia del Pino Machado [1,†]

[1]Soil and Water Department, Agronomy College, University of the Republic, Av. Garzon 780, CP, Montevideo 12900, Uruguay; chperdom@fagro.edu.uy (C.P.V.); andreabentosguimaraes@gmail.com (A.B.G.); amabelia@fagro.edu.uy (A.d.P.M.)

[2]Biometrics, Statistics and Computing Department, Agronomy College, University of the Republic, Av. Garzón 780, CP, Montevideo 12900, Uruguay; pablog@fagro.edu.uy

*Correspondence: cristinamori@fagro.edu.uy

† These authors contributed equally to this work.

Abstract: A substantial amount of phosphorus (P) in the soil is not readily available for plant uptake. Certain species may enhance P availability from poorly soluble P forms. This study focused on improving our comprehension of the effect of two lupine species (*L. albus* and *L. angustifolius*) on soil's P mobilization and its link with soil acidity variations, comparing the response of the lupine species in terms of plant traits (i.e., aboveground biomass and nutrient uptake) with that of oats (*Avena strigosa* L.) in four contrasting soils (i.e., available P in soil, soil acidity, soil fertility, and texture). The phosphorus solubilization capacity was assessed on variations of P availability (PBray1) at four points in time, comparing soils with lupine to oat-containing soils and their baseline values. Compared to soils containing oats, at harvest, lupine soils had significantly increased PBray1 concentrations; the maximum average increment was around 5.3 mg kg^{-1}, with *L. albus* in Sites 1 and 2, which presented higher organic matter (OM) contents than the other two sites. Lupine-induced soil acidification did not fully explain that P increase. Oats exhibited the highest increase in shoot dry weight in response to soil's P availability, while lupine was the least affected. Nevertheless, *L. albus* showed similar or higher nutrient uptake than oats across all soils. The manganese (Mn) concentration was high in both lupine species' shoot biomass; however, within each lupine species, across all soil types tested, these legumes had different Mn accumulation levels depending on the soil acidity. *Lupinus albus* had a higher ability to mobilize non-labile P in the light-textured soil with a high OM content, achieving comparable and higher plant P status than oats and providing N through biological N fixation (BNF), positioning it as a suitable crop for diversifying Uruguay's agricultural crop rotation systems.

Keywords: Lupine; soil–plant interactions; soil P availability; acidification; nutrient uptake

4.1. Introduction

The adoption of continuous cropping (CC), primarily soybean (*Glycine max* L. Merr.), has increased in Uruguayan agricultural systems since the early 2000s [1], replacing the traditional crop–pasture rotation [2,3]. While grazing persists in agricultural systems, its duration within the rotation has been significantly reduced. According to the Ministry of Livestock, Agriculture and Fisheries of Uruguay, the 2021 agricultural survey [4], estimated that pastures associated with wheat cropping constituted less than 8% of the total area that was planted with this winter crop. In the past 15 years, the proportion of pastures associated with winter crops has significantly decreased, from 28% in 2005 to 4% in 2021. Despite the increase in grain production due to pasture phase exclusion, recent studies have confirmed persistent wheat yield gaps in Uruguay [3], which cannot be explained by nutrient deficiency and remain unaddressed by correcting nutrient deficiencies. The issue is exacerbated because the soil quality has been compromised, which affects the sustainability of the system [2,5].

In this scenario, there is significant concern regarding the impact of a permanent negative nitrogen (N) balance in the CC rotation; thus, balancing N within this system becomes necessary [6]. In contrast to crop–pasture rotation, the CC systems have experienced an increase in the quantities of both N and P fertilizers due to agricultural intensification. However, for P in particular, due to the accumulated yield’s increased extraction of P from the soil, the amount of remaining P decreased, especially when highly extractive crops were the main components of the sequences in the rotation, such as soybean and corn [7]. Moreover, applying P fertilizer above crop requirements leads to a slightly positive soil P balance, as P gradually accumulates in the soil over time [7]. This accumulation is affected by the low P use efficiency in most crops, which typically ranges from 15 to 30% [8].

Although the majority of residual P is scarcely assimilable by plants, certain species may be able to make this P available [9,10]. The lupine genus may be able to mobilize residual P through root exudates, releasing phosphatases [11], acidifying [12], and chelating compounds or carboxylates [13–15], which can enhance their growth and possibly that of subsequent crops [16,17]. However, this genus has not yet been incorporated into crop rotations in Uruguay, which typically favor winter cereal crops like wheat or barley and summer crops such as soybean or maize. Additionally, further investigations [18] on lupine cultivation and its effects on soil nutrient dynamics must to be conducted.

Accordingly, for competitive crop production, the integration and diversification of functional groups—such as annual winter legumes like lupine—may be a key strategy to reverse soil deterioration processes [19–21]. This lupine genus is recognized for its high potential as a pulse crop in Australia, Chile, and other countries for a variety of production purposes [17,22–24]. Integrating crops that are capable of N fixation from the atmosphere and P solubilization from unavailable forms provides an alternative to excessive or improper fertilizer applications. Moreover, the root activity of this annual winter legume contributes to soil OM as a carbon source and enhances soil’s N content through its N-fixing ability, while also improving physical, chemical, and biological soil properties.

Given the growing interest in diversifying crops within agricultural systems and reducing fertilizer use, there is a timely opportunity to evaluate lupine cultivation. Species such as white lupine (*L. albus*) and narrow-leaf lupine (*L. angustifolius*) are renowned in regions of Australia and Chile as N fixers and P solubilizers, particularly in mildly acidic or neutral soils of light-to-medium texture [25]. Consequently, we hypothesize that the availability of plant P in contrasting soils (pH, texture, OM, and soil P concentration) increases by acidification of the soil surrounding roots by those lupine species, enhancing the solubility of P from sparingly available soil P sources. This enhanced plant P availability is expected to improve the dry matter (DM) yield and the P and N biomass content of lupine, potentially having a residual effect on subsequent crops.

This study focused on improving our comprehension of the impacts of two lupine species (*L. albus* and *L. angustifolius)* on the mobilization of P in soil and its association with soil acidification, comparing the response of aboveground biomass productivity and nutrient (i.e., P, N, base cation, Mn, and Fe) uptake effectiveness to these lupine species with the response to oats (*Avena strigosa*) in four contrasting (i.e., available P in soil, soil acidity, soil fertility, texture) soil types. Hence, the aims were to assess the changes in soil's P availability and its link with soil acidification and to establish which type of lupine has a more substantial P solubilization capacity and which soil–lupine combination yields the most effective P mobilization effect.

4.2. Material and Methods

4.2.1. Soil Collection and Preparation Prior to Experiment Installation

Soil samples were collected from the topsoil layer (0–20 cm depth) from four Uruguayan agroecological areas under different soil uses with the following geographic coordinates: agricultural—Site 1 (33°59’05.4” S and 57°43’42.7” E); livestock grassland—Site 2 (32°49’1.20” S and S 54°25’28.27” O); grassland—Site 3 (34°50’15.61” S and 56°13’21.62” O); forestry—Site 4 (31°23′43.44” S and 55°41′39.37” O). The sites have contrasting soil textures, pH values, organic matter (OM) levels, and P concentrations (PBray1). Table 1 displays the collection sites and USDA soil classification [26] for each of the four soils analyzed in this study; these references (Site 1 to Site 4) were used throughout the text to identify each soil treatment.

Before planting, each soil was sieved through a 1 cm mesh sieve to homogenize the size of the aggregates and discard coarse plant material. After this screening, for each soil type, the granulometric composition (texture) and the chemical characterization (i.e., PBray1, inorganic N forms, exchangeable bases, exchangeable Al, and soil pH) were determined (Supplementary Materials, Table S1).

Table 1. Site location, soil type, dominant geological material, physical characterization, and soil texture of the soil samples collected from four sites in Uruguay.

Site (Location)	**Soil Type (USDA Classification System) †**	**Geological Material**	**OM**	**Sand**	**Silt**	**Clay**	**Texture ‡**
			%				
Site 1 (Colonia)	Pachic Argiudoll	Clay silt sediment/Crystalline basement	4.8	16.3	38.8	44.9	C
Site 2 (T. and Tres)	Typic Dystrutepts	Crystalline basement	3.7	23.5	44.2	32.3	CL
Site 3 (Montevideo)	Typic Argiudolls	Libertad clayey silt sediment	1.7	19.3	52.3	28.4	SiCL
Site 4 (Rivera)	Typic Hapludults	Colluvial material (Sandy soils)/Tacuarembó sandstones	1.4	84.7	1.4	13.9	SL

† USDA source: Keys to soil taxonomy. Soil Conservation Service. 2014. ‡ Texture: C = clay; SiCL = silty clay loam; SL = sandy loam; CL = clay loam.

4.2.2. Greenhouse Experiment

4.2.2.1. Experimental Design and Plant Growth Conditions

A pot experiment was conducted under natural light conditions in a greenhouse at the Agronomy College in Montevideo (34° 50’ 18.20” S, 56°13 ‘16.36” W) to evaluate the efficacy of two lupine species in enhancing soil’s P availability. A total of 48 three-liter pots (15 cm in diameter) were filled with the different soil types. The treatments were arranged in a factorial design with two factors, four soil types and three species, in a completely randomized design (CRD) with four replications.

The evaluated species were *Lupinus angustifolius* L. var. Lavalle, a non-cluster root-forming lupine (narrow leaf lupine or blue lupine), and *L. albus* L. Blu25, a cluster root-forming legume (white lupin). Additionally, an annual grass, black oat (*Avena strigosa* L., var. Agroplanalto), was included in the study as a comparative measure to assess the increase in soil P that was induced by lupines and to evaluate BNF. A seed company based in Uruguay (Fadisol S.A.) supplied all seed species that were utilized in this study, constituting those of lupine, the single plant material available by Fadisol during the research period of the current work. The white lupine accession was provided by Seeds Baer (Chile), a breeder who conducted formal identification and permitted using it for research purposes; blue lupine and black oat were developed and protected by Fadisol S.A. Oats were chosen as the control in our study because they are the most widely cultivated and regionally adapted cover crops in Uruguay due to their precocity and high growth rate during the winter season [27]. Furthermore, according to Wang et al. [28], this genus would be a good candidate for evaluating soil’s P utilization, given its extraordinary cover’s fibrous root system promoting key roles, such as preventing erosion and scavenging excess nutrients [29]. Both lupine species were inoculated with a non-specific commercial inoculant (Bradyrhizobium sp., strains U-612 and U620), known to be effective for these species. The planting date was 10 July 2019, with two or three lupine seeds per pot, leaving only one plant per pot after emergence. There were five oat plants per pot.

Plants were irrigated using potable water, supplemented once a week during the first month of the experiment with a nutrient solution that contained (μM): KCl, 200; CaCl, 150; $MgSO_4$, 100; H_3BO_3, 2.0; $MnSO_4$, 0.4; $ZnSO_4$, 0.4; and $CuSO_4$, 0.2, Na_2MoO_4, 0.05; Fe Edta, 0.005, free of N and P and using 200 mL per pot. Throughout the trial, each pot was maintained around 60% of its field capacity (FC), estimated by gravimetric determination and considering the water content at FC (based on mass) of each soil type (35, 30, 28, and 13% for Sites 1 to 4, respectively). The experiment was conducted from June to October 2019, encompassing the mid-winter and early spring seasons. The average temperatures during this period varied between 8 and 25 °C. There was no need to control weeds and pests during the experiment.

4.2.3. Soil and Plant Sampling

Soil samples were collected at four different time points: 48, 76, 87, and 103 days after planting (dap). The initial soil samples were obtained on September 9, 2019, two months after the start of the experiment, when the crop was in the vegetative stage. The second sampling took place on October 2, 2019, during flowering stage, the third sampling on October 14, 2019, at the beginning of the lupine grain filling, which was the harvest time, and the final sampling on October 30, 2019. The soil pH was measured on September 9 and October 14, 2019. The soil's exchangeable acidity was assessed to confirm any connection between changes in the solubility of inorganic P or Al and soil acidity. A hand drill, 1.9 cm in diameter, was used to collect a single soil sample from each pot, reaching a depth of 0 to 10 cm.

The plants were harvested at 87 dap at ground level using pruning shears to remove all aerial biomass per pot. At this sampling time, the oat plants had reached full maturity, while *L. angustifolius* was in the initial phase of grain filling, and *L. albus* exhibited pods in an early stage of development. Compared to *L. albus*, *L. angustifolius* had more mature pods, which accounted for around ten percent of the total P absorbed in the aboveground biomass. Consequently, the study separated the analytical determination of *L. angustifolius* pods from the remaining aerial plant parts. However, except for P concentration, the other analyses on these pod components were not achievable due to the sample size being insufficient for accomplishing this. The shoot dry weight was expressed per pot (g pot^{-1}), and in some instances, it was converted to its equivalent per hectare (kg ha^{-1}) based on the pot's surface area.

4.2.4. Sample Processing and Analytical Determinations

4.2.4.1. Soil Measurements

The soil samples were dried in a forced-air oven at 40 °C for at least 48 h and were ground to a 2 mm sieve size. For the initial characterization, the determined parameters were granulometric composition, organic matter (OM), pH, labile P (P Bray N°1, henceforth PBray1), mineral nitrogen (ammonium and nitrate), exchangeable cations (Ca, Mg, K, and Na), and exchangeable acidity. OM was determined by the Walkley–Black method [30], while exchangeable cations Ca and Mg were determined by atomic absorption and K and Na by flame spectrophotometry, following extraction with 1 M ammonium acetate at pH 7 [31]. Soil pH was measured in deionized water (1: 2.5 soil/ deionized water ratio) using a pH probe (Orion Research 701 pH electrode), while exchangeable acidity was determined using the potassium chloride method [31]. The labile P or available P content was measured using the Bray N° 1 method extraction system [32], which is the most widely used method in Uruguay for evaluating plants' P availability in most agricultural soils in the country [33]. Nitrate–N (NO_3-N) concentration was determined using the Griess–Ilosvany method [34], and ammonium–N (NH_4-N) was determined using the colorimetric method [35]. Phosphorus, ammonium, and nitrate readings were taken in a spectrophotometer at 880, 650, and 540 nm, respectively, using the MRC microplate reader for the first two elements and the UNICAM spectrophotometer for nitrate. The hydrometer method [36] was employed to analyze the granulometric soil composition. During the growing season, soil samples were analyzed for PBray1 and pH using the same techniques described for soil characterization analysis.

4.2.5. Plant Measurements

The harvested plants were oven-dried at 65 °C for a minimum of 48 h until the mass remained constant. Then, initially, dried plant materials were ground with a stationary and mobile knife mill (Marconi MA-580) until the particulate size was less than 2 mm. Plant samples of this granulometric size were analyzed for total P and K contents. Afterward, previously ground subsamples were newly ground by a rotary mill (SampleTek 200 vial Rotator) to a fine powder (typically a consistency approaching that of the talcum powder), which was necessary for ^{15}N analysis by mass spectrometry. Total C and N concentration and $^{15}N/^{14}N$ of the samples (at the natural abundance) were determined by mass spectrometry in a US laboratory "https://csi.unm.edu, accessed on 10 November 2020"). The following formula by Shearer and Kohl [37] was used to calculate the proportion of N fixed from the air (% Ndfa) for each lupine species:

$$\mathbf{Ndfa}(\%) = \left(\frac{\boldsymbol{\delta^{15}N_{ref} - \delta^{15}N_{fix}}}{\boldsymbol{\delta^{15}N_{ref} - B}}\right) \times \mathbf{100}$$

where the following abbreviations are used:

Ndfa is the proportion of plant N derived from BNF;

δ^{15}Nref is the $\delta^{15}N$ value of the reference plant (non-fixing);

δ^{15}Nfix is the $\delta^{15}N$ value of the fixing plant (lupine);

B is the $\delta^{15}N$ value of a fixing plant growing in a medium without N.

The B value was estimated as the mean $\delta^{15}N$ value from pure lupine growing in the sand, with a value of +1.6‰ and −0.6‰ of $\delta^{15}N$ for *L. albus* and *L. angustifolius*, respectively. The reference plant used was oat, and all $\delta^{15}N$ values were determined under the same conditions as lupine legumes.

The total concentrations of P and potassium (K), calcium (Ca), magnesium (Mg), Mn, and iron (Fe) were also determined after calcination at 550 °C for 5 h. Phosphorus concentration was determined using the ascorbic acid method [38] after extraction with diluted HCl (20% *v*/*v*) [39]. In the ash extracts, the remaining elements (Ca, Mg, K, Fe, and Mn) were determined by spectrometry as described for soil samples. Shoot's P and N contents per pot (mg pot^{-1}) were calculated by P or N concentrations (mg g^{-1} dw) × shoot dry weight (g pot^{-1}), respectively. In certain instances, the plant nutrient content was also expressed per hectare, considering the pot's surface area.

4.2.6. Statistics

The changes in soil PBray1, at four points in time, were evaluated by repeated measures analysis of variance using a MIXED procedure of SAS (between-subject factors were species and soil type, and within-subject factor was sampling time). Additionally, two-way ANOVAs performed at two time points (i.e., at 87 and 103 dap) were used to study the factors of interest (i.e., soil, species, and their interaction as fixed effects) on the evaluated soil and plant variables. Datasets that did not follow assumptions of normality of residuals and homogeneity of variance were log10 transformed. Two additional soil variables were estimated: 1: the difference between PBray1 (and pH) at a specific sampling time and the corresponding parameter, measured at the beginning of the experiment, is denoted as ΔPBray1−initial and ΔpH−initial, respectively; 2: for each soil parameter (PBray1 and pH values), the difference between the soil samples containing lupine and those containing oats (ΔPBray1_$_{\text{Lup-Oat}}$ and ΔpH_$_{\text{Lup-Oat}}$) was estimated.

The further analyses included orthogonal contrasts (C1: lupine vs. oat, C2: *L. albus* vs. *L. angustifolius*) that were performed to identify differences between groups of treatments and Pearson correlation and linear regression to describe and explain the association between soil and plant variables. A Tukey's test with a confidence level of 5% was used to compare the means of treatments between species, across soils, and within each soil type. All analyses were conducted using SAS statistical software version 9.04 (SAS Institute) and R software (version 4.0.4) [40].

4.3. Results

4.3.1. Species, Soil, and Sampling Time Effects on PBray1 Concentration

The effects of species and soil type were highly significant ($p = 0.001$) on the soil's PBray1, but their interaction was not (Table 2). Considering the effect of species, *L. albus* had the highest mean values (Figure 1), while concerning the soil factor, its effect on PBray1 reflected, on average, the initial P concentrations of each soil, and this was observed as early as 76 dap, after which the concentration tended to stabilize.

The C1 (lupine vs. oat) was significant at 48 dap, but only in the soil of Site 1 ($p = 0.0029$), whereas C2 (*L. albus* vs. *L. angustifolius*) was significant in Site 1 and Site 3 ($p = 0.0028$ and $p = 0.0445$, respectively). At 76 dap, both contrasts were no longer statistically significant in any of the soils, indicating that the differences between species diminished. At harvest (87 dap), C1 was significant in Site 3 ($p = 0.0331$), Site 4 ($p = 0.029$), and Site 2 ($p = 0.0001$), but differences between lupine species were not detected. Fourteen days after harvesting (103 dap), C1 was highly significant in all the soils, while C2 was significant in all soils except Site 4, where no differences in PBray1 between the lupine species were observed.

Table 2. Repeated measures analysis of variance model for soil PBray1 concentration.

Treatment Effect	DF	F Value	p > F †
Species	2	23.05	**<0.0001**
Soil type	3	101.56	**<0.0001**
Species x soil type	6	1.36	0.2564
Sampling time	3	91.85	**<0.0001**
Species × sampling time	6	2.29	0.0518
Soil type × sampling time	9	6.66	**<0.0001**
Species × soil type × sampling time	18	2.17	**0.0133**

† Significant effects ($p < 0.05$) are in bold. *Note*: The within-subject factor was sampling time. Results are from 48, 76, 87, and 103 days after planting, and the between-subject factors were species and soil type.

