Aplicación de efluentes de tambo: una perspectiva microbiológica sobre fertilidad de suelo e impacto ambiental

Gabriela Illarze Dive

Ciencias Agrarias

Opción Ciencias Vegetales

Julio, 2024

Aplicación de efluentes de tambo: una perspectiva microbiológica sobre fertilidad de suelo e impacto ambiental

Gabriela Illarze Dive

Doctora en Ciencias Agrarias

Opción Ciencias Vegetales

Julio, 2024

Tesis aprobada por el tribunal integrado por el Ing. Agr. (PhD) Ariel Szogi, la Ing. Quim. (Dra.) Ángela Cabezas y la Ing. Agr. (Dra.) Carolina Leoni el 20 de agosto de 2024. Autora: MSC, Gabriela Illarze Dive. Directora: Ing. Agr. (Dra.) Pilar Irisarri. Codirectora: Ing. Agr. (Dra.) Amabelia del Pino.

Dedico este trabajo a mi hijo Salvador.

Agradecimientos

Un agradecimiento muy especial al equipo de investigación que colaboró en este proyecto, Rebeca Gonnet, Ana Laura Rivero, Dana Montedónico, Ana María Bianco, Yamila Figueroa y compañeros de laboratorio, Andrea Rodríguez, Germán Pérez, Gastón Azziz, Natalia Rodríguez, Yolanda Fernández.

A los estudiantes que se comprometieron con el arduo trabajo de campo y confiaron en mí para orientarlos en su último tramo académico, Guillermo Gordillo, Martín López y Bianca Collazi.

A los funcionarios y docentes del Centro Regional Sur de la Facultad de Agronomía, por su apoyo en la logística de la colecta de efluentes de tambo y la instalación del ensayo de campo, en especial a José Pedro Dieste y Fabián Milsevich.

Al Comité de Seguimiento del doctorado, Lucía Ferrando y Laura Astigarraga, por sus aportes y ponerse a disposición durante el desarrollo de la investigación.

A Eiko Kuramae y Kesia Lourenzo, por la colaboración en el análisis de datos y asistencia durante mi estadía en el Instituto de Ecología NIOO-KNAW, Wageningen, Países Bajos.

A Mariana Illarze, por su colaboración en el armado de scripts y manejo del programa R.

A mis orientadoras, Pilar Irisarri y Amabelia del Pino, que confiaron plenamente en mí para llevar a cabo este proyecto y me permitieron desarrollarlo en total libertad, aportando y acompañado cada etapa.

Al Departamento de Biología Vegetal de la Facultad de Agronomía, por el apoyo institucional, y a los colegas que siempre apoyaron mi trayectoria.

A la Comisión Académica de Posgrado, que otorgó la beca de posgrado nacional, y a CSIC-Udelar, que financió la beca de pasantía en el exterior y el proyecto de investigación I+D en el cual se desarrolló esta tesis.

Tabla de contenido

Página

Página de aprobación……..III
Agradecimientos V
Resumen.....XI
Summary XII
1. **Introducción** 1
1.1. **Justificación** 1
1.2. **Hipótesis de investigación** 3
1.3. **Objetivos**..... 4
1.3.1. Objetivo general..... 4
1.4. **Esquema general de la tesis** 5
2. **Revisión bibliográfica**..... 7
2.1 **La producción lechera en Uruguay** 7
2.1.1 Desafíos ambientales de la producción lechera 7
2.2 **Efluentes de tambo** 8
2.2.1 Valor nutricional de los efluentes de tambo..... 9
2.2.2. Manejo de los efluentes de tambo..... 10
2.3. **Riego con efluentes de tambo** 11
2.3.1. Aplicación de efluentes de tambo: efecto en la fertilidad del suelo y la productividad forrajera 12
2.3.2. Gases de efecto invernadero derivados de la aplicación de efluentes de tambo al suelo 15
2.3.3. Efecto de la aplicación de efluentes de tambo sobre la microbiota del suelo 18
2.3.4. Riesgo sanitario asociado a la aplicación de efluentes de tambo al suelo 19
3. **Application of dairy effluents to pastures affects soil nitrogen dynamics and microbial activity**..... 22
3.1. **Resumen** 22
3.2. **Summary** 23
3.3. **Introduction** 24
3.4. **Materials and methods**..... 26
3.4.1. Dairy effluent and Soil Collection..... 26

3.4.2. Experimental Design 27
3.4.3. Characterization of Farm Dairy Effluents (FDE) 28
3.4.4. Soil Chemical Analysis 28
3.4.5. Forage Yield and Nitrogen Fertilizer Replacement Value 29
3.4.6. Nitrous Oxide Emission 29
3.4.7. Potentially Mineralizable Nitrogen 30
3.4.8. Soil Potential Nitrification Activity 30
3.4.9. Soil Microbial Enzyme Activities 30
3.4.10. Microbial Community Physiological Profiling 31
3.4.11. Statistical Analysis 32
3.5. **Results** 33
3.5.1. Effluent Characterization 33
3.5.2. Yield and N Uptake of *F. arundinacea* 35
3.5.3. N_2O Fluxes and N Dynamics in Soils 35
3.5.4. Soil microbiological activities 39
3.6. **Discussion** 43
3.6.1. Dairy effluents as N-fertilizers 43
3.6.2. Effect of farm dairy effluent application on soil N dynamics and N2O emission 45
3.6.3. Effect of farm dairy effluent application on soil microbial functional diversity and activity 46
3.7. **References** 48
4. **Effect of untreated and stabilized dairy effluent applications on soil fertility and associated health risks** 54
4.1. **Resumen** 54
4.2. **Summary** 55
4.3. **Introduction** 56
4.4. **Materials and methods** 58
4.4.1. Experimental design 58
4.4.2. Chemical soil analysis 59
4.4.3. Soil basal respiration 59
4.4.4. Chemical analysis of tall fescue 60
4.4.5. Quantification of pathogenic bacterial indicators 60

4.4.6. DNA extraction and quantification of ARGs 61
4.4.7. Statistical analysis 62
4.5. **Results and Discussion** 62
4.5.1. The effect of DE applications on soil chemical properties and plant biomass and nutrition 62
4.5.2. Bacterial indicators of soils with DE applications 66
4.5.3 Occurrence of ARGs in DE and amended soil 69
4.6. **Conclusions** 71
4.7. **References** 72
5. **Differences in bacterial communities and pathogen indicators of raw and lagoon-stabilized farm dairy effluents** 76
5.1. **Resumen** 76
5.2. **Summary** 77
5.3. **Introduction** 78
5.4. **Materials and methods** 80
5.4.1. Dairy effluents collection and Farm characteristics 80
5.4.2. Characterization of dairy effluents 81
5.4.3. Quantification of pathogenic bacterial indicators 81
5.4.4. Dairy effluents DNA extraction and 16S rRNA qPCR and gene amplicon sequencing 82
5.4.5. Statistical analysis 83
5.5. **Results** 84
5.5.1. Farm Dairy Effluents Physicochemical Characterization 84
5.5.2. Quantification of pathogenic indicator bacteria in Farm Dairy Effluents 85
5.5.3. Bacterial abundance, composition, and diversity of Farm Dairy Effluents 87
5.5.4. Linking bacterial community with farm dairy effluents characteristics ... 92
5.6. **Discussion** 94
5.6.1. Bacterial community composition changed with Farm Dairy Effluent treatment and season 94
5.6.2. Variability in physicochemical properties of Farm Dairy Effluents and their relation with bacterial community structure 97
5.6.3. Risk of pathogen loads from Farm Dairy Effluent 98
5.7. **Supplementary material** 101

5.8. **References** 103

6. **Dairy Effluent applications to a pasture enhance soil fertility and microbial activity without impacting soil bacterial and fungal community composition**. 110

6.1. **Resumen** 110

6.2. **Summary** 111

6.4. **Introduction** 113

6.4. **Materials and Methods** 115

6.4.1. Field experiment and sample collection 115

6.4.2. Soil chemical analysis 117

6.4.3. Soil enzyme activities and basal respiration 117

6.4.4. Nitrogen potential activities 118

6.4.5. Microbial community physiological profiling 118

6.4.6. Soil DNA extraction, qPCR, and 16S rRNA gene and ITS region amplicon sequencing 119

6.4.7. Statistical analysis 121

6.5. **Results** 122

6.5.1. Soil chemical analysis 122

6.5.2. Soil microbial activity 123

6.5.3. Microbial community physiological profiling 125

6.5.4. Effect of dairy effluents on soil bacterial and fungal microbial community abundance, composition, and diversity 128

6.5.5. Data integration of soil nutrient content and microbial community composition, abundance, and activity 129

6.6. **Discussion** 132

6.6.1. FDE soil application increases soil microbial activity and nutrient availability 132

6.6.2. Linking the soil microbiome with soil properties and microbial functioning as affected by FDE applications 134

6.7. **Conclusions** 136

6.8. **Supplementary material** 137

6.9. **References** 142

7. **Farm dairy effluents as an alternative for pasture fertilization and greenhouse gas emissions** 149

7.1. **Resumen** 149

7.2. **Summary** 150
7.3. **Introduction** 150
7.4. **Materials and methods** 153
7.4.1. Study site 153
7.4.2. Experimental design and setup 154
7.4.3. Farm Dairy Effluent collection and characterization 155
7.4.4. Gas sampling and analysis 156
7.4.5. Soil sampling and analysis 157
7.4.6. Soil potential microbial activities 158
7.4.7. Pasture yield and nutrient content analysis 158
7.4.8. Statistical analyses 159
7.5. **Results** 159
7.5.1. Pasture Yield and Nutrient Uptake 159
7.5.2. Soil N dynamics and GHG fluxes 161
7.6. **Discussion** 169
7.6.1. Dairy effluents as biofertilizers 169
7.6.2. Effect of dairy effluent applications on GHG emissions and N cycling 171
7.7. **Conclusion** 173
7.8. **References** 173
8. **Discusión general** 187
8.1. **Aportes del presente trabajo** 187
8.2. **Conclusiones y perspectivas** 193
9. **Bibliografía** 195

Resumen

La intensificación de la lechería produce una gran cantidad de efluentes de tambo (FDE). La disposición final de los FDE es generalmente al suelo como fertilizante. Si bien este uso de los FDE tiene numerosos beneficios, como reducir la aplicación de fertilizantes sintéticos o mejorar la calidad del suelo, hay preocupación por los riesgos ambientales y de salud asociados con los patógenos microbianos provenientes de los desechos animales, la propagación de genes de resistencia antimicrobiana, las pérdidas de nutrientes y las emisiones de gases de efecto invernadero (GEI). Además, aún no se ha dilucidado cómo esta práctica agrícola impacta la composición y funcionalidad de las comunidades microbianas del suelo. El objetivo de esta tesis fue evaluar el impacto de las aplicaciones de FDE en las emisiones de GEI del suelo, la composición, actividad y diversidad de su comunidad microbiana y los riesgos sanitarios. Los FDE se recolectaron crudos y de un sistema de doble laguna de estabilización. La tasa de aplicación de N normalizada a 200 kg N ha^{-1} se dividió en cuatro aplicaciones estacionales en comparación con la fertilización con urea o un control sin agregados a la pastura festuca (*Festuca arundinacea* Schreb.). Se tomaron muestras de suelo después de cada aplicación para la caracterización microbiana y fisicoquímica, y se midieron los flujos de GEI hasta veinte días después de cada aplicación. La pastura se cosechó según los manejos de pastoreo típicos. La aplicación repetida de ambos tipos de FDE aumentó el contenido nutricional del suelo y mejoró la actividad microbiana. La producción de festuca y su contenido de macronutrientes fueron mayores con FDE que para el control, posicionando la aplicación de FDE al suelo como una alternativa de disposición. Sin embargo, las emisiones de N_2O aumentaron después de aplicar ambos FDE, más con el FDE crudo. La composición de la comunidad microbiana del suelo no fue significativamente influenciada por las aplicaciones de FDE, pero la diversidad funcional cambió con el FDE crudo. El almacenamiento en lagunas redujo efectivamente el C orgánico y el N total junto con las bacterias indicadoras patógenas y cambió la composición de la comunidad bacteriana, salvo en invierno. Se detectaron genes de resistencia a beta-lactámicos en ambos FDE, pero no en el suelo después de aplicaciones repetidas.

Palabras clave: efluentes líquidos de tambo, fertilidad del suelo, gases de efecto invernadero, diversidad microbiana, actividades enzimáticas

Farm dairy effluent application: A microbiological perspective of soil fertility and environmental impact

Summary

Dairy intensification produces a large amount of farm dairy effluents (FDE). The final disposal of FDE is generally to the soil as fertilizer. While this use of FDE as fertilizer has numerous benefits, such as reducing the application of synthetic fertilizers or improving soil quality, there are concerns about the environmental and health risks associated with microbial pathogens from animal waste, the spread of antibiotic resistance genes, nutrient losses and greenhouse gases (GHG) emissions. Furthermore, how this agricultural practice impacts the composition and functionality of soil microbial communities has not yet been elucidated. This thesis aimed to evaluate the impact of FDE applications on soil GHG emissions, the composition, activity and diversity of its microbial community, and health risks. The FDE were collected raw and from a two-stage stabilization lagoon. The N application rate normalized to 200 kg N ha^{-1} was divided into four seasonal applications compared to urea fertilization or a no-aggregate control to tall fescue (*Festuca arundinacea* Schreb.). Soil samples were taken after each application for microbial and physicochemical characterization and GHG fluxes were measured up to twenty days after each application. The pasture was harvested according to typical grazing management. Repeated application of both types of FDE increased the soil nutrient status and enhanced microbial activity. Higher fescue pasture production and macronutrient content with FDE than the control positioned FDE application to soil as an alternative disposal. However, N_2O emissions increased after applying both FDE's, most notably with raw FDE. Soil microbial community composition was not significantly influenced by FDE applications, but functional diversity shifted with raw FDE. Lagoon storage effectively reduced organic C and total N along with pathogenic indicator bacteria and changed the bacterial community composition except in winter. Beta-lactam resistance genes were detected in both FDEs, but not in the soil after repeated applications.

Keywords: liquid farm dairy effluents, soil fertility, greenhouse gasses emission, microbial diversity, enzyme activities

1. Introducción

1.1. Justificación

En los últimos treinta años, Uruguay ha experimentado un proceso de intensificación en la producción lechera, que ha resultado en un incremento significativo de los efluentes generados durante las operaciones de ordeñe (Chilibroste, 2021). Los efluentes de tambo se generan por el lavado de las áreas de ordeñe y se componen principalmente de heces, orina, microorganismos fecales, restos de leche, residuos de ración, barro, detergentes y otros productos químicos (Charlón, 2009). La gestión de estos residuos representa un desafío tanto desde la perspectiva ambiental como sanitaria.

La normativa actual propone el uso de efluentes de tambo para riego en pasturas y cultivos (Correa et al., 2016). Así, se pretende eliminar cualquier vertido a las aguas superficiales y controlar la filtración hacia napas de agua subterránea. Esta política asigna al suelo una doble función: en primer lugar, reducir la carga contaminante y, en segundo lugar, el ciclado de nutrientes, que hacen aprovechables aquellos contenidos en las deyecciones para nuevos ciclos productivos. De este modo, los efluentes de tambo pueden visualizarse como un recurso debido al aporte de nutrientes para satisfacer los requerimientos de las plantas, principalmente en forma de nitrógeno (N), fósforo (P) y potasio (K).

Los efluentes de tambo son en general aplicados al suelo directamente luego del ordeñe y limpieza de las instalaciones (efluentes crudos) o desde pozos de decantación o posterior tratamiento en sistema de lagunas de estabilización en serie. Esta variabilidad en el manejo de los efluentes de tambo hace difícil poder predecir su uso agronómico. Además, cuando la composición fisicoquímica de los efluentes y sus transformaciones en el suelo se desconocen, no se puede predecir si existen dificultades para su reciclado en el predio ni su valor agronómico para sustituir la aplicación de fertilizante comercial. Por otra parte, tampoco se puede asegurar que esta práctica no presente riesgos

ambientales asociados al exceso de nutrientes, al movimiento de microorganismos patógenos o a la reducción de la calidad del aire, entre otros (Hawke y Summers, 2006; Heuer et al., 2011; Pandey et al., 2014; Saggar et al., 2004).

Los microorganismos son los grandes descomponedores de la materia orgánica (MO) del suelo y juegan un papel clave en el ciclado de los nutrientes, entre otros servicios brindados (Philippot et al., 2023). La fracción carbonada de la MO de los efluentes provee mayoritariamente energía a los microorganismos que la mineralizan y el N mineralizado no solamente es absorbido por las plantas sino que es susceptible de perderse por lixiviación, volatilización de amoníaco (NH_3) o retornar a la atmósfera en forma de N_2O, NO o N_2 como resultado de los procesos biológicos de nitrificación y desnitrificación (Conrad, 1996). Reducir las pérdidas de N luego de la aplicación de efluentes de tambo requiere, por lo tanto, comprender los procesos y las comunidades microbianas que regulan la retención y el ciclo de nutrientes del suelo.

Las comunidades microbianas en el estiércol pueden influir en el microbioma del suelo, ya sea directamente a través de la competencia o indirectamente al propagar la resistencia antimicrobiana (Laconi et al., 2021; Lu et al., 2021), lo que afecta el funcionamiento del agroecosistema (van der Heijden y Wagg, 2013). Se sabe muy poco sobre la comunidad microbiana y su diversidad en efluentes de tambo y cómo cambia con los distintos manejos (Pandey et al., 2018).

La fertilización nitrogenada orgánica y mineral ha evidenciado impactar la composición de las comunidades microbianas que prosperan en la rizosfera y desempeñan un papel crítico en la movilización de nutrientes para la absorción por las plantas (Alegria Terrazas et al., 2016; Caradonia et al., 2019). Sin embargo, queda por dilucidar cómo la práctica del riego con efluentes de tambo impacta en la composición y funciones de las comunidades microbianas del suelo (Laconi et al., 2021; Liu y Haynes, 2011; Pandey et al., 2018; Shi et al., 2017).

Por otra parte, los niveles de patógenos en los efluentes de tambo dependen tanto del estado sanitario de los animales como de la forma en que son almacenados o tratados (Oun et al., 2014). Algunos patógenos son particularmente riesgosos para la salud de los animales; por lo tanto, se recomienda no pastorear suelos regados con efluentes hasta después de treinta días de la aplicación (Correa et al., 2016). También se sugiere no pastorear con terneros en sitios con aplicación de efluentes, ya que estos son más sensibles a los patógenos. Sin embargo, no hay en Uruguay estudios de evaluación del número de microorganismos indicadores en suelo y agua, luego del riego con efluentes. El ingreso de estas bacterias fecales al suelo puede a su vez generar cambios en la comunidad microbiana de este (Gerba y Smith, 2005).

Los antibióticos beta-lactámicos como la penicilina son de uso frecuente para el tratamiento de mastitis bovina en Uruguay y se han reportado cepas patógenas aisladas de leche con alta susceptibilidad a estos antibióticos (De Los Santos et al., 2017; Gianneechini et al., 2014) y portadoras de genes de resistencia a estos en las heces (Coppola et al., 2020), que podrían diseminarse al suelo incluso luego del tratamiento de estos residuos (Dungan et al., 2018; Lu et al., 2021).

El conocimiento de los factores que influencian la persistencia y el destino de microorganismos patógenos y los genes de resistencia antimicrobiana (ARG) por la aplicación de los efluentes de tambo al suelo sentaría las bases para el desarrollo de prácticas del manejo que minimicen los riesgos sanitarios.

Esta tesis de doctorado propone contribuir a la comprensión de los impactos del uso productivo de efluentes de tambo y avanzar en la valoración de estos no como un desecho, sino como un elemento útil para proveer de nutrientes a las pasturas y el suelo.

1.2. Hipótesis de investigación

La aplicación de efluentes de tambo a una pastura, crudos o estabilizados, a través del aporte de nutrientes y microorganismos, producen una respuesta

diferencial en el suelo, en términos de la productividad forrajera, la fertilidad del suelo, la actividad y la diversidad microbiana, la emisión de gases de efecto invernadero (GEI) y la persistencia de indicadores microbianos de sanidad.

1.3. Objetivos

1.3.1. Objetivo general

Este estudio busca mejorar la utilización productiva de efluentes de tambo a través del conocimiento de sus características y de procesos que ocurren una vez aplicados al suelo, asociados a la fertilidad, la actividad y diversidad de la comunidad microbiana, la productividad forrajera y el potencial de contaminación ambiental.

1.3.2. Objetivos específicos

1. Comparar el efecto de la aplicación de efluentes crudos y estabilizados en sistema de doble laguna con la equivalente fertilización nitrogenada en términos de
    a. El aporte de nutrientes y la productividad de festuca (*Festuca arundinacea* Schreb.).
    b. La fertilidad del suelo.
    c. La actividad microbiana del suelo.
    d. Los flujos de emisión de GEI.
2. Conocer la composición y diversidad de la comunidad microbiana de los efluentes, su cambio con el manejo y la estación, y evaluar el efecto de su aplicación al suelo sobre la población microbiana presente en este para conocer si su diversidad se ve afectada.
3. Determinar el riesgo sanitario asociado a la aplicación de efluentes de tambo.

1.4. Esquema general de la tesis

El **capítulo 2** consta de una revisión bibliográfica sobre el contexto nacional de la producción lechera y el manejo de efluentes y se contrasta los distintos trabajos que abordaron el efecto de la aplicación de efluentes de tambo al suelo tanto desde el punto de vista de su uso agronómico en pasturas y cultivos como desde el punto de vista de las externalidades ambientales asociadas a la emisión de GEI, la diseminación de patógenos y resistencia antimicrobiana y el impacto sobre las comunidades microbianas.

La estrategia de investigación utilizada para llevar a cabo los objetivos de este trabajo consistió en un ensayo de macetas en invernáculo y otro a campo sembrado con la pastura perenne festuca. El experimento de invernáculo se desarrolló en las instalaciones del Departamento de Suelos y Aguas, Facultad de Agronomía, sede Sayago-Montevideo, y el experimento a campo se realizó en el Centro Regional Sur (CRS) de la Facultad de Agronomía, Juanicó-Canelones. Los efluentes de tambo fueron obtenidos del tambo del CRS. El diseño experimental consistió en cuatro aplicaciones de fertilización nitrogenada para lograr una dosis anual de N equivalente a 200 kg/ha con los siguientes tratamientos:

1. Efluentes de tambo crudos (colectados enseguida del lavado de las instalaciones de ordeñe).
2. Efluentes de tambo estabilizado en sistema de doble laguna (colectado de la laguna facultativa).
3. Urea.
4. Control sin aplicaciones.

En los capítulos 3 y 4 se presentan los resultados del experimento de invernáculo, organizados en dos artículos que fueron publicados en revistas arbitradas. El **capítulo 3**, «La aplicación de efluentes de tambo a una pastura afecta la dinámica de N y la actividad microbiana del suelo» fue publicado en la revista *Agronomy*. El **capítulo 4**, «Efecto de la aplicación de efluentes de tambo,

sin tratar y estabilizados, sobre la fertilidad del suelo, el contenido de nutrientes de la pastura y los riesgos sanitarios asociados», fue publicado en la revista *Agrociencia Uruguay*.

En los capítulos 5, 6 y 7 se presentan los resultados del experimento a campo, organizado en tres artículos. El **capítulo 5** presenta los resultados publicados en la revista *Microorganisms*, «Diferencias en la comunidad de bacterias y el número de indicadores de patógenos entre efluentes de tambo crudos y estabilizados en lagunas». El **capítulo 6**, «La aplicación de efluentes de tambo a una pastura mejora la fertilidad del suelo y la actividad microbiana sin impactar la composición de las comunidades de bacterias y hongos», fue aceptado en la revista *European Journal of Soil Biology*. En el **capítulo 7** se incluyen los resultados de «Efluentes de tambo como una alternativa para la fertilización en pasturas y gases de efecto invernadero», versión preliminar, que será enviado a publicar en la revista *Journal of Integrative Agriculture*. Finalmente, en el **capítulo 8** se realizan consideraciones finales en las cuales se discute y sintetiza el trabajo y sus proyecciones.

Los capítulos 3 a 7 han sido redactados en inglés, ya que comprenden los artículos preparados para revistas internacionales. Por este motivo, se respetan los formatos de citado y referenciado de cada revista. Al inicio de cada uno de estos capítulos se presenta un resumen en español. La sección de bibliografía al final contiene las referencias de las citas de los capítulos 1, 2 y 8.

2. Revisión bibliográfica

2.1 La producción lechera en Uruguay

La lechería en Uruguay alcanza valores de exportación correspondientes a un 70 % de la producción, convirtiéndose en uno de los sectores más dinámicos de la agropecuaria nacional. Se concentra en el 5 % del territorio, principalmente en el sureste del país (INALE, 2023). Los departamentos de Colonia, San José y Florida representan el 84 % de la producción nacional y el 64 % de los establecimientos, y contienen la mayoría de las empresas industrializadoras (DIEA, 2023).

En los últimos treinta años, la producción lechera en Uruguay pasó de producir 700 millones de litros a 2.200 millones (DIEA, 2023). Esta evolución no ha sido exclusiva de nuestro país, sino una tendencia generalizada en el mundo. El aumento en la producción de leche se explica por una mayor intensificación de la producción, debido a aumentos tanto en la carga (1,6 veces) como en la producción por vaca (2,6 veces) (Fariña y Chilibroste, 2019), en una superficie total de tierras para lechería que pasó de un millón de hectáreas en 2001 a 649.000 hectáreas en 2023 (DIEA, 2023).

La producción de leche en Uruguay está basada en un sistema pastoril con suplementaciones estratégicas de concentrados y ensilados (Fariña y Chilibroste, 2019). Actualmente, cerca del 60 % de la superficie dedicada a la lechería cuenta con pasturas mejoradas (DIEA, 2023). Esto permite obtener costos medios de producción por litro de leche que se ubican entre los más bajos del mundo (IFCN, 2017). Sin embargo, la industria lechera nacional enfrenta el desafío de aumentar la productividad para mantener su competitividad (Chilibroste, 2021).

2.1.1 Desafíos ambientales de la producción lechera

La intensificación de la producción lechera también ha resultado en efectos ambientales adversos, tanto local (R. Arocena et al., 2013; Barreto Wagner

et al., 2017; Chilibroste, 2021) como internacionalmente (Cameron et al., 2002; Foote et al., 2015). Estos están asociados a que los tambos ordeñan un número de animales mayor que para lo que fueron diseñados y a la falta de planificación sobre la disposición final de los residuos. Este hecho genera problemas de manejo por grandes volúmenes de estiércol, de contaminación y de traslado de los nutrientes (La Manna et al., 2004; Sosa et al., 2016). Los impactos ambientales incluyen, entre otros, problemas asociados a la pérdida de calidad del agua, la emisión de GEI y problemas emergentes de bienestar y salubridad animal.

En Uruguay, el riesgo de contaminación puntual, más que la contaminación difusa, aparece como un desafío fuerte para la creciente producción lechera (Fariña y Chilibroste, 2019). Esta contaminación puntual se asocia principalmente al excedente de los nutrientes, N y fósforo (P) que pueden perderse hacia cursos de agua por la lixiviación de nitrato (NO_3^-) o escorrentía. Estudios locales han atribuido parcialmente el enriquecimiento de P encontrado en el agua de la cuenca del Río Santa Lucía al escurrimiento del suelo y la descarga directa de efluentes de las explotaciones lecheras (R. Arocena et al., 2013; Barreto Wagner et al., 2017; Chalar et al., 2013). El cuarenta por ciento de las explotaciones lecheras en Uruguay se encuentran cerca de la cuenca de este río. Debido a esto, ha habido una fuerte atención pública sobre las operaciones lecheras y se han lanzado programas gubernamentales para apoyar las mejores prácticas de gestión y de infraestructura en los sistemas lecheros para el manejo de los efluentes (Correa et al., 2016; MVOTMA, 2013).

2.2 Efluentes de tambo

Los efluentes de tambo están conformados por una fracción sólida (materia fecal, restos de alimentos y barro) y una líquida (agua, orina, restos de leche y soluciones de limpieza del equipamiento de ordeño) (Charlón et al., 2009). Generalmente, el 10 % es representado por las excretas y el 90 % por el agua de lavado (Gibson, 1995), por lo cual es un efluente muy diluido que contiene

menos del 1 % de sólidos (Hawke y Summers, 2006). Además, los efluentes de tambo contienen concentraciones variadas de organismos entéricos como bacterias, arqueas, virus, protozoarios y helmintos, que tienen su origen en las excretas del ganado. El principal grupo son las bacterias, que en el estiércol pueden alcanzar hasta 10^{10}, representadas principalmente por los coliformes fecales y estreptococos (Jensen y Sommer, 2013). Las características fisicoquímicas y microbiológicas de cada efluente dependerán, entre otros factores, del manejo del tambo y del tratamiento previo a su vertido.

Se estima que en Uruguay se generan cuatro litros de efluentes por cada litro de leche producida. Por tanto, con la producción actual se generarían unos 9000 millones de litros de efluente anualmente (DIEA, 2023). El manejo adecuado de estos resulta determinante para limitar su efecto negativo sobre el ambiente y dar cumplimiento a normativas internacionales y de exigencias comerciales en materia ambiental (Taverna et al., 2007).

2.2.1 Valor nutricional de los efluentes de tambo

Los efluentes de tambo se caracterizan por contener nutrientes como N, P, potasio (K), azufre (S) y altos contenidos de MO (Hawke y Summers, 2006; B. Longhurst et al., 2017). Los animales excretan casi el 70 % del N y P que ingieren a través de la orina y las heces. Entre el 60 y 85 % del total del N presente en los efluentes se presenta bajo forma orgánica. La fracción soluble es en su mayoría la orina que también contribuye a la fracción orgánica, pero rápidamente se hidroliza a N amoniacal (La Manna, 1995). Se estima que anualmente una vaca puede generar excretas con contenidos de 5,9 kg de N, 0,7 kg de P, 5,4 kg de K, 0,8 kg de S, 2,2 kg de Ca, 1 kg de Mg y 0,7 kg de Na (Heatley, 1995).

De todos modos, la composición y, por ende, el valor fertilizante de los efluentes de tambo dependerá de factores asociados al manejo de las operaciones durante el ordeñe, tales como: sistema de estabulación, alimentación, sistema de limpieza, forma de recolección, almacenaje y tratamiento, y también de factores

como el momento de lactancia, la edad y raza del animal (La Manna et al., 2004; R. D. Longhurst et al., 2000). Esta variabilidad en la composición de los efluentes tiene además un marcado efecto estacional, que se vuelve aún más pronunciado en los sistemas pastoriles de Uruguay dado por la variación en la cantidad de forraje producido (Gutiérrez y Cabrera, 2012). La mayor ingesta se corresponde con una mayor producción de leche y esta se correlaciona con un mayor volumen de excretas recogidas por vaca. Parámetros como la demanda química de oxígeno (DQO), que expresa la concentración de MO en los efluentes, mostró este patrón estacional, que fue mayor durante invierno-primavera que durante verano-otoño (Gutiérrez y Cabrera, 2012).

Salazar et al. (2010) recopilaron información para Uruguay mediante encuestas a 621 productores y toma de muestras de 20 tambos de la cuenca lechera de Uruguay y también para Argentina y Chile. Respecto a la composición de los efluentes, el contenido de materia seca (MS) mostró similitudes entre todos los países analizados y comparado a otros sistemas lecheros de base pastoril, pero se registró un contenido de nutrientes más bajo (aunque variable), explicado por cortos o nulos períodos de confinamiento, sumado a grandes cantidades de agua provenientes del lavado y lluvia. En las muestras analizadas de los tambos de Uruguay, el contenido de MS promedió 1,05 %, N total (Kjeldahl) 270 mg/L, N amoniacal 190 mg/L, P 130 mg/L y K 490 mg/L.

2.2.2. Manejo de los efluentes de tambo

Hasta hace pocos años los efluentes de tambo en Uruguay eran canalizados sin tratamiento directamente a cursos de agua. Posteriormente, se adoptó el uso de lagunas o piletas como tratamiento de efluentes; sin embargo, el líquido sobrenadante era vertido a cursos de agua. Estos sistemas de almacenaje y estabilización, si bien permiten una reducción importante de la carga orgánica, no lo logran con el contenido de N, P y patógenos, que superan los límites requeridos para su vertido a cauce (Gutiérrez y Cabrera, 2012). Esto, sumado a los altos niveles de nutrientes constatados en aguas superficiales y profundas

(Arocena et al., 2013; Barreto Wagner et al., 2017), implicó regular su vertido, sugiriéndose su uso para riego (Correa et al., 2016). Así se pretende eliminar los vertidos de todo tipo a las aguas superficiales y controlar la filtración hacia napas de agua subterránea. Sin embargo, se constató que existe poca planificación de distribución de los efluentes líquidos, ya sea crudos o estabilizados, en lagunas y son más bien eliminados que utilizados por su valor fertilizante (Houlbrooke, 2015).

Para la gestión de los efluentes de tambo, se propone como mínimo un sistema que cuente con trampa para retención de sólidos, una o varias piletas o lagunas de almacenamiento y un sistema de aplicación al terreno (Correa et al., 2016). Los sistemas de almacenaje de los efluentes son muy variables y dependerán de las condiciones operativas de cada establecimiento y de los objetivos buscados: los hay desde pozos estercoleros con capacidad limitada de almacenamiento (días a pocas semanas), lo que requiere una recolección y aplicación frecuente al suelo, hasta sistemas de doble o triple laguna de tratamiento con tiempos de retención que superan los cien días (Correa et al., 2016). El 69 % de los establecimientos lecheros realizan un manejo de los efluentes en sistema de lagunas y el 80 % de estas descargan al suelo por desagüe natural en el terreno (Bianco, 2016). Si bien existen diversas opciones para el sistema de laguna de tratamiento, usualmente consta de al menos dos lagunas conectadas en serie, la primera anaeróbica y la segunda facultativa. En la primera laguna de tratamiento biológico se produce la sedimentación y degradación anaeróbica de la MO y, por lo tanto, se reduce la fracción sólida del efluente. Luego, el efluente pasa a una segunda laguna facultativa, donde continúa el proceso de degradación combinando procesos de respiración aerobia y fotosíntesis en capa superficial y anaerobia en el fondo.

2.3. Riego con efluentes de tambo

El riego con efluentes de tambo es una práctica agrícola de uso frecuente en el mundo y es una forma efectiva de manejo de modo de reducir la

incorporación de contaminantes al ambiente, mejorar la fertilidad de los suelos y aumentar la sustentabilidad de los sistemas productivos (Hagare et al., 2022; Houlbrooke et al., 2004; Pagliari et al., 2020). Hay poca información en Uruguay respecto a las interacciones suelo-efluentes que son importantes para las decisiones de manejo y la reutilización de los recursos para una productividad sostenible (Bianco, 2016). Houlbrooke (2015) señala la importancia de definir la carga/ha de efluente para minimizar las pérdidas de nutrientes y ajustarla según el tipo de suelo y su déficit hídrico para las condiciones locales de producción. Por otro lado, existe dificultad asociada a la implementación de la aplicación de efluentes de tambo al suelo dada por su gran heterogeneidad y condiciona la utilización a su caracterización previa.

Idealmente, la aplicación de efluentes al suelo contrabalancearía la extracción de nutrientes por las pasturas, aportaría MO y fomentaría la actividad biológica. Sin embargo, los resultados nacionales son variables y no han considerado o son incipientes en evaluar los riesgos sanitarios, su efecto sobre los procesos microbianos del suelo o su contribución a la huella de carbono de la leche (Casanova et al., 2007; Del Pino et al., 2014; La Manna et al., 2004).

2.3.1. Aplicación de efluentes de tambo: efecto en la fertilidad del suelo y la productividad forrajera

La aplicación de efluentes de tambo aporta nutrientes al suelo, aumenta la productividad de las pasturas y cultivos y mejora la calidad del suelo (Bhogal et al., 2018; Ghezzehei et al., 2014; Hawke y Summers, 2006; Houlbrooke et al., 2004; B O Manono et al., 2016; McDowell y Nash, 2012; Niraula et al., 2021; Wallenstein et al., 2006). Pero también existen resultados en donde la aplicación de estos no ha sido totalmente efectiva (La Manna et al., 2004; Saunders et al., 2012; Sosa et al., 2016). La variabilidad en los resultados encontrados es debida a que el valor del efluente como fertilizante es afectado por el sistema de tratamiento, las dosis aplicadas al suelo y las condiciones climáticas, entre otros factores.

Una vez aplicados al suelo, la fracción orgánica de los efluentes de tambo debe ser mineralizada por los microorganismos. Este proceso es clave, ya que hace disponible los nutrientes en forma inorgánica para ser asimilados por las plantas, pero es muy difícil estimar con precisión si estos nutrientes quedarán disponibles en los momentos de mayor absorción por las plantas, sumado a que las tasas de mineralización están sujetas a las condiciones climáticas y edáficas y al manejo del efluente en el predio. En la medida que un efluente es estacionado por mayor tiempo, o los procesos biológicos son acelerados, el material a ser aplicado tendrá menor relación C/N, que dejará mayor disponibilidad de N mineral (Bolan et al., 2004). El efluente fresco se caracteriza por una relación C/N más elevada; por lo tanto, es rápidamente atacado por los microorganismos del suelo al ser aplicado y produce, en algunos casos, un efecto momentáneo de inmovilización de N. Los efluentes de tambo también proveen otros macro- y micronutrientes esenciales además del N, pero la relación en la que se encuentran en los efluentes difiere de la absorbida por las plantas. Existe, entonces, la preocupación de que la aplicación a largo plazo de efluentes de tambo, a tasas que cumplen con las pautas establecidas para el N, pueda estar agregando P y K por encima de los requisitos de la planta (ya que son los nutrientes que se encuentran en mayor proporción en los efluentes). Si bien la acumulación de K en el suelo no se considera una preocupación ambiental, la mayor captación de este nutriente por las pasturas puede inducir problemas metabólicos en el ganado, particularmente deficiencia de Mg y Ca (Bolan et al., 2004; Tillman y Surapaneni, 2002). La acumulación de P en aguas superficiales de microcuencas ubicadas en la cuenca del río Santa Lucía se asocia claramente al tipo de explotación, registrándose las mayores concentraciones de este nutriente en la zona de tambos, donde los niveles de P lábil del suelo son más elevados (R. Arocena et al., 2013). Además, la aplicación de efluentes puede estimular la mineralización del CO del suelo y así aumentar el contenido de P de este (Angers et al., 2010). Un estudio reciente sobre aplicación continua de estiércol de ganado lechero en un suelo calcáreo mostró una acumulación excesiva de P en el suelo que puede contribuir a la disponibilidad del nutriente (Hu et al., 2024).

Castagna et al. (2024) desarrollaron una herramienta para analizar escenarios en la cuenca lechera que reduzcan la exportación de P y recomiendan aumentar el área de pasturas perennes y planificar el uso del suelo.

En Uruguay, Casanova et al. (2007) evaluaron el riego con efluentes de una pileta de retención de sólidos y los del vaciado de una laguna anaeróbica. En el primer caso no se observaron efectos positivos, mientras que en el segundo la productividad aumentó hasta un 23 %. En un experimento a corto plazo llevado a cabo por Mello y Bonino (2010) en el CRS de la Facultad de Agronomía, se sembró raigrás anual en un brunosol eútrico típico de la unidad Tala Rodríguez y se aplicaron 620.000 l/ha de efluentes del tambo con composición: 0,71 % MS, 0,5 g/l de N y 0,05 g/l de P. Los autores encontraron que hubo un incremento del 21 % en la producción inicial de materia seca por hectárea.

En Nueva Zelanda, varios estudios mostraron un marcado aumento en la productividad de pasturas en respuesta al aporte de nutrientes de los efluentes en suelos con baja fertilidad (sobre todo N) (Houlbrooke et al., 2004). Habteselassie et al. (2006), en un ensayo de cinco años, encontraron que los efluentes líquidos de tambo dejaban el N casi inmediatamente disponible para las plantas y aumentaban la actividad nitrificante del suelo.

Mientras tanto, Bhogal et al. (2018) sostienen que la aplicación de enmiendas orgánicas, entre las que se encontraban los efluentes de tambo, aumentan el contenido de nutrientes (N, P K, Mg) de los suelos en el corto plazo (menos de tres años), pero aquellos con bajo contenido de sólidos, como los efluentes de tambo, tienen una capacidad limitada para mejorar las propiedades biológicas y físicas de los suelos. Los mismos autores señalan que la calidad de las enmiendas orgánicas depende del grado de descomposición de la materia orgánica de estas, que a su vez determina el C disponible para la microbiota y la composición de esta última y de la red trófica del suelo. Las enmiendas líquidas se aplican mayoritariamente para reciclar nutrientes en el sistema suelo-planta y reducir la necesidad de fertilizante sintético más que para aumentar el COS, aunque dependerá del tiempo durante el que continúen las aplicaciones.

2.3.2. Gases de efecto invernadero derivados de la aplicación de efluentes de tambo al suelo

El riego con efluentes de tambo puede aumentar la volatilización de NH_3 y las emisiones de N_2O y CH_4, estos últimos dos GEI, que contribuyen al cambio climático global (Bolan et al., 2004; Luo et al., 2008; Saggar et al., 2004). Las pérdidas gaseosas debidas al manejo de los efluentes de tambo fueron responsables del 15,2 % de las emisiones de la agricultura en E.E.U.U (USEPA, 2015).

El N_2O, con un potencial de calentamiento 298 veces mayor al del CO_2 y 33 veces mayor al del CH_4 (IPCC, 2007), se considera muy importante en la destrucción de la capa de ozono estratosférica (Ravishankara et al., 2009) y tiene una vida media de 114 años, por lo cual su producción tendrá influencia varias generaciones después (Tian et al., 2020), mientras que el CH_4 contribuye unas veintiún veces más que el CO_2 al cambio climático y su vida media en la atmósfera es más corta (unos doce años) (IPCC, 2007).

Las emisiones de GEI en los tambos están dominadas por CH_4 y N_2O y el manejo de efluentes líquidos ha aumentado ambos gases (Petersen, 2018). Las pérdidas de amoniaco durante el manejo de los efluentes son una fuente indirecta pero significativa de N_2O.

2.3.2.1 Emisiones de N_2O

La principal fuente de N_2O en el suelo son los procesos microbianos de desnitrificación o nitrificación (Conrad, 1996). La nitrificación es un proceso aerobio, pero cuando la provisión de O_2 es limitada, se genera N_2O como subproducto, mientras que la desnitrificación se activa una vez que el O_2 ha sido consumido en los microambientes anóxicos generados dentro del perfil del suelo (Smith et al., 2003). Estos dos procesos pueden ocurrir de forma simultánea en agregados del suelo (micrositios), debido al gradiente espacial de O_2 producido (Stevens et al., 1997).

Se pensaba que la nitrificación era realizada exclusivamente por bacterias quimiolitoautótrofas; sin embargo, el proceso es más complejo e involucra también a arqueas nitrificantes autótrofas, nitrificantes heterótrofos y bacterias anammox y comammox (Norton y Stark, 2011; van Groenigen et al., 2015).

Los microorganismos nitrificantes producen N_2O por nitrificación y por desnitrificación nitrificante. Por otra parte, los microorganismos desnitrificantes son en su mayoría heterótrofos, anaerobios facultativos y a bajas concentraciones de oxígeno usan NO_3^- o NO_2^- como aceptor final de electrones. Son un grupo de microorganismos muy diversos (bacterias, arqueas y hongos) y dispersos en varias ramas filogenéticas, aunque se encuentran con mayor frecuencia entre las α y β proteobacterias. Si bien la ruta metabólica completa de la desnitrificación comprende la conversión de NO_3^- a N_2, se lleva a cabo en etapas sucesivas por distintas enzimas reductasas e incluye los siguientes intermediarios: NO_2^-, NO^- y N_2O, por lo que puede provocar pérdidas de N del suelo (Groffman, 2012). Además, no todos los microorganismos capaces de desnitrificar tienen toda la vía enzimática hasta N_2 y, en caso de tenerla, su expresión depende en gran medida de la cantidad de O_2 disponible (Jones, 2010; Zumft, 1997).

Los procesos de nitrificación y desnitrificación en conjunto regulan gran parte de la dinámica del N en los suelos y son afectados tanto por factores climáticos como las lluvias y temperaturas, así como por factores intrínsecos del suelo como el espacio poroso del suelo ocupado por agua, el pH y la disponibilidad de N y C (Bolan et al., 2004; Saggar et al., 2013; Tiedje, 1988). Definir, entonces, las condiciones locales es clave para una estimación más precisa de la emisión.

La contribución a la emisión de N_2O por la aplicación efluentes de lechería al suelo ocurre principalmente por el aporte de N y C disponible que promueve la actividad microbiana y por el aumento de la humedad del suelo que induce condiciones de anaerobiosis (Laubach et al., 2015). Se estima que los efluentes de tambo tienen una concentración de N que varía de 70 a 200 mg de N L^{-1} (Barnett et al., 1994) y una alta disponibilidad de C orgánico que favorecen la nitrificación y la desnitrificación (Cameron et al., 2013). Cameron et al. (2002)

encontraron que, en pasturas mixtas regadas con efluentes de tambo durante tres años, las pérdidas de N por lixiviación y volatilización fueron inferiores al 1 % del N aplicado, mientras que la desnitrificación fue del 7 a 12 %, del que entre 10 y 30 % se perdió como N_2O y el resto como N_2. Luo et al. (2008) encontraron que, en general, las praderas que recibieron efluentes líquidos emitieron más GEI y el factor de emisión de N_2O de aplicar estiércol fue menor que aplicando fertilizante químico. Sin embargo, Barton y Schipper (2001) sugieren que el aumento en el N_2O enseguida del riego con efluentes es mayor al de la fertilización inorgánica debido a la desnitrificación, ya sea por la mayor disponibilidad de C o por una disminución de la aireación del suelo, producto de un aumento en la respiración microbiana. Estos picos de emisión de N_2O concomitante a la aplicación de efluentes han sido reportados por varios investigadores (Bhandral et al., 2010; Li et al., 2015; Saggar et al., 2004). Los efluentes líquidos infiltrarían el suelo parcialmente en respuesta a los gradientes de potencial hídrico de este y establecerían en un corto plazo un gradiente de C y N disponible (Petersen, 2018).

La alta variabilidad de emisión de N_2O en suelos regados con efluentes ha llevado al reporte de un amplio rango de factores de emisión de este gas, desde <0,1% a 6 % (Bhandral et al., 2004; Chadwick et al., 2011; Li et al., 2014; Luo et al., 2008). En las condiciones actuales del manejo de efluentes de tambo para riego en Uruguay, es esperable que se estén aplicando altas tasas de efluentes al suelo y esto podría aumentar el factor de emisión de N_2O (Saggar et al., 2004).

Bhandral et al. (2007) comparó las emisiones de N_2O producidas en una pradera regada con efluentes que ingresan y salen de una laguna aerobia en la estación seca y la estación húmeda. El suelo regado con los efluentes tratados emitió los mayores flujos de N_2O en otoño, a pesar de que los efluentes sin tratar presentaron mayor contenido de N total y C orgánico disponible. Los autores sugieren que la variación en la relación C/N y la disponibilidad de C y N fácilmente asimilables de cada efluente afecta tanto las tasas de desnitrificación como la relación N_2O: ($N_2O + N_2$). Así, el efluente fresco, con mayor relación C/N, pudo haber retardado la liberación de N por mineralización y, además, por

su mayor contenido de C, favoreció una desnitrificación completa hasta N_2. De forma contraria, Li et al. (2014) estimaron un factor de emisión de N_2O mayor para efluentes frescos que para estacionados (cuatro meses). Si bien el contenido de C fue similar para ambos efluentes, el fresco presentó mayor N bajo forma de NH_4^+, lo que sugiere que hubo mayores tasas de nitrificación en el suelo. Las pérdidas de N_2O, expresadas como el factor de N_2O, fueron 0,13 y 0,14 % para las excretas y los efluentes líquidos frescos, respectivamente. Los efluentes estacionados presentaron un factor de emisión bajo de 0,03 %. Además, este estudio demostró los cambios estacionales de la emisión de N_2O, principalmente asociados a los cambios en la temperatura y la humedad del suelo. Houlbrooke et al. (2004) encontraron que aplicar efluentes durante la primavera resulta en mayores concentraciones de N asimilable en el suelo como resultado de las temperaturas altas y la alta tasa de mineralización. Laubach et al. (2015) revisaron las emisiones del almacenamiento y riego con efluentes de tambo en Nueva Zelanda y reportan que la emisión de N_2O en las lagunas anaerobias es despreciable, mientras que la volatilización de amonio es muy variable con el pH y composición de los efluentes. Por otra parte, estos autores señalan que el riego con efluentes de tambo puede ser una fuente importante de emisión de N_2O.

2.3.3. Efecto de la aplicación de efluentes de tambo sobre la microbiota del suelo

La aplicación de efluentes de tambo al suelo puede afectar a las comunidades microbianas, al cambiar su diversidad y actividad que en última instancia alteran el funcionamiento del ecosistema (Semenov et al., 2021; Sun et al., 2015; Urra et al., 2019; Zhang, 2018). A través del aporte de nutrientes y MO de los efluentes, ocurre la mineralización por microorganismos del suelo, como se evidencia por el aumento de la respiración, la biomasa microbiana y actividades enzimáticas (La Manna et al., 2004; Liu y Haynes, 2011; Manono, 2014; Shi et al., 2017; Zaman et al., 2002). Como las enzimas del suelo

desempeñan un papel vital en la descomposición y transformación de la MO que libera compuestos minerales (Lladó y Baldrian, 2017), contribuyen significativamente al ciclo de los nutrientes. Las actividades enzimáticas del suelo han sido señaladas como indicadores confiables de la descomposición de la MO de los efluentes de tambo (Dungan et al., 2022). Las enmiendas orgánicas pueden llevar a la activación de muchos microorganismos usualmente poco activos en el suelo, que responden con rapidez a la entrada adicional de sustrato (He et al., 2012; Zelenev et al., 2006). Sin embargo, aún no está claro si estos efectos son causados principalmente por la activación del microbioma del suelo o por la supervivencia de microbios introducidos con la enmienda orgánica y, además, estos cambios en la microbiota del suelo parecen ser transitorios (Semenov et al., 2021) y los resultados encontrados en la literatura son contradictorios.

En este sentido, algunos estudios indican que mientras la biomasa y la actividad microbiana responden a la aplicación de efluentes de tambo, la diversidad de la comunidad se mostró resiliente (Laconi et al., 2021; Liu y Haynes, 2011) o cambia dependiendo el tipo de enmienda y las tasas de aplicación (Shi et al., 2017). En cambio, otros indican una respuesta a la aplicación prolongada de efluentes de tambo tanto en la biomasa y actividad microbiana como en la estructura de la comunidad (Neufeld et al., 2017). Además, estudios que se centraron en grupos microbianos específicos, por ejemplo, relacionados con el ciclo de N, encontraron una mayor abundancia y cambios en la comunidad en los suelos que recibieron efluentes (Habteselassie et al., 2013).

2.3.4. Riesgo sanitario asociado a la aplicación de efluentes de tambo al suelo

El ganado transporta en las excretas microorganismos patógenos que pueden contaminar alimentos y aguas y afectar la salud humana y animal. Si bien el riesgo del transporte de patógenos aumenta con el uso de excretas o efluentes vacunos frescos como fertilizante (Pandey et al., 2018; Peyton et al., 2016; Unc

y Goss, 2004; van Bruggen et al., 2019), su persistencia en el suelo y el transporte hacia cursos de agua depende en gran medida de las variables climáticas y agronómicas (Lang et al., 2003). Varios estudios reportan que los patógenos pueden sobrevivir por largos períodos en el suelo luego de la aplicación de efluentes (Gilchrist et al., 2007; Nicholson et al., 2005; Rogers et al., 2011; Sahlström, 2003). Algunos estudios postulan que *E. coli* y los enterococos pueden sobrevivir hasta diecinueve semanas luego del incorporado de excretas al suelo, mientras que otros indican sobrevivencias mayores, 42 y 49 semanas (Avery et al., 2004; Lau y Ingham, 2001; Manshadi et al., 2013).

En general, el riesgo de contaminación de recursos acuáticos aumenta si luego de que se aplicaron excretas o efluentes vacunos al suelo ocurrieron eventos de lluvias (Peyton et al., 2016). Además, las bajas temperaturas y la humedad favorecen la persistencia de la contaminación microbiológica (Franz et al., 2008; Goss et al., 1998; Jamieson et al., 2002).

Las formas de aplicación de los efluentes de tambo al suelo y el tipo de efluente (sólido o líquido) también inciden en la sobrevivencia de patógenos. En general, los efluentes líquidos permiten el movimiento de los microorganismos y, por lo tanto, tienden a contaminar más uniformemente que los sólidos. Además, se ha visto que, en efluentes estacionados por largos períodos de tiempo, la cantidad de bacterias fecales disminuye (Crane et al., 1983; Elliott y Ellis, 1977; Patni et al., 1985). Por otro lado, si estos se incorporan en el suelo, es menos probable que los microorganismos se mueran por acción de los rayos solares ultravioletas que si los efluentes se depositan en la superficie (Unc y Goss, 2004). Franz et al. (2008) modelaron factores del suelo que afectan la sobrevivencia de *E. coli* enterohemorrágica O157:H7 y sugieren que aplicar efluentes con mayor relación C:N afectaría negativamente la sobrevivencia de esta bacteria por crear ambientes más oligotróficos (por inmovilización temporal de N).

La mastitis es la principal enfermedad del rodeo lechero y se trata con antibióticos, como penicilina, si el agente es *Staphylococcus aureus* (De Los Santos et al., 2017). El contenido microbiano del estiércol tiene el potencial de

diseminar al ambiente patógenos zoonóticos y genes de resistencia antibiótica (Black et al., 2021).

En 2017 Umpiérrez et al. reportaron por primera vez en Uruguay la ocurrencia de genes de resistencia antibiótica en *E. coli* aislada de ganado. También se detectaron genes de resistencia antibiótica transferibles en *E. coli* aisladas de heces de terneros en nuestro país (Coppola et al., 2020). Los genes plasmídicos de resistencia a quinolonas detectados fueron *qnrS1* y *qnrES1* y, resistente a oximino-sulfonamidas, el gen de β-lactamasa $bla_{\text{CTX–M–15}}$. Dungan et al. (2018) evaluaron por qPCR la persistencia de genes de resistencia antibiótica en suelos regados con efluentes de tambo. Encontraron que los efluentes aumentaron el reservorio de genes de resistencia antibiótica en los suelos, mientras que esto no ocurría en aquellos regados con agua del canal. Algunos estudios sostienen que las bacterias derivadas del estiércol no persisten en el suelo mucho tiempo y que la aplicación de estiércol no afecta radicalmente el microbioma del suelo (Laconi et al., 2021).

3. Application of dairy effluents to pastures affects soil nitrogen dynamics and microbial activity

G. Illarze [1], A. del Pino [2], A. Rodríguez-Blanco [1] and P. Irisarri [1*]

[1] Laboratorio de Microbiología, Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

[2] Departamento de Suelos y Aguas, Facultad de Agronomía, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

3.1. Resumen

El uso de efluentes de lechería se ha convertido en una alternativa prometedora para aumentar el rendimiento de pasturas, al tiempo que se reduce el impacto ambiental de la acumulación de este deshecho en cursos de agua; no obstante, se deben considerar otras implicaciones ambientales. El presente estudio tuvo como objetivo evaluar el efecto de la aplicación de efluentes de tambo crudo o estabilizados en lagunas, en comparación con urea disuelta o un control, sobre la emisión de N_2O, la dinámica del nitrógeno en el suelo, la actividad microbiana, y el rendimiento y eficiencia de uso de nitrógeno de una pastura de festuca. La tasa normalizada de aplicación de N de 200 kg N ha^{-1} se dividió en cuatro eventos estacionales en un experimento de invernáculo. La producción forrajera de festuca similar entre los efluentes de tambo y la urea posicionó a los efluentes como una alternativa de disposición al suelo. La aplicación repetida del efluente crudo proporcionó más C orgánico al suelo, lo que indujo un aumento en las actividades enzimáticas, un cambio en la actividad catabólica de la comunidad microbiana del suelo y un mayor potencial de mineralización de N. Por otro lado, la aplicación de urea disminuyó la actividad funcional de la comunidad microbiana del suelo. Sin embargo, las emisiones de N_2O fueron mayores para el efluente crudo, por lo que el efluente estabilizado en lagunas es una alternativa para gestionar los efluentes de tambo en estas condiciones, evitando la aplicación de urea y mejorando la actividad metabólica del suelo.

Palabras clave: efluentes de tambo, óxido nitroso, mineralización de nitrógeno, CLPP, impacto ambiental

3.2. Summary

The use of farm dairy effluents (FDE) has become a promising alternative to increase pasture yield while reducing the environmental impact of waste accumulation into streams, but other environmental implications should be considered. The present study aimed to assess the effect of application of either raw FDE or lagoon-stored FDE compared to dissolved urea or a non-amended control on N_2O emission, soil N dynamics, functional microbial activity, and the yield and N-use efficiency of a fescue pasture. The normalized N application rate of 200 kg N ha^{-1} was divided into four seasonal events in a greenhouse experiment. Similar fescue forage production with FDE or urea positioned FDE application to soil as an alternative disposal. The repeated application of raw effluent delivered more organic C to the soil which induced an increase in enzyme activities, a shift in the catabolic activity of the soil microbial community, and greater N mineralization potential. On the other hand, urea addition decreased the functional activity of the soil microbial community. However, N_2O emissions were greater for the raw effluent, so lagoon-stored effluent is an alternative to manage FDE under these conditions, avoiding urea addition and enhancing soil metabolic activity.

Keywords: dairy effluents, nitrous oxide, nitrogen mineralization, CLPP, environmental impact

3.3. Introduction

Uruguay has a dairy herd of 759,000 and an annual milk production of 2.3 million tons, exporting 70% of its production [1]. Milk production in the country is predominantly pasture-based and managed with the input of N fertilizers [2]. In pasture-based systems, cows spend a few hours a day in the milking parlor or standing in the waiting yard. As a result, around 8100 million liters of farm dairy effluents (FDE) are generated annually in the country [3]. FDE contain a mixture of dairy cow feces and urine deposited during milking and subsequently diluted with wash-down water during the cleaning of the milking parlor [4].

In this predominantly pasture-based system, the dairy industry is increasingly attempting to maximize returns achievable through better utilization of grass with lesser inputs. Land application of FDE is a recommended practice in order to avoid direct discharge to waterways [5] and due to its recognized nutrient value, it reduces the need for purchased commercial fertilizer. This practice can provide organic matter for soil and pastures, recovering the nutrients from animal feeding within a framework of circular economy [6].

Milk production based mainly on grazing systems is important in Oceania and South American countries [7] and generates FDE with low dry matter content. The application of FDE for fertilization is a recommended practice to minimize reliance on synthetic chemical inputs [8] and to irrigate pastures or crops. In South America, FDE are used without treatment or with solid sedimentation and are sometimes stored in a two-lagoon system [9]. Generally, FDE are applied to the field by surface application (tanks or irrigation pumps) throughout the year [10]. In Australia, land application of FDE increased plant productivity [11] and injection and surface application were less prone to contaminating groundwater due to N losses [12].

Two-stage stabilization lagoons can remove significant amounts of suspended solids and organic contaminants, but may not be very effective in the removal of N and P [13]. Only considering N supply, inappropriate application rates or timing can lead to poor utilization by plants, causing nitrate leaching and

groundwater contamination, or surface water contamination. In addition, soil nitrogen imbalances can lead to gaseous losses as nitrous oxide (N_2O) emissions and volatilization of ammonia [14]. Agriculture is the principal source of N_2O in Uruguay [15], and so the abatement of this potent greenhouse gas emission is essential for the country and the sustainability of the dairy industry to continue exporting products [16].

FDE are a significant source of available N and labile C with a high water content that can induce anaerobiosis in soil after their application [17]. These aspects are some of the factors that control the emission of N_2O [18]. Hence, FDE application as an organic soil amendment can affect the soil's organic C pool, nutrient content, and microbial activities [19]. The quantity and quality of organic C in FDE, depending on biodegradability and C:N ratio, may influence soil microbial activity and function and, thus, the turnover of mineral N after FDE application to the soil-plant system [20].

Since the majority of soil functions are microbially mediated, the diversity of the microbial community is essential in maintaining functioning terrestrial ecosystems [21]. Microbial functional diversity is defined as "the sum of the ecological processes, and/or capacity to use different substrates developed by the organisms of a community" [22]. In the past decades, different methodological approaches have been used for monitoring microbial functional diversity; among them, the most common are enzyme activities and community-level physiological profiling (CLPP) techniques [23]. Soil CLPP analysis with Biolog ECO-platesTM has demonstrated repeatability, discriminating power, and sensitivity for many factors [24]. Functional diversity measures derived from using enzyme activities inform us on general soil biological functioning, including not only the actual living microbial activity, but also the past biochemical activity still operating within the soil matrix, while CLPP provides an instant photograph of microbial physiology [23,25].

Studies about dairy effluents' application to substitute N fertilization and the effect on the functional diversity of soil communities are scarce. Neufald et al. [26] reported that FDE, when compared to mineral fertilizer application,

increased soil microbial biomass and activity and changed the ratio of bacterial to fungal biomass and soil microbial community structure. Applying FDE can increase microbial biomass and activity in soils, while community structure and catabolic capability remain resilient [27] or change depending on the type of manure treatment and rate of irrigation [28]. Therefore, determining the changes in the soil microbial functional diversity and activity resulting from FDE application is important to evaluate the effect of effluents on soil health [29].

The present work aimed to improve the understanding of the impact of the productive use of dairy effluents on pasture yield, microbial activity, and functional diversity and assess the environmental concern related to nutrient losses as N_2O emissions. Through a greenhouse experiment, we compared the effects of seasonal application of equal amounts of total N as raw dairy effluent (RDE), lagoon-stored dairy effluent (LDE), or urea in pots planted with tall fescue (*Festuca arundinacea* Schreb.). We hypothesized that FDE application would promote microbial activity and soil N dynamics and, thus, increase pasture yield compared to synthetic fertilization. Simultaneously, RDE and LDE soil application may produce larger N_2O emissions than urea.

3.4. Materials and methods

3.4.1. Dairy effluent and Soil Collection

Dairy effluents were collected from the dairy farm of Centro Regional Sur from the Faculty of Agronomy (Universidad de la República) with geographic coordinates 34◦36047.8300 S and 56◦12054.0000 W. The RDE was flushed from the barn floor and sampled fresh from a holding tank after solid sedimentation. For the LDE sampling, a total of 2 L was collected from four different points (north, south, east, and west) of a facultative lagoon system on the same day. The samples were stored in a plastic container and refrigerated at 4 ◦C once they reached the laboratory. The FDE were collected at the start of the experiment (September 2018) and before each application to the greenhouse pot experiment

(November 2018, January 2019, and March 2019). Total N content was determined for both effluents at each sampling date.

The soil was collected from the topsoil layer (0–15 cm depth) of a field in the Faculty of Agronomy (34◦50 S, 56◦13 W). The soil was a Mollisol with 5.0%, 70.9%, and 24.1% proportions of sand, silt, and clay, respectively. The soil pH was 6.4, and the exchangeable cation contents were K, 2.0; Ca, 14.0; Mg, 3.1; and Na 0.3 cmol kg^{-1}.

3.4.2. Experimental Design

The greenhouse pot experiment was maintained for eight months. Pots containing 4 kg of homogenized soil were sown with *Festuca arundinacea* cv. Tacuabé (four plants per pot), whose seeds were supplied by the National Institute for Seeds (INASE). The experiment had a randomized block design with three replicates for soil analysis and five replicates for gas sampling. The N fertilizer treatments included four applications to pasture soil at an equivalent rate of 50 kg N ha^{-1}, i.e., 20 mg N kg of pot $soil^{-1}$ consisting of (1) LDE, (2) RDE, (3) synthetic nitrogen fertilizer (Urea), and (4) a non-amended control (Control). All applications were performed in solution adjusted to the same final volume per pot; the same amount of tap water (pH: 6.9, electric conductivity (EC): 2 mS cm^{-1}) was added to the control. The first application was at seeding. Consecutive fertilization/harvesting cycles of 45 days, simulating the typical management of fescue forage cuts, were performed three times. The final cut was performed two months after the last application of treatments. The fescue forage was cut manually at a height of 5 cm. The leachate was collected in trays beneath the pots and replenished to prevent nutrient loss by percolating the excess water when irrigated. During the whole trial period, each pot was weighed and watered to maintain 50% of the water-holding capacity when needed. Additionally, soil samples for some microbial activities and mineral N determinations were collected on the day of each treatment application and 7, 30, and 45 days after applications. The number of pots was considered to be

destructively sampled after 45 days of each treatment application. The samples were collected from ten random soil cores from depths of 0 to 10 cm of the upper soil layer from each replicated pot, homogenized by hand, composited, mixed thoroughly, and sieved (2 mm mesh). Soil samples were kept at 4 ◦C for enzymatic activities and microbial physiological profiling at the end of the experiment.

3.4.3. Characterization of Farm Dairy Effluents (FDE)

The pH and EC were measured in a mixture of effluent and deionized water (1:2.5 v/v) by potentiometry. Total solids (TS) and suspended solids (SS) were analyzed following the standard procedure of APHA [30]. Total organic carbon (TOC) was analyzed by oxidation with potassium dichromate following Mebius's technique [31]. Total nitrogen (TN) was analyzed by the Kjeldahl method [32]. Ammoniacal N (NH_4^+–N) in filtered suspensions was determined by colorimetric analysis according to Rhine et al. [33]. Organic N was calculated as TN content − NH_4^+–N.