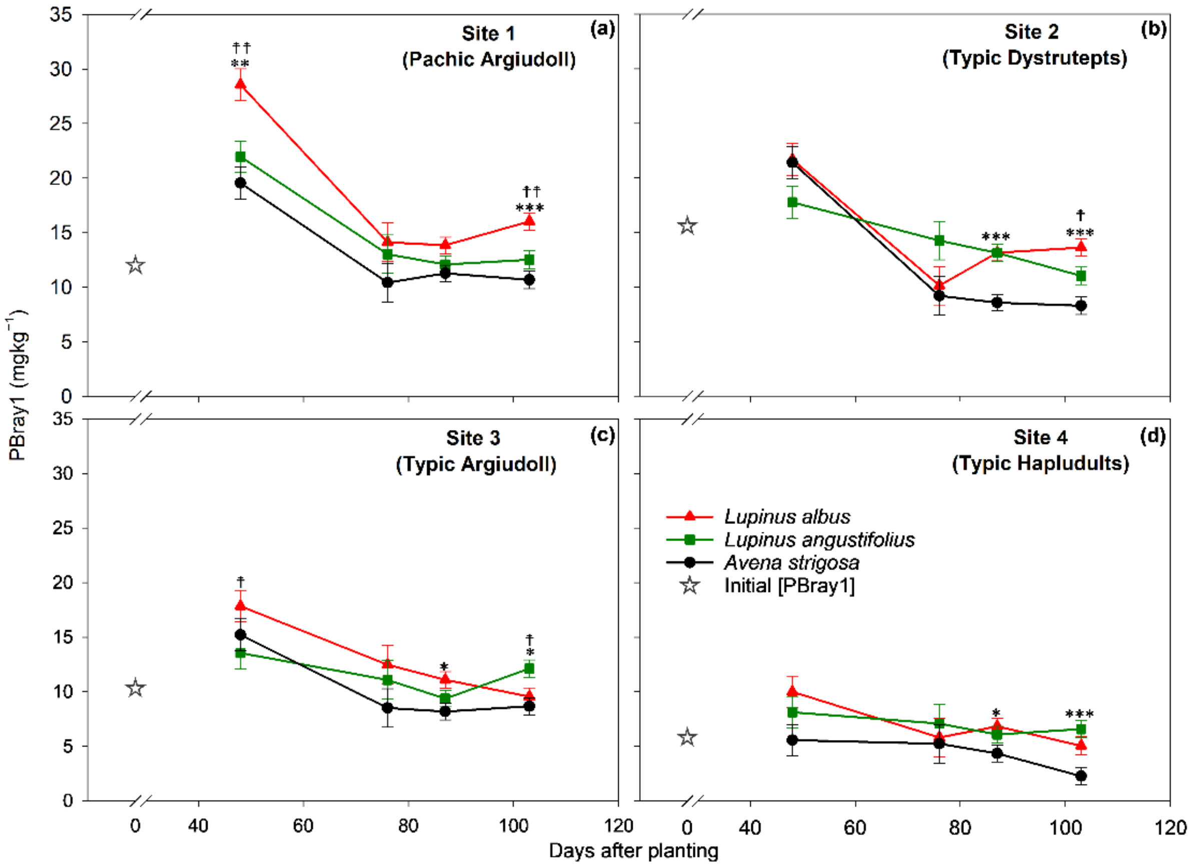


Figure 1. Soil PBray1 concentrations under two legumes (*L. albus* and *L. angustifolius*) and one grass (*Avena strigosa*) across Sites (soil type) 1 (**a**), 2 (**b**), 3 (**c**), and 4 (**d**). The vertical bars indicate the standard error, and the symbols "*" and "†" indicate the sampling time in which Contrast 1 (lupine vs. oat) and

Contrast 2 (*L. albus* vs. *L. angustifolius*) were significant (* † $p < 0.05$; ** †† $p < 0.01$; *** ††† $p < 0.001$), respectively.

At harvest time (87 dap), the two-way ANOVA showed significant effects of the main factors, species and soil, but not of their interaction (Supplementary Materials, Table S2). The contrast analysis between lupine vs. oats (C1) was significant, the estimated difference being 2.6 mg kg^{-1}, while the contrast between the lupine species (C2) was not significant on this sampling date. Multiple comparisons of means using the Tukey method revealed a significant difference between the lupine species, with higher values for *L. albus*. For this sampling time, the difference in PBray1 compared to the initial values was almost null in the soils with lupine; with oats, meanwhile, there was a mean decrease of 2.8 mg kg^{-1}. At 87 dap, there were also statistically significant differences in the concentrations of PBray1 between soils, primarily reflecting the P analysis values of the tested soils before the start of the experiment. The concentration of PBray1 did not differ statistically between Site 1 and Site 2. However, the P levels in these soils were substantially higher and statistically different from those of the other soils (p = 0.05). In the heavy-textured soils and Site 4, the ΔPBray1-initial was minimal in this sampling period; however, Site 2 had a mean decrease of 4.0 mg kg^{-1}. Additionally, a significant percentage increase in PBray1 of 57% was observed in the loamy soil containing lupine relative to this soil containing oat, reaching 4.6 mg kg^{-1}.

At 103 dap, a significant effect of the main factors (p < 0.0001) but also of the species x soil type interaction (p = 0.0048) was detected (Figure 2; Supplementary Materials, Table S3). The C1 was significant in all the soils, the difference being 4.0, 3.6, 3.6, and 2.2 mg kg^{-1} in Site 2, Site 1, Site 4, and Site 3, respectively. The C2 was also significant in three soils, especially for *L. albu*s in Site 1 and Site 2, with a higher P content. Site 3 was the other soil with a significant difference between the lupine species. In this soil, however, *L. angustifolius* stood out over *L. albus*, with a difference from the oat of 3.4 mg kg^{-1}; meanwhile, the difference between *L. albus* and oats was statistically non-significant and estimated at 0.9 mg kg^{-1}. In summary, the ΔPBray1_$_{\text{Lup-Oat}}$, depending on the lupine species and the soil, ranged from 0.9 (non-significant difference) to 5.4 mg kg^{-1} (p < 0.0001). In addition, a significant difference was found between both species of lupine to oats only in light-textured soils (Site 2 and Site 4). In the other soils, meanwhile, that difference was significant only in one species of lupine. This was *L. albus* in Site 1 and *L. angustifolius* in Site 3.

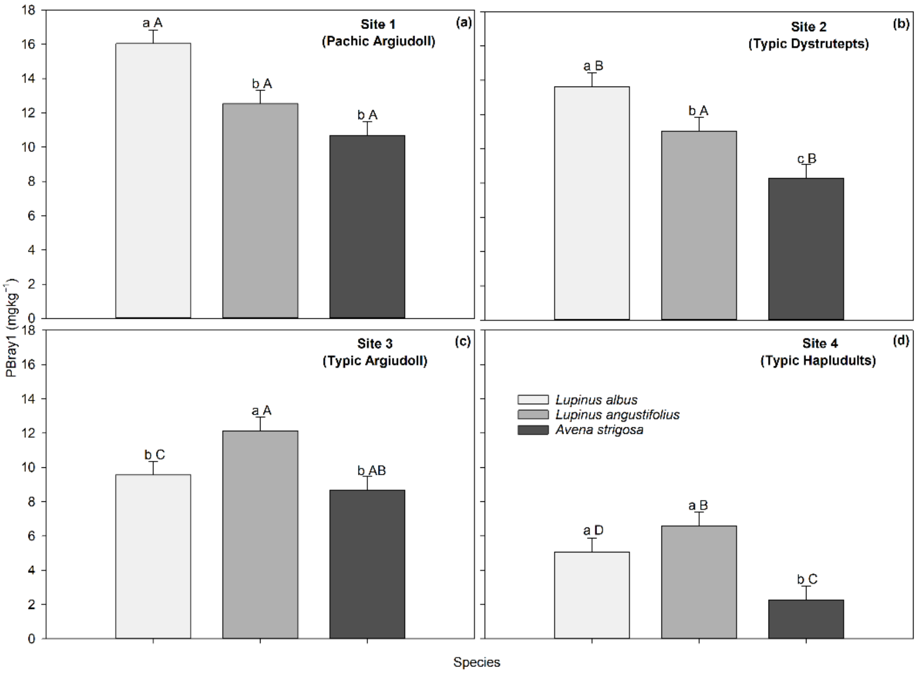


Figure 2. PBray1 concentration according to species across Sites (soil types) 1 (**a**), 2 (**b**), 3 (**c**), and 4 (**d**) at 103 days after planting. The vertical bars indicate the standard error. Different lowercase letters indicate significant differences between species within each soil type, and different capital letters indicate differences between sites across species, according to Tukey's test, with a $p \leq 0.05$.

4.3.2. Species and Soil Type Effects on Soil pH

The pH of the soil at harvest differed considerably between soils with lupine and those with oats, by 0.5 pH units on average (Figure 3). Compared to the pH values at 48 dap, the values were lower at harvest, although this decrease was only observed in soils with lupine (0.1 pH unit) and not in soils with oat. When the soil's pH at harvest was compared with the initial pH of each soil, it was found that oats caused a mean 0.25 units of pH increase, while both species of lupine caused a decrease in pH, but without statistically significant differences. The effect of soil on pH changes was also highly significant, with an average increase of 0.57 units in Site 4 and a decrease of a similar magnitude (−0.62 units) in Site 2. The species x soil type interaction on soil pH measured at harvest was significant (p = 0.0365; Supplementary Materials, Table S4). As shown in Figure 3, oat-containing soils consistently recorded the lowest acidity levels. In addition, the soil pH of this species differed significantly from that of lupine in all soils except Site 1, where the differences between species were not statistically significant. Based on the statistical differences between soils across species, Figure 3 reveals that Site 2 had the lowest soil pH at harvest, while Site 3 had the highest (coincidentally with the highest initial soil pH).

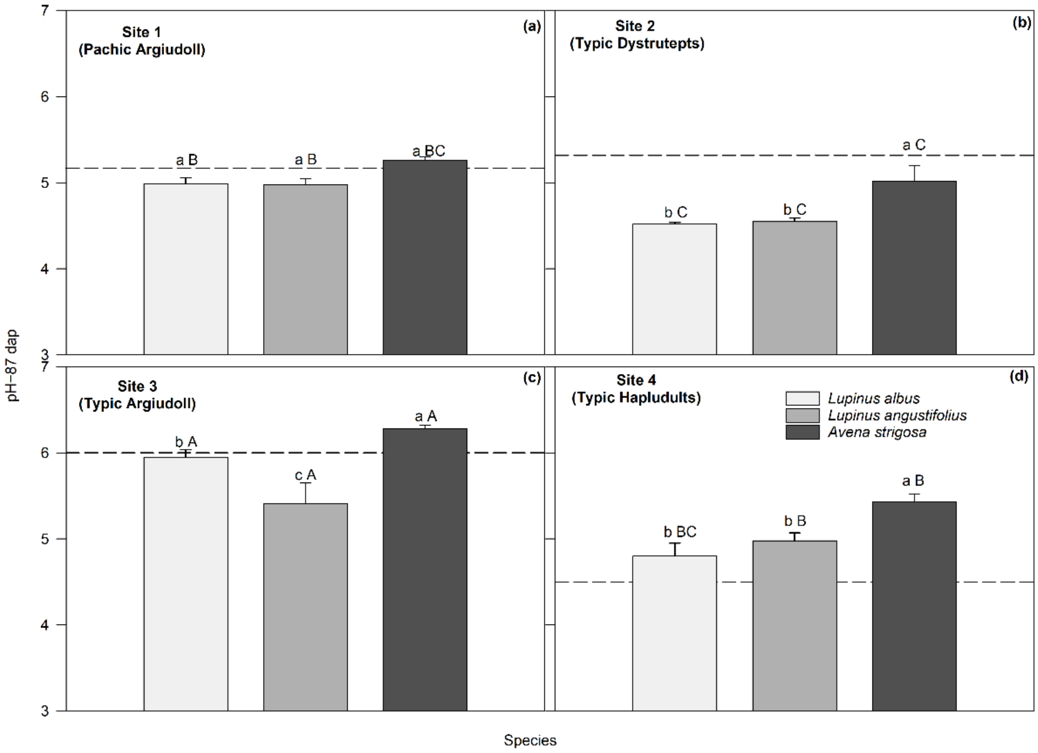


Figure 3. Soil pH measured 87 days after planting of the two legumes (*L. albus* and *L. angustifolius*) and the grass (*Avena strigosa*) across Sites (soil type) 1 (**a**), 2 (**b**), 3 (**c**), and 4 (**d**). The dotted line represents the soil's pH value at the beginning of the experiment. Different lowercase letters indicate significant differences between species within each soil type, and different capital letters indicate differences between sites across species, according to Tukey's test, with a $p \leq 0.05$.

At 87 dap, in Site 3, Site 4, Site 2, and Site 1, the $\Delta pH\_{Lup\text{-}Oat}$ was −1.2, −1.1, −1.0, and −0.6 units, respectively. According to the contrast analysis, the difference between the pH values of lupine and oat (C1) was statistically significant in all soils. The C2 was only significant in Site 3, with *L. angustifolius* standing out with a decrease in pH of −0.6 units from the initial value. Figure 3 also shows that the ΔpH−initial fluctuated within a more negative range for soils with lupine (−0.8 to 0.5) and a more positive range for soils with oat (−0.3 to 0.9). Likewise, the exchangeable acidity values corresponded to those of the pH; when the pH decreased, the soil acidity increased. However, this increase in acidity was not associated with exchangeable aluminum but rather with an increase in the hydrogen ion concentration. On average, the highest increase in exchangeable aluminum was 0.14 $cmol_c\ kg^{-1}$ at 87 dap in Site 2; this concentration would not impose production limitations (Table 3).

Additionally, sandy soils at Sites 2 and 4 displayed more significant pH fluctuations. At Site 2, where the ΔpH−initial was the most pronounced, the difference in exchangeable acidity between soil holding lupine and soil holding oats was most noticeable and statistically significant. In contrast, at Site 4, the exchangeable acidity with oat-bearing soil was only statistically distinct from soil containing *L. albus*. On the other hand, the exchangeable acidity between species was not statistically significant at Sites 1 and 3 (Table 3).

Table 3. Soil’s exchangeable acidity and exchangeable aluminum according to species and soil type. Values are means ± standard error.

Site	**Species**	**Exchangeable Acidity**	**Exchangeable Aluminum**
		$\text{cmol}_c\ \text{kg}^{-1}$	
1	*Lupinus albus*	0.07 ± 0.01ns C	0.01 ± 0.03ns C
	Lupinus angustifolius	0.08 ± 0.02ns C	0.04 ± 0.03ns ns
	Avena strigosa	0.07 ± 0.01ns BC	0.04 ± 0.01ns AB
2	*Lupinus albus*	0.69 ± 0.04a A	0.18 ± 0.03ns A
	Lupinus angustifolius	0.56 ± 0.09a A	0.10 ± 0.03ns ns
	Avena strigosa	0.33 ± 0.04b A	0.13 ± 0.03ns A
3	*Lupinus albus*	0.08 ± 0.03ns C	0.06 ± 0.02ns BC
	Lupinus angustifolius	0.05 ± 0.00ns C	0.06 ± 0.02ns ns
	Avena strigosa	0.03 ± 0.01ns C	0.03 ± 0.03ns B
4	*Lupinus albus*	0.32 ± 0.08a B	0.11 ± 0.03ns AB
	Lupinus angustifolius	0.23 ± 0.04ab B	0.10 ± 0.03ns ns

Avena strigosa	0.17 ± 0.07b B	0.08 ± 0.03ns AB
Significance of treatment effect		
Species	**0.0008**	0.6089
Soil type	**<0.0001**	**0.0008**
Species * soil type	**0.0179**	0.6339

Significant effects ($p < 0.05$) are in bold. Different lowercase letters within a column indicate differences between species within each soil type, and different capital letters within a column indicate differences between soils across species being significant at a p-level of 0.05.; ns means no significant difference.

4.3.3. Aboveground Biomass and Nutritional Status: Effects of Species and Soil Type

4.3.3.1. Shoots' Dry Weight and P and N Content

As shown in Table 4, the species, soil, and their interaction significantly influenced the shoots' dry weight and P content. The maximum shoot dry weight was produced by oat, followed by *L. albus* and *L. angustifolius*. Regarding soils, Site 4 had the lowest yield, heavier soils offered an intermediate yield, and Site 2 presented the highest yield, which was three times that of Site 4. The yield of each species was also differentially affected by the soil type (soil x species interaction; $p < 0.0001$). Except for Site 3, oats outyielded lupines, while *L. albus* produced roughly twice as much as *L. angustifolius* across all soil types.

The plants' P content in oats and *L. albus* was not statistically different, except in Site 3, where *L. albus* absorbed more P than oats (Table 4). The absorption of P and the yield of the aboveground biomass varied substantially between the lupine species, with the lowest values detected in *L. angustifolius*. In terms of P concentrations, there were fewer differences within each species across sites, because they were already similar in all soils except for Site 4, where the concentrations were half of those that were registered in the other sites, whatever the species considered. Oats displayed the most noticeable variation in P content between sites. In soils with lupine, such differences between soils diminished, although in Site 4, the P content of lupine was substantially lower than in the other sites. The contrast analysis between lupine and oat (C1) was significant only in Site 1 and Site 2, where the plant P content in oats was markedly higher than that of lupines (Supplementary Materials, Table S5). Contrast 2 was not significant in Site 4; the remaining soils had higher P contents in *L. albus* than in *L. angustifolius*. All data that are displayed in Table 4 exclude the pod components of L. *angustifolius*. However, upon their inclusion, the effect of species became not significant, eliminating the difference between the P content that was reached by both lupine species in Sites 1, 2, and 4. In contrast, *L. albus* continued to attain a higher plant P content than *L. angustifolius* in Site 3. On average, the P concentration of *L.*

angustifolius plants in Sites 1, 2, and 3, both with and without pods, was 1.6 and 1.4 mg g^{-1} dry weight, respectively.

Similar to the shoots' dry weight, the content of N in the biomass significantly differed between species and soils, with the lowest values in Site 4 for both lupines and in Sites 1 and 3 for oats. In contrast to the lupines' P content, the values of N content differed less between sites, as the soil type effect was not statistically significant in terms of N concentration (Table 4). The ANOVA results revealed no significant difference between lupine species in Ndfa (Supplementary Materials, Table S6). However, there were statistically significant differences in the N biomass and the amount of N that was fixed, with average values of 347 and 237 mg N pot^{-1}, respectively, registering the highest values in *L. albus*. These variables, expressed as total N and N fixed content in shoots' dry weight per hectare to determine their agronomic significance to N entering the soil system, represent approximately 190 kg N ha^{-1} and 130 kg ha^{-1} of N, respectively.

Table 4. Shoots' dry weight, plants' P and N concentration, and plants' P and N content of different species, as affected by soil type. Values are means ± standard error.

Site	Species	Shoots' Dry Weight	Plants' P Concentration	Plants' N Concentration	Plants' P Content	Plants' N Content
		g pot^{-1}	mg g^{-1} Dry Weight		mg pot^{-1}	
1	*Lupinus albus*	12.5 ±1.5aA	1.3 ±0.1 aAB	29.3 ±1.0ans	15.5±1.7 aA	366.5±46.5 aA
	Lupinus angustifolius	6.7 ± 0.3 bA	1.4 ± 0.1 aA	26.1 ±1.6ans	9.0 ± 0.6 bA	174.4±15.3 bA
	Avena strigosa	14.5 ±1.1aB	1.2 ± 0.1 bA	5.7 ± 0.2bB	16.8±1.3 aB	81.9 ± 4.8 cB
2	*Lupinus albus*	13.9 ±1.3bA	1.4 ± 0.1 aA	31.9 ±1.3ans	19.5±1.3 aA	436.9±24.7 aA
	Lupinus angustifolius	7.8 ± 1.1 cA	1.4 ± 0.1 aA	24.5 ±0.4bns	10.6±1.6 bA	189.8±25.8 bA
	Avena strigosa	27.0 ±2.7aA	0.9 ± 0.0 bB	8.4 ± 1.0cAB	23.5±1.4 aA	222.1±19.1 bA
3	*Lupinus albus*	14.0± 1.3aA	1.1 ± 0.1cB	28.8 ± 1.9ans	15.4 ± 2.1aA	402.4 ± 37.0aA
	Lupinus angustifolius	7.0±1.9 bA	1.5 ± 0.1aA	29.4 ± 1.4ans	10.8 ± 2.9bA	203.6 ± 52.9bA

	Avena strigosa	8.1 ± 0.4 bC	1.3 ± 0.1bA	6.5 ± 0.3bB	10.2 ± 0.9bC	52.9 ± 3.01cB
4	*Lupinus albus*	6.5 ± 1.1 bB	0.9 ± 0.1aC	27.5 ± 2.4ans	5.6 ± 1.2nsB	180.7 ± 38.6aB
	Lupinus angustifolius	1.7 ± 0.4 cB	0.6 ± 0.2bB	30.2 ± 0.0ans	1.1 ± 0.6nsB	38.1 ± 0.0bB
	Avena strigosa	11.0 ±0.4aBC	0.5 ± 0.0bC	10.2 ± 0.6bA	5.3 ± 0.3nsD	111.8 ± 2.8abB
Significance of treatment effect						
Species		**<0.0001**	**0.0004**	**<0.0001**	**<0.0001**	**<0.0001**
Soil type		**<0.0001**	**<0.0001**	0.3286	**<0.0001**	**<0.0001**
Species ×soil type		**<0.0001**	**0.0017**	**0.0088**	**0.0062**	**0.0009**

Significant effects ($p < 0.05$) are in bold. Different lowercase letters within a column indicate differences between species within each soil type, and different capital letters within a column indicate differences between sites across species being significant at *a p*-level of 0.05; ns means no significant difference.

4.3.3.2. Plants’ Ca, Mg, K, and Micronutrient (Fe and Mn) Concentrations in Aboveground Biomass

The ANOVA of the plant nutrient concentration revealed that only the K concentration was similar between species. The K concentration in *L. albus* showed differences between soils, with the highest concentration values found in Site 3 and the lowest in Site 4. On the other hand, there were significant variations in Ca and Mg levels among species, with *L. angustifolius* consistently exhibiting the highest concentrations across all soil types (Table 5).

The impact of different species on the concentration of micronutrients in plants varied depending on the soil type (notably, a significant interaction effect was found between soil type x species). Across most soils, *L. angustifolius* exhibited the highest concentrations of Mn and Fe, with two exceptions. Specifically, *L. albus* had statistically higher Mn content at Site 3 compared to oats, and oats displayed the highest concentrations of Fe at Site 4 compared to lupines (Table 5). In all soils, the Mn concentration was substantially higher in lupines than in oats. The concentrations of Mn and Fe in the aboveground biomass also differed among soils within each species, with oats showing no significant differences in their Mn concentration across soils. Similarly, *L. angustifolius* did not show significant differences in their Fe concentration. When examining the Fe concentrations of each species across soils, both lupine species consistently exhibited higher values than oats in all soils except in Site 4, as previously mentioned.

Table 5. Plants' Ca, Mg, K, Mn, and Fe concentration of different species, as affected by soil type. Values are means ± standard error.

Site	Species	Plants' Ca Concentration	Plants' Mg Concentration	Plants' K Concentration	Plants' Mn Concentration	Plants' Fe Concentration
			mg g^{-1}		mg kg^{-1}	
1	*Lupinus albus*	5.6 ± 0.4bAB	1.4 ± 0.1bAB	14.3 ± 1.7nsB	482.6 ± 51.2 bB	97.2 ± 12.7bBC
	Lupinus angustifolius	26.5 ± 1.6ans	4.7 ± 0.3ans	16.2 ± 0.2nsns	1067.0±150.2 aB	214.2±23.5a ns
	Avena strigosa	3.1 ± 0.2cB	1.2 ± 0.1cB	15.5 ± 0.6nsns	216.7 ± 27.6 bns	36.9 ± 8.6b B
2	*Lupinus albus*	3.9 ± 0.4bB	1.1±0.1bB	13.7 ± 1.1nsB	537.6 ± 218.2bB	54.0 ± 5.5abC
	Lupinus angustifolius	22.5 ± 1.1ans	4.4 ± 0.2ans	14.7 ± 1.9nsns	3161.0±259.1aA	142.1 ± 4.8a ns
	Avena strigosa	2.9 ± 0.3bB	1.5 ± 0.1bB	13.0 ± 0.9nsns	214.9 ± 21.66bns	28.7 ± 2.6b B
3	*Lupinus albus*	7.2 ± 0.1b A	2.0 ± 0.3b A	17.1 ± 0.7nsA	1191.2±21.51a A	165.3±21.5aAB
	Lupinus angustifolius	26.7 ± 1.3a ns	4.5 ± 0.2a ns	17.4 ± 1.1ns ns	791.9 ± 184.0aB	214.6±36.6a ns
	Avena strigosa	4.4 ± 0.6b AB	1.0 ± 0.2b B	15.1 ± 0.2ns ns	209.8 ± 96.0b ns	47.7 ± 18.7b B

4	*Lupinus albus*	4.3 ± 0.1b B	1.2 ± 0.2b B	13.2 ± 1.3ns B	628.6 ± 210.0ab B	189.0±28.5b A
	Lupinus angustifolius	12.0 ± 0.0a ns	3.1 ± 0.0a ns	10.8 ± 0.0ns ns	1021.5 ± 0.0a B	218.6 ± 0.0b ns
	Avena strigosa	4.1 ± 0.2a A	2.9 ± 0.2a A	14.7 ± 0.2ns ns	403.0 ± 47.3b ns	440.1±88.7aA
Significance of treatment effect						
Species		**<0.0001**	**<0.0001**	0.7014	**<0.0001**	**0.0342**
Soil type		0.1651	0.4564	**0.0453**	**<0.0001**	**<0.0001**
Species × soil type		**0.0237**	**0.0026**	0.4818	**<0.0001**	**<0.0001**

Significant effects ($p < 0.05$) are in bold. Different lowercase letters within a column indicate differences between species within each soil type, and different capital letters within a column indicate differences between sites across species being significant at *a p*-level of 0.05. ns: means no significant difference.

4.3.4. Relationships between Soil and Plant Parameters

The correlations between the soil and plant variables revealed a moderate, yet significant ($p = 0.0001$) positive relationship between plants' N and P contents (Table 6). This correlation increased to 0.90 ($p = 0.0001$) and 0.65 ($p = 0.0068$) when the legumes and oats datasets were separated. The shoots' dry weight and shoots' P and N contents were also positively related to the soil's PBray1 availability at 48 dap and negatively related to the ΔpH-initial (Table 6). This last finding suggests that a pH drop, relative to its initial value, increased the P and N uptake. In addition, the relationships between ΔpH−initial and ΔPBray1−initial, depicted in Figure 4, displayed a positive association for each species, showing that when the pH decreased (soil acidification), the PBray1 at harvest diminished compared to its initial value. This outcome can be linked to the significant negative correlation (−0.59, $p < 0.001$) between plants' P content and ΔpH−initial across all combinations of species and soils (Table 6) and within each species across all four soils types (Supplementary Materials, Table S7).

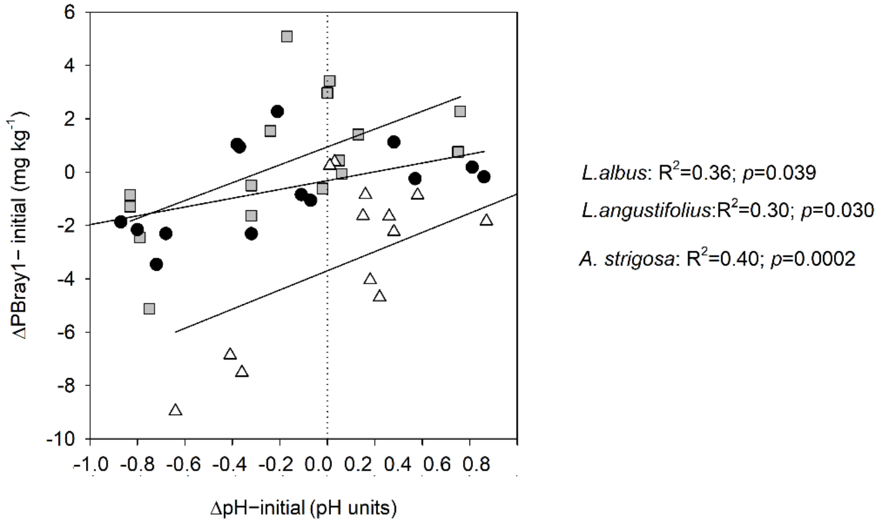


Figure 4. Relationships between ΔpH−initial (difference between soil pH at harvest and soil pH at the beginning of the experiment) and ΔPBray1−initial (difference between soil PBray1 at harvest and soil PBray1 at the beginning of the experiment). The dotted line splits the data from soil acidification to alkalinization relative to the initial pH. Gray squares, black circles, and white triangles represent *Lupinus albus*, *Lupinus angustifolius,* and *Avena strigosa* species, respectively.

Table 6. Matrix of Pearson correlation coefficients (r) for the relationships between soil (PBray1 and pH) and plant variables (P and N content, Fe and Mn concentration) for all combinations of species (lupines and oats) and soils at the harvest time of the experiment (87dap).

	ΔpH−Initial †	**PBray1−48dap**	**PBray1−87dap**	**Shoots' dry Weight**	**Plants' N Content**	**Plants' P Content**	**Plants' Fe Concentration**
PBray1−48dap	−0.56 ***						
PBray1−87dap	−0.61 ***	0.76 ***					
Shoots' dry weight	−0.24	0.39 **	0.06				
Plants' N content	−0.56 ***	0.49 ***	0.50 ***	0.32 *			
Plants' P content	−0.59 ***	0.66 ***	0.45 **	0.83 ***	0.54 ***		
Plants' Fe concentration	0.53 ***	−0.57 ***	−0.42 **	−0.36 *	−0.17	−0.61 ***	
Plants' Mn concentration	−0.36 *	0.02	0.36 *	−0.28	0.14	−0.13	0.15

† ΔpH-initial: difference between soil pH at harvest and soil pH at the beginning of the experiment. Significant at * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

This study also revealed a notable inverse relationship between plants' Fe concentration and PBray1 and a direct relationship between PBray1 and plants' P content. Additionally, a significant positive association was detected between plants' Fe concentration and the soil's ΔpH−initial. The first two associations establish a positive relationship between the soil's P availability and the plants' P content, while indicating a negative relationship between plants' Fe concentration and the soil's P availability. Conversely, the final association suggests that an increase in the soil pH was associated with an increase in plants' Fe concentration. The concentration of Mn in plants, on the other hand, exhibited a weak and inverse correlation with the soil's ΔpH-initial, suggesting that as the soil becomes more acidic within the range of the examined pH values (4.5–6.4 units), the soil's Mn solubility will increase (Table 6). Nevertheless, when analyzing within each species (Supplementary Materials, Table S7), the correlation was statistically significant and positive just for oats (r = 0.52, *p* = 0.037). Meanwhile, the soil pH at harvest only correlated with plants' Mn concentrations for both lupines, but it was positive (r = 0.66, *p* = 0.006) for *L. albus* and negative (−0.76, *p* = 0.011) for *L. angustifolius*. In the case of oats, the significant correlations that were observed between plants' Mn concentrations and PBray1−48 dap (r = −0.58) or plants' Mn concentrations and plant's P concentrations (r = −0.59) might be due to the influence of data from Site 4, in which the soil's plant Mn concentration was more than double compared to oats that were cultivated in the other soils (Table 5). For *L. albus*, there was a negative trend (r = −0.5, *p* = 0.1, excluding Site 4) between the Mn concentration (as an index of the organic anion concentration) and plant's P concentrations (Supplementary Materials, Table S8). In contrast, there was a significant correlation at a higher soil PBray1 at 48 dap (r = −0.63, *p* = 0.048, excluding Site 4, Supplementary Materials, Table S8). These correlations were not significant for *L. angustifolius* (Supplementary Materials, Table S8).

4.4. Discussion

4.4.1. Effects of Species and Soil Types: Variations in Soil's PBray1 Concentration and Soil pH

The changes in the soil's PBray1 relative to its initial concentration were agronomically negligible in soils that were planted with lupine. These results suggest that this species could solubilize plant-unavailable residual P or mobilize organic P forms, consequently maintaining an equilibrium level and not being depleted by plant absorption. In contrast, the absorption by oats reduced the amount of available P. When analyzing ΔPBray_$_{\text{Lup-Oat}}$, the values ranged from 0.9 (not a significant difference) to 5.4 mg kg^{-1}, and assuming an equivalent fertilizer value of 10 kg ha^{-1} of P_2O_5 (the average value used as equivalent fertilizer for Uruguayan soils according to Hernández and Zamalvide [41]), the highest increase in P availability due to lupines occurred in Site 2, which represents an equivalent of 54 kg ha^{-1} of P_2O_5. This increase in soil's PBray1 availability would significantly contribute to systems with a high proportion of P-extractive crops, leading to a considerable reduction in the application of P fertilizers. However, the species effect was also affected by the soil type, as lupine plants grew better in sites with higher PBray1 availability (Sites 1, 2, and 3) and greater soil fertility (high OM and high exchangeable cations).

The increase in PBray1 at 48 dap may be primarily attributed to the mineralization of organic P. The OM content would explain the extent of this increase, with the most significant increase in Site 1 and Site 2, which had higher OM contents than the other two sites. Interestingly, by 76 dap, the available P in the soil began to stabilize, i.e., returning to pre-experimental levels. This finding suggests that the mineralized organic P was either absorbed by the plants, retrograded in the soil, or immobilized by microorganisms [42]. The soil's biological activity may positively influence the mobilization (accessibility) of organic P forms [43,44] or negatively hamper the P mobilization efficiency of carboxylates by way of microbial degradation [13] and biochemical factors, such as the hydrolysis rates of extracellular phosphatase enzymes [11,43,45], which play a role in plant P acquisition [46]. Other physical factors, such as variations in the soil moisture, drying conditions, and aggregate stability, may have facilitated the mineralization of organic P [46].

The higher soil buffering capacity in Site 1 and Site 3 (higher clay content, OM, and cation exchange capacity) may account for the lower PBray1 variation compared with their baseline values at 87 and 103 dap. Conversely, the minimal change in Site 4 could be due to the generally poor performance of the lupine, particularly *L. angustifolius*, which exhibited the lowest shoot dry weight and P uptake. The soil of Site 2, however, demonstrated the most remarkable differences in ΔPBray_$_{\text{Lup-Oat}}$, which could be attributed to the higher P biomass that was attained by oats. The loamy texture, moderate clay, and OM content of Site 2 suggest a lower P adsorption capacity, leading to a higher equilibrium concentration of labile P forms in this soil compared to Sites 1 and 3, which possibly boosts the efficiency of organic anions in solubilizing inorganic P [42]. Moreover, this site had the highest PBray1 concentration at the start of the experiment, which can be partially attributed to organic P sources, ensuring a more consistent and substantial P supply for plants.

Even though lupine increased the PBray1 availability in all soils, the species effect alone could not entirely explain the increase in P by soil acidification, suggesting that other factors associated with the species could account for the differences [12]. It is crucial to recognize that variations in a species' capacity to obtain P can be attributed to the inherent traits of each species. Furthermore, morphological modifications in the root architecture and the development of specialized structures such as proteoid or cluster roots can also contribute to these differences [14,47]. While our study did not directly measure the contribution and composition of the root exudates of organic acids, we consider this trait indirectly based on the Mn concentration, which is proposed as an index of the carboxylate concentration in the rhizosphere under low P availability conditions in soil [21,48]. The subsequent section of this paper will delve further into this matter.

As demonstrated in our study, the BNF process contributed to variations in the soil pH during the experiment, leading to higher plant Mn concentrations in lupines than in oats. Legumes also absorb more cations than anions, resulting in the rhizosphere's exudation of protons and acidification [49]. It was further reported that

P deficiency in *L. albus* stimulates proton release and citrate root exudation by the proteoid roots of this species, along with the inhibition of nitrate uptake [50,51]. Pearse et al. [14] showed that seven lupine species had a greater acidification capacity of the soil rhizosphere than grasses and even than other legume crops, arguing that this could be an adaptation to increase the solubility of acid-soluble Ca-phosphates. In the present study, both lupine species contributed to pH decreases in different soil types, showing comparable trends in acidification levels, except for in Site 3, where *L. albus* had a minimal influence on the soil pH. This discrepancy could be attributed to differences in the inherent characteristics of the species, as mentioned above. *Lupinus albus* is a cluster root-forming legume and grows well in strongly acidic soils and mildly acid to neutral soils; *L. angustifolius,* on the contrary, does not form cluster roots and is sensitive to calcareous soil, displaying a preference for acidic soil conditions. Consequently, the growth of *L. angustifolius* in Site 3 may have induced changes in the rhizosphere that resulted in a decrease in the soil pH by an average of 0.6 units. This decline in pH, caused by a net release of protons, may have occurred as a compensatory mechanism to counterbalance the elevated uptake of cations. The high concentration of cations that was observed in the aboveground biomass of *L. angustifolius* in this study was consistent with previous studies [52,53].

When oats were cultivated, all soils (except Site 2) experienced an increase in pH compared to the initial values, with the Site 4 soil experiencing an increase of nearly one pH unit. Wang et al. [28], using the rhizotron technique with *Avena sativa*, also found that the soil pH increased compared to the control (without plants). In that study, despite having the lowest acid phosphatase activity, oats absorbed the same amount of P as other species, a result that was attributed to the extensive root mass and high mycorrhizal colonization, which contributed to the high P absorption [28]. We did not investigate the morphology or other aspects of the roots in our study, but this explanation would be valid for our experiment. Additionally, we can partially explain the pH change trends in oat-bearing soils based on an imbalance of the cation–anion uptake (mainly influenced by the N source). In Site 2, ammonium was the predominant form of N, and its absorption by oats would account for the soil acidification in this case [54]. However, the increased uptake of N in the form of nitrate would increase the absorption of H+, consequently promoting soil alkalinization [54]; this would explain the increase in the soil pH with oats in the acidic soil of Site 4.

4.4.2. Effects of Species and Soil Types: Plant Growth and Nutrient Uptake

While the availability of PBray1 differed among Sites 1, 2, and 3, there were no significant differences in the shoot biomass within each lupine species across these soils. However, the P concentrations were comparable to or higher than those of oats across these soils. In addition, these sites exhibited significant differences in aboveground biomass and plants' N and P contents for oats, indicating that the P supply and an additional factor (N availability) impacted the performance of this species across soil types differently. These findings align with a prior study that indicated that *L. albus* was the least impacted species by P supply, but that it exhibited the greatest P concentration in its shoots among all plant species [11].

Regarding P concentrations, whatever the species considered, there were differences across sites, particularly for Site 4, where the concentrations were, on average, half of those registered in the other sites. Previous studies have reported that the critical threshold for optimum development for *L. albus* and *L. angustifolius* is 2.2 and 2.3 mg g^{-1} of P, respectively [55]. In our work, the shoots' P concentrations of lupine species was below 2.0 mg g^{-1}, a level that typically stimulates cluster root development [56]. Concerning oats, the plant's P concentration would be adequate for their mature stage, which oscillates between 1.0 and 1.5 mg g^{-1} [57]. Furthermore, all concentrations of P, regardless of the species, were lower than 2.0 mg P g^{-1} dry weight, implying that the shoot biomass decay will probably lead to limited P availability in the soil due to P immobilization in the microbial biomass, as Hallama et al. noted [21].

The lowest plant growth, P uptake, and BNF values were observed at Site 4, suggesting that even though lupine species have adapted to acidic soils, Site 4 had several growth-limiting factors that prevented them from thriving as well as they did at the other sites. *Lupinus angustifolius* exhibited reduced growth and accelerated senescence of leaves, and both lupine species showed lower P and K concentrations in plants in Site 4 compared to other sites with higher soil PBray1, OM, and K concentration. The poor performance of lupines in Site 4, which experienced severe P deficiency, can be linked to the disruption in their ability to balance the carbon costs that are involved in cluster root development and nodulation [58,59].