3.4.4. Soil Chemical Analysis

At the end of the experiment, two months after the fourth application of FDE, soil samples were collected from each pot and chemical properties were determined. The soil was dried at 45 ◦C until a constant weight was reached. The soil's mineral N (NO_3^-–N and NH_4^+–N) was extracted with a 2 M KCl solution, and colorimetric analysis was used to determine NH_4^+–N according to Rhine et al. [33] and NO_3^-–N according to Mulvaney [34]. Soil organic carbon was quantified by the Walkley–Black wet combustion method [35]. N mineralization (sum N_min) was estimated by the sum of NO_3^-–N and NH_4^+–N content (mg pot^{-1}) plus the total N content in the aboveground biomass of fescue plants (mg pot^{-1}) as per Arló et al. [36].

3.4.5. Forage Yield and Nitrogen Fertilizer Replacement Value

The aboveground biomass obtained at the final forage cut was dried at 65 ◦C for 48–72 h (until a constant weight was reached) and ground to pass through a 0.5 mm mesh. After that, the total N content was determined in forage biomass by the Kjeldahl method [32].

N uptake was calculated as the sum of total N content in the forage biomass of each cut. As the same amount of N was applied in both forms of the amendment (either synthetic fertilizer or FDE), the N-use efficiency (NUE) per pot was calculated as the subtraction of N uptake of the control from those of each treatment divided by the applied N [37].

Nitrogen fertilizer replacement value (NFRV %) was calculated as NUE effluent/NUE synthetic fertilizer × 100 [37].

3.4.6. Nitrous Oxide Emission

N_2O fluxes were measured by the static chamber–gas chromatography method. The sampling chambers (diameter: 20 cm, height: 20 cm) were inserted 5 cm deep into the pot's soil. The lid was fitted with a sampling port with a three-way valve and placed on top of the box at the beginning of each gas sampling day, when temperatures were recorded. Headspace gas samples were obtained with airtight 20 mL propylene syringes and were immediately transferred to pre-evacuated 12 mL glass Exetainer® vials (Labco Ltd., Buckinghamshire, UK). The sampling times were 0 min, 20 min, and 40 min. The sampling was carried out periodically after every N addition event for at least 20 days. The N_2O concentrations were measured by gas chromatography on a GC-FID-mECD 7890 Agilent gas chromatograph with a HayeSep Q 80/100 mesh 1/8 column. To calculate gas flux rates from the soil's surface to the chamber atmosphere, a linear increase in gas concentration over time was assumed as described previously [38]. The cumulative N_2O emissions after each cut/N-added application were calculated by adding all mass flux values during the

measurement period (20 days after application). The net N_2O mass flux between two measurement dates was calculated as the mean flux values of the two dates multiplied by the number of days between these dates [39].

Yield-scaled N_2O flux for each N-added application refers to cumulative N_2O emissions divided by forage biomass production.

3.4.7. Potentially Mineralizable Nitrogen

Potentially mineralizable nitrogen (PMN) was determined using the anaerobic method [40]. The soil was made into a slurry and incubated at 40 ◦C and accumulation of soil PMN was determined after one week.

3.4.8. Soil Potential Nitrification Activity

The assay for potential nitrification activity (PNA) was carried out using a microscale method based on ISO 15685 [41]. Briefly, 2.5 g of fresh soil was placed in a 50 mL flask, and a solution with 300 mM KH_2PO_4, 700 mM K_2HPO_4, 10 mM sodium chlorate, and 1.5 mM $(NH_4)_2SO_4$ was added to create a 10 mL slurry. The reaction was incubated at 25 ◦C and shaken at 175 rpm. After 24 h, 1 mL samples were collected and added to 1 mL 2 M KCl, briefly vortexed, and centrifuged to separate soil particles. The resulting supernatant was removed and NO_2^- concentration was determined spectrophotometrically at 540 nm with Griess–Ilosvay reagent.

3.4.9. Soil Microbial Enzyme Activities

Dehydrogenase activity was determined using [2-(p-iodophenyl)-3-(p-nitrophenyl)- 5-phenyl tetrazolium chloride] solution (INT) as substrate, incubated for 2 h at 40 ◦C. The reduced iodonitrotetrazolium formazan (INTF) was extracted and photometrically measured at 464 nm [42].

The alkaline phosphatase enzyme was determined following Margesin [43] and expressed as µg p-nitrophenol (pNP) per gram of dry soil and incubation time.

Urease activity was determined by a modified method according to Kandeler [44]. Briefly, 5 g soil samples were incubated with 2.5 mL of urea solution at 37 ◦C for 2 h and the released NH_4^+ (extracted with 2 M KCl) was determined.

3.4.10. Microbial Community Physiological Profiling

CLPP of soil microbial populations was performed by the BIOLOG EcoPlates™ (Biolog®, Hayward, CA, USA) method based on carbon substrate utilization. Briefly, 10 g of soil was shaken for 30 min in 90 mL of sterile 0.01 M PBS and then allowed to settle for 10 min. Then, 125 µL of the supernatant was diluted 100-fold in sterile PBS, mixed, and finally used to inoculate the wells of the BIOLOG EcoPlates™. The plates were incubated at 25 ◦C and read every 24 h with a microtiter plate reader Multiskan FC (Thermo Scientific, Waltham, MA, USA) at 590 nm for six days. The optical density (OD) for each well was calculated by subtracting the control well values of each plate from the OD value of the well [45]. Microbial activity in each microplate/time point was expressed using average well color development (AWCD), calculated as the sum of wells with activity per plate, divided by the 31 carbon sources, and the general bacterial activity for each plate was calculated as the area under the curve (AUC).

Substrate richness, microbial functional diversity, and growth efficiency were calculated based on 96 h worth of data, using an OD of 0.5 as a threshold for positive response. The OD data for a given well were normalized by dividing by the mean absorbance for all wells [46]. Growth efficiency (GE) was calculated as total growth divided by richness.

The substrates were classified into six substrate guilds, namely amines, amino acids, carbohydrates, carboxylic acids, polymers, and phenolics,

according to Insam [47]. Then, mean well color development for each guild after 96 h was calculated.

3.4.11. Statistical Analysis

A general linear mixed model was fitted to N dynamics variables (mineral N, PMN, and PNA) and N_2O fluxes to compare differences between treatments and application dates, using treatment as a fixed effect in the analysis of variance with Infostat software [48]. Data were tested for normality using the Shapiro–Wilk test and, when not normally distributed, were log-transformed to meet normality assumptions. When log data did not have a normal distribution, they were analyzed using the non-parametric Kruskal–Wallis test followed by a Wilcoxon rank sum test to determine significant differences between treatments.

One-way ANOVA was used to analyze the effect of FDE on pasture yield and quality, and on the soil chemical and microbiological parameters at the end of the experimental period.

Differences between CLPP were analyzed with a multivariate analysis of variance (MANOVA) model using the ANOSIM test. In addition, the CLPP was further analyzed by principal component analysis (PCA) using a correlation similarity matrix with the ten carbon substrates that accounted for 50% of the variability (SIMPER test). PCA was carried out using the "prcomp" function in the ggplot2 package [49] of R software (version 4.1.0) (R Foundation for Statistical Analysis, Viena, Austria).

The least significant differences (LSD) at the level $p \leq 0.05$ was used to determine significance for all analyses. Pearson correlation was used to calculate the correlation coefficients of inorganic N ($NH_4^+ + NO_3^-$)–N, PMN, and PNA with N_2O fluxes at the third and fourth application of treatments.

3.5. Results

3.5.1. Effluent Characterization

Data on the physical properties and chemical composition of the FDE applied to the pots are summarized in Table 1.

The pH of both effluents was slightly basic and the RDE always presented lower pH values (7.3–8.4) for each sampling date than the LDE (8.3–8.5), which was more stable, but differences between FDE types were only significant in January and March ($p < 0.05$). Electrical conductivity was more or less stable for both dairy effluents across all sampling dates. RDE had significantly higher EC than LDE (on average 3.8 vs. 2.8 mS cm^{-1}), except for the September sample. Dry matter content in both FDE was generally low (less than 2.5%) and differed slightly between dates.

Table 1. Characterization of Raw dairy effluent (RDE) and Lagoon dairy effluent (LDE) applied to pots with fescue pasture (mean n = 3 ± S.E).

Parameter	Application dates							
	September		November		January		March	
	RDE	LDE	RDE	LDE	RDE	LDE	RDE	LDE
pH	8.1± 0.0 a	8.3± 0.0 a	8.5± 0.1 a	8.5± 0.0 a	8.1± 0.1 a	8.5± 0.1 b	7.3± 0.0 a	8.3± 0.1 b
Total solids %	0.24± 0.01 a	0.16± 0.03 a	1.06± 0.06 b	0.33± 0.01 a	0.97± 0.03 b	2.13± 0.20 a	0.61± 0.04 a	0.69± 0.19 a
Suspended solids %	0.11± 0.01 a	0.06± 0.01 a	0.46± 0.07 b	0.08± 0.01 a	1.23± 0.07 b	0.54± 0.01 a	0.40± 0.04 a	0.72± 0.16 b
CE mS cm^{-1}	2.8± 0.2 a	3.1± 0.3 a	4.0± 0.1 a	3.2± 0.3 b	4.6± 0.1 a	3.2± 0.0 b	2.8± 0.3 a	1.9± 0.1 b
Organic C mg L^{-1}	848± 097 b	316± 016 a	1825± 157 b	202± 072 a	1436± 068 a	1242± 135 a	1191± 139 b	340± 081 a
Kjeldahl N mg L^{-1}	205± 09 a	202± 03 a	399± 09 b	137± 16 a	517± 09 b	241± 22 a	364± 07 b	195± 13 a
Organic N mg L^{-1}	142± 04	92± 14	286± 05	91± 15	396± 09	188± 23	268± 07	151± 12
NH_4^+-N mg L^{-1}	63± 02 a	111± 17 b	80± 41 b	46± 02 a	122± 02 b	53± 01 a	96± 08 b	44± 01 a
C:N	6.0 b	3.7 a	6.4 b	2.4 a	3.6 a	7.0 b	4.5 b	2.3 a

Different letters for each parameter and sampling date indicate significant differences at $p < 0.05$ between RDE and LDE.

Total N was always higher for RDE than LDE (205–517 vs. 137–241 mg L^{-1}), although not significantly different in September; the highest N content was in the summer sample. The organic N fluctuated between 64 and 94% of the total N for the RDE and 70–82% for the LDE. The lowest % organic N/total N corresponded to the warmest sampling dates (January and March for the southern hemisphere). Most of the mineral N was presented as NH_4^+. The NH_4^+–N content in RDE was significantly higher than that in LDE, but in the September sample, the highest content was for LDE.

As is typical of RDE, organic C content was higher, with an average of 1325 mg L^{-1} (848–1825 mg L^{-1}), while LDE had an average of 287 mg L^{-1}, excluding the January sample, which exhibited an unexpectedly higher concentration (1242 mg L^{-1}). For the September sample (end of the cool season), RDE registered less total C than the rest of the sampling dates and the highest C content was registered in November samples. C:N ratios of RDE were high for the September–November samples compared to the January–March sampling dates and tended to be higher than those of LDE, excluding the January sampling date. In the summer samples, the higher organic C content was also correlated with higher dry matter content and total N and, thus, a higher C:N ratio.

Data regarding FDE characteristics corrected with the volume applied to the pots in each application season appear in Table S1.

3.5.2. Yield and N Uptake of *F. arundinacea*

The effect of FDE applications to fescue pasture on biomass production is shown in Table 2. Neither accumulated biomass nor N uptake of total pasture cuts were significantly different between treatments, although RDE and Urea presented higher average values of yield and N uptake.

At the end of the experiment, with data from the last application, though not significantly different ($p = 0.07$), the average NUE of LDE was the lowest (0.15 g N pot^{-1}) while that of Urea was 0.63 and that of RDE was 0.38 (Table 2).

The NFRV value was not significantly different between both FDE ($p = 0.13$), on average, it was 60% for RDE and 22% for LDE (Table 2).

Table 2. Total yield of *F. arundinacea,* N uptake, N use efficiency (NUE), and N fertilizer replacement values (NFRV%) from raw (RDE) and lagoon dairy effluents (LDE) or urea fertilizer amendment soil (mean n = 3 ± S.E).

Treatments	Total pasture yield (g DM pot^{-1}) *[1]	N uptake (mg pot^{-1}) *[1]	NUE[1]	NFRV (%)[1]
Control	26.44± 1.11	106.2± 0.06		
LDE	28.01± 0.53	117.6± 0.02	0.15 ± 0.03	22.4 ± 5.5
RDE	33.15± 1.02	136.8± 0.09	0.38 ± 0.19	60.5 ± 30.1
Urea	30.92± 5.18	156.8± 0.20	0.63 ± 0.43	

* Sum of four forage cuts [1] Not significantly different at $p < 0.05$

3.5.3. N_2O Fluxes and N Dynamics in Soils

N_2O fluxes peaked immediately after effluent application for up to 5 days for all the dates of application measured (Figure 1). For the first application, there was no detected difference in N_2O flux within treatments. After January applications, N_2O emissions increased significantly ($p < 0.05$) only in treatment RDE compared with the others on the day of application and remained elevated compared to control 4 and 6 days after application. Notably, N_2O fluxes from LDE were similar to those of RDE at 4 and at 6 days after application and were only greater than the control (Figure 1). After the last application (March), all N-amended treatments had greater N_2O emissions than the control.

Nevertheless, when considering cumulative emissions of N_2O-N for the 20 days after each application, only in the March application (the latter) RDE increased the N_2O fluxes over the other treatments (p = 0.03; Table S3). The yield-scaled N_2O emission for the March application ranged from 1.1 to 5.1 mg N_2O-N g forage^{-1} with RDE treatment presenting the highest value whereas there were no significant differences between treatments (Table S2).

There was a significant interaction effect between the sampling date and treatment on soil PMN, NH_4^+-N, and NO_3^--N content, indicating a varying response to the seasonal addition of N amendments measured over the 219-day experimental period (Figure 2).

LDE's first application significantly increased PMN but this effect disappeared thereafter (Figure 2a). Only after the fourth application, RDE significantly increased PMN with respect to the rest of the treatments. When considering cumulative PMN during the last application, RDE increased PMN with respect to control, urea and LDE and PMN declined in these treatments with respect to the rest of the applications (Table S3).

PNA was greater in soils with FDE compared with urea fertilization irrespective of the sampling date ($p < 0.0001$, Figure 2b). LDE increased PNA with respect to control soil, but no significant differences were detected between control and RDE-amended soil ($p < 0.05$). PNA significantly increased after the third and fourth application of treatments compared to the previous ones. When considering cumulative PNA at each application date, differences between soils with FDE and urea fertilization were more important in the last two applications (Table S3). Meanwhile, LDE increased cumulative PNA with respect to urea at the third and fourth application dates, RDE only did it at the fourth application, and no differences between FDEs and non-amended soil were detected (Table S3).

The soil NH_4^+-N content peaked immediately after treatment application but significantly increased in FDE with respect to the control only at the first and second applications (Figure 2c). In the third application, the NH_4^+-N content significantly increased only in RDE treatment and showed the highest levels.

The soil NO_3^--N content was significantly higher for the first two N amendment applications compared with the successive ones (Figure 2d). The application of RDE significantly increased the soil NO_3^--N content immediately after the third application, but no differences in NO_3^--N content between treatments were further detected.

Both NH_4^+-N and NO_3^--N content were higher in soils with N amendments 45 days after sowing with the first application of treatments, but this increase in soil mineral N disappeared following the successive amendment events. When considering accumulated mineral N during each application there was more mineral N available in the soil for the first application date than during the last two applications (Table S3). Accumulated mineral N in the first two application dates was higher for urea and LDE than RDE, and all the N amendment treatments increased mineral N compared to non-amended soil. On the other hand, at the second application, mineral N was significantly higher in urea and RDE compared to non-amended soil and there were no differences between both FDEs (Table S3). During the last two applications, there were no differences between treatments on accumulated soil mineral N.

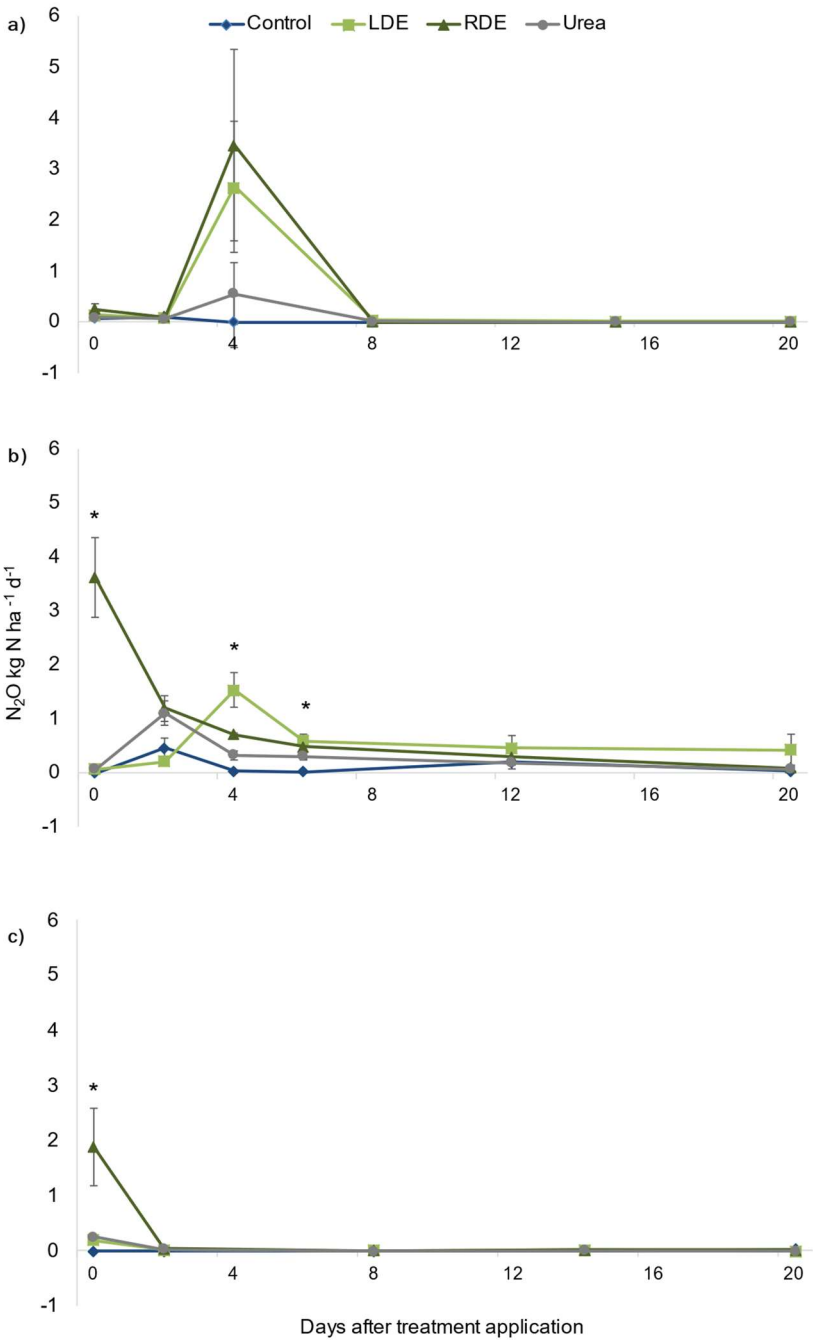

Figure 1. Evolution of N_2O fluxes after September (a), January (b) and March (c) application of Dairy effluents (DE), urea or non-amended control (mean n = 5 ± S.E). (November application was not measured). Asterisk indicate sampling dates with significant differences within treatments at $p < 0.05$.

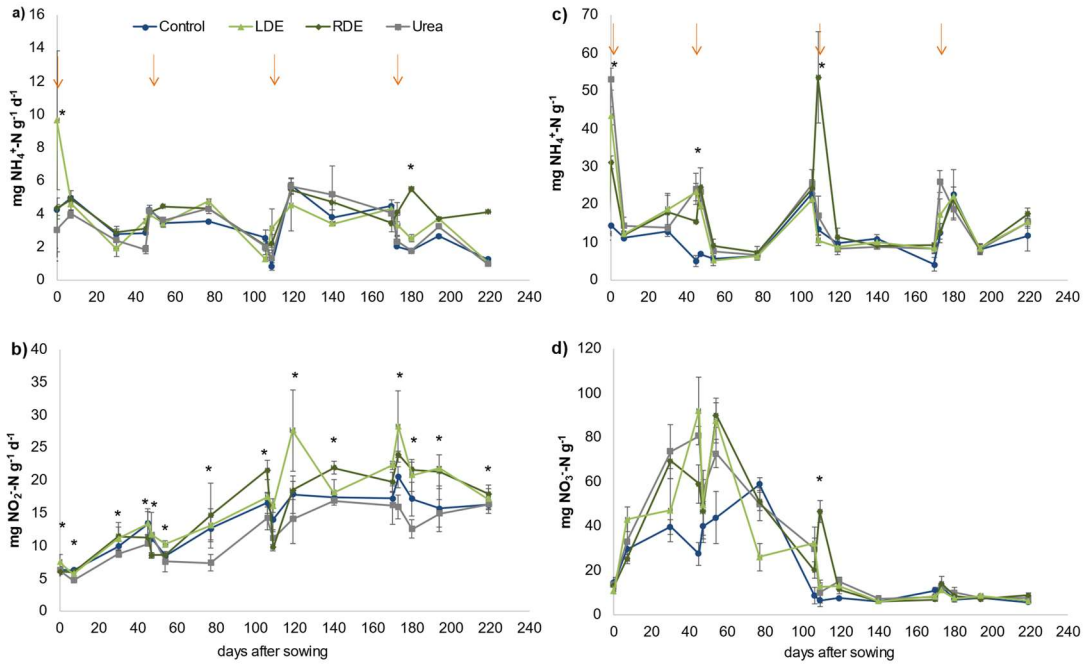

Figure 2. Nitrogen dynamics in soil with fescue pasture with application of Raw (RDE) or Lagoon Dairy effluent (LDE)), urea, and non-amended control (mean n = 3 ± S.E).) Potentially mineralizable nitrogen (a), Potential nitrification activity (b), Ammonium (NH_4^+-N) content (c), Nitrate (NO_3^--N) content (d). Arrows indicate treatment application. Asterisks indicate sampling dates with significant differences within treatments at $p < 0.05$.

Pearson's correlation analysis showed that inorganic N concentrations had a strong positive relationship with N_2O and NH_4^+-N with NO_3^--N (Table S4). Significant positive relationships were also observed between PMN and PNA (Table S4).

When considering the soil N mineralization as the total N content in pasture biomass plus the mineral N in the soil, RDE treatment increased the sum N_min compared to the control (Table S2).

3.5.4. Soil microbiological activities

The CLPP analysis carried out at the end of the experiment showed that the microbial activity of soil with urea addition was lower than those treated with FDE or control soil (AWCD, AUC, and GE, Table 3). FDE application did not increase the microbial activity with respect to the control soil. However, the indices of richness and diversity of FDE amended soil and the other treatments

were similar. Only, lower Shannon's evenness (E) in carbon source utilization was observed in LDE (Table 3).

Table 3. Substrate average colour development (AWCD), the area under the curve (AUC), growth efficiency (GE), Shannon diversity index (H´), substrate richness (SR), and Shannon's evenness (E) of the soil bacterial community of fescue pots after incubation for 96 h in Biolog EcoPlates with the application of RDE or LDE, urea and control (mean n = 3 ± S.E). Different letters beside the numbers of each column indicate significant differences at $p < 0.05$.

Treatments	AWCD	AUC	GE	H´	SR	E
Control	1.72± 0.06a	2.11± 0.09a	2.16± 0.05a	3.17± 0.03a	24.66± 0.67a	0.97± 0.00ab
LDE	1.75± 0.00a	2.21± 0.07a	2.06± 0.06a	3.23± 0.03a	26.33± 0.67a	0.96± 0.00a
RDE	1.73± 0.02a	2.22± 0.03a	2.06± 0.03a	3.23± 0.02a	26 ± 0.58a	0.98± 0.00b
Urea	1.51± 0.06b	1.8± 0.07b	1.9± 0.08b	3.17± 0.04a	24.66± 0.88a	0.97± 0.00ab

The PCA visualization of general patterns was done for the CLPP in order to determine the extent of differentiation of FDE application with regard to carbon source utilization. The PCA showed a shift in the pattern of substrate utilization along PC1 with RDE clearly separated from the other treatments (Figure 3). The first and second PC explained 68.9% of the data variance.

ANOSIM analysis supported this shift in carbon source utilization in response to soil RDE application (R = 0.73, p = 0.0002), indicating different functional diversity of soil microbiota.

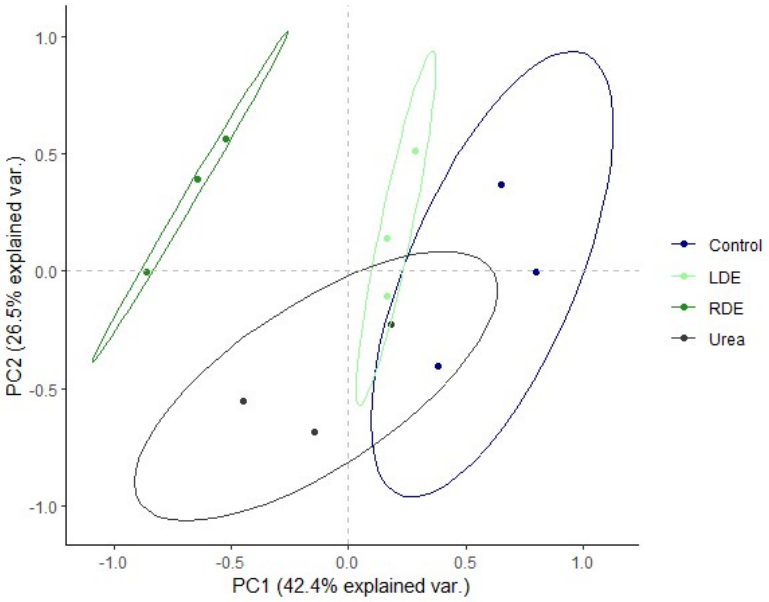

Figure 3. Principal component analysis of Biolog EcoPlates incubated for 96 h of pot soils with fescue pasture with the application of raw dairy effluent (RDE) or lagoon-stored (LDE), urea, or no fertilization (control). Ellipses indicate 95% confidence intervals.

To gain a better understanding of microorganism metabolism in response to FDE application, we characterized microbial community function based on sole carbon source group utilization patterns. Figure 4 shows the utilization of compounds in each of the grouped chemical classes.

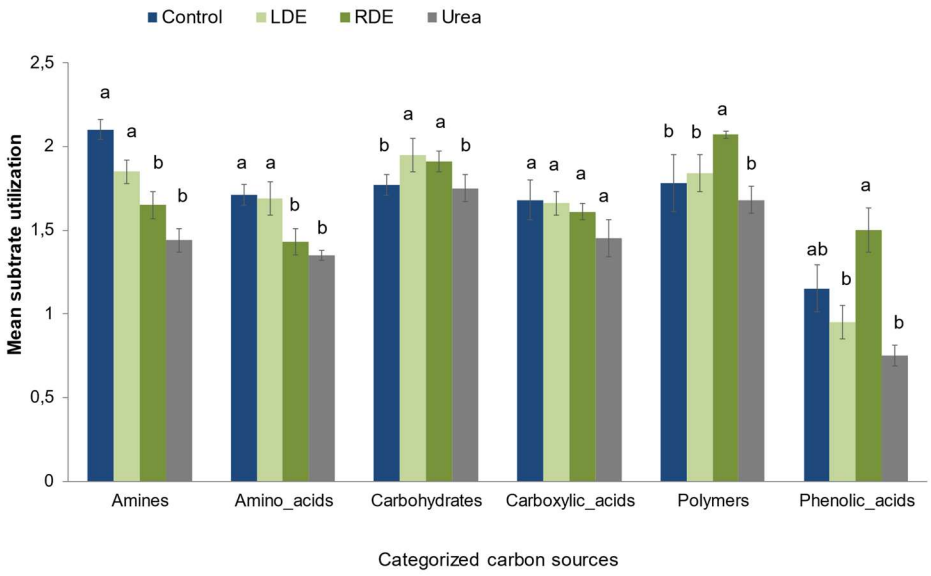

Figure 4. Substrate utilization patterns of different carbon sources by soil microbial community after 96 h of incubation in the Biolog EcoPlates with the application of RDE and LDE, urea or control to pots with fescue pasture. Values represent means ± standard error (n = 3). Significant differences between treatments in each carbon substrate` group utilization are indicated with different letters ($p < 0.05$).

There was a different pattern of carbon substrate utilization depending on the treatment applied to the soil. RDE application presented the soil microbial community with the greatest polymers and phenolic compounds utilization. On the other hand, LDE and non-amended soil significantly increased amines and amino acids utilization. Both RDE and LDE applications showed greater carbohydrate utilization by the soil microbial community. Except for RDE, the rest of the treatments showed that the phenolic acids were the microorganisms´least used carbon source ($p < 0.05$).

The microbial functional activity of fescue soil treated with FDE was also assessed by key enzymatic activities. Compared with the control soil, applications of RDE increased enzymatic activities, while LDE did not differ (Figure 5). Urease activity was also increased in urea-fertilized soil compared to control and LDE soils.

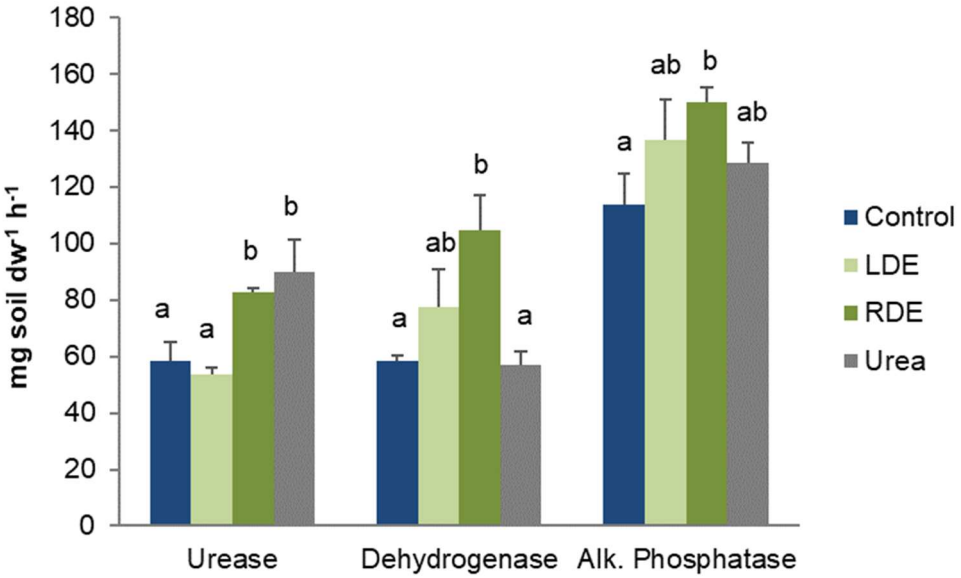

Figure 5. Enzyme activities of soil with fescue pasture with application of raw (RDE) or lagoon stored dairy effluent (LDE), urea, or control to pots with fescue pasture. Values represent means ± standard error (n = 3). Different letters between treatments indicate statistically significant differences ($p < 0.05$).

3.6. Discussion

As an alternative to discharge to waterways, the application of dairy effluents to agricultural land is encouraged by the regulatory organisms of Uruguay. However, few studies in the country focus on the effects of dairy effluent application to pastures on N dynamics including N_2O emission, and soil microbial activity.

3.6.1. Dairy effluents as N-fertilizers

One of the challenges of FDE reutilization as a pasture N-supplier is its variable composition, complicating the prediction of its agronomic value. Compositional variations of FDE are likely due to the time of milking, the age and breed of the herd, feed quality, wash water management, and the time relative to lactation [4]. The RDE presented the highest C:N ratio except for the summer sample (Table S1). An increase in the nutrient content of dairy-effluent lagoons in summer (January) has been previously reported [50]. Anyway,

organic C content was generally higher in RDE than in LDE, accounting for one of the expected lagoon purposes. The organic N in both effluents was higher than ammonia-N except for LDE in September (Table 1), being organic N, the main N source added to the soil with FDE applications. The FDE composition had similar characteristics as other dairy grazing systems like those in New Zealand [51].