The proportion and amount of N in the lupine's aerial biomass that are derived from the air fall within the reported ranges of 44 to 95% and 147 to 400 kg ha^{-1}, respectively [60]. The amount of N that is fixed by *L. albus* is, in most cases, higher than that of *L. angustifolius*, as evidenced by our research [23,61]. The wide variation between soils in the quantity of fixed N (60–240 mg pot^{-1}, or approximately 30–140 kg N ha^{-1}) reflects the varying levels of natural fertility among soils. The N that is fixed in biomass also varies between species (105–240 mg pot^{-1}, approximately 60–130 kg N ha^{-1}; $p = 0.0001$), indicating that lupines have different soil requirements that mainly affect the shoots' dry weight. Besides solubilizing non-labile forms of P, the N that is derived from the BNF process is an additional benefit of the lupine genus, because this contribution of N to the system might facilitate the balancing of N losses that occur during agricultural cycles [62].

4.4.3. Effects of Species and Soil Types: Links between Traits of Soil and Plants

The high PBray1 at harvest in soil containing *L. albus* might suggest a P-sparing effect of this species. However, during the growing phase, the amount of P that was absorbed by *L. albus* was, as was previously mentioned, comparable to and even higher than those taken up by oats. Although higher amounts of PBray1 were present at harvest in soils under *L. albus* than under oats, the differences are probably not due to variations in P uptake but instead to the "rhizosphere effect" that is induced *L. albus*. Neumann et al. [63] stated that *L.albus* employs diverse mechanisms to fulfill its P requirements in nutrient-deficient environments. This species exudes organic acids (citric and malic) that are sufficient to mobilize scarcely available P sources (Ca, Al, and Fe phosphates), primarily via chelation of the bound cations to P or by competition for P adsorption sites in the soil matrix [64]. However, the role of these carboxylates was not consistent in several studies that have reported that in other species, and even *L. albus*, the carboxylate concentration was not explained by any variation in soil or plant traits [11,15,48], leading to the conclusion in those studies that organic anions play a minor role in P acquisition strategies.

The concentration of PBray1 at harvest showed a negative correlation ($r = -0.61$) with the difference in pH from the initial pH across all combinations of species and soils. This result suggests that the increase in the acidification level within the tested soil pH range (4.5 to 6.4 units) was more pronounced in soils with high PBray availability. This result was unexpected in acidic soils, given that it has been reported that the P adsorption capacity of Fe and Al oxides increases with a decreasing pH [49]. However, other studies have shown that lowering the pH of acidic soils can also increase the solubility of soil P [65]. The proposed explanation is that competitive adsorption of sulfate ions would increase with a decreasing pH, leading to a higher equilibrium P concentration [49]. In the same way, the negative relationship between the amount of P biomass and the ΔpH−initial indicated that the P absorption increased with the degree of acidification, which is consistent with other studies [12,65,66]. On average, the plant P content was higher in *L. albus* and oats (14 mg pot^{-1}) than in *L. angustifolius* (7.9 mg pot^{-1}), mainly due to the low biomass yield of the latter species. The positive association of accumulated N and P amounts with the availability of PBray1 ($r = 0.50$ and 0.45, respectively) at harvest (87 dap) and the negative association with the ΔpH−initial ($r = -0.56$ and -0.59, respectively) show that N and P absorption increased with the available soil P and with soil acidification. This last association explains the negative correlation between the ΔpH−initial and the PBray1 that is available in the soil ($r = -0.61$).

Lupinus angustifolius reached higher levels of base cation than *L. albus*, as observed by other researchers [53], although, for K, there were no statistically significant differences between species at each site. The total cation concentrations in plants within each species also differ across soils. These differences were exclusively observed in L. albus, showing the lowest concentrations in plants that were cultivated in Sites 2 and 4, whose sites corresponded to soils that were strongly desaturated in base cations. Nevertheless, these last two soils differed widely in their pH and OM content. For the plant cation concentration in the lupine genus in our experiment, and based on previous research [53,67], this would indicate that plant nutrition was adequate in Sites 1, 2, and 3, and insufficient to cover lupine's requirements in Site 4.

The lupine-induced soil acidification remarkably impacted plants' micronutrient concentrations such as Mn and Fe. The Mn concentrations in *L. albus* and *L. angustifolius* reached mean values of 710 and 1510 mg kg^{-1}, respectively, which represent approximately three and seven times the concentration in oats (228 mg kg^{-1}), as was reported in previous studies [47,68]. These high concentrations of foliar Mn may be deleterious to other organisms or species, but the lupine genus tolerates them [48]. It has been found that plants using a P mobilization strategy based on the release of carboxylates have elevated Mn concentrations in their leaves [68]**,** because carboxylates mobilize both inorganic and organic P from the soil, as well as micronutrients [69]. Modifications in soil acidity or the oxidation/reduction conditions of the rhizosphere can also enhance the increased uptake of Mn [68]. It was further confirmed that lupines' exudation of organic acid anions may or may not be linked to soil acidification, as these processes self-regulate independently [70]. Further, a negative correlation between citrate exudation and plants' P status has been established, which has been perceived clearly with *L. albus* species [71]. Pearse et al. [14] and Wang et al. [72] also observed the variable response among lupine species, who found variation in both their P uptake and sensitivity to external P supply on the formation and development of proteid roots. These authors highlighted that the variations across species within the lupine genus open the possibility of selecting species with high plasticity regarding P supply [14]. In those studies, under pot experiments and using river sand as a growth medium, it was also reported that *L. albus* developed the most cluster roots with the lowest P concentration in shoots. Considering this context and under similar plant cultivation conditions, but using soils as a growth medium, in the present study, the Mn concentration (as an index of the organic anion concentration) in L. albus was downregulated at a greater P concentration in plants. However, this association was barely a trend ($r = -0.5$, $p = 0.1$, excluding Site 4). In addition, there was a significant correlation at a higher soil PBray1—48 dap ($r = -0.63$, $p = 0.048$, excluding Site 4), suggesting that the organic anion exudation increased when the growth medium had a low PBray1 concentration. These results are in accordance with previous studies [15,28], which found no simple linear relationships between plant and soil traits. In our work, *L.albus* showed a low

Mn concentration in plants under severe P stress (conditions under Site 4), maximum under moderate stress (conditions under Site 3), and low again at a high P availability (conditions under Sites 1 and 2). In contrast, the *L. angustifolius* plants with the highest concentration of Mn were those growing in the soil of Site 2, which exhibited the highest initial PBray1 concentration. These findings suggest that the exudation of carboxylates by *L. angustifolius* in the soil is constitutive, similar to what was reported by Pang et al. [73] for chickpea species (*Cicer arietinum* L.), a non-cluster root-forming crop. In contrast, the exudation of carboxylates by *L. albus* would be inducible, because the plants' Mn concentration changed in response to the soil's PBray1 availability [72]. Monei et al. [74], who also noted a divergent response in these lupine species, found that *L. albus* exhibited a high release of carboxylates in conditions of P deficiency, whereas *L. angustifolius* responded with the highest release of carboxylates when the soil had a high P supply. Although there was no clear relationship between Mn concentrations and plants' P contents or soil's P availability in both lupines across all sites in the current study, there was a significant association between the soil acidity at harvest and the Mn concentration; however, it was noticeably different between the lupine species. The correlation between these last variables was statistically significant, but positive ($r = 0.66$) for *L. albus* and negative ($r = -0.76$) for *L. angustifolius*, suggesting that the exudation of organic acid anions was concomitant with proton extrusion in soils containing *L. angustifolius,* while in *L.albus,* this was the case with base cations [48].

4.5. Conclusions

This research contributes to understanding how the species and soil type affect P mobilization and nutrient uptake and helped us inquire about the relationships between plant and soil factors that might explain or give us a clue to increase the comprehension of the processes of controlling P demand relative to its availability in the soil. *Lupinus albus* had a more substantial P solubilization capacity in the light-textured soil with a high OM content. However, the plant growth and nutrient uptake of *L. albus* in this site were comparable to those in the heavy-textured soils. The degree of soil acidity had a direct relationship with the P and N uptake within each species, across all soil types tested. The soil pH differed among species, with the lowest values found in soils that were cultivated with lupine species. In contrast, the soils under oats tended to maintain or even increase their pH compared to the original values. Our investigation revealed a significant variation in Mn accumulation (used as an index of the organic anion concentration) within species across the tested soils. The soil acidity and changes in P supply that were induced by lupines could explain such variation, particularly in soils with *L. albus.* Comparing these results with those obtained with these species in the field would be necessary to determine the robustness of the identified patterns in the soil's available P by the presence of lupine and to analyze them from the perspective of long-term stability, because the interaction and feedback with other soil–plant factors and microbial activity processes could hamper the increase in soil's P availability that is facilitated by lupine species. Hence, field research should be carried out for at least two growing seasons to ascertain whether the advantageous impacts of lupine last with the same magnitude over time and whether these variations result in enhanced soil health and crop production. Additionally, the future recommendations that emerge from the findings of our study include the need to measure soil's phosphatase activity, as this is an essential indicator for evaluating the effectiveness of P that is taken out by plants and analyzing the associated changes in organic P sources, such as the immobilized P within the living soil microbial biomass.

Supplementary Materials: The following supporting information can be downloaded at www.mdpi.com/xxx/s1: Table S1: Initial soil properties before the establishment of the experiment; Table S2: ANOVA for soil PBray1 concentration at 87 days after planting; Table S3: ANOVA for soil PBray1 concentration at 103 days after planting; Table S4: ANOVA for soil pH at 87 days after planting; Table S5: Orthogonal contrasts for biomass measurements; Table S6: Mean values of BNF proportion (% BNF) and N fixed content of lupines as affected by species and soil type; Table S7: Pearson correlation coefficients (r) within each species across all four soil types; Table S8: Pearson correlation coefficients (r) within each species across three soils types (excluding Site 4).

Author Contributions: Conceptualization, C.M.A. and C.P.V.; methodology, C.M.A. and A.B.G.; formal analysis, C.M.A., C.P.V., P.G.B., and A.d.P.M.; investigation, C.M.A. and A.B.G.; resources, C.M.A.; data curation, C.M.A., C.P.V., P.G.B., and A.d.P.M.; writing—original draft preparation, C.M.A., C.P.V., P.G.B., A.B.G., and A.d.P.M.; writing—review and editing, C.M.A., C.P.V., P.G.B., and A.d.P.M.; visualization, C.M.A. and A.d.P.M.; supervision, A.d.P.M. and C.P.V.; project administration, C.M.A.; funding acquisition, C.M.A., C.P.V., and A.d.P.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research and Innovation Agency of Uruguay, with the following funds: María Viñas, grant number ANII: FMV_1_2017_1 135487. The research was also supported by C.M.A., a recipient of a doctoral fellowship from CAP (ComisiónAcadémica de Posgrado), Universidad de la República (UdelaR). The APC was funded by the National Research of our Institution University of the Republic (UdelaR, Uruguay). This work is part of the Doctoral Dissertation of the senior author at the Doctoral Program in Agricultural Sciences, Agronomy College, UdelaR, Uruguay.

Data Availability Statement: The data presented in this study are available upon request from the corresponding author.

Acknowledgments: The authors express their gratitude to N. Di Muro and B. Romano for their assistance in conducting the greenhouse experiment and laboratory work.

Conflicts of Interest: The authors declare no conflicts of interest.

4.6. References

1. Vassallo, M. Dinámica y competencia intrasectorial en la agricultura uruguaya: Los cambios en la última década. *Agrociencia* **2013**, *17*, 170–179. https://doi.org/10.31285/agro.17.488.
2. Ernst, O.R.; Dogliotti, S.; Cadenazzi, M.; Kemanian, A.R. Shifting crop-pasture rotations to no-till annual cropping reduces soil quality and wheat yield. Field Crops Res. 2018, 217, 180–187. https://doi.org/10.1016/j.fcr.2017.11.014.
3. Ernst, O.R.; Kemanian, A.R.; Mazzilli, S.R.; Cadenazzi, M.; Dogliotti, S. Depressed attainable wheat yields under continuous annual no-till agriculture suggest declining soil productivity. Field Crops Res. 2016, 186, 107–116. https://doi.org/10.1016/j.fcr.2015.11.005.
4. Ministerio de Ganadería, Agricultura y Pesca. DIEA presenta los resultados de la Encuesta Agrícola “Invierno 2021”. Available online: https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/datos-y-estadisticas/estadisticas/diea-presenta-resultados-encuesta-agricola-invierno-2021 (accessed on October 7, 2021).
5. Beretta-Blanco, A.; Pérez, O.; Carrasco-Letelier, L. Soil quality decrease over 13 years of agricultural production. Nutr. Cycl. Agroecosystems 2019, 114, 45–55. https://doi.org/10.1007/s10705-019-09990-3.
6. Ernst, O.; Siri-Prieto, G. Impact of perennial pasture and tillage systems on carbon input and soil quality indicators. Soil Tillage Res. 2009, 105, 260–268. https://doi.org/10.1016/j.still.2009.08.001.
7. Cano, J.D.; Ernst, O. Balance aparente de fósforo en rotaciones agrícolas del litoral oeste del Uruguay 1. Inf. Agronómicas 2005, 32, 8–11.

8. Syers, J.K.; Johnston, A.E.; Curtin, D. Efficiency of soil and fertilizer phosphorus use: Reconciling changing concepts of soil phosphorus behaviour with agronomic information. In FAO Fertilizer and Plant Nutrition Bulletin; FAO: Rome, Italy, 2008; p 128.
9. Menezes-Blackburn, D.; Giles, C.; Darch, T.; George, T.S.; Blackwell, M.; Stutter, M.; Shand, C.; Lumsdon, D.; Cooper, P.; Wendler, R.; et al. Opportunities for mobilizing recalcitrant phosphorus from agricultural soils: A review. Plant Soil 2018, 427, 5–16. https://doi.org/10.1007/s11104-017-3362-2.
10. Richardson, A.E.; Barea, J.M.; McNeill, A.M.; Prigent-Combaret, C. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 2009, 321, 305–339. https://doi.org/10.1007/s11104-009-9895-2.
11. Touhami, D.; McDowell, R.W.; Condron, L.M. Role of organic anions and phosphatase enzymes in phosphorus acquisition in the rhizospheres of legumes and grasses grown in a low phosphorus pasture soil. Plants 2020, 9, 1185. https://doi.org/10.3390/plants9091185.
12. Barrow, N.J.; Hartemink, A.E. The effects of pH on nutrient availability depend on both soils and plants. Plant Soil 2023, 487, 21–37. https://doi.org/10.1007/s11104-023-05960-5.
13. Schack-Kirchner, H.; Loew, C.A.; Lang, F. The Cumulative Amount of Exuded Citrate Controls Its Efficiency to Mobilize Soil Phosphorus. Front. For. Glob. Change 2020, 3, 550884.
14. Pearse, S.J.; Veneklaas, E.J.; Cawthray, G.R.; Bolland, M.D.A.; Lambers, H. Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. Plant Soil 2006, 288, 127–139. https://doi.org/10.1007/s11104-006-9099-y.
15. Veneklaas, E.J.; Stevens, J.; Cawthray, G.R.; Turner, S.; Grigg, A.M.; Lambers, H. Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil 2003, 248, 187–197. https://doi.org/10.1023/A:1022367312851.

16. Kamh, M.; Abdou, M.; Chude, V.; Wiesler, F.; Horst, W.J. Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. J. Plant Nutr. Soil Sci. 2002, 165, 566–572. https://doi.org/10.1002/1522-2624(200210)165:5<566::AID-JPLN566>3.0.CO;2-O.
17. Lambers, H.; Clements, J.C.; Nelson, M.N. How aphosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (Lupinus, Fabaceae). Am. J. Bot. 2013, 100, 263–288. https://doi.org/10.3732/ajb.1200474.
18. Ayala, W.; Barrios, E.; Macedo, I.; Sawchik, J.; Terra, J. A. Scouting benefits and developing innovations in temperate grassland to sustainable agriculture production. In Proceedings of the 23rd International Grassland Congress on Sustainable Use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection; New Delhi, India, 20-24 November 2015; pp 208–214.
19. Fontaine, S.; Abbadie, L.; Aubert, M.; Barot, S.; Bloor, J.M.G.; Derrien, D.; Duchene, O.; Gross, N.; Henneron, L.; Le Roux, X.; et al. Plant–soil synchrony in nutrient cycles: Learning from ecosystems to design sustainable agrosystems. Glob. Change Biol. 2023, 30, e17034. https://doi.org/10.1111/gcb.17034.
20. Kirkegaard, J.; Christen, O.; Krupinsky, J.; Layzell, D. Break crop benefits in temperate wheat production. Field Crops Res. 2008, 107, 185–195. https://doi.org/10.1016/j.fcr.2008.02.010.
21. Hallama, M.; Pekrun, C.; Lambers, H.; Kandeler, E. Hidden miners—The roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. Plant Soil 2019, 434, 7–45. https://doi.org/10.1007/s11104-018-3810-7.
22. Mera, M.; Lizana, X.C.; Calderini, D.F. Cropping systems in environments with high yield potential of southern Chile. In Crop Physiology: Applications for Genetic Improvement and Agronomy, 2nd ed. V. Sadras, D. Calderini,Eds.; Academic Press, 2015; pp 111–140. https://doi.org/10.1016/B978-0-12-417104-6.00006-6.

23. Espinoza, S.; Ovalle, C.; Zagal, E.; Matus, I.; Tay, J.; Peoples, M.B.; del Pozo, A. Contribution of legumes to wheat productivity in Mediterranean environments of central Chile. Field Crops Res. 2012, 133, 150–159. https://doi.org/10.1016/j.fcr.2012.03.006.
24. Nuruzzaman, M.; Lambers, H.; Bolland, M.D.A.; Veneklaas, E.J. Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. Aust. J. Agric. Res. 2005, 56, 1041–1047. https://doi.org/10.1071/AR05060.
25. Wolko, B.; Clements, J.C.; Naganowska, B.; Nelson, M.N.; Yang, H. Lupinus; Kole, C., Ed.; Springer: Berlin/Heidelberg, Germany, 2011. https://doi.org/10.1007/978-3-642-14387-8_9.
26. Soil Survey Staff. *Claves para la Taxonomía de Suelos,* 12th ed. USDA-Natural Resources Conservation Service, Washington, DC. 2014, p 399.Available online: https://www.nrcs.usda.gov/resources/guides-and-instructions/keys-to-soil-taxonomy (accessed on September 15, 2018).
27. Sawchik, J.; Siri, G.; Ayala, W.; Barrios, E.; Bustamante, M.; Ceriani, M.; Gutiérrez, F.; Mosqueira, J.; Otaño, C.; Perez, M.; et al. El sistema agrícola bajo amenaza: ¿qué aportan los cultivos de cobertura y/o laspasturas cortas? In IV Simposio Nacional de Agricultura, Paysandú, Uruguay, 28 y 29 Octubre, 2015. A. Ribeiro, M. Barbazán, Eds.; Editorial Hemisferio Sur, Montevideo, Uruguay; pp. 149–168. http://www.ainfo.inia.uy/digital/bitstream/item/5164/1/Simposio-Nacional-Agricultura-2015-Sawchik-p.149-168.pdf
28. Wang, Y.; Krogstad, T.; Clarke, J.L.; Hallama, M.; Øgaard, A.F.; Eich-Greatorex, S.; Kandeler, E.; Clarke, N. Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. Front. Plant Sci. 2016, 7, 1664. https://doi.org/10.3389/fpls.2016.01664.
29. Suzuki, L.E.A.S.; Amaral, R. de L. do; Almeida, W.R. da S.; Ramos, M.F.; Nunes, M.R. Oat Straw Mulching Reduces Interril Erosion and Nutrient Losses Caused by Runoff in a Newly Planted Peach Orchard. Soil Syst. 2023, 7, 8. https://doi.org/10.3390/soilsystems7010008.

30. Nelson, D.W.; Sommers, L.E. Total Carbon, Organic Carbon, and Organic Matter. In Methods of Soil Analysis; Sparks, D.L., Page, A.L., Helmke, P.A., Loeppert, R.H., Eds.; Soil Science Society of America and American Society of Agronomy: Madison, WI, USA, 1996. https://doi.org/10.2136/sssabookser5.3.c34.
31. Thomas, G.W. Exchangeable Cations. In Methods of Soil Analysis; Page, A., Ed.; Wiley: Hoboken, NJ, USA, 1983; pp 159–165. https://doi.org/10.2134/agronmonogr9.2.2ed.c9.
32. Lajtha, K.; Driscoll, C.T.; Jarrell, W.M.; Elliott, E.T. Soil phosphorus: Characterization and total element analysis. In Standard Soil Methods for Long Term Ecological Research; Oxford University Press: New York, NY, USA, 1999; pp. 115–143.
33. Rabuffetti, A. La Fertilidad del Suelo y Su Manejo; Hemisferio Sur: Montevideo, Uruguay, 2017.
34. Mulvaney, R. Nitrogen-inorganic forms. In Methods of Soil Analysis: Chemical Methods—Part 3; Sparks, D.L., Ed. Soil Science Society of America, Inc.: Madison, WI, USA, 1996.
35. Rhine, E.D.; Mulvaney, R.L.; Pratt, E.J.; Sims, G.K. Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. Soil Sci. Soc. Am. J. 1998, 62, 473–480. https://doi.org/10.2136/sssaj1998.03615995006200020026x.
36. Beretta, A.N.; Silbermann, A.V.; Paladino, L.; Torres, D.; Bassahun, D.; Musselli, R.; García-Lamohte, A. Soil texture analyses using a hydrometer: Modification of the Bouyoucos method. Cienc. Investig. Agrar. 2014, 41, 25–26. https://doi.org/10.4067/s0718-16202014000200013.
37. Shearer, G.; Kohl, D.H. N2-fixation in field settings: Estimations based on natural 15N abundance. Aust. J. Plant Physiol. 1987, 13, 699–756. https://doi.org/10.1071/pp9860699.
38. Murphy, J.; Riley, J.P. A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 1962, 27, 31–36. https://doi.org/10.1016/S0003-2670(00)88444-5.

39. Jones, J.B.; Case, V.W. Sampling, handling, and analyzing plant tissue samples. In Soil Testing and Plant Analysis, Westerman, R. L. Ed., pp 389–427. SSSA. 1990. https://doi.org/10.2136/sssabookser3.3ed.c15.

40. R Core Team. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. Available online: http://www.R-project.org (accessed on February 15, 2021).

41. Hernández, J.; Zamalvide, J. Procesos de retención de fosforo por los suelos evaluados a través de parámetros de suelo y planta. Agrociencia 1998, 2, 48–63.

42. Oburger, E.; Jones, D.L.; Wenzel, W.W. Phosphorus saturation and pH differentially regulate the efficiency of organic acid anion-mediated P solubilization mechanisms in soil. Plant Soil 2011, 341, 363–382. https://doi.org/10.1007/s11104-010-0650-5.

43. Renella, G.; Landi, L.; Valori, F.; Nannipieri, P. Microbial and hydrolase activity after release of low molecular weight organic compounds by a model root surface in a clayey and a sandy soil. Appl. Soil Ecol. 2007, 36, 124–129. https://doi.org/10.1016/j.apsoil.2007.01.001.

44. Ai, J.; Banfield, C.C.; Shao, G.; Zamanian, K.; Stürzebecher, T.; Shi, L.; Fan, L.; Liu, X.; Spielvogel, S.; Dippold, M.A. What controls the availability of organic and inorganic P sources in top- and subsoils? A 33P isotopic labeling study with root exudate addition. Soil Biol. Biochem. 2023, 185, 109129. https://doi.org/10.1016/j.soilbio.2023.109129.

45. Janes-Bassett, V.; Blackwell, M.S.A.; Blair, G.; Davies, J.; Haygarth, P.M.; Mezeli, M.M.; Stewart, G. A meta-analysis of phosphatase activity in agricultural settings in response to phosphorus deficiency. Soil Biol. Biochem. 2021, 165, 108537. https://doi.org/10.1016/j.soilbio.2021.108537.

46. Condron, L.; Turner, B.; Cade-Menun, B. Chemistry and Dynamics of Soil Organic Phosphorus. In Phosphorus: Agriculture and the Environment. Thomas Sims, J., Sharpley A.N. and D.T. Westermann, Eds. Wiley: Hoboken, NJ, USA, 2005. https://doi.org/10.2134/agronmonogr46.c4

47. Watt, M.; Evans, J.R. Phosphorus acquisition from soil by white lupin (Lupinus albus L.) and soybean (Glycine max L.), species with contrasting root

development. Plant Soil 2003, 248, 271–283. https://doi.org/10.1023/A:1022332700686.

48. Wang, Y.; Lambers, H. Root-released organic anions in response to low phosphorus availability: Recent progress, challenges and future perspectives. Plant Soil 2020, 447, 135–156. https://doi.org/10.1007/s11104-019-03972-8.
49. Hinsinger, P. Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: A review. Plant Soil 2001, 237, 173–195. https://doi.org/10.1023/A:1013351617532.
50. Neumann, G.; Römheld, V. Root excretion of carboxylic acids and protons in phosphorus-deficient plants. Plant Soil 1999, 211, 121–130. https://doi.org/10.1023/A:1004380832118.
51. Shen, J.; Li, H.; Neumann, G.; Zhang, F. Nutrient uptake, cluster root formation and exudation of protons and citrate in Lupinus albus as affected by localized supply of phosphorus in a split-root system. Plant Sci. 2005, 168, 837–845. https://doi.org/10.1016/j.plantsci.2004.10.017.
52. Brand, J.D.; Tang, C.; Rathjen, A.J. Adaptation of Lupinus angustifolius L. and L. pilosus Murr. to calcareous soils. Aust. J. Agric. Res. 1999, 50, 1027–1033. https://doi.org/10.1071/AR98177.
53. Kerley, S.J.; Shield, I.F.; Huyghe, C. Specific and genotypic variation in the nutrient content of lupin species in soils of neutral and alkaline pH. Aust. J. Agric. Res. 2001, 52, 93–102. https://doi.org/10.1071/AR00060.
54. Kirkby, E.A.; Le Bot, J.; Adamowicz, S.; Römheld, V. Nitrogen in physiology—An agronomic perspective and implications for the use of different nitrogen forms. Proc. Int. Fertil. Soc. 2009, 653, 1–48.
55. Bolland, M.D.A.; Brennan, R.F. Comparing the phosphorus requirements of wheat, lupin, and canola. Aust. J. Agric. Res. 2008, 59, 983–998. https://doi.org/10.1071/AR07430.
56. Li, H.; Shen, J.; Zhang, F.; Tang, C.; Lambers, H. Is there a critical level of shoot phosphorus concentration for cluster-root formation in Lupinus albus? Funct. Plant Biol. 2008, 35, 328–336. https://doi.org/10.1071/FP07222.

57. Mengel, K.; Kirkby, E.A.; Kosegarten, H.; Appel, T. Principles of Plant Nutrition; Mengel, K., Kirkby, E.A., Kosegarten, H., Appel, T., Eds.; Springer: Dordrecht, The Netherlands, 2001. https://doi.org/10.1007/978-94-010-1009-2.
58. Uhde-Stone, C. White Lupin: A Model System for Understanding Plant Adaptation to Low Phosphorus Availability. In: Sulieman, S.; Tran, L.S., Eds. Legume Nitrogen Fixation in Soils with Low Phosphorus Availability: Adaptation and Regulatory Implication; Springer, Cham., 2017. pp 243–280. https://doi.org/10.1007/978-3-319-55729-8_13.
59. Pueyo, J.J.; Quiñones, M.A.; Coba de la Peña, T.; Fedorova, E.E.; Lucas, M.M. Nitrogen and Phosphorus Interplay in Lupin Root Nodules and Cluster Roots. Front. Plant Sci. 2021, 12, 184. https://doi.org/10.3389/fpls.2021.644218.
60. Unkovich, M.J.; Baldock, J.; Peoples, M.B. Prospects and problems of simple linear models for estimating symbiotic N2 fixation by crop and pasture legumes. Plant Soil 2010, 329, 75–89. https://doi.org/10.1007/s11104-009-0136-5.
61. Unkovich, M.J.; Pate, J.S. An appraisal of recent field measurements of symbiotic N2 fixation by annual legumes. Field Crops Res. 2000, 65, 211–228. https://doi.org/10.1016/S0378-4290(99)00088-X.
62. McLauchlan, K. The nature and longevity of agricultural impacts on soil carbon and nutrients: A review. Ecosystems 2006, 9, 1364–1382. https://doi.org/10.1007/s10021-005-0135-1.
63. Neumann, G.; Massonneau, A.; Martinoia, E.; Römheld, V. Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. Planta 1999, 208, 373–382. https://doi.org/10.1007/s004250050572.
64. Fink, J.R.; Inda, A.V.; Bavaresco, J.; Barrón, V.; Torrent, J.; Bayer, C. Adsorption and desorption of phosphorus in subtropical soils as affected by management system and mineralogy. Soil Tillage Res. 2016, 155, 62–68. https://doi.org/10.1016/j.still.2015.07.017.
65. Barrow, N.J. The effects of pH on phosphate uptake from the soil. Plant Soil 2017, 410, 401–410. https://doi.org/10.1007/s11104-016-3008-9.
66. Bouray, M.; Moir, J.L.; Lehto, N.J.; Condron, L.M.; Touhami, D.; Hummel, C. Soil pH effects on phosphorus mobilization in the rhizosphere of Lupinus

angustifolius. Plant Soil 2021, 469, 387–407. https://doi.org/10.1007/s11104-021-05177-4.

67. Martínez-Alcalá, I.; Clemente, R.; Bernal, M.P. Metal availability and chemical properties in the rhizosphere of lupinus albus l. growing in a high-metal calcareous soil. Water Air Soil Pollut. 2009, 201, 283–293. https://doi.org/10.1007/s11270-008-9944-0.

68. Lambers, H.; Hayes, P.E.; Laliberté, E.; Oliveira, R.S.; Turner, B.L. Leaf manganese accumulation and phosphorus-acquisition efficiency. In Trends in Plant Science; Elsevier: Amsterdam, The Netherlands, 2015, 20 (2), pp 83–90. https://doi.org/10.1016/j.tplants.2014.10.007.

69. Ding, W.; Cong, W.F.; Lambers, H. Plant phosphorus-acquisition and -use strategies affect soil carbon cycling. Trends Ecol. Evol. 2021, 36, 899–906. https://doi.org/10.1016/j.tree.2021.06.005.

70. Lambers, H.; Shane, M.W.; Cramer, M.D.; Pearse, S.J.; Veneklaas, E.J. Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. Ann. Bot. 2006, 98, 693–713. https://doi.org/10.1093/aob/mcl114.

71. Dessureault-Rompré, J.; Nowack, B.; Schulin, R.; Luster, J. Spatial and temporal variation in organic acid anion exudation and nutrient anion uptake in the rhizosphere of Lupinus albus L. Plant Soil 2007, 301, 123–134. https://doi.org/10.1007/s11104-007-9427-x.

72. Wang, X.; Pearse, S.J.; Lambers, H. Cluster-root formation and carboxylate release in three Lupinus species as dependent on phosphorus supply, internal phosphorus concentration and relative growth rate. Ann. Bot. 2013, 112, 1449–1459. https://doi.org/10.1093/aob/mct210.

73. Pang, J.; Bansal, R.; Zhao, H.; Bohuon, E.; Lambers, H.; Ryan, M.H.; Ranathunge, K.; Siddique, K.H.M. The carboxylate-releasing phosphorus-mobilizing strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. New Phytol. 2018, 219, 518–529. https://doi.org/10.1111/nph.15200.

74. Monei, N.; Hitch, M.; Heim, J.; Pourret, O.; Heilmeier, H.; Wiche, O. Effect of substrate properties and phosphorus supply on facilitating the uptake of rare earth elements (REE) in mixed culture cropping systems of Hordeum vulgare, Lupinus albus and Lupinus angustifolius. Environ. Sci. Pollut. Res. 2022, 29, 57172–57189. https://doi.org/10.1007/s11356-022-19775-x.

Supplementary Material

Lupine Cultivation Affects Soil's P Availability and Nutrient Uptake in Four Contrasting Soils

Table S1. Initial soil properties before the establishment of the experiment.

Site (soil type†)	PBray1	NH^+_4-N	NO^-_3-N	Ca^{2+}	Mg^{2+}	K^+	Na^+	Al^{3+} exch.	pH
	mg kg^{-1}			cmolc kg^{-1}					
Site 1 (Pachic Argiudoll)	12.0	16.4	21.9	14.5	3.0	1.0	0.2	0.05	5.17
Site 2 (Typic Dystrutepts)	15.6	25.7	0.3	4.6	1.2	0.3	0.1	0.05	5.32
Site 3 (Typic Argiudoll)	10.3	7.8	0.9	17.7	2.8	1.4	0.2	0.02	6.00
Site 4 (Typic Hapludults)	5.8	10.9	12.1	1.4	0.5	0.1	0.1	0.16	4.50

†USDA source: Keys to soil taxonomy. Soil Conservation Service. 2014. https://www.nrcs.usda.gov/resources/guides-and-instructions/keys-to-soil-taxonomy

Table S2. ANOVA for soil PBray1 concentration at 87 days after planting.

Factor	gl	Valor de F	P > F†
Especie	2	17,77	**< 0,0001**
C1: Lupino vs. avena	1		**< 0,0001**
C2: L. albus vs. L. angustifolius	1		0,0556
Tipo de suelo	3	46,40	**< 0,0001**
Tipo de suelo x Especie	6	1,32	0,2721

† Significant effects (p < 0.05) are in bold.

Note: For this model species and soil type were considered as fixed factors.

Table S3. ANOVA for soil PBray1 concentration at 103 days after planting.

Treatment effect	DF	F Value	Pr > F†
Species	2	23.40	**<0.0001**
Soil type	3	60.09	**<0.0001**
Species x Soil type	6	3.81	**0.0048**
C1: Lupine vs. Oat in Site 1	1		**0.0008**
C1: Lupine vs. Oat in Site 2	1		**0.0002**
C1: Lupine vs. Oat in Site 3			**0.0340**
C1: Lupine vs. Oat in Site 4	1		**0.0009**
C2: L. albus vs L.angustifolius in Site 1	1		**0.0039**
C2: L. albus vs L.angustifolius in Site 2	1		**0.0290**
C2: L. albus vs L.angustifolius in Site 3	1		**0.0301**
C2: L. albus vs L.angustifolius in Site 4	1		0.1849

† Significant effects (p < 0.05) are in bold.

Note: For this model species and soil type were considered as fixed factors.

Table S4. ANOVA for soil pH at 87 days after planting.

Treatment effect	DF	F Value	Pr > F†
Species	2	28.25	**<0.0001**
Soil type	3	65.05	**<0.0001**
Species x Soil type	6	2.56	**0.0365**
C1: Lupine vs. Oat in Site 1	1		**0.0372**
C1: Lupine vs. Oat in Site 2	1		**0.0005**
C1: Lupine vs. Oat in Site 3			**<.0001**
C1: Lupine vs. Oat in Site 4	1		**0.0001**
C2: L. albus vs L.angustifolius in Site 1	1		0.9322
C2: L. albus vs L.angustifolius in Site 2	1		0.8250
C2: L. albus vs L.angustifolius in Site 3	1		**0.0017**
C2: L. albus vs L.angustifolius in Site 4	1		0.2385

† Significant effects ($p < 0.05$) are in bold.

Note: For this model species and soil type were considered as fixed factors.

Table S5. Orthogonal contrasts for biomass measurements.

Constrast Label	Aboveg. biomass	Plant P conc.	Plant P content	Plant N conc.	Plant N content
C1: Oat vs lupine in Site 1	0.0058	0.2001	0.0201	<.0001	<.0001
C1: Oat vs lupine in Site 2	<.0001	<.0001	<.0001	<.0001	0.0173
C1: Oat vs lupine in Site 3	0.1525	0.6802	0.1277	<.0001	<.0001
C1: Oat vs lupine in Site 4	0.0005	0.0399	0.3391	<.0001	0.9579
C2:L.albus vs L. angustifolius in Site 1	0.0046	0.4438	0.0040	0.0853	<.0001
C2:L.albus vs L. angustifolius in Site 2	0.0032	0.7289	0.0002	0.0002	<.0001
C2:L.albus vs L. angustifolius in Site 3	0.0009	0.0008	0.0354	0.7395	<.0001
C2:L.albus vs L. angustifolius in Site 4	0.0491	0.0876	0.0906	0.336	0.0394

Significant effects (p<0.05).

Table S6. Mean values of BNF proportion (Ndfa) and N fixed content of lupines as affected by species and soil type.

	Ndfa		N fixed content	
Species	%		mg pot-1	
Lupinus albus	66.2	ns	237.4	a
Lupinus angustifolius	60.3	ns	105.3	b
Site (Soil type)				
Site 1 (Pachic Argiudoll)	71.7	b	197.0	a
Site 2 (Typic Dystrutepts)	59.4	c	187.9	a
Site 3 (Typic Argiudoll)	82.5	a	244.7	a
Site 4 (Typic Hapludults)	39.3	d	55.9	b
Significance of treatment effect				
Species	<0.1206		<.0001	
Soil type	<0.0001		0.0011	
Species*soil type	<0.0644		0.8933	

Significant effects (p < 0.05) are in bold. Different lowercase letters within a column indicate differences between species or sites, at p-level of 0.05. ns: means no significant difference.

Table S7. Pearson correlation coefficients (r) within each species across four soils types.

Variables		L. albus		L. angustifolius		A. strigosa	
ΔpH- initial	Shoot dry weight	-0.61	*	-0.75	*	-0.71	**
ΔpH- initial	Plant N content	-0.72	***	-0.71	*	-0.42	
ΔpH- initial	Plant P content	-0.75	**	-0.71	*	-0.91	***
pH_87 dap	Plant Mn concentration	0.66	**	-0.76	*	-0.06	
pH_48 dap	Plant Mn concentration	0,52	*	-0,77	**	-0.36	
PBray1-48dap	Plant Mn concentration	-0.21		0.30		-0.58	*
PBray1-48dap	Plant P concentration	0.56	*	0.16		0.63	*
PBray1-48dap	ΔpH- initial	-0.51	*	-0.12		-0.86	***
PBray1-48dap	Plant N content	0.60	*	0.16		0.34	
PBray1-48dap	Plant P content	0.80	**	0.22		0.87	***
PBray1-48dap	Shoot dry weight	0.51	*	0.31		-0.57	*
Plant P concentration	Plant Mn concentration	-0.26		0.13		-0.59	*
Plant N content	Plant P content	0.94	***	0.93	***	0.65	**

Significant at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

Table S8. Pearson correlation coefficients (r) within each species across three soils types (excluding Site 4).

Variables		L. albus		L. angustifolius		A. strigosa	
ΔpH- initial	Shoot dry weight	-0.18		-0.51		-0.88	***
ΔpH- initial	Plant N content	-0.49		-0.40		-0.69	*
ΔpH- initial	Plant P content	-0.56		-0.40		-0.86	***
pH_48 dap	Plant Mn concentration	0.68	*	-0.85	**	-0.10	
PBray1-48dap	Plant Mn concentration	-0.63	*	0.27		-0.05	
PBray1-48dap	Plant P concentration	0.07		-0.35		-0.33	
PBray1-48dap	ΔpH- initial	-0.09		0.38		-0.55	
PBray1-48dap	Plant N content	-0.05		-0.22		0.62	*
PBray1-48dap	Plant P content	-0.00		-0.16		0.73	**
PBray1-48dap	Shoot dry weight	-0.07		-0.02		0.63	*
Plant P concentration	Plant Mn concentration	-0.50		0.03		-0.17	
Plant N content	Plant P content	0.83	***	0.92	***	0.87	***

Significant at $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.