Luo et al. [52] reported in a meta-analysis that organic amendments increase crop yields compared to mineral-only fertilization. In the current study, although control pots had the lowest biomass yield and N uptake at the end of the experiment, those values were not significantly different from the fertilized ones, either with urea or with FDE (Table 2). The high fescue yield of the control treatment was likely due to soil characteristics such as high organic matter and nutrient content. This kind of soil with high initial N and P content can be found in the dairy basin of Uruguay. Nevertheless, there was a clear tendency to higher yield values with the application of urea and RDE compared to the control. In accordance with these results, FDE application to land may be an alternative final destination without affecting forage production. On the other hand, FDE application to pastures may be used as a fertilizer substitute if farmers take into account the nutrient content of the effluents. Pagliari et al. [8] recommend to test FDE for nutrient availability as close to field application as possible. It is worthwhile to note that being mainly a source of organic N, RDE showed a similar performance in maintaining pasture production and N uptake than urea, although organic, behaves in the soil as mineral fertilizer. This further indicates that RDE has the potential to be used as a nutrient source, especially of N [53] with similar NUE as urea (Table 2) and a similar nitrogen replacement value (NFRV). However, the great variability between pots may not have registered the differences while LDE tended to have lower NUE than RDE.

Otherwise, the application of FDE to pastures may also cause micronutrients or Cu and Zn accumulation from manure or food additives. Manure is also a potential source of pathogens, antibiotics and antibiotic resistant bacteria [54].

3.6.2. Effect of farm dairy effluent application on soil N dynamics and N_2O emission

The organic N added to the soil with the 4 repeated FDE applications was greater for RDE than LDE (Table S1) and only RDE showed higher estimated net N-mineralization (sum N_min) than the control (Table S2). The different organic nitrogenous compounds present in both effluents and their different organic C content can partially explain this result as well. The potential N-mineralization activity of the soils was highest for RDE after the repeated application (Figure 4c and Table S2). The pulse of this effluent addition to the soil can elicit responses in the decomposer community that can use this new organic matter substrate without inducing a 'positive priming' effect on native soil organic matter [55] as soil organic C did not decrease at least in the short period evaluated (Table S2). The narrow C:N ratio within the FDE assured available N to plants after their application, as N immobilization may not be predominant [56].

Both, RDE and LDE had higher PNA than urea and nitrification was particularly noticeable after 3 or 4 repeated FDE applications where the rhizosphere:bulk soil ratio may be higher, according to Rudisill et al. [57]. However, potential activity differs from the actual *in situ* rate due to the broad physiological diversity of ammonia oxidizers [58]. Furthermore, soil higher NO_3^- content in RDE-treated soils was measured at one of the sampling dates (Figure 2b), leaving more N available for lixiviation, leaching, or denitrification apart from plant uptake [59]. Adequate use of dairy effluents must aim at optimal use of their nutrients that in the case of N, also may be lost via gaseous emissions of which NH_3 and N_2O are of particularly environmental concern. Although NH_3 volatilization was not measured in the current study, the liquid application of FDE with low dry matter content and rapid infiltration in the soil make NH_3 losses less prone in this case [60]. Soil NO_3^- and NH_4^+ content was also highly

related to N_2O emissions (Table S3) which suggests N_2O was the main source of N losses in this case.

Oxidation of NH_3 by bacteria and archaea is partially responsible for global emissions of N_2O directly as a by-product of NH_3 oxidation, and indirectly through the provision of nitrite that is oxidized to NO_3^- and can be denitrified [61]. Nitrification and denitrification, the main processes associated with N_2O emission in agricultural soils, are influenced by agricultural management practices [62]. One of the main reasons for N_2O emissions from agricultural soils is the application of N-fertilizer or amendments when plants cannot uptake all the applied nitrogen. An increase in soil N content correlated with subsequent N_2O losses (Table S2). Denitrification seems to be more relevant as a N_2O source than nitrification when studying different agroecosystems [63] and in grassland soils following manure application [64]. The presence of available C in FDE may enhance N_2O fluxes, as most denitrifiers are heterotrophs. The peak of N_2O registered immediately after treatment application could be a product of the O_2 limitation caused by irrigation [65]. The highest N_2O emission with repeated irrigation with RDE coincided with the highest mineralization. This latter process is mainly an O_2 -demanding heterotrophic process and has a higher dependence on the rhizosphere because its oxic/anoxic interface offers a favourable habitat for coupled nitrification–denitrification [66].

However, greenhouse gases emitted during FDE storage have to be taken into account to recommend the type of effluent to be used for land application [67], showing the need for life-cycle assessments. In the case of replacing urea with FDE as N supply for plants, the fossil energy required for urea synthesis and the consequent greenhouse gasses emissions will be avoided.

3.6.3. Effect of farm dairy effluent application on soil microbial functional diversity and activity

Our study showed that soil microbial community functional activity with addition of synthetic fertilizer was lower than that with FDE application. This is

in accordance with Bonanomi et al. [68] who reported higher AWCD values in the soil treated with organic materials than with synthetic fertilizers. Urea application also decreased enzymatic activity with respect to FDE (Fig 5) except for urease. Meanwhile, RDE increased dehydrogenase and alkaline phosphatase enzyme activities compared to the control soil (Figure 5). The higher dehydrogenase enzyme activity, which is a representation of total microbial activity, was probably a result of the more readily available C pool because of RDE addition to the soil [69]. Luo et al. [52] reported in a meta-analysis that organic amendments increase enzymatic activity compared to mineral-only fertilization and Shi et al. [28] demonstrated that raw dairy effluent application increased soil microbial biomass and enzyme activity. Soil enzymes mediate organic matter decomposition, transformation, and mineralization and play an important role in nutrient cycling [70]. In this case, enzyme activities were good indicators of organic matter decomposition, being higher for RDE which also stimulates N mineralization. However, LDE application was not associated with higher enzyme activity as has been reported for organic amendments [52,53].

The soil after repeated RDE application presented a differentiated substrate utilization pattern (Figure 3). As has been previously reported [71], soil management can influence belowground microbial communities through multiple factors including the type and amount of C inputs. The addition of RDE to the soil with higher organic C content than LDE can keep a more even distribution (Table 3) of the catabolic structure of the soil community [72]. Frąc et al. [73] reported that the Biolog EcoPlates^{TM} assay allowed the detection of changes in the microbial functional diversity after dairy sewage sludge application. Yet, the limitations of the Biolog assay may be considered and better referred to as the catabolic activity of fast-growing or eutrophic bacteria [72].

RDE stimulated polymers and phenolic compounds` use (Figure 4). Gallet et al. [74] showed phenolic compounds were 15% of manure DOC and among them, syringic acid, p- hydroxybenzoic acid, and p-hydroxy benzaldehyde were the main components. This capacity of the microbial community to mineralize

specific C substrates may reflect differences in the type, abundance, and availability of C sources present in the amendment [75].

3.7. Conclusions

The current study covered four complete growth–cut fescue cycles. The repeated application of RDE delivered more organic C and N to the soil than LDE. These inputs induced a shift in the catabolic activity of the soil microbial community and, thus, a greater N mineralization potential in RDE-amended soils. These indicators pointed to RDE as an amendment that can improve nutrient cycling and soil quality. Nevertheless, the trade-offs with other sustainability indicators such as N_2O emission, which was greater during RDE treatment, must be considered. The application of FDE following partial treatment in stabilization lagoons to a fescue pasture substitutes urea as a N source without increasing N_2O emissions and enhances soil biological activity compared to synthetic fertilizers.

3.8. References

1. INALE. Situación y perspectiva de la lechería Uruguaya. 2021. Available online: https://www.inale.org/informes/informe-2021/ (accessed on 1 December 2022).
2. Fariña, S. R.; Chilibroste, P. Opportunities and Challenges for the Growth of Milk Production from Pasture: The Case of Farm Systems in Uruguay. *Agric. Syst.* **2019**, *176*, 102631. https://doi.org/10.1016/j.agsy.2019.05.001.
3. DIEA. Anuario Estadístico Agropecuario. Montevideo. MGAP. 2021. Available online: https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/comunicacion/publicaciones/anuario-estadistico-agropecuario-2021 (accessed on 1 December 2022).
4. Hawke, R. M.; Summers, S. A.; Summers, S. A. Effects of Land Application of Farm Dairy Effluent on Soil Properties: A Literature Review. **2010**, *8233*. https://doi.org/10.1080/00288233.2006.9513721.
5. DINAMA. Manual para la gestión ambiental de tambos. Miniesterio de Ambiente. 2016. Available online: https://www.gub.uy/ministerio-ambiente/comunicacion/publicaciones/manual-para-gestion-ambiental-tambos (accessed on 1 December 2022).
6. Ellen McArthur Foundation. Towards the circular economy: an economic and business rationale for an accelerated transition. 2013. Available online: http://www.ellenmacarthurfoundation.org/publications (accessed on 1 December 2022).
7. Herrero, M. A.; Palhares, J. C. P.; Salazar, F. J.; Charlón, V.; Tieri, M. P.; Pereyra, A. M. Dairy Manure Management Perceptions and Needs in South American Countries. *Front. Sustain. Food Syst.* **2018**, *2*, 1–15. https://doi.org/10.3389/fsufs.2018.00022.
8. P.H, P.; M, W.; H.M, W.; Z, H. Nitrogen and Phosphorus Characteristics of Beef and Dairy Manure. In *Animal Manure: Production, Characteristics, Environmental*

Concerns, and Management; HM, W., PH, P., Z, H., Eds.; ASA Special Publications 67: Madison, WI USA, 2020; pp 45–62.
9. INALE. Cartilla sobre criterios de aplicación de efluente a terreno y su implicancia práctica en el diseño e implementación. 2020. Available online: https://www.inale.org/informes/criterios-de-aplicacion-de-efluente-a-terreno-y-su-implicancia-practica-en-el-diseno-e-implementacion/ (accessed on 1 December 2022).
10. Salazar, F.; Herrero, M. A.; Charlón, V.; La Manna, A. Slurry Management in Dairy Grazing Farms in South American Countries. *Proc. 14th Int. Conf. FAO ESCORENA Netw. Recycl. Agric. Munic. Ind. Redidues Agric.* **2010**, 1–4.
11. Hagare, D.; Hong, W. T.; Siddiqui, Z.; Natarajan, S. K.; Fyfe, J. Effect of Dairy Pond Sludge/Supernatant Application on Ryegrass Dry Matter Yield and Phosphorus Fractions in Soil. *Agric.* **2022**, *12*, 1–12. https://doi.org/10.3390/agriculture12030351.
12. Cameron, K. C.; Rate, A. W.; Noonan, M. J.; Moore, S.; Smith, N. P.; Kerr, L. E. Lysimeter Study of the Fate of Nutrients Following Subsurface Injection and Surface Application of Dairy Pond Sludge to Pasture. *Agric. Ecosyst. Environ.* **1996**, *58*, 187–197. https://doi.org/10.1016/0167-8809(96)01029-8.
13. Wang, H., Magesan, G. N., & Bolan, N. S. An Overview of the Environmental Effects of Land Application of Farm Effluents. *New Zeal. J. Agric. Res.* **2004**, *47*, 389–403. https://doi.org/https://doi.org/10.1081/ESE-120039387.
14. Saggar, S.; Bolan, N. S.; Bhandral, R.; Hedley, C. B.; Luo, J. A Review of Emissions of Methane, Ammonia, and Nitrous Oxide from Animal Excreta Deposition and Farm Effluent Application in Grazed Pastures. *New Zeal. J. Agric. Res.* **2004**, *47*, 513–544. https://doi.org/10.1080/00288233.2004.9513618.
15. Watch, C. Agriculture. 2020. Available online: https://www.climatewatchdata.org/sectors/agriculture#drivers-of-emissions (accessed on 1 December 2022).
16. Chilibroste, P. A Major Challenge for the Uruguayan Dairy Industry Sustainable Growth. *Agrociencia Uruguay* **2021**, *25*, 25–27. https://doi.org/10.31285/agro.25.970.
17. Bhandral, R.; Bolan, N. S.; Saggar, S.; Hedley, M. J. Nitrogen Transformation and Nitrous Oxide Emissions from Various Types of Farm Effluents. *Nutr. Cycl. Agroecosystems* **2007**, *79*, 193–208. https://doi.org/10.1007/s10705-007-9107-5.
18. Chirinda, N.; Carter, M. S.; Albert, K. R.; Ambus, P.; Olesen, J. E.; Porter, J. R.; Petersen, S. O. Emissions of Nitrous Oxide from Arable Organic and Conventional Cropping Systems on Two Soil Types. *Agric. Ecosyst. Environ.* **2010**, *136*, 199–208. https://doi.org/10.1016/J.AGEE.2009.11.012.
19. Ge, G.; Li, Z.; Fan, F.; Chu, G.; Hou, Z.; Liang, Y. Soil Biological Activity and Their Seasonal Variations in Response to Long-Term Application of Organic and Inorganic Fertilizers. *Plant Soil* **2010**, *326*, 31–44. https://doi.org/10.1007/s11104-009-0186-8.
20. Iocoli, A.; Orden, L.; Fernando, M. L.; Marisa, A. G.; Villamil, B.; Zabaloy, C. Towards Sustainable Dairy Production in Argentina: Evaluating Nutrient and CO2 Release from Raw and Processed Farm Waste. *Agronomy* **2021**, *11*, 1–19. https://doi.org/https://www.mdpi.com/2073-4395/11/12/2595.
21. Delgado-Baquerizo, M.; Maestre, F. T.; Reich, P. B.; Jeffries, T. C.; Gaitan, J. J.; Encinar, D.; Berdugo, M.; Campbell, C. D.; Singh, B. K. Microbial Diversity Drives Multifunctionality in Terrestrial Ecosystems. *Nat. Commun.* **2016**, *7*, 1–8. https://doi.org/10.1038/ncomms10541.
22. Insam, H.; Parkinson, D.; Domsch, K. H. Influence of Macroclimate on Soil Microbial Biomass. *Soil Biol. Biochem.* **1989**, *21*, 211–221. https://doi.org/10.1016/0038-0717(89)90097-7.
23. Moscatelli, M. C.; Secondi, L.; Marabottini, R.; Papp, R.; Stazi, S. R.; Mania, E.; Marinari, S. Assessment of Soil Microbial Functional Diversity: Land Use and Soil Properties Affect CLPP-MicroResp and Enzymes Responses. *Pedobiologia (Jena).* **2018**, *66*, 36–42. https://doi.org/10.1016/j.pedobi.2018.01.001.
24. Rutgers, M.; Wouterse, M.; Drost, S. M.; Breure, A. M.; Mulder, C.; Stone, D.; Creamer, R. E.; Winding, A.; Bloem, J. Monitoring Soil Bacteria with Community-

Level Physiological Profiles Using Biolog™ ECO-Plates in the Netherlands and Europe. *Appl. Soil Ecol.* **2016**, *97*, 23–35. https://doi.org/10.1016/j.apsoil.2015.06.007.
25. Nannipieri, P.; Giagnoni, L.; Renella, G.; Puglisi, E.; Ceccanti, B.; Masciandaro, G.; Fornasier, F.; Moscatelli, M. C.; Marinari, S. Soil Enzymology: Classical and Molecular Approaches. *Biol. Fertil. Soils* **2012**, *48*, 743–762. https://doi.org/10.1007/s00374-012-0723-0.
26. Neufeld, K. R.; Grayston, S. J.; Bittman, S.; Krzic, M.; Hunt, D. E.; Smukler, S. M. Long-Term Alternative Dairy Manure Management Approaches Enhance Microbial Biomass and Activity in Perennial Forage Grass. *Biol. Fertil. Soils* **2017**, *53*, 613–626. https://doi.org/10.1007/s00374-017-1204-2.
27. Liu, Y. Y.; Haynes, R. J. Influence of Land Application of Dairy Factory Effluent on Soil Nutrient Status and the Size, Activity, Composition and Catabolic Capability of the Soil Microbial Community. *Appl. Soil Ecol.* **2011**, *48*, 133–141. https://doi.org/10.1016/j.apsoil.2011.03.014.
28. Shi, Y.; Ziadi, N.; Hamel, C.; Bittman, S.; Hunt, D.; Lalande, R. Soil Microbial Biomass, Activity, and Community Composition as a Ff Ected by Dairy Manure Slurry Applications in Grassland Production. *Appl. Soil Ecol.* **2017**, *125*, 97–107. https://doi.org/10.1016/j.apsoil.2017.12.022.
29. Fox, E. M.; Fanning, S.; Corsetti, A.; Jordan, K. Microbial Food Safety along the Dairy Chain. *Front. Microbiol.* **2017**, *8*, 1–2. https://doi.org/10.3389/fmicb.2017.01612.
30. APHA. *Standard Methods for the Examination of Water and Wastewater.*, 21th ed.; American Public Health Association: Washington (DC), 2005.
31. Mebius, L. J. A Rapid Method for the Determination of Organic Carbon in Soil. *Anal. Chim. Acta* **1960**, *22*, 120–124. https://doi.org/10.1016/S0003-2670(00)88254-9.
32. Bremner, J. M.; Mulvaney, C. S. Total Nitrogen. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; Page, AL, RH Miller, and D. K., Ed.; Amer. Soc. Agron.: Madison, WI USA, 1996; pp 595–624.
33. 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. https://doi.org/10.2136/sssaj1998.03615995006200020026x.
34. Mulvaney, R. L. Nitrogen—Inorganic Forms. In *Methods of soil analysis. Part 3. Chemical Methods*; Page, AL, RH Miller, and D. K., Ed.; Amer. Soc. Agron.: Madison, WI USA, 1996; pp 1123–1184.
35. Nelson, D. W.; Sommers, L. E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of soil analysis: Part 3 Chemical methods*; Sparks, D. L., Ed.; Soil Science Society of America and American Society of Agronomy: Madison, WI USA, 1996; pp 961–1010.
36. Arlo, L.; Beretta, A.; Szogi, A. A.; del Pino, A. Biomass Production, Metal and Nutrient Content in Sorghum Plants Grown on Soils Amended with Sewage Sludge. *Heliyon* **2022**, *8*, e08658. https://doi.org/10.1016/j.heliyon.2021.e08658.
37. Baral, K. R.; Labouriau, R.; Olesen, J. E.; Petersen, S. O. Agriculture, Ecosystems and Environment Nitrous Oxide Emissions and Nitrogen Use Ef Fi Ciency of Manure and Digestates Applied to Spring Barley. *"Agriculture, Ecosyst. Environ.* **2017**, *239*, 188–198. https://doi.org/10.1016/j.agee.2017.01.012.
38. Tarlera, S.; Capurro, M. C.; Irisarri, P.; Scavino, A. F.; Cantou, G.; Roel, A. Yield-Scaled Global Warming Potential of Two Irrigation Management Systems in a Highly Productive Rice System. *Sci. Agric.* **2016**, *73*, 43–50. https://doi.org/10.1590/0103-9016-2015-0050.
39. Bowden, D.; Melillo, M.; Steudler, A. Effects of Nitrogen Additions on Anual Nitrous Oxide Fluxes from Temperate Forest Soils in the Northeastern United States. *J. Geophys. Res.* **1991**, *96*, 9321–9328.
40. Kandeler, E. Nitrogen Mineralization. In *Methods in soil biology*; Schinner, F., Ohlinger, R., Margesin, R., Kandeler, E., Eds.; Springer: Berlin, Heidelberg, 1996; pp 135–143.
41. Hoffmann, H.; Schloter, M.; Wilke, B. M. Microscale-Scale Measurement of Potential

Nitrification Rates of Soil Aggregates. *Biol. Fertil. Soils* **2007**, *44*, 411–413. https://doi.org/10.1007/s00374-007-0227-5.
42. von Mersi, W.; Schinner, F. An Improved and Accurate Method for Determining the Dehydrogenase Activity of Soils with Iodonitrotetrazolium Chloride. *Biol. Fertil. Soils* **1991**, *11*, 216–220. https://doi.org/10.1007/BF00335770.
43. Margesin, R. Acid and Alkaline Phosphomonoesterase Activity with the Substrate P-Nitrophenyl Phosphate. In *Methods in soil biology*; Schinner, F., Ohlinger, R., Margesin, R., Kandeler, E., Eds.; Springer: Berlin, Heidelberg, 1996; pp 2013–2016.
44. Kandeler, E. Urease Activity by Colorimetric Technique. In *Methods in soil biology*; Schinner, F., Ohlinger, R., Margesin, R., Kandeler, E., Eds.; Springer: Berlin, Heidelberg, 1996; pp 171–174.
45. Garland, J. L.; Mills, A. L. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. *Appl. Environ. Microbiol.* **1991**, *57*, 2351–2359. https://doi.org/10.1128/aem.57.8.2351-2359.1991.
46. Christian, B. W.; Lind, O. T. Key Issues Concerning Biolog Use for Aerobic and Anaerobic Freshwater Bacterial Community-Level Physiological Profiling. *Int. Rev. Hydrobiol.* **2006**, *91*, 257–268. https://doi.org/https://doi.org/10.1002/iroh.200510838.
47. Insam, H. A New Set of Substrates Proposed for Community Characterization in Environmental Samples. In *Microbial communities: functional versus structural approaches*; Insam, H., Rangger, A., Eds.; Springer: Berlin, Heidelberg, 1997; pp 259–260. https://doi.org/10.1016/B978-008045405-4.00652-2.
48. Di Rienzo, J. A.; Casanoves, F.; Balzarini, M. G.; González, L.; Tablada, M.; Robledo, Y. C. InfoStat Versión 2020. Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina: Córdoba 2017. http://www.infostat.com.ar.
49. Wickham, H.; Chang, W.; Wickham, M. H. Package 'Ggplot2.' *Create elegant data visualisations using the grammar of graphics.* 2016, pp 1–189.
50. Demanet, R.; Aguilera, M.; Mora, M. Efecto de La Aplicación de Purines Sobre El Sistema Suelo-Planta. *Front. Agrícola* **1999**, *5*, 87–94.
51. Longhurst, R. D.; Roberts, A. H. C.; O'Connor, M. B. Farm Dairy Effluent: A Review of Published Data on Chemical and Physical Characteristics in New Zealand. *New Zeal. J. Agric. Res.* **2000**, *43*, 7–14. https://doi.org/10.1080/00288233.2000.9513403.
52. Luo, G.; Li, L.; Friman, V. P.; Guo, J.; Guo, S.; Shen, Q.; Ling, N. Organic Amendments Increase Crop Yields by Improving Microbe-Mediated Soil Functioning of Agroecosystems: A Meta-Analysis. *Soil Biol. Biochem.* **2018**, *124*, 105–115. https://doi.org/10.1016/J.SOILBIO.2018.06.002.
53. Zaman, M.; Cameron, K. C.; Di, H. J.; Inubushi, K. Changes in Mineral N, Microbial Biomass and Enzyme Activities in Different Soil Depths after Surface Applications of Dairy Shed Effluent and Chemical Fertilizer. **2002**, 275–290.
54. H, Z.; F, V.; J, A.; C, G. Temporal Changes of Manure Chemical Compositions and Environmental Awareness in the Southern Great Plains. In *Animal Manure: Production, Characteristics, Environmental Concerns, and Management*; H.M, W., P.H, P., Z, H., Eds.; ASA Special Publications 67: Madison, WI USA, 2020; pp 15–26.
55. Kok, D. D.; Scherer, L.; de Vries, W.; Trimbos, K.; van Bodegom, P. M. Relationships of Priming Effects with Organic Amendment Composition and Soil Microbial Properties. *Geoderma* **2022**, *422*, 115951. https://doi.org/10.1016/J.GEODERMA.2022.115951.
56. Amon, B.; Kryvoruchko, V.; Amon, T.; Zechmeister-Boltenstern, S. Methane, Nitrous Oxide and Ammonia Emissions during Storage and after Application of Dairy Cattle Slurry and Influence of Slurry Treatment. *Agric. Ecosyst. Environ.* **2006**, *112*, 153–162. https://doi.org/10.1016/J.AGEE.2005.08.030.
57. Rudisill, M. A.; Turco, R. F.; Hoagland, L. A. Fertility Practices and Rhizosphere Effects Alter Ammonia Oxidizer Community Structure and Potential Nitri Fi Cation Activity in Pepper Production Soils. *Appl. Soil Ecol.* **2016**, *99*, 70–77. https://doi.org/10.1016/j.apsoil.2015.10.011.

58. Hazard, C.; Prosser, J. I.; Nicol, G. W. Use and Abuse of Potential Rates in Soil Microbiology. *Soil Biol. Biochem.* **2021**, *157*, 108242. https://doi.org/10.1016/J.SOILBIO.2021.108242.
59. Calderón, F. J.; McCarty, G. W.; Reeves, J. B. Analysis of Manure and Soil Nitrogen Mineralization during Incubation. *Biol. Fertil. Soils* **2005**, *41*, 328–336. https://doi.org/10.1007/s00374-005-0843-x.
60. Clough, T. J.; Balaine, N.; Cameron, K. C.; Petersen, S. O.; Sommer, S. G. Effects of Dairy Shed Effluent Dry Matter Content on Ammonia and Nitrous Oxide Emissions from a Pasture Soil. *J. Agric. Sci.* **2018**, *156*, 1070–1078. https://doi.org/10.1017/S0021859618001028.
61. Prosser, J. I.; Hink, L.; Gubry-Rangin, C.; Nicol, G. W. Nitrous Oxide Production by Ammonia Oxidizers: Physiological Diversity, Niche Differentiation and Potential Mitigation Strategies. *Glob. Chang. Biol.* **2020**, *26*, 103–118. https://doi.org/10.1111/gcb.14877.
62. Hofstra, N.; Bouwman, A. F. Denitrification in Agricultural Soils: Summarizing Published Data and Estimating Global Annual Rates. *Nutr. Cycl. Agroecosystems* **2005**, *72*, 267–278. https://doi.org/10.1007/s10705-005-3109-y.
63. Liang, D.; Robertson, G. P. Nitrification Is a Minor Source of Nitrous Oxide (N2O) in an Agricultural Landscape and Declines with Increasing Management Intensity. *Glob. Chang. Biol.* **2021**, *27*, 5599–5613. https://doi.org/10.1111/gcb.15833.
64. Nguyen, Q. Van; Wu, D.; Kong, X.; Bol, R.; Petersen, S. O.; Jensen, L. S.; Liu, S.; Brüggemann, N.; Glud, R. N.; Larsen, M.; Bruun, S. Effects of Cattle Slurry and Nitrification Inhibitor Application on Spatial Soil O2 Dynamics and N2O Production Pathways. *Soil Biol. Biochem.* **2017**, *114*, 200–209. https://doi.org/10.1016/J.SOILBIO.2017.07.012.
65. Bourdin, F.; Sakrabani, R.; Kibblewhite, M. G.; Lanigan, G. J. Effect of Slurry Dry Matter Content, Application Technique and Timing on Emissions of Ammonia and Greenhouse Gas from Cattle Slurry Applied to Grassland Soils in Ireland. *Agric. Ecosyst. Environ.* **2014**, *188*, 122–133. https://doi.org/10.1016/J.AGEE.2014.02.025.
66. Wei, X.; Zhu, Z.; Wei, L.; Wu, J.; Ge, T. Biogeochemical Cycles of Key Elements in the Paddy-Rice Rhizosphere: Microbial Mechanisms and Coupling Processes. *Rhizosphere* **2019**, *10*, 100145. https://doi.org/10.1016/J.RHISPH.2019.100145.
67. Laubach, J.; Heubeck, S.; Pratt, C.; Woodward, K. B.; Guieysse, B.; Van Der Weerden, T. J.; Chung, M. L.; Shilton, A. N.; Craggs, R. J. Review of Greenhouse Gas Emissions from the Storage and Land Application of Farm Dairy Effluent. *New Zeal. J. Agric. Res.* **2015**, *58*, 203–233. https://doi.org/10.1080/00288233.2015.1011284.
68. Bonanomi, G.; Filippis, F. De; Zotti, M.; Idbella, M. Repeated Applications of Organic Amendments Promote Bene Fi Cial Microbiota , Improve Soil Fertility and Increase Crop Yield. *Appl. Soil Ecol.* **2020**, *156*, 103714. https://doi.org/10.1016/j.apsoil.2020.103714.
69. Watts, D. B.; Torbert, H. A.; Feng, Y.; Prior, S. A. Soil Microbial Community Dynamics as Influenced by Composted Dairy Manure, Soil Properties, and Landscape Position. *Soil Sci.* **2010**, *175*, 474–486. https://doi.org/10.1097/SS.0b013e3181f7964f.
70. Lladó, S.; Baldrian, P. Community-Level Physiological Profiling Analyses Show Potential to Identify the Copiotrophic Bacteria Present in Soil Environments. *PLoS One* **2017**, *12*, e0171638. https://doi.org/10.1371/journal.pone.0171638.
71. Liao, J. D.; Boutton, T. W. Soil Microbial Biomass Response to Woody Plant Invasion of Grassland. *Soil Biol. Biochem.* **2008**, *40*, 1207–1216. https://doi.org/10.1016/J.SOILBIO.2007.12.018.
72. Dan, W. E. I.; Qian, Y.; Jun-zheng, Z.; Shuang, W.; Xue-li, C.; Xi-lin, Z. Bacterial Community Structure and Diversity in a Black Soil as Affected by Long-Term Fertilization. *Pedosph. An Int. J.* **2008**, *18*, 582–592. https://doi.org/10.1016/S1002-0160(08)60052-1.
73. Frąc, M.; Oszust, K.; Lipiec, J. Community Level Physiological Profiles (CLPP), Characterization and Microbial Activity of Soil Amended with Dairy Sewage Sludge.

2012, 3253–3268. https://doi.org/10.3390/s120303253.
74. Gallet, C.; Boissier, J.-M.; Berlandis, M. Short-Term Effects of Manure Application on Soil Leachates in a Mountain Catchment. *Agronomie* **2003**, *23*, 335–344. https://doi.org/10.1051/agro:2003006.
75. Banning, N. C.; Lalor, B. M.; Cookson, W. R.; Grigg, A. H.; Murphy, D. V. Analysis of Soil Microbial Community Level Physiological Profiles in Native and Post-Mining Rehabilitation Forest: Which Substrates Discriminate? *Appl. Soil Ecol.* **2012**, *56*, 27–34. https://doi.org/10.1016/J.APSOIL.2012.01.009

4. Effect of untreated and stabilized dairy effluent applications on soil fertility and associated health risks

Illarze G[1], del Pino A[2], Azzíz G[1], Irisarri P[1*]

[1] Laboratorio de Microbiología, Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

[2] Departamento de Suelos y Aguas, Facultad de Agronomía, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

4.1. Resumen

La intensificación de la producción lechera en Uruguay genera grandes volúmenes de residuos orgánicos de difícil manejo y con potenciales riesgos sanitarios. El riego con efluentes de tambo (DE) es una práctica recomendada para mejorar la fertilidad del suelo. Este estudio evaluó la aplicación de DE crudos (RDE) y estabilizados en dos lagunas (LDE) sobre *Festuca arundinacea,* comparado con la fertilización con urea y un control, sobre el contenido de nutrientes de suelo y planta, indicadores de bacterias patógenas y persistencia de genes de resistencia antimicrobiana. En un ensayo de invernadero se realizaron cuatro aplicaciones estacionales con una dosis total equivalente a 200 kg N ha^{-1}. El contenido de Na del suelo aumentó después de las aplicaciones. La aplicación de DE no aumentó el contenido de nutrientes de festuca. La persistencia de *E. coli* fue baja pero detectable con RDE. Los genes beta-lactámicos *bla*TEM y *bla*OXA presentes en ambos DE, no se detectaron en el suelo. La aplicación de DE mostró efectos comparables a los del control y la urea sobre el contenido de nutrientes de la festuca y modificó levemente la química del suelo. LDE redujo la carga de bacterias patogénicas, subrayando la seguridad de su aplicación.

Palabras clave: aplicación de efluentes de lechería, bacterias indicadoras de patogenicidad, fertilidad del suelo, genes de resistencia antimicrobiana, biomasa vegetal

4.2. Summary

The intensification of dairy production in Uruguay generates increasing volumes of organic waste that are difficult to handle and may entail health risks. Irrigation with farm dairy effluents (DE) is a recommended agricultural practice to improve soil fertility. The objective of this work was to evaluate the effect of the application of raw (RDE), two-lagoon stabilized effluents (LDE), urea fertilization, or non-amendment to *Festuca arundinacea* on soil and plant nutrient content, as well as pathogenic bacterial indicators and persistence of antimicrobial resistance genes. In a greenhouse trial, four seasonal applications were made with a total dose equivalent to 200 kg N ha^{-1}. Soil Na content increased after DE applications. There was no increase in the nutrient content of tall fescue caused by DE applications. The persistence of *E. coli* was low but still detected in soil receiving RDE. Beta-lactam genes *bla*TEM and *bla*OXA were detected in both DE, being highest in LDE. However, they were not detected in the soil. DE applications demonstrated effects comparable to those of control and urea fertilization on tall fescue biomass and nutrient content and slightly altered the soil's chemical status. LDE reduced pathogenic bacteria loading to the soil underlining the safety of its application.