5. Discusión general

5.1. Estrategias de diversificación en los sistemas agrícolas y su importancia

Hufnagel et al. (2020) plantean que la diversificación de los sistemas agrícolas involucra procesos orientados a aumentar la heterogeneidad genética, funcional y espacial de los cultivos dentro de un sistema de producción. Este objetivo puede alcanzarse mediante la implementación de diversas estrategias, como la incorporación de cultivos con ciclos de vida diferentes (anuales y perennes) en una rotación a lo largo del tiempo, lo que promueve la diversidad temporal. Ejemplos de esta estrategia incluyen la rotación de cultivos con pasturas o la integración de cultivos de servicio, los cuales pueden pertenecer a distintos grupos funcionales y desempeñar roles complementarios, lo que incrementa la diversidad funcional. En el capítulo 1 de esta tesis se evaluó la productividad del trigo en sistemas de siembra directa a través de la comparación de dos esquemas con niveles similares de intensificación, pero con estrategias de diversificación contrastantes: 1) trigo cultivado como primer cultivo después de una fase larga (3,5 años) de pastura perenne en un sistema de rotación cultivo-pastura (CP) y 2) trigo cultivado dentro de una rotación continua de cultivos anuales (CC). En este estudio, el término *intensidad* se define siguiendo el criterio tomado por Cerecetto et al. (2024), refiriéndose a los años dedicados al cultivo de granos. Este período dentro del ciclo de rotación se caracteriza por generar los mayores disturbios (físico-químicos y biológicos) en el suelo y requerir una mayor cantidad de insumos. En el caso del sistema CP, la intensificación es menor debido a la mayor diversidad vegetal que aporta la inclusión de pasturas en la rotación. El posible beneficio de incorporar pasturas depende en gran medida tanto de la cantidad como de la calidad de la biomasa que estas aportan al suelo durante su ciclo de vida, así como de la eficacia en el reciclaje de nutrientes que ocurre durante esta etapa, especialmente al considerar la influencia de la actividad de pastoreo (Hendrickson, 2020).

Además de las pasturas, los cultivos de servicio son una alternativa valiosa para diversificar los sistemas agrícolas. En el capítulo 2 de esta tesis, se aborda la diversificación mediante el uso de cultivos de cobertura (CC), destacando su papel como herramienta esencial para optimizar beneficios agroecológicos y agronómicos, como la mejora en la dinámica de nutrientes, el control de malezas y la sostenibilidad agrícola. Este análisis pone especial atención en el papel de las leguminosas, como trébol alejandrino y lupino, utilizadas en cultivos puros o mezclas, por su capacidad de aportar N al cultivo sucesor. En el capítulo 3, se profundiza en cómo las especies vegetales (con enfoque en dos especies de lupino en comparación con la avena) y el tipo de suelo (contrastante en textura, pH, fertilidad natural) influye en la movilización de P y la absorción de nutrientes, además de permitir explorar las interacciones entre suelos y especies.

5.1.1. Pasturas perennes: importancia en la dinámica de N desde la MOS y el residuo remanente de la pastura

En el sistema CP, la integración de pasturas que incluyen leguminosas en la rotación agrícola aportó una ventaja significativa en la acumulación de N en el suelo a través de la fijación biológica. Sin embargo, esta ventaja se vio limitada por factores como la competencia de especies invasoras, como *Cynodon dactylon*, que posiblemente incrementaron la relación C:N de los residuos, lo que promovió la inmovilización del N y redujo su disponibilidad para el cultivo sucesor (Dang et al., 2020). Estas pasturas, en su etapa final, presentaban una proporción muy baja de leguminosas, lo que resultó en una entrada de N prácticamente insignificante al sistema durante el último año de la pastura. Sin embargo, se observó que la proporción de N fijado tendió a incrementarse a medida que la pastura envejecía, debido al aumento relativo de gramíneas y malezas en su composición. Este incremento en la fijación de N ocurre porque las gramíneas suelen ser más eficientes que las leguminosas en la competencia por el N disponible en el suelo (Guinet et al., 2018; Schipanski y Drinkwater, 2012). Además, el efecto del pisoteo por el ganado puede haber deteriorado las propiedades físicas del suelo (Barreto et al., 2022), al afectar el crecimiento del cultivo sucesor a la pastura y la dinámica del N en el suelo, así como su eficiencia de uso (Cotrufo y Lavallee, 2022; Mosier et al., 2021;).

Por otro lado, en el sistema AC, aunque dependió más del N aplicado mediante fertilización, se observó una mayor eficiencia de uso del fertilizante (eficiencia agronómica y eficiencia de recuperación) debido a la menor competencia por N del suelo (menor inmovilización) y una mineralización neta más constante en ausencia de pasturas. Este efecto pudo haber sido promovido por una mayor demanda de N por el cultivo que se acompañó de una mayor actividad de las raíces (rizodeposición), que estimula la descomposición microbiana de la MOS y la liberación de nutrientes solubles a través del llamado *efecto de priming* en la materia orgánica asociada a la fracción mineral (MOAM) o *priming* por N (Fontaine et al., 2023). Sin embargo, la dependencia de N externo y la ausencia de leguminosas en la rotación podrían limitar la sostenibilidad del sistema a largo plazo debido a la disminución potencial de la materia orgánica del suelo (Pravia et al., 2019; Rubio et al., 2022).

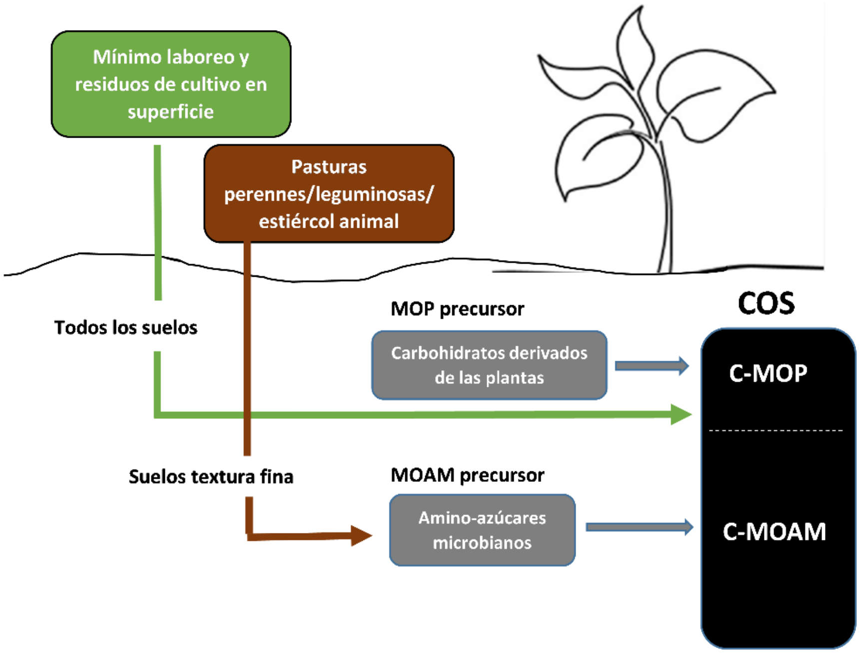
En relación con el desempeño del trigo, en términos de rendimiento de grano (RG) y de concentración de proteína en grano (PG), el sistema AC mostró rendimientos más altos de manera consistente, probablemente debido a condiciones físicas del suelo más favorables y una sincronización más efectiva entre la oferta y la demanda de N (Allen et al., 2021). Sin embargo, la calidad del grano (PG) fue mayor en el sistema CP, lo que sugiere una compensación inherente entre rendimiento y calidad bajo diferentes niveles de disponibilidad de N (Ghimire et al., 2021). En particular, en el sistema CP, se observó una correlación negativa más pronunciada entre RG y PG, probablemente debido a una liberación tardía de N desde los residuos de pastura y limitaciones físicas del suelo que restringieron la capacidad de absorción del cultivo durante las etapas críticas de llenado de grano (Giordano et al., 2023).

Las diferencias en los índices de eficiencia de uso del nitrógeno (EUN) entre sistemas también reflejan la dinámica de los procesos de inmovilización y liberación de N en el suelo. En CP, la inmovilización del N en el suelo fue más alta, lo que explica los valores generalmente menores de eficiencia agronómica (EA) y la mayor variabilidad observada en respuesta a las dosis de fertilización (Liang et al., 2023). En contraste, en AC, la menor inmovilización y la mayor dependencia de N fertilizante resultaron en índices EUN más consistentes, aunque estos índices pueden también incluir errores de sobreestimación respecto de la contribución real del fertilizante al rendimiento debido al uso del N nativo del suelo (Fontaine et al., 2023). De acuerdo con Fontaine et al. (2023), las diferencias observadas entre CP y AC podrían entenderse a través de modelo conceptual basado en sinergias y compensaciones que regulan el funcionamiento de los agroecosistemas. Según estos autores, los suelos con una acumulación considerable de MOS, como ocurre en los sistemas CP, pueden mostrar una disminución en la disponibilidad de N para las plantas, lo que genera un entorno limitante para su productividad. Esto se debe a la alta acumulación de C y otros nutrientes (rizodeposición), la cual es impulsada por la defoliación a través del pastoreo (Xu et al., 2024) y por una alta asimilación microbiana de N, lo que conlleva una respuesta limitada a la fertilización nitrogenada debido a la baja demanda de las plantas en estas condiciones (Cotrufo y Lavallee, 2022). Por el contrario, se espera que los sistemas AC presenten una menor inmovilización microbiana de N en el suelo y una mayor degradación de la MOS, particularmente de la fracción asociada a los minerales (MOAM) (Cotrufo y Lavallee, 2022). Esto sugiere que la inmovilización de N sería mayor en los sistemas CP, lo que explica la menor eficiencia agronómica en CP en comparación con AC. En estos sistemas, la regulación de la actividad microbiana, así como la composición de las entradas de C y de otros nutrientes que entran al sistema a través de la descomposición de residuos de cultivo/pasturas, estiércol animal, también influirán en cómo se distribuye la MOS entre las fracciones MOP y MOAM (Samson et al., 2020; Cotrufo y Lavallee, 2022). En este sentido, la incorporación de estiércol animal, junto con las leguminosas y su rizodeposición en CP, aporta compuestos orgánicos simples, solubles y ricos en N en cantidades considerables. Esta incorporación modifica significativamente la dinámica de descomposición de los residuos (McDaniel et al., 2016), lo que favorece la formación de MOAM pero afecta la acumulación de POM, especialmente en suelos de textura fina, donde la interacción órgano-mineral es más intensa (Cotrufo y Lavallee, 2022; Mosier et al., 2021; Stanley et al., 2024). Asimismo en estos sistemas, la intensidad de remoción forraje por pastoreo y el pisoteo animal pueden obstaculizar la formación de MOP, una situación que se vuelve aún más crítica ante eventos climáticos extremos como la sequía (Xu et al., 2024). Por otro lado, los residuos de cultivos con una alta proporción de componentes de C estructural, como la lignina, implican un mayor costo energético para su descomposición. Esto se traduce en una menor eficiencia de uso de ese C y, por ende, en una menor transferencia de C desde los residuos vegetales hacia

MOAM, por lo cual en sistemas AC podría incrementar proporcionalmente más la POM (figura 1), particularmente en los primeros centímetros del suelo (Osborne et al., 2014; Salvo et al., 2010). Esta situación podría aplicarse al sistema AC, donde la cantidad de biomasa residual proveniente de especies C4 con una alta relación C:N y sin remoción de residuos como en CP por pastoreo sería mayor en AC que en CP. Trabajos previos han subrayado el papel fundamental de la MOP en la formación y estabilidad de los agregados del suelo, lo cual contribuye a mejorar su estructura y funcionalidad (Cotrufo y Lavallee, 2022; Osborne et al., 2014; Six et al., 1998).

Figura 1

Esquema gráfico de la influencia del manejo en las fracciones de carbono orgánico del suelo según la textura (0-10 cm).



Fuente: tomado y adaptado de Samson et al. (2020).

En síntesis, los sistemas CP que combinan una fase de secuestro de C y otros nutrientes en el suelo, como la fase de pastura, con una fase agrícola donde ocurre la descomposición de la MOS han demostrado un desempeño comparable con sistemas de agricultura continua que incluyen especies C4, una fase agrícola de intensificación moderada (número de cultivos por año en la fase agrícola) y períodos de descanso del suelo, pero protegidos con residuos de cultivos con alta relación C:N, como el sorgo en este caso (capítulo 1, tabla 1). En los sistemas CP, suele observarse un incremento en la fertilidad del suelo y en la productividad de los cultivos (Alvarez y Ernst, 2024; Ernst et al., 2018; Pravia et al., 2019; Rubio et al., 2022). Sin embargo, para alcanzar estos beneficios, es fundamental ajustar el manejo durante la fase de pastura para favorecer la formación de ambas fracciones de la MOS, lo que mejora las propiedades físicas del suelo y optimiza la sincronización entre la oferta y la demanda de nutrientes para la pastura, los cultivos posteriores y el microbioma del suelo. Estudios previos (Gosling et al., 2013; Li et al., 2020) demuestran que la retención de residuos es el principal factor que determina la formación de MOS, particularmente la fracción correspondiente a la MOP (Gosling et al., 2013) y su impacto en la estabilidad de los agregados y, por ende, en otras propiedades inferidas (escurrimiento, permeabilidad, aireación y profundidad efectiva, entre otras). Asimismo es esencial lograr una adecuada sincronización entre el suministro y la demanda de nutrientes, tanto en el tiempo como en el espacio. Por lo tanto, es necesario desarrollar estrategias de manejo específicas para los sistemas AC y CP que optimicen esta sincronización. Esto no solo permitirá un uso más eficiente de los nutrientes, sino que también potenciará los servicios ecosistémicos proporcionados por el suelo, como el secuestro de C atmosférico y la mitigación del cambio climático.

5.1.2. Cultivos de servicio: importancia en la dinámica del ciclo de N y de P

El segundo capítulo de esta tesis aborda la diversificación de los sistemas agrícolas a través del empleo de cultivos de servicio o CC. Estos cultivos se presentan como una herramienta clave para optimizar los beneficios agroecológicos y agronómicos derivados de su implementación, como la mejora en la dinámica de nutrientes, el control de malezas y la sostenibilidad del sistema agrícola. La efectividad de los CC en la provisión de servicios depende en gran medida de sus características químicas y bioquímicas, las cuales controlan la mineralización del N y la persistencia de los residuos en el suelo. Los cultivos de leguminosas (como trébol alejandrino y lupino) mostraron mayores niveles de carbohidratos solubles y menor contenido de celulosa, lo que favoreció la mineralización neta de N, en contraste con gramíneas como la avena, que promovieron la inmovilización del N debido a su alta relación C:N. Estos efectos fueron consistentes con las observaciones experimentales, donde los cultivos predecesores con mayor biomasa, menor relación C:N y residuos ricos en N (particularmente en el sitio con suelos de textura más gruesa y de menor fertilidad natural) contribuyeron a una mayor disponibilidad de N para los cultivos sucesores. Estos resultados coinciden con los obtenidos por Carciochi et al. (2023), quienes también observaron que los efectos del cultivo de servicio —en su caso, la vicia— como cultivo predecesor del maíz fueron siempre más pronunciados en ambientes con menor potencial de rendimiento.

Los CC leguminosos ofrecieron ventajas significativas en términos de rendimiento y absorción de N por parte del maíz, atribuidas a la calidad de los residuos ricos en N. En contraste, las gramíneas (avena) y mezclas con alta proporción de avena estimularon una inmovilización neta de N disminuyendo su disponibilidad, lo que afectó negativamente el rendimiento del maíz sin aplicación de fertilizante nitrogenado. Sin embargo, en maíz fertilizado, las diferencias en rendimiento se minimizaron. Se destacó que la sincronización entre la liberación de N de los CC y las etapas de mayor demanda de N del maíz (por ejemplo, entre los estados fenológicos V6 y V10) es crucial para optimizar la eficiencia en el uso de N. La avena demostró ser más efectiva en el control temprano de malezas, mientras que los CC de leguminosas como el trébol alejandrino tuvieron una menor capacidad de supresión de malezas, lo que podría explicar pérdidas de N hacia las malezas en cultivos de maíz sembrados sobre este CC. Nuestros resultados coincidieron con los hallazgos de Cafaro La Menza y Carciochi (2023), quienes analizaron datos desde 52 trabajos de investigación en la región pampeana argentina encontraron que las gramíneas, excepto el pasto raigrás, acumularon más materia seca y C, lo que las hace muy aporpiados para proteger el suelo y mejorar los balances de C. Por su parte, la vicia destacó por su alto contenido de nutrientes y baja relación C:nutriente, lo que la hace adecuada para reciclar nutrientes y aportar N. Las mezclas mostraron un equilibrio entre estos beneficios, lo que resalta la importancia de seleccionar especies de CC según los servicios ecosistémicos deseados.

Las diferencias entre los suelos (textura, disponibilidad de nutrientes) de los sitios experimentales y el efecto año (régimen hídrico) influenciaron marcadamente la producción de biomasa de los CC y su efecto residual en el suelo. Por ejemplo, condiciones más secas y con mayor disponibilidad de K promovieron mayor concentración de lignina en los residuos, lo que puede ralentizar la descomposición y limitar la liberación de N, aunque también podrían haber mejorado otros aspectos no evaluados específicamente en nuestro estudio, como la mejora en la retención del agua del suelo. El efecto adicional de los CC más allá del aporte o inmovilización de N fue evaluado con la metodología de Carciochi et al. (2023). Estos investigadores propusieron una procedimiento de cálculo novedoso para estimar el impacto de los CC al cultivo subsiguiente discriminando el aporte nutricional de N de otros factores, identificándolo como *efecto sin N*. Este concepto hace referencia al efecto de los CC sobre otras propiedades del suelo que influyen en el crecimiento y desarrollo del cultivo, tales como la disponibilidad de agua y cambios en las propiedades físicas y biológicas del suelo. Los análisis de este parámetro mostraron que, en el sitio con menor fertilidad y problemas de malezas (sitio 1), la avena se destacó como el CC más adecuado para las condiciones y desafíos agroecológicos de ese entorno. Sin embargo, su impacto positivo no logró superar el rendimiento del maíz fertilizado en la parcela sin CC (anexo 1). Esto sugiere que una inmovilización temporal de N causada por la avena pudo haber limitado la disponibilidad de N para el maíz durante las etapas iniciales de crecimiento, lo que afectó su vigor y rendimiento. En este sitio, el efecto más significativo asociado al uso de CC sobre la disponibilidad de N se observó en el maíz cultivado tras trébol alejandrino. Por otro lado, en el segundo sitio-año, el efecto sin N fue aún más pronunciado, destacándose los CC de lupino, ya sea en monocultivo o en mezcla con avena. Esto podría atribuirse a un incremento en la disponibilidad de P en el suelo, especialmente bajo las condiciones de sequía extrema presentes en ese año, lo cual pudo haber mejorado la eficiencia de uso de N tanto del derivado del suelo como la de los fertilizantes. Los resultados destacan la relevancia de elegir cuidadosamente los CC según las necesidades específicas del sistema agrícola (por ejemplo, provisión de N, control de malezas, conservación de agua).