Keywords: antibiotic resistance genes, dairy effluent application, plant biomass, pathogenic bacterial indicators, soil fertility

4.3. Introduction

In Uruguay, a large part of dairy production is carried out in small farms, where the family develops most of the activities. These farmers need more investment capacity to install complex effluent handling systems, which include time-consuming management[1][2]. In parallel, commercial fertilizers are applied to crops and pastures intended for animal feed. In this context, the productive use of effluents and their contribution to nutrients can play a crucial role in ensuring the sustainability of the dairy production system, avoiding environmental risks derived from inadequate management practices[1][2].

The effluents are generated during cleaning facilities (milking parlor, waiting, and feeding pens). Because a large volume of water is used (between 30 and 50 L/cow/day)[3], the effluents have low concentrations of organic matter (OM) and nutrients[4]. However, since much of the nutrients are in readily available forms, effluent irrigation can meet the nutritional requirements of crops and pastures through repeated applications[5][6]. It should also be noted that the effluents provide small amounts of secondary nutrients and micronutrients, which can contribute to the sustainability of the production system, replacing the exports made with products[7].

One of Uruguay's most common effluent management systems consists of lagoon storage, to be later used for irrigation. After washing the facilities, the effluents are generally channeled to a solids retention system and are then led to the pools or lagoons. Treatment systems with several lagoons were used in the past, the first being very deep for the anaerobic treatment of effluents. This treatment aimed to reduce the organic load of the effluents, which were discharged into watercourses. Direct discharge into watercourses is not allowed, lessening the need for treatment. Due to the high cost involved in constructing and maintaining large pools or lagoons, storage capacity is often limited, and therefore, storage time is relatively short. It should be noticed, however, that a wide range of situations can be found, from applying effluents immediately after generation to storing effluents in lagoons that are removed after several months.

The characteristics of the storage system, especially the residence time, influence the biological processes and, therefore, the characteristics of the material that will be utilized for irrigation. While fresh effluents generally have higher OM contents, effluents stored for some time have undergone stabilization processes. During storage, the mineralization of the most labile fractions of organic substances occurs, releasing inorganic forms of the nutrients and decreasing carbon content[8][9]. Indeed, it has been reported that by treating effluents in anaerobic lagoons, the pathogen load and the viability of weed seeds can be reduced, although with variable results, depending on the management[10].

Due to its origin, with a significant manure component, dairy effluents carry many microorganisms, some of them pathogens. This represents a dissemination risk when crops and pastures are irrigated with effluents, especially those intended for direct grazing with lactating cows[11]. Generally, it is recommended to impose restrictions to observe a specific waiting time between applying effluents and grazing[12]. However, measurements of pathogen survival in the dairy production systems in Uruguay are scarce, and given the expected climate and soil influence, it is important to provide local assessments.

Bacterial resistance to antibiotics has also become a severe problem among pathogenic bacteria, which has led to an increased concern surrounding environmental risks and the potential spread of antibiotic resistance among microorganisms[13]. Resistance is typically common, where antibiotics are heavily used. However, in Uruguay, the use of antibiotics as growth promoters for cattle is banned, and they are used in dairy farms mainly for mastitis cases[14]. Antibiotics are not completely metabolized in the body, and a significant fraction of the antibiotics administered to humans and animals are excreted unchanged (17%–90%)[15]. Once an antibiotic is released into the environment, its behaviour and fate will be determined by its intrinsic properties, soil characteristics, and weather conditions.

Antibiotic resistance selection occurs among gastrointestinal bacteria, which are also excreted in manure and stored in lagoon systems. Although it is well known that dairy manure storage reduces pathogen number, it is unclear

what effect storage treatments have on antibiotic resistance genes (ARGs) persistence in dairy effluents.

This work aimed to evaluate the effects of the application of untreated dairy effluents and a two-lagoon stabilized system to tall fescue in a greenhouse experiment examining the soil and plant biomass nutrient content, the potential sanitary risks, by microbial measurements and the persistence of some ARGs.

4.4. Materials and methods

4.4.1. Experimental design

The greenhouse pot experiment was maintained for eight months. The soil was collected from the topsoil layer (0-15cm depth) of a field in the Faculty of Agronomy (34°36' 48" S, 56° 12' 54" W). The soil was a Mollisol with 5.0 %, 70.9 %, and 24.1 % of sand, silt, and clay, respectively. The soil pH was 6.4, and the exchangeable cation content was K 2.0, Ca 14.0, Mg 3.1, and Na 0.3 cmolc kg^{-1}.

Pots containing 4 kg of homogenized soil were sown with *Festuca arundinacea* cv. Tacuabé (four plants per pot), whose seeds were supplied by the National Institute for Seeds (INASE). The experiment had a randomized block design with three replicates, according to Illarze [(4)]. The N fertilizer treatments included four applications to the soil at an equivalent rate of 50 kg N ha^{-1}, i.e., 20 mg N kg of pot $soil^{-1}$ consisting of (1) two-lagoon stabilized dairy effluent (LDE), (2) raw dairy effluent (RDE), (3) synthetic nitrogen fertilizer (urea) and (4) a non-amended soil (control). All applications were performed in solution, adjusting the same final volume per pot, and the same amount of tap water (pH 6.9, electric conductivity (EC) 2 mS cm^{-1}) was added to the control. The first application was at seeding. Consecutive fertilization/harvesting cycles of 45 days were performed three times, simulating the typical management of tall fescue forage cuts. The final cut was done two months after the last application of treatments. The tall fescue forage was cut manually at 5 cm height.

Dairy effluents were collected from the dairy farm of Centro Regional Sur from the Faculty of Agronomy with geographic coordinates 34°36' 47.83" S and 56° 12' 54.00" W. The collection of effluents and their characterization is described in Illarze [4]. Although variable in composition throughout the year, dairy effluents had a total solid content lower than 2% and suspended solids less than 1%. The organic carbon (OC) content of RDE was higher than that of LDE except in the January samples [4]. The RDE applied in the experiment presented an average pH of 8.0, 1325 mg L^{-1} of total carbon, 371 mg L^{-1} of total N, and 98 mg L^{-1} of total ammoniacal N, and LDE had an average pH 8.4, 425 mg L^{-1} of total carbon, 194 mg L^{-1} of total N, and 63 mg L^{-1} of total ammoniacal N.

The selected farm followed the usual production practices in the country and used beta-lactam antibiotic agents to treat bovine mastitis.

4.4.2. Chemical soil analysis

At the end of the experiment, after two months of the fourth application of DE, soil samples were collected from each pot, and chemical properties were determined. The soil was dried at 45 °C until a constant weight was reached. The total exchangeable bases, Ca, Mg, K, and Na, were extracted with ammonium acetate buffered at pH 7[16]. The K and Na were determined by atomic emission spectrometry, and Ca and Mg by absorption spectrometry. The available P content was determined by the Bray 1 method [17].

4.4.3. Soil basal respiration

Soil samples were collected immediately after and at 7, 30, and 45 days of DE applications and stored at 5°C before analysis.

The soil basal respiration was measured by titration according to Öhlinger [18] with slight modifications. 15 g of soil were incubated in a closed vessel for 10 days at 25 °C in the dark. The CO_2 was trapped in NaOH solution (0.25 M) and titrated against 0.1 HCl after freshly prepared saturated $BaCl_2$ (0.5 M) was

added. The respiration rate was expressed as mg of CO_2 g^{-1} dry soil d^{-1}. Cumulative basal respiration until 45 days of each application date was calculated by adding all mg CO_2 g^{-1} dry soil d^{-1} mean values for each sampling date multiplied by the number of days between these dates.

4.4.4. Chemical analysis of tall fescue

The aboveground biomass obtained at the final herbage cut was dried at 65 °C for 48–72 h (until constant weight) and ground to pass through a 0.5-mm mesh. After that, the total N content was determined by the Kjeldahl method [19]. P, K, Ca, Mg, and Na were extracted with dilute HCl (20 %) in samples calcined at 550 °C for 5 h. The molybdate blue method was used to determine the total P with ascorbic acid [20]. The Ca and Mg elements were determined by atomic absorption spectrometry, and K and Na by atomic emission spectrometry [21].

4.4.5. Quantification of pathogenic bacterial indicators

Total coliforms and *E. coli* numbers were determined in DE by a ten-fold dilution series of 10 mL of effluent in 90 mL of 0.01 M phosphate buffer followed by agitation for 30 min in an orbital shaker at 200 rpm. The diluted samples were plated in triplicate on 3M™ *E. coli*/ Coliform Petrifilm™. After incubation at 37 °C for 24 h, characteristic blue colonies were counted as *E. coli* and red as total coliforms (CFU/100 mL).

Total coliforms and *E. coli* numbers were determined in the soil immediately after DE application and 45 days after each application and expressed as $\log_{10}$ CFU g^{-1} dry soil. For data analysis, the number of viable bacteria below the detection limit (10 CFU g^{-1} soil) was set at 1 log CFU g^{-1} dry soil. The *E. coli* and Total coliforms survival results were calculated as C/Co values, where Co is the applied concentration in the irrigation solution and C is the concentration in the soil after 45 days of each application of dairy effluents.

4.4.6. DNA extraction and quantification of ARGs

Total DNA was extracted from the dairy effluents and soil samples using the Qiagen DNeasy PowerSoil Pro Kit (Qiagen, Carlsbad, CA, USA). For soil, 0.25 g samples were processed according to the manufacturer's instructions. In dairy effluent samples, 50 mL were centrifuged in 50 mL Falcon tubes at 10,000 × g for 10 minutes at room temperature to obtain pellets before bead beating.

The DNA concentration and purity were determined with NanoDrop® 2000c UV–vis spectrophotometry (Thermo Scientific, USA). The extracted DNA samples were stored at − 20 °C before PCR amplification.

Three beta-lactam resistance genes (*bla*TEM, *bla*OXA, and *bla*SHV) were chosen to assess the presence of the genes in the samples and their quantification.

Primers for each gene were those published in Dallenne and others [22]. For *bla*TEM: forward primer 5'-CAT TTC CGT GTC GCC CTT ATT C-3' and reverse primer 5'- CGT TCA TCC ATA GTT GCC TGA C-3'; for *bla*OXA: forward primer 5'-GGC ACC AGA TTC AAC TTT CAA G-3' and reverse primer 5'-GAC CCC AAG TTT CCT GTA AGT G-3'; for *bla*SHV: forward primer 5'-AGC CGC TTG AGC AAA TTA AAC-3' and reverse primer 5'-ATC CCG CAG ATA AAT CAC CAC-3'.

Purified PCR products for each of the genes analyzed were used as templates for the calibration curves. The calibration curves consisted of five ten-fold dilutions of the template; each dilution point was amplified by triplicate. The DNA concentration of the templates was obtained by spectrofluorometry (QuBit™, ThermoFischer).

The qPCRs for each gene were run separately and not in a multiplex fashion. The Maxima SYBR Green qPCR Master Mix was used (ThermoFischer). The mix for each qPCR assay contained 2 μL of the extracted DNA and 10 mM of each primer. Runs were performed on a Rotor-Gene-Q Pure Detection machine manufactured by QIAGEN. The cycling conditions were an initial denaturing step of 10 minutes at 95 °C, followed by 40 cycles of 15 seconds at 95 °C, 20 seconds at 60 °C, and 20 seconds at 72 °C; the fluorescence

was measured at the end of each cycle. A melting curve was performed at the end of the run beginning fluorescence measurement at 72 °C and incrementing 1 °C in each step until 95 °C.

4.4.7. Statistical analysis

One-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test was used to examine the effects of treatments on the soil chemical composition, plant yield and nutrition, bacterial indicators, and the abundance of ARGs. Each parameter was tested for normality of distribution and variance homogeneity using Shapiro-Wilk's test and Levene's test, respectively. When transformed data, such as pH, was also not normal, it was analyzed using the non-parametric Kruskal-Wallis test followed by a Wilcoxon Rank Sum test to determine significant differences between treatments.

Data were expressed as means ± standard error. All statistical analyses were performed using Infostat[23], and the significance level was considered at $p< 0.05$.

4.5. Results and Discussion

4.5.1. The effect of DE applications on soil chemical properties and plant biomass and nutrition

In general, the soil application of organic amendments like DE increases its fertility, especially N, P, and K [24][25][26][27][28]. In this pot trial, with an N fertilization dose equivalent to an annual 200 kg N ha^{-1}, the content of exchangeable cations, such as Na, K, and Mg, increased in the soil after LDE applications relative to the control (Table 1). Additionally, Na and NO_3^--N content increased in soil with RDE applications relative to the control soil (Table 1).

Table 1. Soil properties of fescue pots with four repeated applications of Raw (RDE) or Lagoon Dairy Effluent (LDE), Urea or non-amended soil at the end of the experiment (mean n=3 ± S.E). Different letters within a column indicate significant differences between treatments ($p< 0.05$).

Treatment	pH	OC%	NO_3^--N (mg kg^{-1})	NH_4^+-N (mg kg^{-1})	P-Bray1 (mg kg^{-1})	Ca^{++} ($cmol_c$ kg^{-1})	Mg^{++} ($cmol_c$ kg^{-1})	K^+ ($cmol_c$ kg^{-1})	Na^+ ($cmol_c$ kg^{-1})
Control	7.4± 0.4 **b**	3.0± 0.1	5.7± 0.1 **a**	11.8± 4.0	78.3± 4.1	14.2± 0.3	3.6± 0.1 **a**	1.5± 0.0 **a**	1.0± 0.0 **a**
Urea	7.0± 0.0 **a**	3.1±0.1	7.5± 0.2 **ab**	15.5± 1.6	80.0± 3.5	14.4± 0.3	3.6± 0.0 **ab**	1.4± 0.0 **a**	1.1± 0.1 **a**
RDE	7.1± 0.2 **ab**	3.1± 0.1	8.7± 1.1 **b**	17.6± 1.4	77.5± 2.2	14.2± 0.2	3.6± 0.1 **ab**	1.5± 0.1 **a**	1.5± 0.0 **b**
LDE	7.1± 0.2 **ab**	3.0± 0.1	6.2± 0.3 **a**	15.4± 1.0	76.7± 9.8	13.8± 0.2	3.8± 0.0 **b**	1.8± 0.1 **b**	1.5± 0.1 **b**

High concentrations of K present a challenge in achieving an agronomic balance of nutrients when applying effluent to land [29], potentially causing an imbalance in Mg and Ca in animals. A study on the land application of two types of DE demonstrated that both plant biomass and soil K levels increased under effluent treatments, as the amount of K applied in DE exceeded tall fescue maintenance requirements at rates above 100 kg N/ha/year [30]. The increase of K levels in soils after the addition of LDE may then pose a problem for land application; however, in the current case, the nutrient content in the plant biomass with applications of DE did not exceed that of the control or the urea treatments (Table 2). The grass stagger index (GSI = $[K^+]/([Ca^{2+}] + [Mg^{2+}])$) used as an index of the nutritive quality of forage, was very low and never exceeded 2.2, pointed as the risk of hypomagnesemia [31].

Long-term application of effluents may lead to the accumulation of exchangeable Na in the soil. Although monovalent cations are generally less strongly held on cation exchange sites than divalent ones and do not pose a risk to the receiving soil, a decrease in exchangeable Ca and Mg has been reported [31]. This effect cannot be detected in this pot trial but must be considered in the field.

While this study focused on N, dairy manure also contained high levels of P, although the final soil P content was not modified by DE addition (Table 1). In this case, the high nutrient status of the soil used for this pot trial could have masked the effect of DE nutrient input on the soil.

Dairy effluents typically exhibit alkalinity, and numerous indicate that effluent irrigation increases soil pH in acid soils [32]. In this study, the soil pH was close to neutrality, and the application of DE did not affect soil pH relative to the control soil (Table 1). Conversely, urea fertilization led to a decrease in soil pH. This acidification effect due to excessive use of synthetic N fertilizer has previously been reported [33].

The percentage of OC input from RDE generally surpassed that from LDE [4], and the application of DE contributes to a long-term increase in the soil OC content [32]. Even though our results did not reveal an increase in soil OC content (Table 1), the cumulative basal respiration at the last application of LDE was significantly higher. There was a similar tendency for RDE in the preceding two applications (Figure 1).

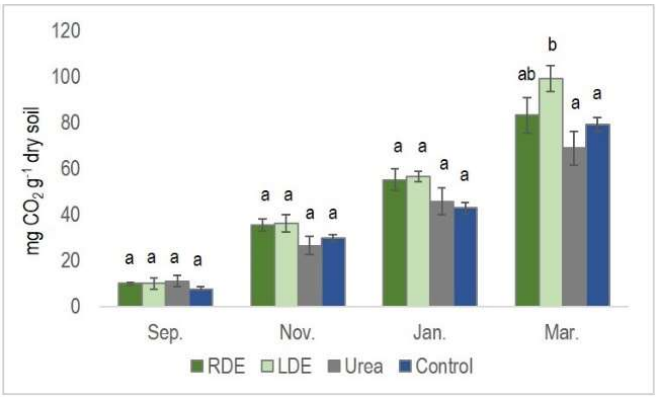

Figure 1. Cumulative basal respiration of soil with the application of Raw Dairy Effluent (RDE) or Lagoon Dairy Effluent (LDE), Urea, and non-amended soil at the four application events. Columns represent means ± standard errors (n=3). Columns with different letters indicate a significant difference between treatment each sampling date ($P< 0.05$).

The cumulative basal respiration of the soil, considering all four applications in the control and urea treatment, averaged less than 40 mg CO_2 g^{-1} dry soil, whereas in the LDE and RDE treatments, it ranged from 46 to 50 mg CO_2 g^{-1} dry soil. This finding aligns with Liu and others [34], who reported that

soil basal respiration with organic amendments like farmyard manure and green manure was higher than with NPK treatment.

Soil respiration, widely employed to quantify microbial activities in soil [35][36], generally exhibits a positive correlation with soil OM content [36]. In accordance, the application of these two DE in the same pot experiment showed an enhancement in microbial activity [4].

The application of organic materials with a low dry matter content, as observed in our case, resulted in few measurable changes in soil properties in the short term [37] and may induce a priming effect, leading to the mineralization of native soil OM and the potential decrease of soil OC [38]. Therefore, the increase in soil basal respiration obtained in this study may be attributed to the priming effect induced by the OC input from DE.

The tall fescue yield at the fourth cut of the experiment was higher for RDE than for LDE application (Table 2). However, neither the N content of the plant biomass nor the N uptake after repeated applications of DE differed from urea fertilization or the control (Table 2). In this same pot trial, the authors of the present study verified that the repeated applications of these DE did not result in detectable changes in N uptake or yield (sum of four herbage cuts) [4]. Furthermore, this study evaluated the chemical composition of other crucial macronutrients in plant biomass that showed similar levels between treatments. These findings once again highlight the basal nutrient status of the soil as the factor responsible for masking the effect of DE or urea fertilization on plant nutrient content and yield.

Table 2. Yield, N uptake and nutrient content of *F. arundinacea* grown in pot with application of Raw (RDE) or Lagoon Dairy Effluent (LDE), urea or non-amended soil (mean n=3 ± S.E), at the last forage cut. Different letters within a column indicate significant differences between treatments ($p< 0.05$).

Treatment	Pasture yield (g DM pot^{-1})	N uptake (g pot^{-1})	N (g kg^{-1})	P (g kg^{-1})	Ca^{++} (g kg^{-1})	Mg^{++} (g kg^{-1})	K^{+} (g kg^{-1})	Na^{+} (g kg^{-1})
Control	17.3± 9.8 **ab**	0.35± 0.03	20.1± 1.9	3.6± 0.3	4.0± 0.0	3.8± 0.2	15.4± 1.1	1.9± 0.3
Urea	20.1± 2.2 **ab**	0.44± 0.07	21.9± 1.2	2.4± 0.4	3.4± 0.3	3.4± 0.2	17.6± 0.4	1.1± 0.1
RDE	20.8± 2.7 **b**	0.41± 0.01	19.7± 0.4	3.0± 0.2	3.7± 0.1	3.7± 0.2	20.3± 2.5	1.5± 0.4
LDE	16.8± 5.2 **a**	0.32± 0.03	19.1± 1.6	3.3± 0.2	3.9± 0.2	3.8± 0.1	17.1± 0.9	1.9± 0.2

4.5.2. Bacterial indicators of soils with DE applications

By determining the number of pathogenic bacterial indicators in DE and assessing the spread and survival in the soil, we aimed to estimate the health risks associated with the land disposal of DE.

Previous research has demonstrated that the number of faecal bacteria in manure slurries can be reduced through long-term storage [39]. Consistent with this, our current study revealed that the Total coliforms and *E. coli* numbers were higher for RDE than for LDE, except for the September sampling date (Figure 2). The difference in Total coliform number between both effluents reached its maximum in November (2.0E+08 vs 1.7E+06), and for *E. coli* in January and March (on average 3.5E+07 vs 2.0E+05). These results indicate a substantial removal of enteric bacterial pathogens through lagoon storage.

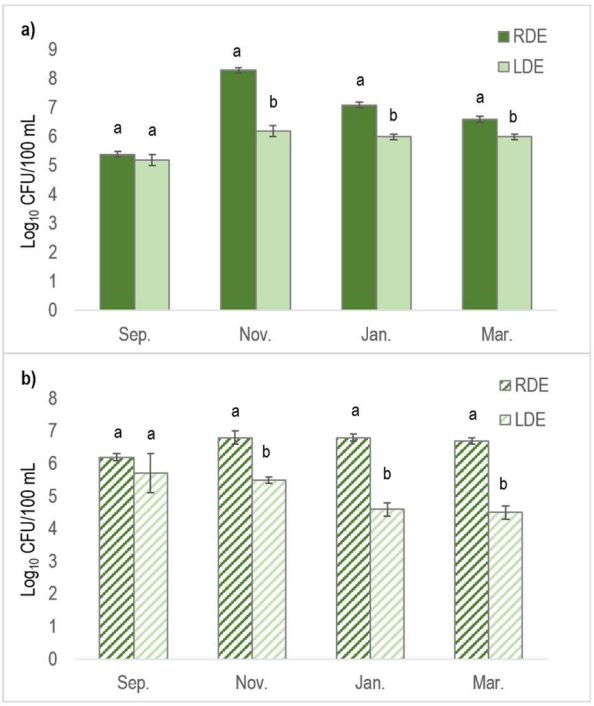

Figure 2. Numbers of Total coliforms (a) and *E. coli* (b) in Raw Dairy Effluent (RDE) or Lagoon Dairy Effluent (LDE) at the four sampling dates. Columns represent means ± standard errors (n=3). Columns with different letters indicate a significant difference between dairy effluent types at each sampling date ($P< 0.05$).

The seasonal variability in the chemical composition of both DE has been reported in our previous work [(4)]. For the September sampling (end of the cool season), RDE did not differ from LDE in dry matter content and recorded lower total C than in the other sampling dates. This may account for the lack of differences in the number of photogenic bacterial indicators at that date.

Upon the application of DE to the soil, significantly higher E. coli numbers were accounted for RDE-treated soil compared to the control and urea treatments (Figure 3).

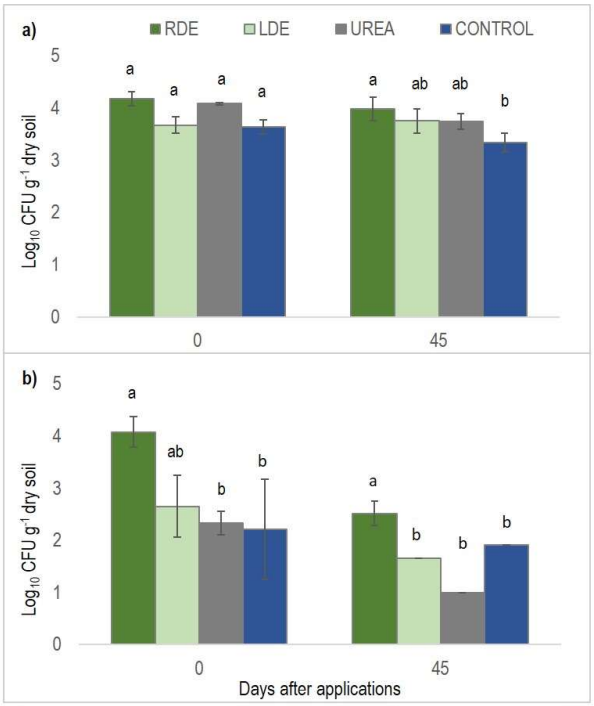

Figure 3. Average number of Total coliforms (a) and *E.coli* (b) in soil with the application of Raw Dairy Effluent (RDE) or Lagoon Dairy Effluent (LDE), Urea or Control, immediately after and 45 days of applications. Columns represent means ± standard errors (n=3). Columns with different letters indicate a significant difference between treatments for each day ($P< 0.05$).

After 45 days of its application, *E. coli* number decreased by nearly two orders of magnitude (from $2.5x10^4$ to $2.3\ x10^2$), yet it remained significantly higher than in the other treatments (Figure 3). On the other hand, the count of Total coliforms did not rise at the time of DE applications and remained nearly constant after 45 days of applications. Nevertheless, soil treated with RDE had a significantly higher number of Total coliforms than the control after 45 days of DE applications (Figure 3).

Table 3 shows the survival of *E. coli* introduced with the effluents in the soil after 45 days of each application. *E. coli* was undetectable after repeated LDE applications but was counted in the pots treated with RDE. No differences were determined in the survival of Total coliform for both DE applications (Table 3).

Several studies have indicated that coliforms can survive and proliferate in extraintestinal environments, and their role as indicators has been questioned [40].

Table 3. Survival of *E.coli* and Total coliforms in fescue pot soil with application of Raw (RDE) or Lagoon Dairy Effluent (LDE) for each of the four applications. Ratio of number of pathogenic bacteria indicators after 45 days of application to number applied with effluents.

	E.coli				**Total Coliforms**			
Treatment/Application date	Sep.	Nov.	Jan.	Mar.	Sep.	Nov.	Jan.	Mar.
RDE	0.46	0	0.85	0.58	0.92	0.88	0.99	1
LDE	0.52	0	0	0	0.90	0.88	1.30	1.05

Results from a field lysimeter study showed that the land application of the treated effluent can lead to significant reductions in *E. coli* leaching compared with the untreated original DE [41].

It has been established that dairy effluent application to soil generally promotes the numbers and survival of enteropathogenic bacteria [30][42]. Our results suggest a low-level persistence of enteric bacteria following RDE application to the soil, indicating that more than 45 days are required for the complete elimination of indicator bacteria.

In Uruguay, a wait time after DE application of 21-30 days before grazing is recommended, and grazing is advised to be avoided with categories less than 1 year old and pre-calving cows [43]. The present results confirm the pertinence of this recommendation.

4.5.3 Occurrence of ARGs in DE and amended soil

The fate of ARGs spread after DE applications to soil was assessed by targeting three beta-lactam ARGs. Resistance to beta-lactam antibiotics is an increasing concern, and beta-lactamase production is the most common mechanism for this drug resistance [22].

A growing number of cases of multidrug-resistant bacteria producing beta-lactamases have been reported in Uruguayan dairy farms [14][44][45].

Numerous studies have proved that DE serves a source of ARGs and may play a key role in the dissemination to the soil [46][47][48].

The presence of *bla*TEM and *bla*OXA were detected in at least one sampling date in RDE or LDE (Table 4) while *bla*SHV was not detected. However, neither of the beta-lactam ARGs could be later observed in the DE-amended soils at the end of the experiment (data not shown). These results indicate that DE applications did not lead in the dissemination of these ARGs in the soil. The decline over time following applications, suggests that microbes possessing beta-lactam ARG had somewhat limited survival potential in the soil environment [46] probably due to their lack of adaptation to the soil characteristics and the inhibition by soil indigenous bacteria [49][50].

Table 4. Abundance of *bla*TEM and *bla*OXA genes in Raw (RDE) or Lagoon Dairy Effluent (LDE) at four sampling dates (mean n=3 ± S.E).

	***bla*TEM (Log$_{10}$ copies mL^{-1})**				***bla*OXA (Log$_{10}$ copies mL^{-1})**			
Treatment/Application date	Sep.	Nov.	Jan.	Mar.	Sep.	Nov.	Jan.	Mar.
RDE	n.d	3.00± 0.05	n.d	3.06± 0.13	n.d	3.21± 0.55	n.d	n.d
LDE	2.93± 0.09	1.98± 1.06	n.d	3.74± 0.12	2.05± 0.21	2.86± 1.11	n.d	n.d

n.d= not detected

In this study, the *bla*TEM gene was present in November and March in RDE samples, and in LDE it was also detected in September (Table 4). On average, the *bla*TEM abundance was 3.5 x 10^3 and 8.8 x 10^2 copies mL^{-1} for LDE and RDE, respectively, with no significant differences. The *bla*OXA was only present in November in RDE samples, and its abundance was not significantly

different from LDE. On average, the *bla*OXA abundance was 1.6 x 10^3 and 2.1 x 10^3 copies mL^{-1} for LDE and RDE, respectively.

Neither of the beta-lactam ARGs were detected in the DE in January, the warmest sampling date (Table 4). According to these findings, Schages and others[51] reported a seasonal effect of ARG abundance in wastewater, with ARG abundance negatively correlated with warmer temperatures. Furthermore, the authors found that antibiotic resistant bacteria were also more prevalent in the colder seasons.

The occurrence of both beta-lactam ARGs was more frequent in LDE than in RDE. This was expected, as the RDE sampling represents one day flush of the dairy barn floor, while the LDE sampling represents annual dairy effluents disposal, where excreted antibiotics can continue exerting selection pressure for antibiotic-resistant genes[52]. So, lagoon storage does not remove ARGs in this case, although qPCR does not provide information on the expression of these genes[53].

Further studies are mandatory to determine the presence of other ARGs that confer resistance to other antibiotics used in the country to treat mastitis. However, our initial work is promising regarding the release of DE to pastures where the beta-lactam ARGs could not be detected.

4.6. Conclusions

No difference in tall fescue nutrient content was observed after DE application in this short-term pot trial performed with rich soil. Besides, DE application to the soil did not result in significant changes in soil nutrient content or pH. However, exchangeable cations increased after lagoon DE applications, while soil Na content was highest with both types of effluent applications.

There was a higher risk of pathogen bacterial indicators dissemination and survival in soil with the application of RDE than that with LDE, accounting for a role in this sense of lagoon storage.

Despite DE being a hotspot for the development of antimicrobial resistance, the fact that at least the ARG evaluated were not detected in the soil underlines the importance of applying DE to a pasture avoiding their disposal to water.

4.7. References

1. Herrero MA, Palhares JCP, Salazar FJ, Charlón V, Tieri MP, Pereyra AM. Dairy manure management perceptions and needs in South American countries. Front Sustain Food Syst. 2018;2:22. Doi: 10.3389/fsufs.2018.00022.

2. Fariña SR, Chilibroste P. Opportunities and challenges for the growth of milk production from pasture: the case of farm systems in Uruguay. Agric Syst. 2019;176:102631. Doi: 10.1016/j.agsy.2019.05.001.

3. Gutiérrez S, Cabrera N, Benítez A, Melani E. Reducing variability in estimating wastewater composition in dairy farms during milking operations. Biosyst Eng. 2009;103(4):497-503. Doi: 10.1016/j.biosystemseng.2009.06.004.

4. Illarze G, del Pino A, Rodríguez-Blanco A, Irisarri P. application of dairy effluents to pastures affects soil nitrogen dynamics and microbial activity. Agronomy. 2023;13(2):470. Doi: 10.3390/agronomy13020470.

5. La Manna A, Mieres J, Acosta Y, Torres I. Utilización de efluentes en tambos: resumen de Investigación. In: Resultados experimentales en lechería. Montevideo: INIA; 2004. p. 35-44.

6. Monaghan RM, Hedley MJ, Di HJ, McDowell RW, Cameron KC, Ledgard SF. Nutrient management in New Zealand pastures-recent developments and future issues. N Z J Agric Res. 2007;50(2):181-201. Doi: 10.1080/00288230709510290.

7. Prado J, Ribeiro H, Alvarenga P, Fangueiro D. A step towards the production of manure-based fertilizers: Disclosing the effects of animal species and slurry treatment on their nutrients content and availability. J Clean Prod. 2022;337:130369. Doi: 10.1016/j.jclepro.2022.130369.

8. Sukias JPS, Tanner CC, Davies-Colley RJ, Nagels JW, Wolters R. Algal abundance, organic matter, and physico-chemical characteristics of dairy farm facultative ponds: implications for treatment performance. N Z J Agric Res. 2001;44(4):279-96. Doi: 10.1080/00288233.2001.9513485.

9. Bolan NS, Laurenson S, Luo J, Sukias J. Integrated treatment of farm effluents in New Zealand's dairy operations. Bioresour Technol. 2009;100(22):5490-7. Doi: 10.1016/j.biortech.2009.03.004.

10. Monaghan RM, Houlbrooke DJ, Smith LC. The use of low-rate sprinkler application systems for applying farm dairy effluent land to reduce contaminant transfers. N Z J Agric Res. 2010;53(4):389-402. Doi: 10.1080/00288233.2010.505943.

11. Pachepsky YA, Sadeghi AM, Bradford SA, Shelton DR, Guber AK, Dao T. Transport and fate of manure-borne pathogens: modeling perspective. Agric Water Manag. 2006;86(1-2):81-92. Doi: 10.1016/j.agwat.2006.06.010.

12. Spiehs M, Goyal S. Best management practices for pathogen control in manure management systems. Minneapolis: University of Minnesota; 2007. 10p.

13. Helt CD, Weber KP, Legge RL, Slawson RM. Antibiotic resistance profiles of representative wetland bacteria and faecal indicators following ciprofloxacin exposure in lab-scale constructed mesocosms. Ecol Eng. 2012;39:113-22. Doi: 10.1016/j.ecoleng.2011.11.007.

14. Gianneechini RE, Concha C, Riveroj R, Gil J, Moreno-Lopez J. Monitoreo de la resistencia de Staphylococcus aureus aislados en rodeos lecheros de la cuenca lechera tradicional de Uruguay. In: XXXIII jornadas Uruguayas de Buiatría. Paysandú: Centro Médico Veterinario de Paysandú; 2005. p. 205-7.

15. Carvalho IT, Santos L. Antibiotics in the aquatic environments: a review of the European scenario. Environ Int. 2016;94:736-57. Doi: 10.1016/j.envint.2016.06.025.

16. Thomas G. Exchangeable cations. In: Page AL, Miller RH, Keeney D, editors. Methods of Soil Analysis: Part 2. Madison: American Society of Agronomy; 1982. p. 159-65.

17. Bray RH, Kurtz LT. Determination of total, organic, and available forms of Phosphorus in soils. Soil Sci. 1945;59(1):39-46.

18. Öhlinger R. Soil respiration by tritation. In: Schinner F, Öhlinger R, Kandeler E, Margesin R, editors. Methodsinsoil biology. New York: Springer Berlin Heidelberg; 1996. p. 95-8.

19. Bremner JM, Mulvaney CS. Total nitrogen. In: Page AL, Miller RH, Keeney D, editors. Methods of Soil Analysis: Part 2. Madison: American Society of Agronomy; 1996. p. 595-624.

20. Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Anal Chim Acta. 1962;27(C):31-6.

21. Isaac RA, Kerber JD. Atomic absorption and flame photometry: techniques and uses in soil, plant, and water analysis. In: Walsh Lm, editor. Instrumental Methods for Analysis of Soils and Plant Tissue. Madison: SSSA; 1971. p. 17-37.

22. Dallenne C, Da Costa A, Decré D, Favier C, Arlet G. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother. 2010;65(3):490-5. Doi: 10.1093/jac/dkp498.

23. Di Rienzo JA, Casanoves F, Balzarini MG, González L, Tablada M, Robledo YC. InfoStat. Versión 2020 [Internet]. Córdoba: Universidad Nacional de Córdoba; 2017 [cited 2024 Feb 22]. Available from: http://www.infostat.com.ar

24. Demanet R, Aguilera M, Mora M. Efecto de la aplicación de purines sobre el sistema suelo-planta. Front Agrícola [Internet]. 1999 [cited 2024 Feb 22];5:87-94. Available from: https://repositorio.uchile.cl/handle/2250/121498

25. Barkle GF, Stenger R, Singleton PL, Painter DJ. Effect of regular irrigation with dairy farm effluent on soil organic matter and soil microbial biomass. Aust J Soil Res. 2000;38(6):1087-97.

26. Hawke RM, Summers SA. Land application of farm dairy effluent: Results from a case study, wairarapa, New Zealand. N Z J Agric Res. 2003;46(4):339-46. Doi: 10.1071/SR99127.

27. Liu YY, Haynes RJ. Long-term irrigation with dairy factory wastewater influences soil quality. World Academy of Science, Engineering and Technology. 2010;4:524-8.

28. Saunders OE, Fortuna AM, Harrison JH, Whitefield E, Cogger CG, Kennedy AC, et al. Comparison of Raw Dairy Manure Slurry and Anaerobically Digested Slurry as N Sources for Grass Forage Production. Int J Agron. 2012;2012:101074. Doi: 10.1155/2012/101074.

29. Fyfe J, Hagare D, Sivakumar M. Dairy shed effluent treatment and recycling: effluent characteristics and performance. J Environ Manage. 2016;180:133-46.

30. Roach CG, Longhurst RD, Ledgard SF. Land application of farm dairy effluent for sustainable dairy farming. Proc New Zeal Grassl Assoc. 2001;(63):53-7. Doi: 10.33584/jnzg.2001.63.2430.

31. Bolan NS, Horne DJ, Currie LD. Growth and chemical composition of legume-based pasture irrigated with dairy farm effluent. N Z J Agric Res. 2004;47(1):85-93. Doi: 10.1080/00288233.2004.9513574.

32. Hawke RM, Summers SA. Effects of land application of farm dairy effluent on soil properties: a literature review. N Z J Agric Res. 2006;49(3):307-20.

33. Goulding KWT. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use Manag. 2016;32(3):390-9.

34. Liu M, Hu F, Chen X, Huang Q, Jiao J, Zhang B, Li H. Organic amendments with reduced chemical fertilizer promote soil microbial development and nutrient availability in a subtropical paddy field: the influence of quantity, type and application time of organic amendments. Appl Soil Ecol. 2009;42(2):166-75.

35. Zibilske LM. Carbon mineralization. In: Weaver R, Angle S, Bottomley P, Bezdicek D, Smith S, Tabatabai A, Wollum A, editors. Methods of Soil Analysis: Part 2 Microbiological and Biochemical Properties. Madison: American Society of Agronomy; 1994. p. 835-63.

36. Alef K. Soil respiration. In: Alef K, Nannipieri P, editors. Methods in Applied Soil Microbiology and Biochemistry. Londres: Elsevier; 1995. p. 193-270.

37. Bhogal A, Nicholson FA, Rollett A, Taylor M, Litterick A, Whittingham MJ, Williams JR. Improvements in the quality of agricultural soils following organic material additions depend on both the quantity and quality of the materials applied. Front Sustain Food Syst. 2018;2:9. Doi: 10.3389/fsufs.2018.00009.

38. Coban H, Miltner A, Elling FJ, Hinrichs KU, Kästner M. The contribution of biogas residues to soil organic matter formation and CO2 emissions in an arable soil. Soil Biol Biochem. 2015;86:108-15.

39. Avery LM, Killham K, Jones DL. Survival of E. coli O157: H7 in organic wastes destined for land application. J Appl Microbiol. 2005;98(4):814-22.

40. Jang J, Hur HG, Sadowsky MJ, Byappanahalli MN, Yan T, Ishii S. Environmental Escherichia coli: ecology and public health implications: a review. J Appl Microbiol. 2017;123(3):570-81.

41. Wang XM, Di HJ, Cameron KC, Li B. Effect of treated farm dairy effluent on E. coli, phosphorus and nitrogen leaching and greenhouse gas emissions: a field lysimeter study. J Soils Sediments. 2019;19(5):2303-12.

42. Manshadi FD, Karpiscak M, Gerba CP. Enteric bacterial contamination and survival on produce during irrigation with dairy wastewater in the field. Water Reuse. 2013;3(2):102-10. Doi: 10.2166/wrd.2013.161.

43. Correa C, Rezzano N, García F. Manual para la gestión ambiental de tambos [Internet]. Montevideo: LATU; 2016 [cited 2024 Feb 23]. 83p. Available from: https://bit.ly/42RRurY

44. Gianneechini RE, Concha C, Franklin A. Antimicrobial susceptibility of udder pathogens isolated from dairy herds in the west littoral region of Uruguay. Acta Vet Scand. 2002;43(1):31-41. Doi: 10.1186/1751-0147-43-31.

45. Gianneechini R, Concha C, Delucci I, Gil J, Salvarrey L, Rivero R. Mastitis bovina, reconocimiento de los patógenos y su resistencia antimicrobiana en la Cuenca Lechera del Sur de Uruguay. Veterinaria (Montevideo). 2014;50(196):4-32.

46. Nõlvak H, Truu M, Kanger K, Tampere M, Espenberg M, Loit E, Raave H, Truu J. Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance and integron-integrase genes in agricultural grassland soil. Sci Total Environ. 2016;562:678-689. Doi: 10.1016/j.scitotenv.2016.04.035.

47. Dungan RS, McKinney CW, Leytem AB. Tracking antibiotic resistance genes in soil irrigated with dairy wastewater. Sci Total Environ. 2018;635:1477-1483. doi: 10.1016/j.scitotenv.2018.04.020.

48. McKinney CW, Dungan RS, Moore A, Leytem AB. Occurrence and abundance of antibiotic resistance genes in agricultural soil receiving dairy manure. FEMS Microbiol Ecol. 2018;94(3). Doi: 10.1093/femsec/fiy010.

49. Chen QL, An XL, Li H, Zhu YG, Su JQ, Cui L. Do manure-borne or indigenous soil microorganisms influence the spread of antibiotic resistance genes in manured soil? Soil Biol Biochem. 2017;114:229-37.

50. Liu P, Jia S, He X, Zhang X, Ye L. Different impacts of manure and chemical fertilizers on bacterial community structure and antibiotic resistance genes in arable soils. Chemosphere. 2017;188:455-64. Doi: 10.1016/j.chemosphere.2017.08.162.

51. Schages L, Wichern F, Kalscheuer R, Bockmühl D. Winter is coming - Impact of temperature on the variation of beta-lactamase and mcr genes in a wastewater treatment plant. Sci Total Environ. 2020;712:136499. Doi: 10.1016/j.scitotenv.2020.136499.

52. Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW. Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. Environ Microbiol. 2007;9(1):143-51. Doi: 10.1111/j.1462-2920.2006.01123.x.

53. Fortunato G, Vaz-Moreira I, Becerra-Castro C, Nunes OC, Manaia CM. A rationale for the high limits of quantification of antibiotic resistance genes in soil. Environ Pollut. 2018;243(Pt B):1696-703. Doi: 10.1016/j.envpol.2018.09.128.

5. Differences in bacterial communities and pathogen indicators of raw and lagoon-stabilized farm dairy effluents

Illarze G. [1], del Pino A. [2], and Irisarri P. [1*]

[1] Laboratorio de Microbiología, Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

[2] Departamento de Suelos y Aguas, Facultad de Agronomía, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

5.1. Resumen

Una práctica para gestionar los Efluentes de tambo consiste en reciclarlos en el suelo con el desafío de equilibrar el compromiso asociado con la contaminación ambiental a través de la carga de nutrientes y microorganismos. Este estudio investigó los cambios estacionales en la composición de la comunidad bacteriana, la diversidad, la abundancia y los indicadores patogénicos en los efluentes no tratados (Crudos) y los estabilizados en sistema de doble laguna (Laguna). También se analizó la correlación entre los perfiles bacterianos y las características fisicoquímicas de los efluentes de tambo. Las bacterias indicadoras de patógenos se estudiaron mediante la enumeración de recuento de viables y la estructura de la comunidad bacteriana mediante el análisis de secuencias del gen 16S ARNr. El almacenamiento en la Laguna redujo de forma efectiva los sólidos totales (64%), los sólidos en suspensión (77%), el carbono orgánico (40%) y el nitrógeno total (82%), así como los coliformes totales, *Escherichia coli* y enterococos. Sin embargo, esta eficiencia se vio comprometida en invierno. Las comunidades bacterianas de la Laguna y de los Crudos presentaron composiciones diferentes, con varias variables ambientales correlacionadas con estas comunidades. Los efluentes tratados en la Laguna exhibieron la comunidad bacteriana más diversa, dominada por Firmicutes (40%), Proteobacteria (30%) y Bacteroidota (7.6%), mientras que los Crudos estaban compuestos principalmente por Firmicutes (76%). Independientemente

de la estación, los géneros dominantes incluyeron *Trichococcus*, *Romboutsia*, *Corynebacterium* y *Paeniclostridium*. En general, el estudio enfatiza la importancia del tratamiento en sistema de doble laguna para la estabilización de los efluentes de tambo, mostrando su papel en cambiar la composición de la comunidad bacteriana y mitigar los riesgos ambientales asociados con la diseminación de patógenos o por exceso de nutrientes, especialmente en verano.

Palabras clave: laguna de estabilización facultativa, secuenciación del 16S ARNr, indicadores de patógenos, composición fisicoquímica, riesgo ambiental

5.2. Summary

One practice for handling Farm Dairy Effluents (DE) comprises recycling them to the soil with the challenge of balancing the trade-off associated with environmental pollution through nutrients and microorganism loading. This study investigated seasonal bacterial community composition, diversity, abundance, and pathogenic indicators in untreated (Raw) and lagoon-stabilized (Lagoon) DE. The correlation between bacterial profiles and DE physicochemical characteristics was also analyzed. Pathogen-indicator bacteria were studied by enumerating viable counts and the bacterial community structure by 16S rRNA gene sequence analysis. Lagoon storage effectively reduced total solids (64%), suspended solids (77%), organic carbon (40%), and total nitrogen (82%), as well as total coliforms, Escherichia coli, and enterococci. However, this efficiency was compromised in winter. Lagoon and Raw bacterial communities presented different compositions, with several environmental variables correlating to microbial community differences. Lagoon-treated DE exhibited the most diverse bacterial community, dominated by Firmicutes (40%), Proteobacteria (30%), and Bacteroidota (7.6%), whereas Raw was mainly composed of Firmicutes (76%). Regardless of the season, dominant genera included *Trichococcus*, *Romboutsia*, *Corynebacterium*, and *Paeniclostridium*. Overall, the study emphasizes the importance of lagoon

treatment for DE stabilization, showcasing its role in altering bacterial community composition and mitigating environmental risks associated with pathogens and nutrients, particularly in summer.

Keywords: facultative stabilization lagoon; 16S rRNA sequencing; pathogen indicators; physicochemical composition; environmental risk

5.3. Introduction

Farm dairy effluents (DE), the wastewater produced from washing down the dairy holding yards during and after milking, is generally managed in South America through direct application to pasture as a fertilizer (raw) or following partial treatment in stabilization lagoons [1]. The development of a circular economy led to the recycling of organic materials in the farm to complete nutrient and C cycles in the system [2]. Nevertheless, careful management is required to use the effluent nutrient and water resources effectively, avoiding environmental impacts. One matter of concern in using DE as liquid organic fertilizer is their potential microbial pollution, as manure is a reservoir of many microbial populations, including pathogens, which can cause contamination and harm public and animal health [3].

Dairy production intensification entails high volumes of effluents, which must be treated and stored to be used as fertilizer for crop and pasture production efficiently. There is a higher risk of pathogen transfer into the food chain when fresh manure is applied to the land than when waste is stored in lagoons, among other reasons to decrease the pathogen number [4]. Two-stage stabilization lagoons (anaerobic followed by facultative lagoon) are one of the most common effluent management systems due to their low operation and maintenance costs to treat dairy effluent to remove significant amounts of suspended solids and organic contaminants [5]. However, this treatment may be ineffective in removing N and P and decreasing the number of bacterial indicators [6],

potentially allowing prolonged survival of fecal indicators and opportunities for frequent re-inoculation from recurring inputs to effluent storage [7,8].

Generally, the surface application of DE (tanks or irrigation pumps) occurs consistently throughout the year [1]. Even though, one of the challenges of DE reutilization as a pasture N supplier is its variable composition, complicating the prediction of its agronomic value [9]. Compositional variations in DE are likely due to the time of milking, the age and breed of the herd, feed quality, wash-water management, the time relative to lactation, and seasonal variations [6,10]. To assess the benefits and potential threats of land application of DE to the environment, it is imperative to quantify the temporal variation of its nutrients and contaminants [11].

More information is needed on the microbial community composition and diversity of untreated and lagoon-stabilized DE and its seasonal variability. Other studies have proposed that the impact and the survival rate of microbes from manure waste-based products on soil microbiomes largely depend on the type of treatment applied to manure [12]. Many studies focus on environmental risk assessment by studying antibiotic-resistant bacteria or their genes. Still, some studies propose that the microbial community of manure waste-based products carries beneficial microorganisms with potential use as biofertilizers [13,14]. Thus, it is essential to expand this knowledge to fully understand how the DE application to land impacts soil microbial dynamics.

There is scarce information regarding the bacterial composition, diversity, and abundance of South American DE, either raw or lagoon-treated. Irazoqui et al. [15] recently analyzed the microbial communities of two serial full-scale stabilization lagoon systems using whole genome shotgun sequencing. The functional analysis of the genomes revealed the central metabolic pathways and attributed them to several key organisms.

The knowledge of DE bacterial composition may help to elucidate ecological and environmental issues as a potential invasion of these microbes into the resident soil microbial community, especially since there is much concern about raw DE pathogenic contamination. This work aimed to assess

microbial community and pathogenic bacterial indicators differences between Raw and Lagoon stabilized DE and to determine the variability with the season and the physicochemical characteristics of DE. We hypothesize that (1) Raw and Lagoon DE harbor different bacterial communities and potential pathogenic risk, and (2) these bacterial communities and pathogenic indicators change in response to the seasonal variability and physicochemical characteristics of DE.

5.4. Materials and methods

5.4.1. Dairy effluents collection and Farm characteristics

DE were collected from the dairy farm of Centro Regional Sur from the Faculty of Agronomy (Universidad de la República) with geographic coordinates 34°36' 47.83" S and 56° 12' 54.00" W. Samples were collected on 5 September 2019, 7 November 2019 (spring-summer season), 11 May 2020 (autumn) and 18 August 2020 (winter), with mean seasonal water temperatures of 21.8 ◦C, 26.4 ◦C, 19.0◦C, and 13.7 ◦C, respectively. Throughout the study, the farm ran a milking herd of 200 cows that grazed temperate pastures all year round. The pastures comprised a diverse array of species of grasses and legumes: Dactylis glomerata with *Trifolium repens*, *Medicago sativa* with *Bromus catharticus*, and *Lolium multiflorum* with *Avena bizantina*. Reserved forage was supplemented mostly in the autumn/winter season due to the typical seasonality of pasture growth. Supplementation with concentrates was done during the milking and the herd was milked twice a day.

The farm site's effluent management system comprises a conventional two-stage dairy shed waste stabilization lagoon system (an anaerobic and a facultative lagoon operated in series) with a solid trap for effluent pre-treatment. The system received the effluent generated by flushing the milking parlor and holding yard and chemically cleaning the milking machinery with sodium hypochlorite. The Raw DE was collected immediately after the cleaning from a holding tank (for solid trap) into a 2 L sterile container. The liquid portion

undergoes gravimetric flushing into the two stabilization lagoons operated in series, with a hydraulic retention time of 119 days. The Lagoon DE was collected in a 2 L sterile container 15 cm below the surface from four different cardinal points of the facultative lagoon of 711 m3 volume and then composited. Three composite replicate samples were obtained from each type of DE and stored refrigerated at 4 °C (chemical analyses) or frozen at −20 °C (molecular analyses).

5.4.2. Characterization of dairy effluents

The pH and electric conductivity (EC) were measured in a mixture of effluent and deionized water (1:2.5 v/v) through potentiometry. Total solids (TS) and suspended solids (SS) were assessed gravimetrically following Standard Methods 2540 [16]. Total organic carbon (TOC) was measured via oxidation using potassium dichromate, following Mebius's technique [17]. Total nitrogen (TN) was analyzed by the Kjeldahl method [18]. Ammoniacal N (NH_4^+-N) was extracted using a 2 M KCl solution and quantified by colorimetric analysis according to Rhine et al. [19]. By subtraction, organic N concentration (Norg) was deduced from total Kjeldhal and NH_4^+.

To determine elements P, K, Ca, Mg, and Na, samples were treated with HCl (20%) and calcined at 550º C for 5h. Total P was determined by the molybdate blue method with ascorbic acid [20]. Ca and Mg were determined by atomic absorption spectrometry, while K and Na were determined by atomic emission spectrometry [21].

5.4.3. Quantification of pathogenic bacterial indicators

A 2-L subsample from each DE was transported on ice to the laboratory for immediate analysis. Total coliforms, *E. coli*, and enterococci numbers were determined by a ten-fold dilution series of 10 mL of each DE in 90 mL of 0.01 M phosphate buffer followed by agitation for 30 min in an orbital shaker at 200

rpm. The diluted samples were triplicated on 3M™ *E.coli*/Coliform Petrifilm™ or Chromocult Enterococci agar (Merck). After incubation at 37 °C for 24 h, characteristic blue colonies were counted as *E. coli* and red ones as total coliforms (CFU/100 mL) or incubated at 41 ºC for 24 hours to enumerate enterococci.

5.4.4. Dairy effluents DNA extraction and 16S rRNA qPCR and gene amplicon sequencing

An aliquot of 100 mL of each DE sample was centrifuged at 10,000 × g for 10 minutes at room temperature to obtain pellets. Total DNA was extracted using the PowerSoil® DNA Isolation kit (Qiagen®) from 0.25 g fresh weight (FW) of each centrifuged sample of the DE following the manufacturer's instruction. The DNA concentration and purity were determined with NanoDrop® 2000c UV–vis spectrophotometry (Thermo Scientific, USA). The extracted DNA samples were stored at − 20 °C before analysis.

Quantitative real-time PCR (qPCR) of the copy number (abundance) of total bacteria was conducted with forward primer 515F (5′-GTGC CAGCMGCCGCGGTAA-3′) and the reverse primer 806R (5′-G GACTACHVGGGTWTCTAAT-3′) with 300-bp in length. An initial incubation of 5 min at 95 °C was followed by 30 cycles of 30 sec at 95 °C, 45 sec at the annealing temperature of 55 °C and 50 sec of extension at 72 °C.

The bacterial community composition was determined based on variable V3-V4 region of the 16S rRNA gene sequencing with forward primer 341F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer 805R (5′-GACTACHVGGGTATCTAATCC-3′). Amplicon library preparation was performed using the DNA extracted from each DE sample and then sequenced by Illumina MiSeq platform (Illumina, San Diego, CA) at Macrogen Inc. Company, South Korea. The generated data (accession number will be provided previous to publication) was processed, demultiplexed, and quality controlled using DADA2 pipeline [22] with the “consensus” method to remove any

remaining chimeric and low-quality sequences. The taxonomic identification (with 99% similarity) was performed using SILVA database (v. 138) [23]. The data were then analyzed in R using Microeco package [24].

5.4.5. Statistical analysis

Data were analyzed in R version 4.1.0 (R Core Team, 2019). One-way analysis of variance (ANOVA) for DE physicochemical characteristics and microbial indicators was performed to evaluate the effect of DE type or sampling date (seasonal effect). To define the statistical significance of the mean, Fisher´s LSD post hoc test was performed with a 95% degree of confidence ($p < 0.05$). The Chao1 and the Shannon Diversity indexes investigated bacterial community richness and evenness. β-diversity comparisons were completed to compare communities using Bray–Curtis index distance method. Visual differences among DE type and sampling date were determined using principal coordinate analysis (PCoA). Statistical differences in β-diversity among DE bacterial communities were tested with PERMANOVA (permutational multivariate analysis of variance) [25], and the PERMANOVA assumption of equal variance between groups was tested with PERMDISP (permutational analysis of multivariate dispersions) [26], using Vegan package in R [27].

The LEfSe (Linear Discriminate Analysis Effect Size) algorithm identifies the most biologically informative features (for example: organisms, genes, or pathways) that consistently explain differences between microbial communities by emphasizing statistical significance, biological consistency, and effect relevance [28]. This study used the LEfSe algorithm to identify microbes that characterized the differences between DE types.

Redundancy discriminant analysis (RDA) to determine the associations between bacterial genus relative abundance and the physicochemical parameters of DE was performed using Vegan package in R [27].

5.5. Results

5.5.1. Farm Dairy Effluents Physicochemical Characterization

The physicochemical characteristics of Raw DE (inlet) and the treated in a two-lagoon storage system were evaluated through seasonal sampling (Table 1). Throughout the study period, there was considerable variation in the physicochemical parameters of Raw relative to Lagoon DE (Table S1). Raw exhibited a pH ranging from 6 to 8.5, while Lagoon was consistently slightly alkaline, on average 7.8. In the coldest seasons (May and August, for the South Hemisphere), Lagoon had significantly higher pH than Raw, whereas the reverse was observed in September; no significant difference was noted in November (Table 1). Electrical conductivity (EC) again exhibited greater variability in Raw, ranging from 1.8 to 6.9. The highest EC value for Lagoon, around 2.6, was recorded in November sampling. Significant differences in EC between DE types were observed only in November and May.

Table 1. Physicochemical characteristics of the Farm Dairy Effluents, obtained immediately after washing off the milking parlor (Raw) or from the lagoon storage system (Lagoon) in four seasonal sampling dates (n=3± E.E).

						mg L^{-1}						
Month	**Effluent**	**pH**	**EC (mS m^{-1})**	**TS (%)**	**SS (%)**	**Corg***	**TKN***	**NH_4^+-N***	**P***	**K^{+*}**	**Na^{+*}**	**Mg^{2+*}**	**Ca^{2+*}**
September	Raw	8.5±0.0 **a**	2.4±0.0 **a**	0.8±0.4 **a**	0.8±0.4 **a**	514±80 **a**	800±39 **a**	216±23 **a**	16±1 **b**	722±88 **a**	217±17 **a**	100±26 **a**	197±61 **a**
	Lagoon	7.7±0.0 **b**	2.1±0.1 **a**	0.1±0.0 **b**	0.1±0.0 **b**	359±12 **b**	137±14 **b**	87±15 **b**	38±1 **a**	150±18 **b**	144±17 **b**	31±4 **b**	48±5 **b**
November	Raw	8.2±0.4 **a**	6.9±1.2 **a**	0.3±1 **a**	0.3±1 **a**	643±35 **a**	538±11 **a**	332±47 **a**	84±19 **a**	436±107 **a**	131±28 **a**	46±9 **a**	76±15 **a**
	Lagoon	7.7±0.0 **a**	2.3±0.1 **b**	0.1±0.0 **b**	0.1±0.0 **b**	414±19 **b**	95±7 **b**	39±1 **b**	36±3 **b**	156±13 **b**	75±8 **b**	26±3 **b**	47±5 **b**
May	Raw	7.3±0.0 **b**	4.8±0.1 **a**	0.6±0.1 **a**	0.6±0.1 **a**	736±145 **a**	539±9 **a**	75±10 **a**	101±5 **a**	277±21 **a**	226±19 **a**	73±5 **a**	87±5 **a**
	Lagoon	7.8±0.1 **a**	3.8±0.0 **b**	0.1±0.0 **b**	0.1±0.0 **b**	275±18 **b**	88±2 **b**	37±1 **b**	24±4 **b**	224±27 **a**	157±15 **b**	38±6 **b**	32±6 **b**
August	Raw	6.0±0.0 **b**	1.8±0.0 **a**	0.1±0.0 **a**	0.1±0.0 **a**	890±22 **a**	281±17 **a**	19±1 **b**	19±2 **b**	69±4 **b**	16±1 **b**	8.3±1 **b**	15±1 **b**
	Lagoon	8.1±0.0 **a**	2.3±0.4 **a**	0.2±0.0 **a**	0.2±0.0 **a**	573±42 **b**	231±5 **b**	53±4 **a**	26±2 **a**	92±12 **a**	52±7 **a**	17±2 **a**	23±3 **a**

Raw generally presented a higher % of TS and SS compared to Lagoon, with significant variability across sampling dates (Table 1, S1). The Raw TS

ranged from 0.3 to 2.3% and SS from 0.1 to 0.8%, whereas Lagoon DE levels were around 0.3% and 0.1%, respectively. Following stabilization in the Lagoon, DE`s TS and SS were substantially reduced, except for winter sampling.

Despite the seasonal variations, the Lagoon-stored DE consistently demonstrated significantly lower TOC and TN than Raw. The reduction in TOC averaged around 40%, and for TN, it was around 82%, except for the August sampling, which accounted for less than 20% of TN removal. Accordingly, NH_4^+ content in winter sampling was notably higher in Lagoon DE than Raw DE; however, this trend was reversed in the other three sampling dates. Organic N fluctuated between 38% and 93% of the total N in Raw DE and 36 to 77% in Lagoon. The highest % organic N/Total N corresponded to the coldest sampling date (August).

P content in Raw DE was highly variable, ranging from 16-19 mg/L in September and August to 84-101 mg/L in November and May, whereas the total P in Lagoon ranged between 24 and 38 mg/L. Significantly higher P content in Lagoon was observed in September and August, while the highest P content for Raw was accounted for in November and May. In September, November, and May, Raw had higher cation content than Lagoon, but again, in winter sampling, Lagoon accounted for the highest cation content (Table S1).

When considering the seasonal effect of the physicochemical characteristics of DE (Table S1), Raw DE generally exhibited its lowest nutrient content in winter sampling. The highest difference in TN and cation content for Raw was observed between winter and spring sampling dates. Conversely, the TOC was higher in winter than in spring. Lagoon DE also had higher TOC in winter, significantly higher than for the rest of the sampling dates (Table S1), and TN was also significantly higher in winter. However, the cation content in Lagoon DE decreased in winter sampling relative to the other three sampling dates.

5.5.2. Quantification of pathogenic indicator bacteria in Farm Dairy Effluents

The total coliforms, *E. coli*, and enterococci numbers were detected at higher levels in Raw than in Lagoon DE (Figure 1). The maximum difference in total coliform and *E. coli* was observed in August (winter), with 10^8 CFU 100 mL^{-1} and 10^6 CFU 100 mL^{-1} for Raw and Lagoon DE, respectively. Conversely, the maximum differences in enterococci were observed for September and November (Spring-summer) with an average concentration of 10^7 CFU 100 mL^{-1} and 10^2 CFU 100 mL^{-1} for Raw and Lagoon DE, respectively.

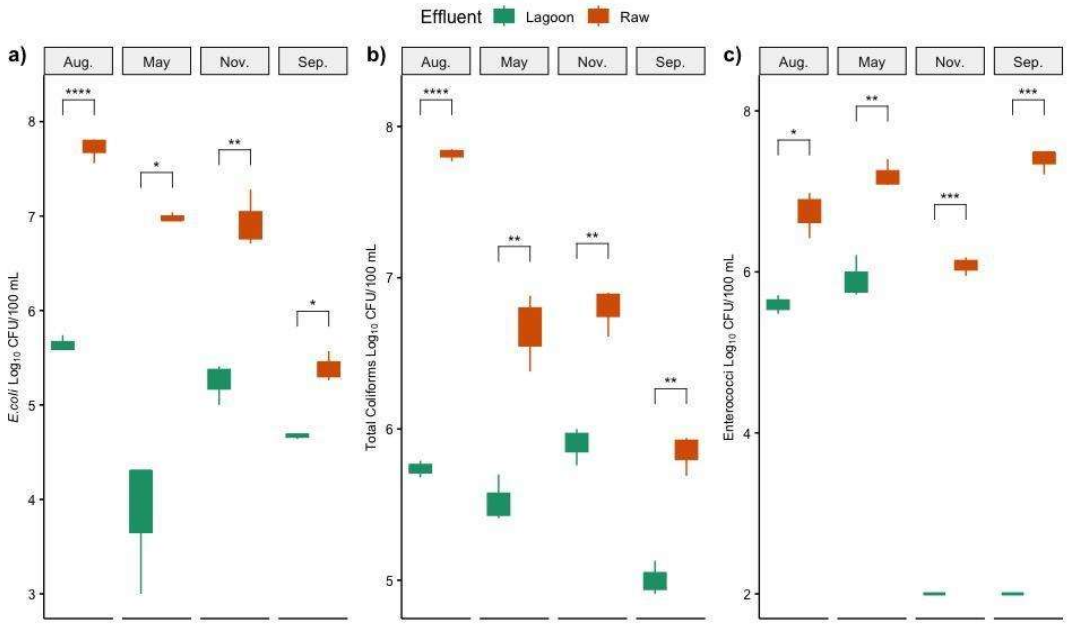

Figure 1. Numbers of E. coli (a), total coliforms (b), and enterococci c) in Raw and Lagoon dairy effluents (Log10 CFU/100 mL) at four sampling dates (September, November, August, and May) (n= 3± E.E). The length of each box represents the interquartile range IQR = Q 3 − Q 1. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

Considering seasonal sampling, the highest counts of *E. coli* and total coliforms in Raw DE were recorded in winter (August sampling), and the lowest was accounted for in spring (September sampling). Similar patterns were observed for Lagoon DE, with *E. coli* and total coliforms significantly elevated in winter but not differing significantly from summer levels. Enterococci were near undetectable in Lagoon DE in spring-summer samplings (<10^2 CFU 100 mL^{-1}) but markedly increased in autumn. On the other hand, enterococci count

in Raw DE was the lowest in winter, and significantly higher counts were recorded in autumn (May sampling) and spring ($p < 0.05$).

5.5.3. Bacterial abundance, composition, and diversity of Farm Dairy Effluents

During the winter sampling, a higher abundance of bacterial copies was recorded for both DE types, and only at this sampling date were the bacterial 16S rRNA gene copy numbers significantly higher in Lagoon than in Raw DE (Figure 2). Bacterial copy numbers of Lagoon ranged from 4.3 x 10^{10} to 5.9 x 10^{11} copies 100 mL^{-1}, whereas Raw DE ranged from 5.3 x 10^{10} to 2.3 x 10^{11} copies 100 mL^{-1}.