El impacto del lupino en la disponibilidad y ciclaje de P en el suelo, como se vislumbra de diversos estudios (Hallama et al., 2019; Tiecher et al., 2012, 2020), fue investigado y se detalla en el capítulo 3 de esta tesis. Este estudio analiza cómo las especies vegetales y las características del suelo afectan la movilización de P y la absorción de nutrientes, además de explorar las interacciones específicas entre plantas y suelos. Los resultados destacan que las plantas pueden influir significativamente en la disponibilidad de P en la rizósfera. Al final del ciclo, los suelos cultivados con lupino (*Lupinus* spp.) no mostraron cambios agronómicamente relevantes en la concentración de P disponible (PBray1) en comparación con las condiciones iniciales pero si a nivel de la cantidad de P absorbido. Este resultado confirma que los lupinos poseen mecanismos para movilizar formas de P orgánico o solubilizar P residual no disponible, lo que mantiene un equilibrio en la disponibilidad del nutriente sin agotarlo. Este comportamiento contrasta con el observado en la avena, donde la disponibilidad de P disminuyó debido a una absorción más intensiva. Aunque, durante el crecimiento, *L. albus* absorbió cantidades de P comparables o superiores a las de la avena, esta mayor absorción no se tradujo en una disminución de PBray1 en el suelo al final del experimento. Más bien, estas diferencias están asociadas a un efecto de rizósfera característico de *L. albus*. Según Neumann et al. (1999), *L. albus* implementa diversos mecanismos para satisfacer sus demandas de P en suelos pobres en este nutriente. Entre ellos, la exudación de ácidos orgánicos como cítrico y málico, que movilizan fuentes de P poco disponibles (por ejemplo, fosfatos de calcio, aluminio y hierro) mediante procesos como la quelación de cationes asociados al P o la competencia por sitios de adsorción en la matriz del suelo (Fink et al., 2016). Sin embargo, el papel de estos carboxilatos no es completamente consistente; investigaciones previas, incluso en *L. albus*, no han identificado una correlación clara entre la concentración de estos compuestos y las características del suelo o de la planta (Veneklaas et al., 2003; Wang y Lambers, 2020).

Los mayores incrementos en PBray1 relacionados con los lupinos se registraron en el sitio 2, donde la disponibilidad inicial de P y la fertilidad general del suelo eran más altas. Este resultado coincide con estudios previos que indican que el lupino es más efectivo en suelos con alta MO y capacidad de intercambio catiónico (CIC) elevada, condiciones que favorecen la mineralización de P orgánico y su solubilización mediante exudados orgánicos (Neumann et al., 1999). Al mismo tiempo, el proceso de FBN en los lupinos contribuyó a la acidificación del suelo, especialmente en *Lupinus albus*, donde se observó una reducción promedio de 0,6 unidades de pH. Esto se atribuye a la exudación de protones en la rizósfera, una estrategia adaptativa que incrementa la solubilidad de fosfatos de calcio y otros compuestos de P unidos a cationes (Pearse et al., 2006). En contraste, en los suelos cultivados con avena, el pH mostró un aumento en la mayoría de los casos. Este fenómeno probablemente se deba a un desequilibrio en la absorción de cationes y aniones, asociado con el predominio del nitrato como fuente principal de N, en lugar del amonio (Wang et al., 2016). Aunque los lupinos demostraron una mayor capacidad para mantener el PBray1 disponible, esta no se tradujo siempre en un aumento proporcional del crecimiento vegetal o la absorción de nutrientes. En sitios con limitaciones severas de P (como el sitio 4), ambos lupinos mostraron una menor biomasa aérea y concentraciones reducidas de P y K en comparación con los otros sitios. Esto coincide con investigaciones que indican que las deficiencias pronunciadas de P limitan el desarrollo de raíces proteoides y la nodulación en leguminosas (Pang et al., 2018). Por otro lado, la avena presentó una mayor biomasa aérea y una absorción eficiente de P en sitios con menor acidificación, posiblemente gracias a su extensa masa radicular y su alta colonización micorrítica, como lo han señalado estudios previos (Wang et al., 2016). La correlación negativa entre el cambio de pH y la concentración de PBray1 (r = -0,61) sugiere que la acidificación contribuyó a la movilización del P en suelos con alta disponibilidad de este nutriente. Asimismo, los contenidos de N y P en las plantas estuvieron positivamente asociados con la disponibilidad de PBray1 y negativamente con el cambio de pH, lo que refuerza el rol de la acidificación en la solubilización de P (Monei et al., 2020).

Los cambios en la disponibilidad de P por efecto del lupino también concuerdan con los observados por Tiecher et al. (2017) en suelos subtropicales fuertemente meteorizados con alta fijación de P en minerales arcillosos y óxidos de hierro. En este estudio se observó un impacto significativo de los CC avena negra y lupino azul (*L. angustifolius*), en un sistema de siembra directa (experimento de largo plazo de veintitrés años de duración), que incrementaron la disponibilidad de P y K en el suelo, lo que se resultó en una mayor capacidad de reciclaje de nutrientes en comparación con otros CC invernales (Tiecher et al., 2017). Este efecto diferencial en el lupino azul se atribuyó a su capacidad para liberar ácidos orgánicos, como citrato, malato y fumarato, incluso en condiciones de alta disponibilidad de P. En cuanto al potasio, la avena negra presentó los niveles más altos de disponibilidad en el perfil del suelo hasta los 20 cm de profundidad en comparación con los demás cultivos de invierno. La elevada disponibilidad de nutrientes observada en avena negra se relacionó con su destacada producción de biomasa con respecto a los demás tratamientos de cobertura invernal. Esta biomasa, al ser depositada en la superficie del suelo, contribuye al reciclaje de nutrientes a través de su descomposición gradual y mejora la fertilidad de las capas superficiales del suelo. En el caso del lupino azul, su capacidad de movilizar P mediante procesos biológicos refuerza su contribución al reciclaje de este nutriente, tras lo cual se posiciona como una opción estratégica para mejorar la fertilidad del suelo. Estos resultados subrayan la importancia del uso de CC como la avena negra y el lupino azul en sistemas agrícolas sostenibles, no solo para incrementar la disponibilidad de P y K, sino también para fortalecer los procesos de ciclado de otros nutrientes a través de mecanismos biológicos y físicos que mejoran la calidad del suelo a largo plazo.

6. Conclusiones y perspectivas

Los resultados de esta tesis destacan la importancia de equilibrar los beneficios y desafíos asociados a los sistemas con y sin integración de pasturas perennes para promover prácticas agrícolas sostenibles. Si bien el sistema CP puede ofrecer ventajas a largo plazo en términos de calidad del suelo y sostenibilidad ambiental, su implementación enfrenta desafíos asociados a la calidad y cantidad de los residuos dejados en superficie y las condiciones físicas del suelo en la terminación de la fase de pastura y el inicio de la fase agrícola y en la sincronización entre suministro y demanda de N. Por el contrario, el sistema AC presenta una mayor consistencia en los rendimientos, pero depende en gran medida de insumos externos de N y podría ser insostenible en el largo plazo. A partir de estos hallazgos, surge la necesidad de desarrollar estrategias integradas que optimicen ambos enfoques. Estas estrategias podrían incluir la diversificación de cultivos mediante la incorporación de leguminosas anuales como el lupino en los sistemas AC y ajustes en las prácticas de manejo de las fases de pastura para maximizar los beneficios de cada sistema y mitigar sus limitaciones.

También es necesario considerar los niveles de C y N del suelo, así como la cantidad y calidad de los residuos, y correlacionarlos con indicadores de salud del suelo. Igualmente importante es utilizar el mejor conocimiento y tecnologías disponibles para reducir el impacto del estrés por nutrientes, plagas y malezas que afectan el establecimiento de los cultivos (por ejemplo, emergencias desiguales, herramientas diagnóstico de deficiencia de nutrientes) en los sistemas agrícolas. La cantidad de residuo generado por los CC depende de características intrínsecas de la especie, aunque también puede ser modulada mediante decisiones de manejo agronómico, como la fecha de siembra, el momento de terminación y la fertilización. En cuanto a la calidad del residuo, esta se puede ajustar a través de la selección de especies específicas o del uso de mezclas en proporciones adecuadas. Además, es posible optimizar la calidad mediante el ajuste de las fechas de terminación de los CC; por ejemplo, en una mezcla, se podría finalizar antes una gramínea para evitar un aumento excesivo de la relación C/N, aunque esto podría implicar un mayor uso de herbicidas. Para reducir el uso de herbicidas, otra opción es ajustar la terminación de las especies mediante diferentes métodos, como el rolado, en lugar de recurrir a productos químicos.

La diversificación de los sistemas de producción agrícola mediante la incorporación CC anuales, especialmente aquellos que incluyen leguminosas, representa una estrategia prometedora para optimizar su sostenibilidad. Sin embargo, la efectividad de los CC depende de factores específicos como las condiciones edafoclimáticas y problemática asociada a un sitio-año espeçifico, la especie de CC y el manejo agronómico. Este enfoque, al combinar beneficios agronómicos y ambientales, refuerza la importancia de integrar los CC en estrategias de diversificación para mejorar el ciclaje de los nutrientes y productividad de los sistemas agrícolas.

Se identifican importantes líneas de investigación futura, como la exploración de los mecanismos subyacentes que explican las diferencias en la respuesta al agregado de N en el rendimiento del trigo entre los sistemas CP y AC. Específicamente, resulta esencial profundizar en los procesos de mineralización e inmovilización y en las posibles variaciones en la comunidad microbiana (relación hongos/bacterias) y su influencia en el ciclo de nutrientes y la dinámica de la MOS. También es relevante analizar factores que alteran dicha comunidad, como cambios en la acidez del suelo y la disponibilidad de nutrientes clave como P y K, particularmente influenciados por la incorporación de estiércol y orina en el sistema CP y por el agregado de fertilizantes sintéticos. Estos factores pueden influir en la formación y distribución de las fracciones de la materia orgánica del suelo (POM y MAOM), así como en su estabilización. Además, tienen un impacto significativo en propiedades físicas esenciales para la conservación del recurso suelo y la sostenibilidad del sistema, como la estabilidad de los agregados y la resistencia a la erosión. En relación con el potencial del lupino como cultivo de servicio (o aún quizás como cultivo de renta), los resultados preliminares sugieren la necesidad de profundizar en este cultivo con datos obtenidos en campo para determinar la robustez de los patrones identificados en la disponibilidad de P en el suelo y analizarlos desde una perspectiva de estabilidad a largo plazo. La posible variación en la disponibilidad de P en el suelo estará influenciada por la interacción y retroalimentación con diversos factores suelo-planta y por los procesos de la actividad microbiana, los cuales podrían dificultar el aumento en la disponibilidad de P facilitado por las especies de lupino. Por ello, es fundamental realizar investigaciones de campo durante al menos dos ciclos de cultivo para confirmar si los efectos beneficiosos del lupino se mantienen con la misma magnitud a lo largo del tiempo y si estas variaciones contribuyen a mejorar la salud del suelo y la producción de cultivos. Entre las perspectivas de investigación más relevantes, se destaca la necesidad de evaluar el impacto del lupino en suelos con alta capacidad de fijación de P y cuantificar no solo los cambios en el P disponible, sino también en los reservorios de P orgánico. Asimismo sería importante medir la actividad de fosfatasa del suelo, ya que constituye un indicador clave para evaluar la eficacia del P absorbido por las plantas y analizar los cambios asociados en las fuentes de P orgánico, como el P inmovilizado en la biomasa microbiana ~~viva~~ del suelo. Estos enfoques proporcionarán una visión más completa del rol del lupino en la sostenibilidad y funcionalidad del recurso suelo.

7. Bibliografía general

Allar, J. y Maltais-Landry, G. (2022). Limited benefits of summer cover crops on nitrogen cycling in organic vegetable production. *Nutrient Cycling in Agroecosystems*, *122*, 119-138.10.1007/s10705-021-10189-8

Alvarez, S. y Ernst, O. (2024). Impact of cropping systems on soil quality. *European Journal of Agronomy, 158*, 127197. 10.1016/j.eja.2024.127197

Ambrosini, V. G., de Almeida, J. L., de Araujo, E. A., Alves, L. A., Filippi, D., Flores, J. P. M., Fostim, M. L., Fontoura, S. M. V., Bortoluzzi, E. C., Bayer, C. y Tiecher, T. (2022). Effect of diversified cropping systems on crop yield, legacy, and budget of potassium in a subtropical Oxisol. *Field Crops Research*, *275*, 108342 10.1016/j.fcr.2021.108342

Arbeletche, P. (2020). El agronegocio en Uruguay: su evolución y estrategias cambiantes en el siglo XXI. *RIVAR*, 7(19), 109-129. 10.35588/rivar.v7i19.4355

Baiyeri, P. K., Foleng, H. N., Machebe, N. S. y Nwobodo, C. E. (2019). Crop-livestock interaction for sustainable agriculture. En M. Farooq y M. Pisante M. (eds), *Innovations in Sustainable Agriculture* (pp. 557-582). Springer. 10.1007/978-3-030-23169-9_18

Baldassini, P., Baethgen, W., Camba Sans, G., Quincke, A., Pravia, V. M., Terra, J., Macedo, I., Piñeiro, G. y Paruelo, J. (2023). Carbon stocks and potential sequestration of Uruguayan soils: a road map to a comprehensive characterization of temporal and spatial changes to assess carbon footprint. *Frontiers in Sustainable Food Systems*, *7*. 10.3389/fsufs.2023.1045734

Barreto, P., Dogliotti, S. y Perdomo, C. (2017). Surface Water Quality of Intensive Farming Areas Within the Santa Lucia River Basin of Uruguay. *Air, Soil and Water Research*, *10*. https://doi.org/10.1177/1178622117715446

Barreto, P., Ernst, O., Pérez Bidegain, M. y Perdomo, C. (2022). Effects of grazing, rotation, and tillage on surface runoff in a heavy textured Uruguayan soil. *Soil Science Society of America Journal*, *86*(4), 1096-1112. 10.1002/saj2.20427

Beretta-Blanco, A., Pérez, O. y Carrasco-Letelier, L. (2019). Soil quality decrease over 13 years of agricultural production. *Nutrient Cycling in Agroecosystems*, *114*, 45-55. 10.1007/s10705-019-09990-3

Berriel, V. y Perdomo, C. H. (2023). Cajanus cajan: a promissory high-nitrogen fixing cover crop for Uruguay. *Frontiers in Agronomy*, *5*. 10.3389/fagro.2023.1214811

Berriel, V., Perdomo, C. y Monza, J. (2020). Carbon Isotope Discrimination and Water-Use Efficiency in *Crotalaria* Cover Crops under Moderate Water Deficit. *Journal of Soil Science and Plant* Nutrition, *20*, 537-545. 10.1007/s42729-019-00142-8

Bolland, M. D. A. y Brennan, R. F. (2008). Comparing the phosphorus requirements of wheat, lupin, and canola. *Australian Journal of Agricultural Research, 59*(11), 983-998. 10.1071/AR07430

Brazeiro, A., Achkar, M., Toranza, C. y Bartesaghi, L. (2020). Agricultural expansion in uruguayan grasslands and priority areas for vertebrate and woody plant conservation. *Ecology and Society*, *25*(1). 10.5751/ES-11360-250115

Cafaro La Menza, F. y Carciochi, W. D. (2023). Catch crops in the Argentinean Pampas: a synthesis-analysis on nutrient characteristics and their implications for a sustainable agriculture. *Frontiers in Agronomy*, *5*. 10.3389/fagro.2023.1244057

Céspedes-Payret, C., Piñeiro, G., Achkar, M., Gutierrez, O. y Panario, D. (2009). The irruption of new agro-industrial technologies in Uruguay and their environmental impacts on soil, water supply and biodiversity: A review. *International Journal of Environmental Health*, *3*(2), 175-197. 10.1504/IJENVH.2009.024877

Cotrufo, M. F. y Lavallee, J. M. (2022). Soil organic matter formation, persistence, and functioning: A synthesis of current understanding to inform its conservation and regeneration. *Advances in Agronomy, 172*, 1-66. 10.1016/bs.agron.2021.11.002

Crews, T. E. y Peoples, M. B. (2005). Can the synchrony of nitrogen supply and crop demand be improved in legume and fertilizer-based agroecosystems? A review. *Nutrient Cycling in Agroecosystems*, *72*, 101-120. 10.1007/s10705-004-6480-1

Dabney, S. M., Delgado, J. a, Collins, F., Meisinger, J. J., Schomberg, H. H., Liebig, M. a, Kaspar, T. y Mitchell, J. (2010). Using cover crops and cropping systems for nitrogen management. En J. A. Delgado y R. F. Follett (eds), *Advances in Nitrogen Management* (pp. 230-281). Soil and Water Conservation Society.

Dang, Y. P., Dalal, R. C. y Menzies, N. W. (2020). *No-till Farming Systems for Sustainable Agriculture: Challenges and Opportunities* (Y. P. Dang, R. C. Dalal y N. W. Menzies, eds.). Springer. 10.1007/978-3-030-46409-7

Del Pino, A., Rodríguez, T. y Andión, J. (2016). Production improvement through phosphorus fertilization and legume introduction in grazed native pastures of Uruguay. *The Journal of Agricultural Science, 154*(2), 347-358. 10.1017/S002185961500101X

Dong, Z., Wu, L., Kettlewell, B., Caldwell, C. D. y Layzell, D. B. (2003). Hydrogen fertilization of soils - Is this a benefit of legumes in rotation? *Plant, Cell & Environment*, *26*(11), 1875-1879. 10.1046/j.1365-3040.2003.01103.x

Ernst, O. R., Dogliotti, S., Cadenazzi, M. y Kemanian, A. R. (2018). Shifting crop-pasture rotations to no-till annual cropping reduces soil quality and wheat yield. *Field Crops Research*, *217*, 180-187. 10.1016/j.fcr.2017.11.014

Ernst, O. R., Kemanian, A. R., Mazzilli, S. R., Cadenazzi, M. y Dogliotti, S. (2016). Depressed attainable wheat yields under continuous annual no-till agriculture suggest declining soil productivity. *Field Crops Research*, *186*, 107-116. 10.1016/j.fcr.2015.11.005

Ernst, O. R., Kemanian, A. R., Mazzilli, S., Siri-Prieto, G. y Dogliotti, S. (2020). The dos and don’ts of no-till continuous cropping: Evidence from wheat yield and nitrogen use efficiency. *Field Crops Research, 257*, 107934. 10.1016/j.fcr.2020.107934

Ernst, O. y Siri-Prieto, G. (2009). Impact of perennial pasture and tillage systems on carbon input and soil quality indicators. *Soil Tillage Research, 105*(2), 260-268. 10.1016/j.still.2009.08.001

Espinoza, S., Ovalle, C., Zagal, E., Matus, I., Tay, J., Peoples, M. B., y Del Pozo, A. (2012). Contribution of legumes to wheat productivity in Mediterranean environments of central Chile. *Field Crops Research*, *133*, 150-159. 10.1016/j.fcr.2012.03.006

Fink, J. R., Inda, A. V., Bavaresco, J., Barrón, V., Torrent, J. y Bayer, C. (2016). Adsorption and desorption of phosphorus in subtropical soils as affected by management system and mineralogy. *Soil Tillage Research*, *155*, 62-68. 10.1016/j.still.2015.07.017

Fontaine, S., Abbadie, L., Aubert, M., Barot, S., Bloor, J. M. G., Derrien, D., Duchene, O., Gross, N., Henneron, L., Le Roux, X., Loeuille, N., Michel, J., Recous, S., Wipf, D., y Alvarez, G. (2023). Plant–soil synchrony in nutrient cycles: Learning from ecosystems to design sustainable agrosystems. *Global Change Biology*, *30*(1), e17034. 10.1111/gcb.17034

García-Préchac, F., Ernst, O., Siri-Prieto, G. y Terra, J. A. (2004). Integrating no-till into crop-pasture rotations in Uruguay. *Soil Tillage Research*, *77*(1), 1-13. 10.1016/j.still.2003.12.002

García-Préchac, F. (2020). Soil management and environmental sustainability of crop-livestock systems. *Agrociencia Uruguay*, *24*(1). 10.31285/agro.24.205

Ghaley, B. B., Porter, J. R. y Sandhu, H. S. (2014). Soil-based ecosystem services: A synthesis of nutrient cycling and carbon sequestration assessment methods. *International Journal of Biodiversity Science, Ecosystem Services & Management*, *10*(3), 177-186. 10.1080/21513732.2014.926990

Ghimire, D., Das, S., Mueller, N. D., Creech, C. F., Santra, D., Baenziger, P. S., Easterly, A. C., Maust, B., y Maharjan, B. (2021). Effects of cultivars and nitrogen management on wheat grain yield and protein. *Agronomy Journal*, *113*(5), 4348-4368. 10.1002/agj2.20836

Giordano, N., Sadras, V. O. y Lollato, R. P. (2023). Late-season nitrogen application increases grain protein concentration and is neutral for yield in wheat. A global meta-analysis. *Field Crops Research*, *290*, 108740. 10.1016/j.fcr.2022.108740

Gosling, P., Parsons, N. y Bending, G. D. (2013). What are the primary factors controlling the light fraction and particulate soil organic matter content of agricultural soils? *Biology and Fertility of Soils*, *49*, 1001-1014. 10.1007/s00374-013-0791-9

Grahmann, K., Rubio Dellepiane, V., Terra, J. A. y Quincke, J. A. (2020). Long-term observations in contrasting crop-pasture rotations over half a century: Statistical analysis of chemical soil properties and implications for soil sampling frequency. *Agriculture, Ecosystems & Environment, 287*, 106710. 10.1016/j.agee.2019.106710

Guinet, M., Nicolardot, B., Revellin, C., Durey, V., Carlsson, G. y Voisin, A. S. (2018). Comparative effect of inorganic N on plant growth and N2 fixation of ten legume crops: towards a better understanding of the differential response among species. *Plant and Soil*, *432*, 207-227. 10.1007/s11104-018-3788-1

Hallama, M., Pekrun, C., Lambers, H. y Kandeler, E. (2019). Hidden miners – the roles of cover crops and soil microorganisms in phosphorus cycling through agroecosystems. *Plant and Soil*, *434*, 7-45. 10.1007/s11104-018-3810-7

Hendrickson, J. R. (2020). Crop-livestock integrated systems for more sustainable agricultural production: A review. *CAB Reviews, 15*(12). 10.1079/PAVSNNR202015012

Hernández, J., Otegui, O., Zalmalvide, J. P. y Zamalvide, J. P. (1995). Formas y contenidos de fósforo en algunos suelos del Uruguay. *Facultad de Agronomía*, (43).