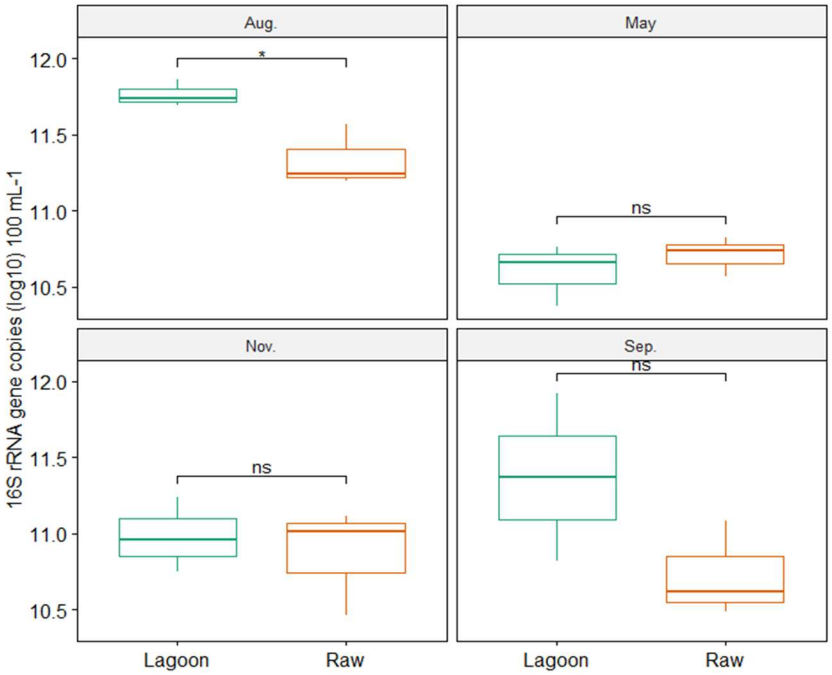

Figure 2. The gene copy numbers for bacterial 16S rRNA in Raw and Lagoon dairy effluents at four sampling dates: August, May, November, and September. The length of each box represents the interquartile range IQR = Q 3 − Q 1, and the horizontal line is the median value. * $p < 0.05$, ns: not significantly different.

Sequencing of the 16S rRNA V3-V4 region generated sequence reads from 24,582 to 39,412 with an average of 32,053 for Lagoon DE. For Raw DE, sequence reads varied from 13,461 to 40,616, averaging of 27,451. In the Lagoon DE, the most abundant phylum was Firmicutes (40%), followed by

Proteobacteria (30%) and Bacteroidota (7.6%), while in Raw, the phylum Firmicutes accounted for 76% of the total relative abundance, followed by Actinobacteriota (10%) (Figure S1).

A heat map of the top 40 genera in a total of 6392 ASVs is shown in Figure 3. Four genera comprised about 30 % of the bacterial genes in DE (Lagoon-Raw): *Trichococcus* (16-20 %), *Romboutsia* (4-8 %), *Corynebacterium* (1-8 %), and *Paeniclostridium* (2-6 %). Other abundant genera in Raw were *Lactococcus* and *Enterococcus*, accounting for 4 and 5%, respectively, while they were marginally detected in Lagoon samples. Certain genera exhibited noticeable seasonal variations in relative abundance. Specifically, *Jeotgalibaca*, *Psychrobacter*, *Exiguobacterium*, and *Planococcus* were more abundant in Lagoon during autumn (May) and winter (August) samplings than in spring-summer (September-November) samplings. In Raw winter samples, however, *Weisella* and *Paucilactobacillus* had higher relative abundance, while *Jeotgalibaca* had lower relative abundance than the rest of the sampling dates.

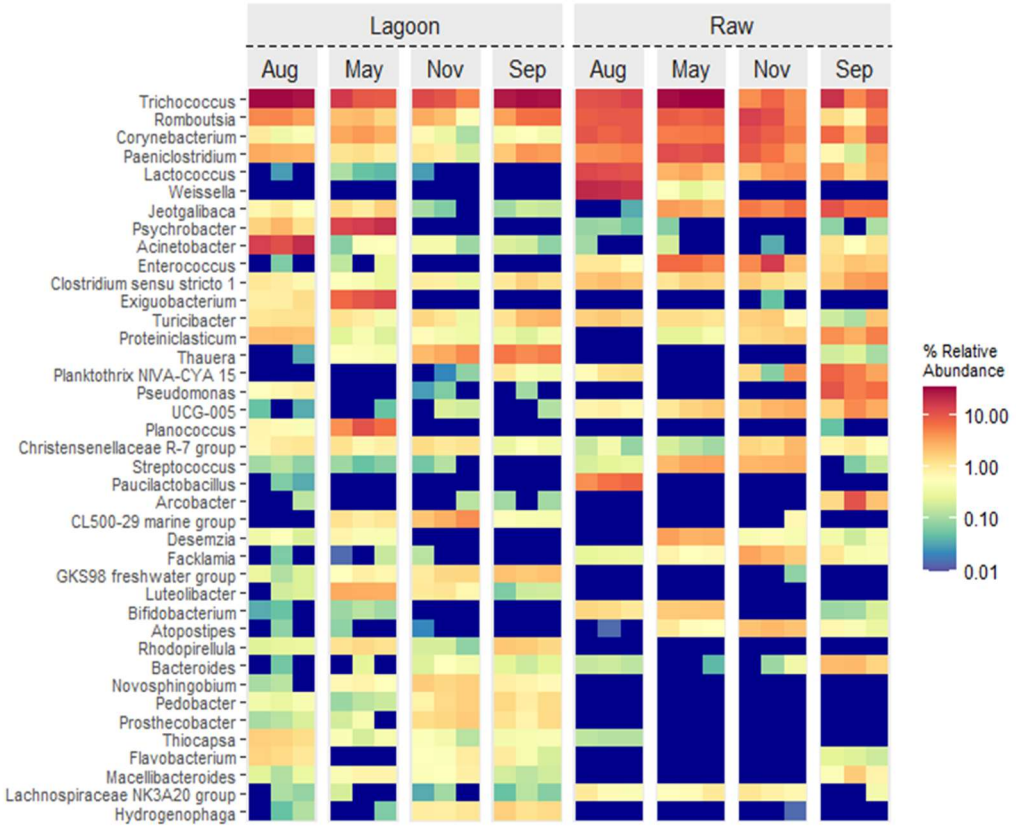

Figure 3. Microbial community heat map of 40 most abundant genera in Lagoon and Raw Dairy Effluents at four sampling dates: September and November (spring-summer), May (autumn), and August (winter).

The PERMDISP analysis revealed no significant difference in dispersion from Raw and Lagoon and sampling dates ($p > 0.05$). This suggests homogeneity of variance, indicating that differences in α- and β-diversities primarily resulted from dissimilarity rather than dispersion between DE type and sampling dates. Regardless of the sampling date, Lagoon DE had a significantly higher α-diversity than Raw DE (Figure 4a,b). Within each sampling date, November sampling (spring-summer) had significantly higher Chao1 and Shannon indexes than the rest of the sampling dates (Figure 4c,d). On the other hand, the August sampling had the lowest α-diversity indexes.

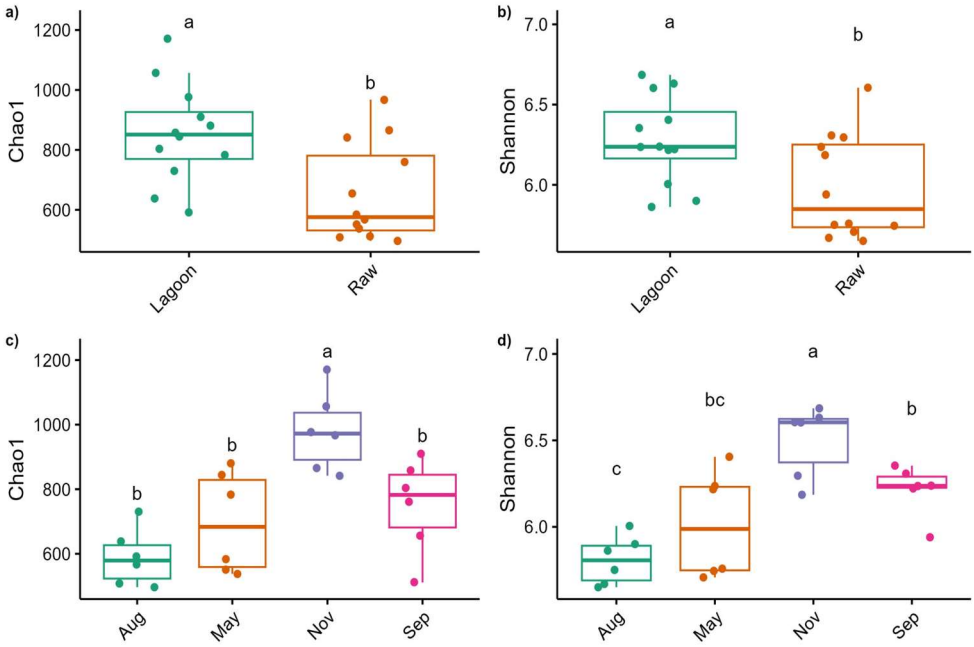

Figure 4. Bacterial richness (Chao1 index) and evenness (Shannon index) according to the type of Farm Dairy Effluent (Raw or Lagoon) (a-b) or the sampling date (c-d). Aug: August, May, Nov: November, Sep: September.

PCoA plots, using a Bray Curtis distance matrix, also showed that bacterial communities grouped into two clusters (Figure 5, PERMANOVA $R = 0.77$, $p < 0.05$). The first two principal coordinates, PC1 and PC2, explained 22.8% and 15.4% of the data variation, respectively, clearly separating the communities of Raw from Lagoon. The separation of bacterial communities by sampling date was also visible in the PCoA (Figure 5). For Raw, the bacterial community was segregated into the spring-summer (September-November) and the autumn-winter (May-August) clusters. For Lagoon, however, the September sampling grouped with August, while the May and November sampling were distant from them and between each other.

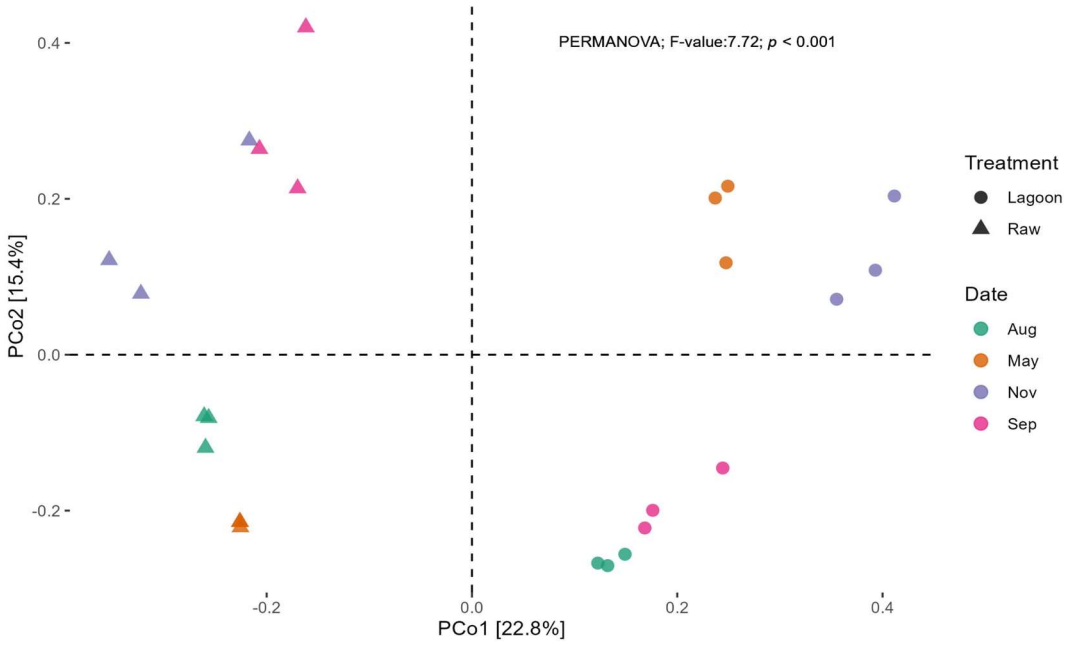

Figure 5. Principal coordinate analysis (PCoA) of bacterial communities based on the ASVs of the 16S rRNA gene sequencing from Raw Dairy Effluent and Lagoon Dairy Effluent at each sampling date. Aug: August, May, Nov: November, Sep: September.

The LEfSe analysis was carried out to identify the genera that predominantly contribute to explaining the greatest differences between bacterial communities of Lagoon and Raw DE (Figure 6). Among the 129 genus/ASVs that showed significant changes in their relative abundances, 13 were particularly representative of Raw DE, encompassing Firmicutes (*Weissella*, *Lactococcus*, *Romboutsia*, *Enterococcus*, *Paeniclostridium*, *Atopostipes*, *Facklamia*, *Streptococcus*, *Clostridium* sensu stricto 1, UCG-005), Actinobacteriota (*Corynebacterium* and *Bifidobacterium*), and Cyanobacteria (*Planktothrix* NIVA-CYA 15). Within Raw DE *Corynebacterium*, *Lactococcus* and *Weissella* emerged as the most significant genera (LDA score $\geq$ 4.4). Whereas, Proteobacteria (*Thauera*, GKS98 freshwater group, *Novosphingobium*, *Thiocapsa*, *Hydrogenophaga*, *Ottowia*), Actinobacteriota (CL500-29 marine group), Verrucomicrobiota (*Luteolibacter*, *Prosthecobacter*), Planctomycetota (*Rhodopirellula*) and Bacteroidota (*Pedobacter*,

Flavobacterium) had a greater abundance in Lagoon, being *Thauera* differentially predominant ((LDA score > 4.0).

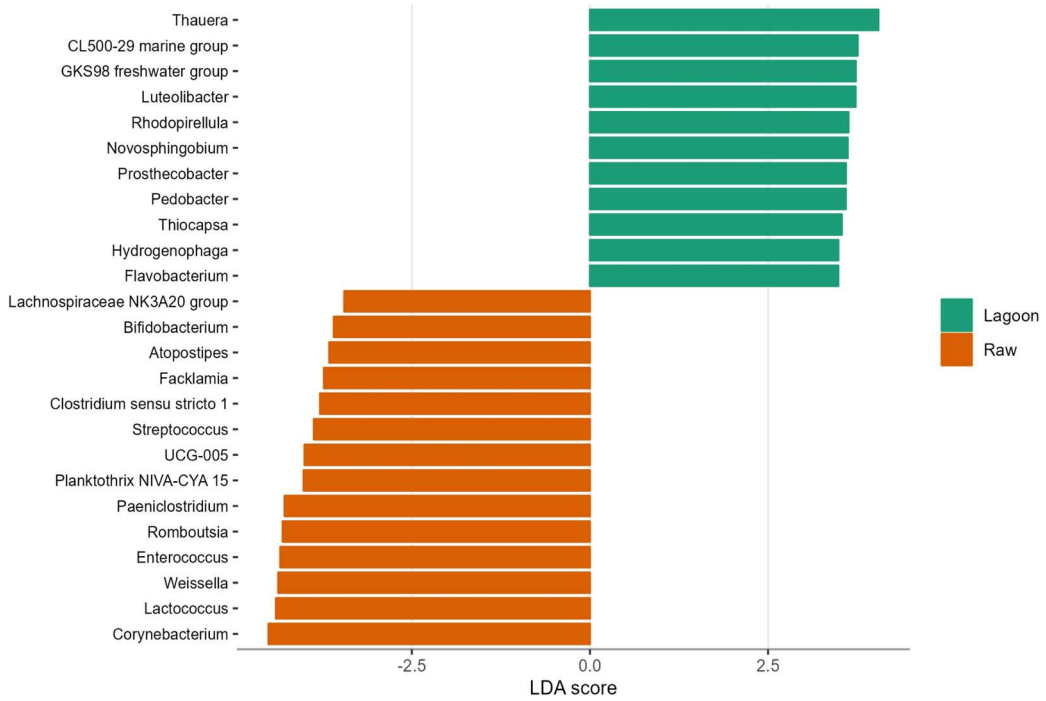

Figure 6. Histogram of Linear Discriminate Analysis Effect Size (LEfSe) analysis of the 25 most abundant genera in Lagoon and Raw dairy effluents. Longer histogram bars indicate a larger impact of certain taxa on the difference between the type of farm dairy effluent ($p < 0.05$).

5.5.4. Linking bacterial community with farm dairy effluents characteristics

RDA was performed to elucidate the potential relationship between the bacterial community composition and the physicochemical properties of DE (Figure 7). The results showed that the first two axes explained 60 % of the total variance. Both DE types are clustered separately, with the Raw DE generally exhibiting the highest values for the physicochemical parameters, as outlined in Table 1. Among the factors influencing microbial community composition, TOC emerged as the most significant, followed by pH, TP, K, and TN. The Mantel test supports these findings, revealing significant correlations with organic N, NH_4, TS, SS, Na, Ca, and Mg (Table S2).

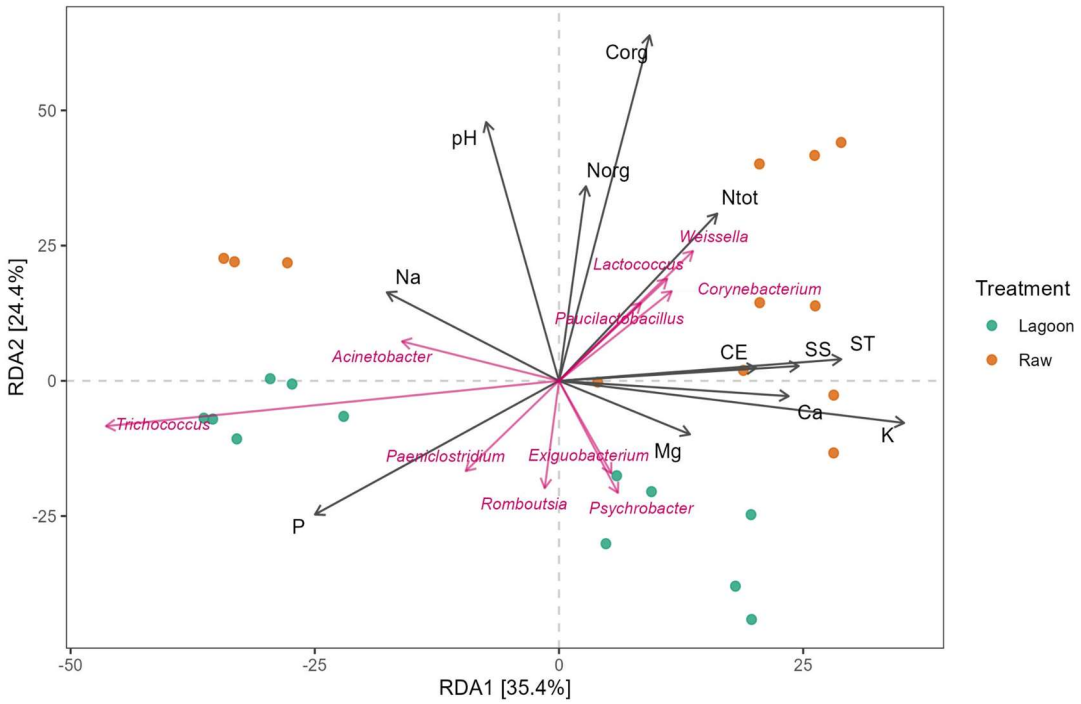

Figure 7. Relationship between bacterial communities and physicochemical parameters of farm dairy effluents (Raw or Lagoon) revealed by redundancy analysis (RDA). Pink arrows: dominant genera; Black arrows: physicochemical properties of dairy effluents. Green and orange points correspond to the bacterial community of Raw and Lagoon Dairy Effluents, respectively. Corg: Total organic Carbon, Ntot: Total Nitrogen, Norg: organic Nitrogen, CE: Electrical Conductivity, ST: Total Solids, SS: Suspended Solids.

Genera, including *Weissella*, *Corynebacterium*, *Lactococcus*, and *Paucilactobacillus*, were mainly associated with TN. In contrast, *Psychrobacter*, *Exiguobacterium*, *Romboutsia*, and *Paeniclostridium* were negatively correlated with TOC, TN, organic N, and pH. On the other hand, the *Paeniclostridium* was correlated with TP, and *Acinetobacter* did it with Na content.

5.6. Discussion

5.6.1. Bacterial community composition changed with Farm Dairy Effluent treatment and season

The dairy industry generates large volumes of effluents that are applied to land as organic fertilizers throughout the year, but it is not without environmental concerns. The study of DE's microbial composition, nutrient-value content, and seasonal variation is imperative to designing best management practices for appropriate DE disposal, and this matter needs to be well-evaluated. This study compared the physicochemical and bacterial composition of untreated (Raw) from that of lagoon-stabilized DE and their variation across seasonal sampling. Our work revealed the first insights into the changes in the microbial community structure of Raw from that of lagoon-stabilized DE. Notably, Lagoon DE exhibited a distinct bacterial community composition, as evidenced by the microbial ASVs, alpha diversity indices, and PCoA (Figures 3, 4, 5). We found phyla Firmicutes and Proteobacteria almost proportionally dominant in Lagoon DE (40-30%), followed by Actinobacteriota and Bacteroidota (Figure S1). On the other hand, Raw was overwhelmingly dominated by Firmicutes (76%). Those phyla were consistently dominant in DE, and the bacterial community was relatively stable at the phylum level; however, the composition of dominant genera varied considerably in different seasons (Figure 3).

Irazoqui et al. [15] performed taxonomic profiling of facultative lagoons from Argentina dairy industries, showing the dominance of phyla Proteobacteria and Actinobacteriota and, in less proportion, Firmicutes and Bacteroidota. Overall, there were no major differences in dominant phyla from our study's bacterial community of the facultative lagoon, suggesting a core microbial selection by this treatment system (Figure S1). In addition, this observation aligns with the findings of Pandey et al. [29], suggesting that lagoon environments develop similar microbial profiles over time. The study of Irazoqui et al. [15] also found members of the *Patescibacteria*, *Verrucomicrobia*, and

Erysipelotrichales to be in lower abundance in facultative lagoons coinciding with our findings (Figure S1, S2).

Differential microbial community composition was also reported through a DE treatment plant [30,31]. These studies found that Firmicutes was the dominant phylum in Raw (inlet) DE, but it decreased after Lagoon treatment while Proteobacteria increased in the anoxic-oxic zone. This difference may be explained by the growth inhibition of many of the obligate anaerobic members of the Firmicutes in aerobic conditions, as suggested by McGarvey et al. [31]. In accordance, Chang et al. [32] reported that treating DE with aeration and microalga and bacteria consortia led to the same shift in the dominance of Firmicutes to Proteobacteria. These findings coincided with our results, suggesting that Proteobacteria is also enriched during treatment in the facultative Lagoon environment. Indeed, genera *Thauera*, GKS98 freshwater group, *Novosphingobium*, *Thiocapsa*, *Hydrogenophaga*, and *Ottowia* within Proteobacteria had the largest effect in differentiating the Lagoon-treated DE from the untread one (Figure 6). These genera have been isolated from several sludge-based anaerobic wastewater treatment plants and identified as key members for removing N [30,33,34].

On the other hand, the genera *Corynebacterium*, *Enterococcus*, *Lactococcus*, and *Weissella* were associated with Raw DE (Figure 3, 6), most of which can be found in raw milk and cattle feces [35,36], which were regular inputs present in fresh effluents.

Among the genera detected, *Trichococcus*, *Romboutsia*, *Clostridium senso stricto* 1, and *Paeniclostridium* were highly abundant in both raw and lagoon-treated DE and at all sampling dates. They were also major genera frequently detected in dairy manure treatments [29,30,31,37,38]. Most Firmicutes have higher environmental adaptability and can degrade various complex organic materials [39]. Furthermore, members of Firmicutes and Bacteroidota have been reported to be present in different reactors because of their ability to degrade organic compounds into monomers [40]. In this sense, Irazoqui et al. [15] identified *Bacteroidetes* and *Clostridiales* as key members of the communities

in facultative ponds involved in polymer and amino acids metabolism, respectively. They suggest that the high amount of carbohydrates and proteins dumped in these ponds would make *Clostridiales* important community members. Other studies based on metagenome-assembled genomes indicate that *Clostridiales* also could play a role in polysaccharide degradation with substrates rich in cellulose [41]. A previous study also reported that biogas slurry application enriched the genera *Paeniclostridium* and *Romboutsia* in the soil, and they were implicated in the transmission of antibiotic resistance genes (ARGs) [42].

Additionally, some archaea families were identified in both DE in a relative abundance of less than 0.1% (data not shown). *Methanosaetaceae*, a family of archaeal acetoclastic methanogens, occurs in Lagoon DE. This is the predominant acetoclastic methanogen in anaerobic digestor systems [43]. *Methanosphaera* and *Methanobacteriaceae,* archaeal families of hydrogenotrophic methane producers were detected in Raw DE. *Methanobrevibacter*, another hydrogenotrophic or formate methane producer, was present in both DE. Identifying these archaeal families is of particular importance, given the interest of the dairy industry in performing anaerobic digestion for energy production [44].

This study observed seasonal changes in the composition of the bacterial community (Figures 3, 4, 5). The bacterial diversity indices increased in the spring/summer sampling (November, Figure 4). This increase might be attributed to the higher temperatures that produce the fastest-growing microorganisms that accelerate organic matter decomposition. Although our study accounted for the higher total bacterial abundance in the winter sampling for both DE (Figure 2), another study also found the lowest bacterial richness and diversity to be in winter in a wastewater treatment plant [45] and higher concentrations of microorganisms were reported in summer than in the winter in air samples collected near the dairy sludge tanks [46].

Although the high-abundant bacterial communities did not vary with the sampling date, some genera showed a remarkable shift between the warm and

cold sampling dates. For example, the genera *Jeotgalibaca*, *Psychrobacter*, *Exiguobacterium*, and *Planococcus* were enriched in Lagoon samples in autumn and winter, *Acinetobacter* did it in autumn, while *Weisella* did it in Raw samples (Figure 3). On the other hand, *Thauera* had a greater relative abundance in the warmest samplings in Lagoon, while *Jeotgalibaca* decreased in Raw samples in winter (Figure 3). Other studies have reported this seasonality in the microbial community of different DE and wastewater treatment systems and attributed these changes mostly to environmental temperatures [38,45,47].

5.6.2. Variability in physicochemical properties of Farm Dairy Effluents and their relation with bacterial community structure

The variation of physicochemical parameters has both direct and indirect influence on the activities of microorganisms. In this study, the Mantel test and RDA analysis (Figure 7) revealed that the community composition of bacteria is significantly affected by different physicochemical parameters of DE. The main factors influencing the bacterial community were TOC, pH, and TN content. The dominant heterotrophic bacteria belonging to Firmicutes and Proteobacteria may be implied in the organic C and N turnover in the DE. Indeed, as indicated in Figure 7, the highly abundant genera *Weissella*, *Corynebacterium*, *Lactococcus*, and *Paucilactobacillus* significantly correlated with TN, indicating that they play key roles in transforming N compounds. TN, K, Na, pH, and suspended solids have been previously demonstrated to correlate with bacterial community structure [48,49].

The RDA analysis also revealed that the stabilization lagoons system had lower levels of organic Carbon (TOC), nitrogen (TN and organic N), solids (ST and SS), and EC (Figure 7), accounting for the expected lagoon purposes. However, there was high variability in nutrient removal efficiency along the seasonal sampling. For example, a lower efficiency of TN removal (20%) at treating DE in winter was observed in the Lagoon (Table S1). Therefore, considering that low temperatures decrease the activity of various

microorganisms and slow the rate of organic matter degradation, it was expected that DE´s TOC would be higher in winter sampling and, accordingly, the higher TN concentration found in Lagoon, while the lower cation content concentration (Table 1, S1). Previous studies showed similar results [38,50]. *Thauera* was identified as a major denitrifying bacteria that occurred in digested DE [51], and its increase in Lagoon in the warmest sampling may suggest a role in N removal, as postulated by Ren et al. [34].

5.6.3. Risk of pathogen loads from Farm Dairy Effluent

Effective management of pathogenic risks linked to DE treatment is critical for public health. Treated effluents from lagoon storage systems may still contain pathogenic bacteria, posing a substantial risk of transporting zoonotic pathogens from land-applied DE through runoff [52]. Our study delves into the risks posed by untreated DE and the efficacy of lagoon stabilization in reducing pathogen loads across varying seasons. This study used pathogenic indicator bacteria such as *E. coli*, total coliforms, and enterococci as biomarkers to infer their reduction through DE treatment. The results demonstrated an overall significant effect of the lagoon stabilization of DE on the microbial concentration of indicator microorganisms. As expected, the number of bacterial biomarkers was highest in Raw DE. After Lagoon stabilization treatment of DE, *E. coli* and total coliform numbers declined almost two orders of magnitude (Figure 1), but still were higher to achieve the levels recommended by the National Council for discharging to waterways (5.0×10^3 UFC/100 mL) [53].

The sequencing analysis of 16S rRNA revealed that Raw DE had dominant genera such as *Enterococcus*, *Streptococcus*, and other possible pathogenic bacteria such as *Facklamia* (Figure 3) [54], all of which are manure-borne microorganisms regularly detected in cattle manures and wastewaters [4,55]. The Lagoon could effectively eliminate the majority of pathogenic bacteria as the occurrence of these genera was not detected or was in very low abundance. Most dominant pathogenic bacteria, such as *Escherichia coli* O157:H7,

Salmonella, *Listeria,* and *Campylobacter* [56,57,58], etc. associated with cow manure and DE may contaminate the forage crops when applied [4]. In our study, these genera were not identified in either type of DE (data not shown).

The season had a crucial effect on the persistence of pathogenic indicators [59]. The fact that the highest counts of *E. coli* and total coliforms were recorded in winter for both types of DE indicate greater consideration should be given to its environmental impact during winter application. Indeed, it has been shown that the mechanism of pathogen removal in natural attenuation lagoons has been associated with effluent exposure to sunlight as a natural source of bacterial inactivation by UV light [60], explaining the higher performance of lagoons in summer to reduce the number of pathogen indicators. A study conducted in covered and open swine lagoons also showed that the reduction in solar radiation contributed to the survival of bacterial indicators [48]. However, contrary to our findings, they reported higher counts of pathogen indicators in swine lagoons occurring in summer and the lowest in winter, but the densities of these bacteria were more associated with the physicochemical composition of the wastewater rather than the season.

5.7. Conclusions

Our results highlight the lagoon system's effectiveness in altering bacterial community composition and reducing pathogen indicator levels, particularly in summer.

The 16S rRNA gene sequencing analysis demonstrated that a large, diverse bacterial population inhabits the Lagoon stabilized DE and changes with the season.

These results emphasize the importance of adopting lagoon systems as a good practice for mitigating the risks associated with pathogen loads from DE. However, the observed decline in treatment efficiency during winter warrants further investigation to enhance the robustness of lagoon systems under diverse environmental conditions.

Knowledge about the DE microbial community composition and its functional potential is key to improving the efficiency and safeness of DE stabilization and application to pastures.

5.7. Supplementary material

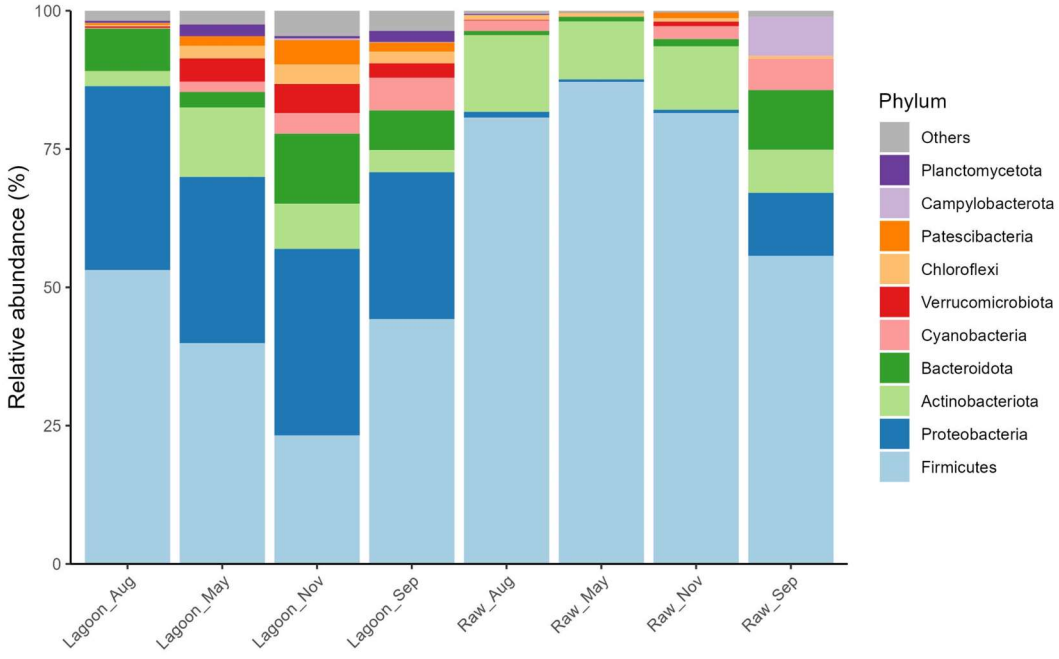

Figure S1. Relative abundances of the main bacterial phyla in Lagoon and Raw dairy effluents at four sampling dates: August, May, November, September.