Herridge, D. F., Giller, K. E., Jensen, E. S. y Peoples, M. B. (2022). Quantifying country-to-global scale nitrogen fixation for grain legumes II. Coefficients, templates and estimates for soybean, groundnut and pulses. *Plant and Soil*, *474*, 1-15. 10.1007/s11104-021-05166-7

Hocking, P. J. (2001). Organic acids exuded from roots in phosphorus uptake and aluminum tolerance of plants in acid soils. *Advances in Agronomy, 74*, 63-97. 10.1016/s0065-2113(01)74031-x

Hu, S., Qiao, B., Yang, Y., Rees, R. M., Huang, W., Zou, J., Zhang, L., Zheng, H., Liu, S., Shen, S., Chen, F., y Yin, X. (2023). Optimizing nitrogen rates for synergistically achieving high yield and high nitrogen use efficiency with low environmental risks in wheat production – Evidences from a long-term experiment in the North China Plain. *European Journal of Agronomy*, *142*, 126681. 10.1016/j.eja.2022.126681

Hufnagel, J., Reckling, M. y Ewert, F. (2020). Diverse approaches to crop diversification in agricultural research. A review. *Agronomy for Sustainable Development*, *40*. 10.1007/s13593-020-00617-4

Iglesias Rossini, G. F. (2022). Soil and subsoil conservation from an environmental perspective. *Revista de Derecho*, *21*(42), 41-52. 10.47274/DERUM/42.3

Kamh, M., Abdou, M., Chude, V., Wiesler, F. y Horst, W. J. (2002). Mobilization of phosphorus contributes to positive rotational effects of leguminous cover crops on maize grown on soils from northern Nigeria. *Journal of Plant Nutrition and Soil Science*, *165*(5), 566-572. 10.1002/1522-2624(200210)165:5<566::AID-JPLN566>3.0.CO;2-O

Koritschoner, J. J., Whitworth Hulse, J. I., Cuchietti, A. y Arrieta, E. M. (2023). Spatial patterns of nutrients balance of major crops in Argentina. *Science of the Total Environment*, *858*, 159863. 10.1016/j.scitotenv.2022.159863

Lambers, H., Clements, J. C. y Nelson, M. N. (2013). How aphosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (*Lupinus*, Fabaceae). *American Journal of Botany*, *100*(2), 263-288. 10.3732/ajb.1200474

Lambers, H., Shane, M. W., Cramer, M. D., Pearse, S. J. y Veneklaas, E. J. (2006). Root structure and functioning for efficient acquisition of phosphorus: Matching morphological and physiological traits. *Annals of Botany*, *98*(4), 693-713. 10.1093/aob/mcl114

Li, G., Yu, C., Shen, P., Hou, Y., Ren, Z., Li, N., Liao, Y., Li, T., y Wen, X. (2024). Crop diversification promotes soil aggregation and carbon accumulation in global agroecosystems: A meta-analysis. *Journal of Environmental Management*, *350*, 119661. 10.1016/j.jenvman.2023.119661

Li, Y., Li, Z., Chang, S. X., Cui, S., Jagadamma, S., Zhang, Q., y Cai, Y. (2020). Residue retention promotes soil carbon accumulation in minimum tillage systems: Implications for conservation agriculture. *Science of the Total Environment*, *740*, 140147. 10.1016/j.scitotenv.2020.140147

Liang, Z., Rasmussen, J., Poeplau, C. y Elsgaard, L. (2023). Priming effects decrease with the quantity of cover crop residues – Potential implications for soil carbon sequestration. *Soil Biology and Biochemistry,* 184, 109110. 10.1016/j.soilbio.2023.109110

Lichtenberg, E. M., Kennedy, C. M., Kremen, C., Batáry, P., Berendse, F., Bommarco, R., Bosque-Pérez, N. A., Carvalheiro, L. G., Snyder, W. E., Williams, N. M., Winfree, R., Klatt, B. K., Åström, S., Benjamin, F., Brittain, C., Chaplin-Kramer, R., Clough, Y., Danforth, B., Diekötter, T., … Crowder, D. W. (2017). A global synthesis of the effects of diversified farming systems on arthropod diversity within fields and across agricultural landscapes. *Global Change Biology*, *23*(11), 4946-4957. 10.1111/gcb.13714

Liebman, M., Helmers, M. J., Schulte, L. A. y Chase, C. A. (2013). Using biodiversity to link agricultural productivity with environmental quality: Results from three field experiments in Iowa. *Renewable Agriculture and Food Systems*, *28*(2), 115-128. 10.1017/S1742170512000300

Lorenz, K., Lal, R. y Ehlers, K. (2019). Soil organic carbon stock as an indicator for monitoring land and soil degradation in relation to United Nations' Sustainable Development Goals. *Land Degradation & Development, 30*(7), 824-838. 10.1002/ldr.3270

Mazzilli, S. R. y Ernst, O. R. (2019). Rapeseed-to-Wheat Yield Ratio in Different Production Environments and Effects on Subsequent Summer Crops Yields. *Agrosystems, Geosciences & Environment*, *2*(1), 1-7. 10.2134/age2019.03.0017

Mcdaniel, M. D., Grandy, A. S., Tiemann, L. K., y Weintraub, M. N. (2016). Eleven years of crop diversification alters decomposition dynamics of litter mixtures incubated with soil. *Ecosphere,* 7(8). 10.1002/ecs2.1426

Mosier, S., Apfelbaum, S., Byck, P., Calderon, F., Teague, R., Thompson, R., y Cotrufo, M. F. (2021). Adaptive multi-paddock grazing enhances soil carbon and nitrogen stocks and stabilization through mineral association in southeastern U.S. grazing lands. *Journal of Environmental Management*, *288*, 112409. 10.1016/j.jenvman.2021.112409

Neumann, G., Massonneau, A., Martinoia, E. y Römheld, V. (1999). Physiological adaptations to phosphorus deficiency during proteoid root development in white lupin. *Planta*, *208*, 373-382. 10.1007/s004250050572

Nuruzzaman, M., Lambers, H., Bolland, M. D. A. y Veneklaas, E. J. (2005). Phosphorus uptake by grain legumes and subsequently grown wheat at different levels of residual phosphorus fertiliser. *Australian Journal of Agricultural Research, 56*(10), 1041-1047. 10.1071/AR05060

Osborne, S. L., Johnson, J. M. F., Jin, V. L., Hammerbeck, A. L., Varvel, G. E. y Schumacher, T. E. (2014). The Impact of Corn Residue Removal on Soil Aggregates and Particulate Organic Matter. *Bioenergy Research*, *7,* 559-567. 10.1007/s12155-014-9413-0

Ovalle, C., Del Pozo, A., Peoples, M. B. y Lavín, A. (2010). Estimating the contribution of nitrogen from legume cover crops to the nitrogen nutrition of grapevines using a 15N dilution technique. *Plant and Soil,* 334(1), 247-259. 10.1007/s11104-010-0379-1

Pang, J., Bansal, R., Zhao, H., Bohuon, E., Lambers, H., Ryan, M. H., Ranathunge, K., y Siddique, K. H. M. (2018). The carboxylate-releasing phosphorus-mobilizing strategy can be proxied by foliar manganese concentration in a large set of chickpea germplasm under low phosphorus supply. *New Phytologist, 219*(2), 518-529. 10.1111/nph.15200

Parihar, C. M., Singh, A. K., Jat, S. L., Dey, A., Nayak, H. S., Mandal, B. N., Saharawat, Y. S., Jat, M. L., y Yadav, O. P. (2020). Soil quality and carbon sequestration under conservation agriculture with balanced nutrition in intensive cereal-based system. *Soil and Tillage Research*, *202*, 104653. 10.1016/j.still.2020.104653

Paruelo, J. M., Camba Sans, G., Gallego, F., Baldassini, P., Staiano, L., Baeza, S., y Dieguez, H. (2024). A comprehensive analysis of the environmental performance of the Uruguayan agricultural sector. *Ecological Indicators*, *166*, 112385. 10.1016/j.ecolind.2024.112385

Pearse, S. J., Veneklaas, E. J., Cawthray, G. R., Bolland, M. D. A. y Lambers, H. (2006). Carboxylate release of wheat, canola and 11 grain legume species as affected by phosphorus status. *Plant and Soil*, *288*, 127-139. 10.1007/s11104-006-9099-y

Peoples, M. B., Hauggaard-Nielsen, H. y Jensen, E. S. (2015). The potential environmental benefits and risks derived from legumes in rotations. En D. W. Emerich y H. B. Krishnan (eds.), *Nitrogen Fixation in Crop Production* (pp. 349-385). American Society of Agronomy. 10.2134/agronmonogr52.c13

Peterson, C. A., Deiss, L. y Gaudin, A. C. M. (2020). Commercial integrated crop-livestock systems achieve comparable crop yields to specialized production systems: A meta-analysis. *PLoS One*, *15*(5), e0231840. 10.1371/journal.pone.0231840

Pinto, P., Rubio, G., Gutiérrez, F., Sawchik, J., Arana, S. y Piñeiro, G. (2021). Variable root:shoot ratios and plant nitrogen concentrations discourage using just aboveground biomass to select legume service crops. *Plant and Soil*, *463*, 347-358. 10.1007/s11104-021-04916-x

Pravia, M. V., Kemanian, A. R., Terra, J. A., Shi, Y., Macedo, I. y Goslee, S. (2019). Soil carbon saturation, productivity, and carbon and nitrogen cycling in crop-pasture rotations. *Agricultural Systems*, *171*, 13-22. 10.1016/j.agsy.2018.11.001

Pypers, P., Huybrighs, M., Diels, J., Abaidoo, R., Smolders, E. y Merckx, R. (2007). Does the enhanced P acquisition by maize following legumes in a rotation result from improved soil P availability? *Soil Biology and Biochemistry*, 39(10), 2555-2566. 10.1016/j.soilbio.2007.04.026

Reiss, E. R. y Drinkwater, L. E. (2022). Promoting enhanced ecosystem services from cover crops using intra- and interspecific diversity. *Agriculture, Ecosystems & Environment*, *323*, 107586. 10.1016/j.agee.2021.107586

Rodriguez, M. P., Carcedo, A. J. P., Correndo, A. A., Crespo, C., Carciochi, W. D., Sainz Rozas, H. R., Ciampitti, I. A., y Barbieri, P. A. (2024). Assessing the nitrogen supply of hairy vetch in a soybean-wheat sequence. *Field Crops Research, 316,* 109496. 10.1016/j.fcr.2024.109496

Rubio, V., Diaz-Rossello, R., Quincke, J. A. y van Es, H. M. (2021). Quantifying soil organic carbon's critical role in cereal productivity losses under annualized crop rotations. *Agriculture, Ecosystems & Environment, 321,* 107607. 10.1016/j.agee.2021.107607

Rubio, V., Núñez, A., Berger, A. y van Es, H. (2025). Biomass inputs drive agronomic management impacts on soil health. *Agriculture, Ecosystems & Environment, 378*. 10.1016/j.agee.2024.109316

Rubio, V., Sawchik, J. y van Es, H. (2022). Soil health benefits from sequence intensification, fertilization, and no-tillage in annual cropping systems. *Soil Secururity*, *9*, 100074. 10.1016/j.soisec.2022.100074

Samson, M. E., Chantigny, M. H., Vanasse, A., Menasseri-Aubry, S., Royer, I. Angers, D. A. (2020). Management practices differently affect particulate and mineral-associated organic matter and their precursors in arable soils. *Soil Biology and Biochemistry*, *148*, 107867. 10.1016/j.soilbio.2020.107867

Schipanski, M. E. y Drinkwater, L. E. (2012). Nitrogen fixation in annual and perennial legume-grass mixtures across a fertility gradient. *Plant and Soil*, *357,* 147-159. 10.1007/s11104-012-1137-3

Silveira, D., Hernández, J., Cadenazzi, M., Del Pino, A., Zanoniani, R. y Boggiano, P. (2015). Efecto de la fertilización fosfatada sobre la composición química de Lotus tenuis y Trifolium repens. *Agrociencia, 19*(2), 49-58. 10.31285/agro.19.301

Six, J., Elliott, E. T., Paustian, K. y Doran, J. W. (1998). Aggregation and Soil Organic Matter Accumulation in Cultivated and Native Grassland Soils. *Soil Science Society of America Journal*, *62*(5), 1367-1377. 10.2136/sssaj1998.03615995006200050032x

Stanley, P. L., Wilson, C., Patterson, E., Machmuller, M. y Cotrufo, M. F. (2024). Ruminating on soil carbon: Applying current understanding to inform grazing management. *Global Change Biology*, *30*(3), e17223. 10.1111/gcb.17223

Takahashi, T. K. S. (2015). Introduction of various cover crop species to improve soil biological P parameters and P uptake of the following crops. *Nutrient Cycling in Agroecosystems, 103*, 15-28. https://doi.org/10.1007/s10705-015-9715-4

Tiecher, T., Calegari, A., Caner, L. y Rheinheimer, D. dos S. (2017). Soil fertility and nutrient budget after 23-years of different soil tillage systems and winter cover crops in a subtropical Oxisol. *Geoderma, 308,* 78-85. 10.1016/j.geoderma.2017.08.028

Tiecher, T., Dos Santos, D. R. y Calegari, A. (2012). Soil organic phosphorus forms under different soil management systems and winter crops, in a long term experiment. *Soil Tillage Research, 124*, 57-67. 10.1016/j.still.2012.05.001

Tiecher, T., Gubiani, E., Santanna, M. A., Veloso, M. G., Calegari, A., Canalli, L. B. dos S., Finckh, M. R., Caner, L., y Rheinheimer, D. dos S. (2020). Effect of 26-years of soil tillage systems and winter cover crops on C and N stocks in a Southern Brazilian Oxisol. *Revista Brasileira de Ciência do Solo*, *44*, e0200029. 10.36783/18069657rbcs20200029

Vanino, S., Di Bene, C., Piccini, C., Fila, G., Pennelli, B., Zornoza, R., Sanchez-Navarro, V., Álvaro-Fuentes, J., Hüppi, R., Six, J., y Farina, R. (2022). A comprehensive assessment of diversified cropping systems on agro-environmental sustainability in three Mediterranean long-term field experiments. *European Journal of Agronomy, 140*, 126598. 10.1016/j.eja.2022.126598

Vassallo, M. (2013). Dinámica y competencia intrasectorial en la agricultura uruguaya: los cambios en la última década. *Agrociencia Uruguay*, *17*(2), 170-179. 10.31285/agro.17.488

Veneklaas, E. J., Stevens, J., Cawthray, G. R., Turner, S., Grigg, A. M. y Lambers, H. (2003). Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. *Plant and Soil*, *248*, 187-197. 10.1023/A:1022367312851

Wang, Y., Krogstad, T., Clarke, J. L., Hallama, M., Øgaard, A. F., Eich-Greatorex, S., Kandeler, E., y Clarke, N. (2016). Rhizosphere organic anions play a minor role in improving crop species' ability to take up residual phosphorus (P) in agricultural soils low in P availability. *Frontiers in Plant Science*, *7*. 10.3389/fpls.2016.01664

Wang, Y. y Lambers, H. (2020). Root-released organic anions in response to low phosphorus availability: recent progress, challenges and future perspectives. *Plant and Soil*, *447*, 135-156. 10.1007/s11104-019-03972-8

Xie, H., Huang, Y., Chen, Q., Zhang, Y. y Wu, Q. (2019). Prospects for agricultural sustainable intensification: A review of research. *Land*, *8*(11), 157. 10.3390/land8110157

Xu, T., Johnson, D., y Bardgett, R. D. (2024). Defoliation modifies the impact of drought on the transfer of recent plant-assimilated carbon to soil and arbuscular mycorrhizal fungi. Plant and Soil. https://doi.org/10.1007/s11104-024-06762-z

8. Anexos

Anexo 1. Incremento en de rendimiento de grano (RG) en maíz (kg ha-1) por efecto de los cultivos de servicio (CS) o por la fertilización N (100 kg N ha^{-1}).

Sitio	Especie	1. Respuesta RG maíz _CS	2. Respuesta RG maíz a N_ desp. CC	3. Respuesta RG maíz a CS no rel. a N	4. Efecto CS rel. a N (1-3)
		kg grano maíz ha^{-1}			
1	Alejandrino	897 ± 156^{a}	-857 ± 1209^{b}	-1566 ± 1687	2463 ± 1650^{a}
	Avena	-2757 ± 337^{b}	3085 ± 380^{a}	-1277 ± 488	-1479 ± 166^{b}
	Lupino	-3105 ± 1379^{b}	2445 ± 912^{ab}	-2266 ± 1350	-839 ± 474^{b}
	Mezcla	-686 ± 988^{ab}	268 ± 1395^{b}	-2024 ± 1373	1338 ± 1250^{ab}
Efecto del tratamiento		*P*-valor			
	Especie	0,0367	0,0802	n. s.	0,0910
2	Alejandrino	146 ± 671	1440 ± 603	708 ± 1758	-563 ± 1237
	Avena	-547 ± 560	2395 ± 1047	970 ± 1188	-1517 ± 1687
	Lupino	745 ± 274	2250 ±1530	2117 ± 1047	-1373 ± 1213
	Mezcla	935 ± 1548	1526 ± 334	1584 ± 1025	-649 ± 787
Efecto del tratamiento		*P*-valor			
	Especie	n. s.	n. s.	n. s.	n. s.

Letras minúsculas diferentes dentro de una columna indican diferencias entre los cultivos de servicio dentro de cada sitio, que fueron significativas en p de 0,1; n. s: significa que no hay diferencias significativas.

Parámetros para estimar el aporte de los CC según Carciochi et al. (2023)

1: Respuesta RG maíz_CS=RG en trat. CS – RG en trat, control (Sin CS)

2: Respuesta RG maíz a N_después de CC= RG en trat CS_$_{\text{Fert}}$ - RG en trat CS_$_{\text{NOfert}}$

3: Respuesta RG maíz a CS_ no relacionado a N= RG en trat CS_$_{\text{Fert}}$ – RG trat. Control (Sin CS)_$_{\text{fert}}$

4: Efecto CS relacionado a N: La diferencia entre los parámetros 1-3