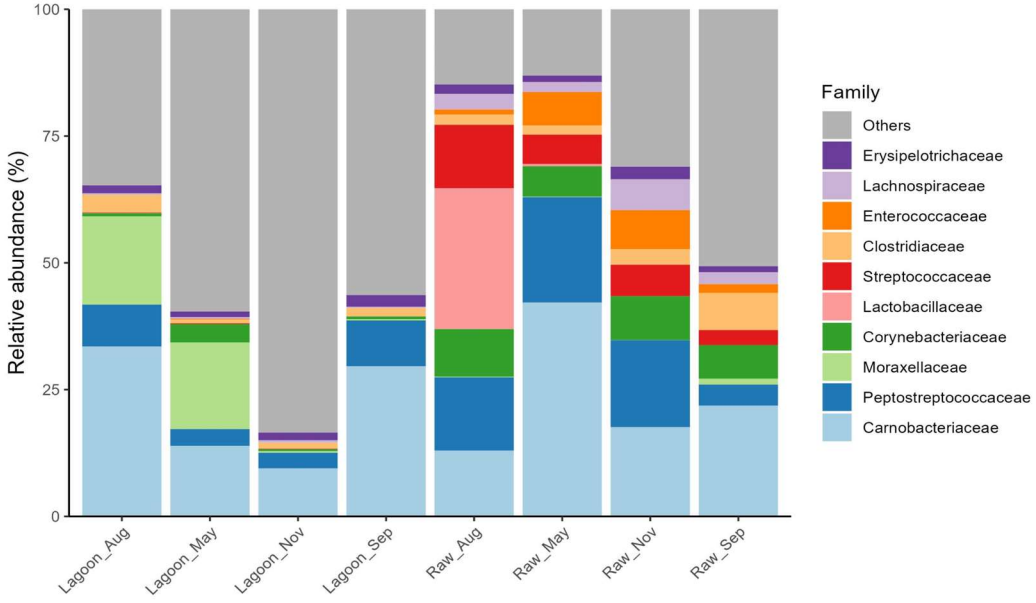

Figure S2. Relative abundances of the main bacterial families in Lagoon and Raw dairy effluents at four sampling dates: August, May, November, September.

Table S1. Seasonal variation in physicochemical characteristics of the Farm Dairy Effluents, obtained immediately after washing off the milking parlor (Raw) or from the lagoon storage system (Lagoon) (n=3± E.E).

						(mg L^{-1})							
Months	Effluent	pH	CE (mS m^{-1})	TS (%)	SS (%)	Corg	TKN	NH_4^+-N	P	K^+	Na^+	Mg^{2+}	Ca^{2+}
September	Raw	8.5±0.0 a	2.4±0.0 c	2.3±0.5a	0.8±0.4a	514±80b	800±39a	216±23b	16±1b	722±88a	217±17a	100±26a	197±61a
November		8.2±0.4 a	6.9±1.2 a	0.8±0.0b	0.3±1b	643±35ab	538±11b	332±47a	84±19a	436±107b	131±28b	46±9c	76±15b
May		7.3±0.0 b	4.8±0.1 b	0.9±0.0b	0.6±0.1b	736±145ab	539±9b	75±10c	101±5a	277±21c	226±19a	73±5b	87±5b
August		6.0±0.0 c	1.8±0.0 c	0.3±0.0b	0.1±0.0b	890±22a	281±17c	19±1d	19±2b	69±4d	16±1c	8.3±1d	15±1c
September	Lagoon	7.7±0.0 b	2.1±0.1 b	0.3±0.0a	0.1±0.0b	359±12c	137±14b	87±15a	38±1a	150±18b	144±17a	31±4ab	48±5a
November		7.7±0.0 b	2.3±0.1 b	0.3±0.0a	0.1±0.0ab	414±19b	95±7c	39±1bc	36±3a	156±13b	75±8b	26±3b	47±5a
May		7.8±0.1 b	3.8±0.0 a	0.3±0.0a	0.1±0.0ab	275±18d	88±2c	37±1c	24±4b	224±27a	157±15a	38±6a	32±6b
August		8.1±0.0 a	2.3±0.4 b	0.2±0.0b	0.2±0.0a	573±42a	231±5a	53±4b	26±2b	92±12c	52±7b	17±2c	23±3b

Table S2. Relationships between bacterial community compositions at genus level and physicochemical properties of farm dairy effluents revealed by Mantel test.

Variable	Correlation coefficient	*p*
pH	0.229	0.013
CE	0.152	0.106
ST	0.344	0.004
SS	0.285	0.010
Corg	0.299	0.003
Ntot	0.508	0.003
Norg	0.367	0.003
NH4	0.323	0.003
K	0.423	0.003
Na	0.181	0.019
Mg	0.293	0.005
Ca	0.352	0.004
P	0.056	0.223

$p < 0.05$ indicates significant correlation

5.8. References

1. Salazar, F.; Herrero, M.A.; Charlón, V.; La Manna, A. Slurry Management in Dairy Grazing Farms in South American Countries. *Proceedings of the 14th International Conference of the FAO ESCORENA Network on the Recycling of Agricultural, Municipal and Industrial Residues in Agriculture* **2010**, 1–4.

2. Bhogal, A.; Nicholson, F.A.; Rollett, A.; Taylor, M.; Litterick, A.; Whittingham, M.J.; Williams, J.R. Improvements in the Quality of Agricultural Soils Following Organic Material Additions Depend on Both the Quantity and Quality of the Materials Applied. *Front Sustain Food Syst* **2018**, *2*, 1–13, doi:10.3389/fsufs.2018.00009.

3. McAllister, T.A.; Topp, E. Role of Livestock in Microbiological Contamination of Water: Commonly the Blame, but Not Always the Source. *Animal Frontiers* **2012**, *2*, 17–27, doi:10.2527/af.2012-0039.

4. Alegbeleye, O.O.; Sant'Ana, A.S. Manure-Borne Pathogens as an Important Source of Water Contamination: An Update on the Dynamics of Pathogen Survival/Transport as Well as Practical Risk Mitigation Strategies. *Int J Hyg Environ Health* **2020**, *227*, 113524, doi:10.1016/j.ijheh.2020.113524.

5. Von Sperling, M. *Wastewater Characteristics, Treatment and Disposal*; IWA publishing, 2007; ISBN 1843391619.

6. Gutiérrez, S.; Cabrera, N. Criterios de Diseño Para Lagunas de Efluentes de Tambos En Condiciones Nacionales. *Revista AIDIS de Ingeniería y Ciencias Ambientales. Investigación, desarrollo y práctica.* **2007**, *1*.

7. Al-Hazmi, H.E.; Mohammadi, A.; Hejna, A.; Majtacz, J.; Esmaeili, A.; Habibzadeh, S.; Saeb, M.R.; Badawi, M.; Lima, E.C.; Mąkinia, J. Wastewater Reuse in Agriculture: Prospects and Challenges. *Environ Res* **2023**, *236*, 116711, doi:10.1016/J.ENVRES.2023.116711.

8. Blaiotta, G.; Di Cerbo, A.; Murru, N.; Coppola, R.; Aponte, M. Persistence of Bacterial Indicators and Zoonotic Pathogens in Contaminated Cattle Wastes. *BMC Microbiol* **2016**, *16*, doi:10.1186/s12866-016-0705-8.

9. Houlbrooke, D.J.; Horne, D.J.; Hedley, M.J.; Hanly, J.A.; Snow, V.O.; Horne, D.J.; Hedley, M.J.; Hanly, J.A.; Snow, V.O. A Review of Literature on the Land Treatment of Farm - Dairy Effluent in New Zealand and Its Impact on Water Quality. **2004**, *8233*, doi:10.1080/00288233.2004.9513617.

10. Hawke, R.M.; Summers, S.A. Effects of Land Application of Farm Dairy Effluent on Soil Properties: A Literature Review. *New Zealand Journal of Agricultural Research* **2006**, *49*, 307–320, doi:10.1080/00288233.2006.9513721.

11. Hill, D.; Morra, M.J.; Stalder, T.; Jechalke, S.; Top, E.; Pollard, A.T.; Popova, I. Dairy Manure as a Potential Source of Crop Nutrients and Environmental Contaminants. *J Environ Sci (China)* **2021**, *100*, 117–130, doi:10.1016/j.jes.2020.07.016.

12. Akari, M.; Uchida, Y. Survival Rates of Microbial Communities from Livestock Waste to Soils: A Comparison between Compost and Digestate. *Appl Environ Soil Sci* **2021**, *2021*, doi:10.1155/2021/6645203.

13. Coelho, J.J.; Hennessy, A.; Casey, I.; Roberto, C.; Bragança, S.; Woodcock, T.; Kennedy, N. Biofertilisation with Anaerobic Digestates: A Field Study of Effects on Soil Microbial Abundance and Diversity. *Applied Soil Ecology* **2019**, 103403, doi:10.1016/j.apsoil.2019.103403.

14. Qi, G.; Pan, Z.; Sugawa, Y.; Andriamanohiarisoamanana, F.J.; Yamashiro, T.; Iwasaki, M.; Kawamoto, K.; Ihara, I.; Umetsu, K. Comparative Fertilizer Properties of Digestates from Mesophilic and Thermophilic Anaerobic Digestion of Dairy Manure: Focusing on Plant Growth Promoting Bacteria (PGPB) and Environmental Risk. *J Mater Cycles Waste Manag* **2018**, *20*, 1448–1457, doi:10.1007/s10163-018-0708-7.

15. Irazoqui, J.M.; Eberhardt, M.F.; Adjad, M.M.; Amadio, A.F. Identification of Key Microorganisms in Facultative Stabilization Ponds from Dairy Industries, Using Metagenomics. *PeerJ* **2022**, *10*, 1–18, doi:10.7717/peerj.12772.

16. APHA *Standard Methods for the Examination of Water and Wastewater.*; 21th ed.; American Public Health Association: Washington (DC), 2005;

17. Mebius, L.J. A Rapid Method for the Determination of Organic Carbon in Soil. *Anal Chim Acta* **1960**, *22*, 120–124, doi:10.1016/S0003-2670(00)88254-9.

18. Bremner, J.M.; Mulvaney, C.S. Total Nitrogen. In *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*; Page, AL, RH Miller, and D.K., Ed.; Amer. Soc. Agron.: Madison, WI USA, 1996; pp. 595–624.

19. 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 Science Society of America Journal* **1998**, *62*, 473, doi:10.2136/sssaj1998.03615995006200020026x.

20. 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, doi:10.1016/S0003-2670(00)88444-5.

21. Isaac, R.A.; Kerber, J.D. Atomic Absorption and Flame Photometry: Techniques and Uses in Soil, Plant, and Water Analysis. *Instrumental Methods for Analysis of Soils and Plant Tissue* **1971**, 17–37, doi:10.2136/1971.instrumentalmethods.c2.

22. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.A.; Holmes, S.P. Dada2. *Nat Methods* **2016**, *13*, 581–583, doi:10.1038/nmeth.3869.

23. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA Ribosomal RNA Gene Database Project: Improved Data Processing and Web-Based Tools. *Nucleic Acids Res* **2013**, *41*, 590–596, doi:10.1093/nar/gks1219.

24. Liu, C.; Cui, Y.; Li, X.; Yao, M. Microeco: An R Package for Data Mining in Microbial Community Ecology. *FEMS Microbiol Ecol* **2021**, *97*, 255, doi:10.1093/femsec/fiaa255.

25. Anderson, M.J. Permutation Tests for Univariate or Multivariate Analysis of Variance and Regression. *Canadian Journal of Fisheries and Aquatic Sciences* **2001**, *58*, 626–639, doi:10.1139/cjfas-58-3-626.

26. Anderson, M. Distance-Based Tests for Homogeneity of Multivariate Dispersions. *Journal of the International Biometric Society* **2006**, *62*, 245–253.

27. Oksanen, J. Vegan: Ecological Diversity. *R Project* **2022**, *368*, 1–11.

28. Segata, N.; Izard, J.; Waldron, L.; Gevers, D.; Miropolsky, L.; Garrett, W.S.; Huttenhower, C. Metagenomic Biomarker Discovery and Explanation. *Genome Biol* **2011**, *12*, doi:10.1186/gb-2011-12-6-r60.

29. Pandey, P.; Chiu, C.; Miao, M.; Wang, Y.; Settles, M.; Del Rio, N.S.; Castillo, A.; Souza, A.; Pereira, R.; Jeannotte, R. 16S RRNA Analysis of Diversity of Manure Microbial Community in Dairy Farm Environment. *PLoS One* **2018**, *13*, 1–18, doi:10.1371/journal.pone.0190126.

30. Yan, W.; Wang, N.; Wei, D.; Liang, C.; Chen, X.; Liu, L.; Shi, J. Bacterial Community Compositions and Nitrogen Metabolism Function in a Cattle Farm Wastewater Treatment Plant Revealed by Illumina High-Throughput Sequencing. *Environmental Science and Pollution Research* **2021**, *28*, 40895–40907, doi:10.1007/s11356-021-13570-w.

31. McGarvey, J.A.; Miller, W.G.; Zhang, R.; Ma, Y.; Mitloehner, F. Bacterial Population Dynamics in Dairy Waste during Aerobic and Anaerobic Treatment and Subsequent Storage. *Appl Environ Microbiol* **2007**, *73*, 193–202, doi:10.1128/AEM.01422-06.

32. Chang, Y.L.; Nagarajan, D.; Chen, J.H.; Yen Chen, C.; Wu, Y.J.; Whang, L.M.; Lee, D.J.; Chang, J.S. Microalgae-Bacteria Consortia for the Treatment of Raw Dairy Manure Wastewater Using a Novel Two-Stage Process: Process Optimization and Bacterial Community Analysis. *Chemical Engineering Journal* **2023**, *473*, doi:10.1016/j.cej.2023.145388.

33. Isobe, K.; Ohte, N. Ecological Perspectives on Microbes Involved in N-Cycling. *Microbes Environ* **2014**, *29*, 4–16, doi:10.1264/jsme2.ME13159.

34. Ren, T.; Chi, Y.; Wang, Y.; Shi, X.; Jin, X.; Jin, P. Diversified Metabolism Makes Novel Thauera Strain Highly Competitive in Low Carbon Wastewater Treatment. *Water Res* **2021**, *206*, 117742, doi:10.1016/J.WATRES.2021.117742.

35. Fusco, V.; Quero, G.M.; Cho, G.S.; Kabisch, J.; Meske, D.; Neve, H.; Bockelmann, W.; Franz, C.M.A.P. The Genus Weissella: Taxonomy, Ecology and Biotechnological Potential. *Front Microbiol* **2015**, *6*, doi:10.3389/fmicb.2015.00155.

36. Velázquez-Ordoñez, V.; Valladares-Carranza, B.; Tenorio-Borroto, E.; Talavera-Rojas, M.; Antonio Varela-Guerrero, J.; Acosta-Dibarrat, J.; Puigvert, F.; Grille, L.; González Revello, Á.; Pareja, L. Microbial Contamination in Milk Quality and Health Risk of the Consumers of Raw Milk and Dairy Products. In *Nutrition in Health and Disease - Our Challenges Now and Forthcoming Time*; Mozsik, G., Figler, M., Eds.; 2019.

37. Habtewold, J.; Gordon, R.; Sokolov, V.; VanderZaag, A.; Wagner-Riddle, C.; Dunfield, K. Targeting Bacteria and Methanogens to Understand the Role of Residual Slurry as an Inoculant in Stored Liquid Dairy Manure. *Appl Environ Microbiol* **2018**, *84*, 1–15, doi:10.1128/AEM.02830-17.

38. Ma, Y.; Shi, F.; Qian, S.; Ma, J. Variations in the Dairy Wastewater Properties and Bacterial Diversity of Each Unit in Membrane-Enclosed Anaerobic Lagoon Treatment Process in Heilongjiang Province. *J Environ Manage* **2023**, *347*, 119035, doi:https://doi.org/10.1016/j.jenvman.2023.119035.

39. Hartmann, M.; Frey, B.; Mayer, J.; Mäder, P.; Widmer, F. Distinct Soil Microbial Diversity under Long-Term Organic and Conventional Farming. *ISME Journal* **2015**, *9*, 1177–1194, doi:10.1038/ismej.2014.210.

40. Sun, L.; Pope, P.B.; Eijsink, V.G.H.; Schnürer, A. Characterization of Microbial Community Structure during Continuous Anaerobic Digestion of Straw and Cow Manure. *Microb Biotechnol* **2015**, *8*, 815–827, doi:10.1111/1751-7915.12298.

41. Blasco, L.; Kahala, M.; Ervasti, S.; Tampio, E. Dynamics of Microbial Community in Response to Co-Feedstock Composition in Anaerobic Digestion. *Bioresour Technol* **2022**, *364*, 128039, doi:10.1016/j.biortech.2022.128039.

42. Lu, Y.; Sun, R.; Zhang, C.; Ding, S.; Ying, M.; Shan, S. In Situ Analysis of Antibiotic Resistance Genes in Anaerobically Digested Dairy Manure and Its Subsequent Disposal Facilities. *Bioresour Technol* **2021**, *333*, doi:10.1016/j.biortech.2021.124988.

43. Nelson, M.C.; Morrison, M.; Yu, Z. A Meta-Analysis of the Microbial Diversity Observed in Anaerobic Digesters. *Bioresour Technol* **2011**, *102*, 3730–3739, doi:10.1016/j.biortech.2010.11.119.

44. Villarroel-Schneider, J.; Höglund-Isaksson, L.; Mainali, B.; Martí-Herrero, J.; Cardozo, E.; Malmquist, A.; Martin, A. Energy Self-Sufficiency and Greenhouse Gas Emission Reductions in Latin American Dairy Farms through Massive Implementation of Biogas-Based Solutions. *Energy Convers Manag* **2022**, *261*, doi:10.1016/j.enconman.2022.115670.

45. Chen, X.; Lang, X.L.; Xu, A.L.; Song, Z.W.; Yang, J.; Guo, M.Y. Seasonal Variability in the Microbial Community and Pathogens in Wastewater Final Effluents. *Water 2019, Vol. 11, Page 2586* **2019**, *11*, 2586, doi:10.3390/W11122586.

46. Szyłak-Szydłowski, M.; Kulig, A.; Miaśkiewicz-Pęska, E. Seasonal Changes in the Concentrations of Airborne Bacteria Emitted from a Large Wastewater Treatment Plant. *Int Biodeterior Biodegradation* **2016**, *115*, 11–16, doi:10.1016/j.ibiod.2016.07.008.

47. Wang, Y.; Sutton, N.B.; Zheng, Y.; Dong, H.; Rijnaarts, H.H.M. Seasonal Variation in Antibiotic Resistance Genes and Bacterial Phenotypes in Swine Wastewater during Three-Chamber Anaerobic Pond Treatment. *Environ Res* **2023**, *216*, 114495, doi:10.1016/j.envres.2022.114495.

48. Ducey, T.F.; Rashash, D.M.C.; Szogi, A.A. Differences in Microbial Communities and Pathogen Survival between a Covered and Uncovered Anaerobic Lagoon. *Environments - MDPI* **2019**, *6*, doi:10.3390/environments6080091.

49. Ducey, T.F.; Hunt, P.G. Microbial Community Analysis of Swine Wastewater Anaerobic Lagoons By next-Generation DNA Sequencing. *Anaerobe* **2013**, *21*, 50–57, doi:10.1016/j.anaerobe.2013.03.005.

50. Illarze, G.; del Pino, A.; Rodríguez-Blanco, A.; Irisarri, P. Application of Dairy Effluents to Pastures Affects Soil Nitrogen Dynamics and Microbial Activity. *Agronomy* **2023**, *13*, 470, doi:10.3390/agronomy13020470.

51. Wang, Y.; Liang, L.; Liu, J.; Guo, D.; Zhu, Z.; Dong, H. Impact of Anaerobic Digestion on Reactive Nitrogen Gas Emissions from Dairy Slurry Storage. *J Environ Manage* **2022**, *316*, 115306, doi:10.1016/J.JENVMAN.2022.115306.

52. Thurston-Enriquez, J.A.; Gilley, J.E.; Eghball, B. Microbial Quality of Runoff Following Land Application of Cattle Manure and Swine Slurry. *J Water Health* **2005**, *3*, 157–171.

53. Decreto Nº 253/979 Artículo 11 Aprobación de Normativa Para Prevenir La Contaminación Ambiental, a Través Del Control de Las Aguas Available online: https://www.impo.com.uy/bases/decretos/253-1979 (accessed on 15 January 2024).

54. Rahmati, E.; Martin, V.; Wong, D.; Sattler, F.; Petterson, J.; Ward, P.; Butler-Wu, S.M.; She, R.C. Facklamia Species as an Underrecognized Pathogen. *Open Forum Infect Dis* **2017**, *4*, doi:10.1093/ofid/ofw272.

55. Dungan, R.S.; Klein, M.; Leytem, A.B. Quantification of Bacterial Indicators and Zoonotic Pathogens in Dairy Wastewater Ponds. **2012**, *78*, 8089–8095, doi:10.1128/AEM.02470-12.

56. Ramos, T.D.M.; Jay-Russell, M.T.; Millner, P.D.; Baron, J.N.; Stover, J.; Pagliari, P.; Hutchinson, M.; Lilley, J.; Rowley, N.; Haghani, V.; et al. Survival and Persistence of Foodborne Pathogens in Manure-Amended Soils and Prevalence on Fresh Produce in Certified Organic Farms: A Multi-Regional Baseline Analysis. *Front Sustain Food Syst* **2021**, *5*, 674767, doi:10.3389/FSUFS.2021.674767/BIBTEX.

57. Rapp, D.; Ross, C.; Hea, S.Y.; Brightwell, G. Importance of the Farm Environment and Wildlife for Transmission of Campylobacter Jejuni in a Pasture-Based Dairy Herd. *Microorganisms* **2020**, *8*, 1–11, doi:10.3390/microorganisms8121877.

58. Ross, C.; Donnison, A. Campylobacter and Farm Dairy Effluent Irrigation. *New Zealand Journal of Agricultural Research* **2003**, *46*, 255–262, doi:10.1080/00288233.2003.9513551.

59. Baker, C.A.; Almeida, G.; Lee, J.A.; Gibson, K.E. Pathogen and Surrogate Survival in Relation to Fecal Indicator Bacteria in Freshwater Mesocosms. *Appl Environ Microbiol* **2021**, *87*, 1–14, doi:10.1128/AEM.00558-21.

60. Wang, M.; Zhu, J.; Mao, X. Removal of Pathogens in Onsite Wastewater Treatment Systems: A Review of Design Considerations and Influencing Factors. *Water (Switzerland)* **2021**, *13*, doi:10.3390/w13091190.

6. Dairy Effluent applications to a pasture enhance soil fertility and microbial activity without impacting soil bacterial and fungal community composition

Illarze G [a], Kuramae E.E [b,c], Illarze M [d], del Pino A [e], Irisarri P [a*]

[a] Laboratorio de Microbiología, Departamento de Biología Vegetal, Facultad de Agronomía, Av. Garzón 780, Montevideo, Uruguay

[b] Netherlands Institute of Ecology (NIOO–KNAW), Department of Microbial Ecology, 6708 PB Wageningen, the Netherlands

[c] Utrecht University, Institute of Environmental Biology, Ecology and Biodiversity, 3584 CH Utrecht, the Netherlands

[d] Departamento de Ecología y Gestión Ambiental, Centro Universitario Regional del Este (CURE), Universidad de la República, Maldonado, Uruguay

[e] Departamento de Suelos y Agua, Facultad de Agronomía, Av. Garzón 780, Universidad de la República, Montevideo, Uruguay

6.1. Resumen

Los efluentes de tambo (FDE, por sus siglas en inglés) provenientes del lavado de la sala de ordeño contienen estiércol, orina y productos químicos, y constituyen una gran cantidad de aguas residuales. La aplicación de FDE como fertilizantes al suelo en pastizales puede aumentar el rendimiento del forraje y mejorar el estado de los nutrientes del suelo. Dado que la industria láctea está intentando cada vez más maximizar los rendimientos mediante una mejor utilización del forraje con menores insumos, hay una demanda de suministro de FDE como fertilizantes. Sin embargo, el impacto de esta práctica en la microbiota del suelo sigue siendo en gran medida desconocido. Debe estudiarse antes de la disposición en el suelo a gran escala para evitar la disminución de la diversidad microbiana o el aumento de la abundancia de patógenos. Este estudio evaluó los efectos de aplicar efluentes de tambo almacenados en lagunas

(Lagoon) y crudos (Raw) a una tasa de 50 kg N ha^{-1} en cuatro dosis iguales, en comparación con la fertilización con urea, sobre el contenido de nutrientes del suelo y la actividad, abundancia y estructura comunitaria de la microbiota del suelo. El efluente crudo (Raw) se obtuvo después de la separación de sólidos, y el de laguna (Lagoon) corresponde al efluente crudo estacionado en un sistema de dos lagunas. La actividad microbiana se evaluó como respiración basal, nitrógeno potencialmente mineralizable, actividad de nitrificación potencial y actividades enzimáticas (deshidrogenasa, fosfatasa alcalina, ureasa). La actividad catabólica de la comunidad microbiana se evaluó utilizando Biolog Ecoplates™. La composición y diversidad de las comunidades bacterianas y fúngicas se analizaron mediante secuenciación de amplicones de 16S rRNA e ITS2. La aplicación de FDE benefició el status nutricional del suelo y la actividad microbiana. El efluente de laguna (Lagoon) tuvo los efectos más potentes sobre el P disponible y los K^+, Na^+, Mg^{2+} y Ca^{2+} extractables del suelo. El suelo tratado con efluente crudo (Raw) mostró mayores actividades microbianas, como deshidrogenasa, respiración basal, ureasa y nitrógeno potencialmente mineralizable, que los otros tratamientos. Los FDE no alteraron significativamente la composición, abundancia o diversidad funcional microbiana. En conclusión, en este ensayo a corto plazo, a pesar de los cambios en las propiedades químicas del suelo y la actividad microbiana, la composición y diversidad de las comunidades bacterianas y fúngicas permanecieron sin cambios por la irrigación con FDE.

Palabras clave: Tambo, perfil fisiológico de la comunidad, comunidades microbianas, fertilizante orgánico, actividades enzimáticas del suelo, efluentes de tambo crudos, efluentes de tambo estacionados en laguna

6.2. Summary

Farm dairy effluents (FDE) from washing the milking parlor contain manure, urine, and chemicals and constitute a large amount of wastewater. Applying FDE

as soil fertilizers to pastures can enhance forage yield and improve soil nutrient status. Since the dairy industry is increasingly attempting to maximize returns through better utilization of forage with lesser inputs, there is demand for a supply of FDE as fertilizers. Nevertheless, the impact of this practice on soil microbiota remains largely unexplored. It must be studied before large-scale soil disposal to avoid diminishing microbial diversity or enhancing pathogen abundance. This study evaluated the effects of applying lagoon-stored (Lagoon) and raw dairy effluents (Raw) at a rate of 50 kg N ha^{-1} in four equal doses, in comparison to urea fertilization, on soil fertility and the activity, abundance, and community structure of soil microbiota. Raw was obtained after solid separation, and Lagoon corresponds to the Raw stationed in a two-lagoon system. Microbial activity was assessed as basal respiration, potentially mineralizable N, potential nitrification activity, and enzymatic activities (dehydrogenase, alkaline-phosphatase, urease). The catabolic activity of the microbial community was evaluated using Biolog Ecoplates™. Bacterial and fungal community composition and diversity were analyzed through amplicon sequencing of 16S rRNA and ITS2. The application of FDE benefited soil fertility and microbial activity. Lagoon had the most potent effects on soil available P and extractable K^+, Na^+, Mg^{2+} and Ca^{2+}. Soil treated with Raw displayed higher microbial activities, such as dehydrogenase, basal respiration, urease, and potentially mineralizable N, than the other treatments. FDE did not significantly alter the microbial composition, abundance, or functional diversity. In conclusion, in this short-term trial, despite changes in soil chemical properties and microbial activity, the composition and diversity of the bacterial and fungal communities remained unaffected by FDE irrigation.

Key words: dairy farm, community-level physiological profiles, microbial communities, organic fertilizer, soil enzyme activities, raw dairy effluents, lagoon-stored dairy effluents.

6.4. Introduction

The intensification of the dairy industry deals with the sustainable management of large volumes of C and nutrient-rich effluents while minimizing the risks generated after their final destination [1]. Land application of farm dairy effluents (FDE) is encouraged to recycle them as organic fertilizers, providing valuable nutrients to pasture or crops and improving soil fertility [2]. The environmental concerns of FDE land application include the possibility of nitrate leaching to groundwater, surface runoff to waterways, excess of P, and accumulation of Na+, possibly promoting soil structural breakdown [3]. FDE application may also trigger the release of gases into the atmosphere, including ammonia and greenhouse gases such as methane and nitrous oxide [4]. This organic amendment can also contain potentially pathogenic organisms such as enteric bacteria, parasites, viruses, and fungi. On the other hand, belowground soil biodiversity, a primary driver of many critical soil processes, needs to be evaluated after FDE application as shifts in microbial community composition might entail changes in soil ecosystem functioning [5].

Wastewater stabilization lagoons are a low-operation and maintenance form of FDE treatment. In these systems, effluents are retained for long periods, allowing the microbial communities present to reduce organic load while increasing inorganic forms of nutrients and decreasing C content [6]. These systems often comprise an anaerobic and a facultative lagoon operated in series [7]. The first lagoon's anaerobic conditions result from solid material accumulation on the surface. The second lagoon is considered facultative because it presents oxic conditions on the surface and is anoxic in depth. The dairy effluent produced after this stabilization treatment differs substantially from the raw dairy effluent generated after cleaning dairy facilities. The most easily degradable C is turned out by the fermentation/oxidation process, which results in lower dry matter compared to untreated effluents [8,9]. These

differences in available C are recognized to change microbial activity [10]. When used as fertilizers, both effluents (Lagoon or Raw) may alter soil's N and C cycle processes and differentially modulate the responses of the soil microbial community and its activity, for example, by increasing or reducing microbial biomass and activity in soil [11]. Moreover, the less organic C available for soil microbial communities in lagoon-stored dairy effluents may affect the turnover of mineral N after its application to the soil-plant system [12]. In a greenhouse pot experiment, Raw FDE but not Lagoon FDE increased soil enzymatic activity and presented a differentiated substrate utilization pattern [13].

Illarze et al. [7] found that the effluents used in this work differed in their organic carbon, nitrogen, solids content, and electrical conductivity. Furthermore, lagoon dairy effluent exhibited a distinct bacterial community composition from raw one. In that work, the bacterial community was more diverse in the lagoon, with Firmicutes and Proteobacteria proportionally dominant, while Raw was mainly composed of Firmicutes. The FDE bacterial composition informs about a potential invasion of these microbes into the resident soil microbial community, especially since there is much concern about raw FDE pathogenic contamination [7].

The concentrations of solids and nutrients of effluents derived from different management systems [14] may result in different volumes applied to the soil to achieve plant N requirements. Thus, the variability in nutrient input concurrent with a shift in soil water status may impact soil microbiota. Most studies have evaluated the effect of solid manure amendments rather than FDE on soil microbial communities. Manure amendments change the bacterial community [15] and increase microbial activity more than mineral N [11,16,17]. Although some studies have found that manure application significantly alters the soil microbiome [18,19], other studies have reported changes limited to a few taxa, whereas the main microbial composition of the soil remains unmodified [17,20]. However, the extent to which FDE influences the soil microbial community composition, diversity, and activity remains unclear [21].

A large volume of water is used to clean parlor facilities in farms that cannot install complex effluent handling systems [22]. These effluents have low concentrations of organic matter (OM) and nutrients [23]. The application of this type of effluent to a pasture has yet to be studied [7,13]. In the present work, we investigated the impact of the application of two forms of effluent, namely treated lagoon-stored dairy effluent (Lagoon) and untread dairy effluent (Raw) to a fescue pasture compared to synthetic N fertilization (Urea) and non-amendment (Control) on the soil fertility, soil microbial community structure, abundance, and activity. We hypothesized that the difference in nutrient and microbial input provided by the two FDE types applications increases soil fertility and differentially modulates the responses of the soil microbial community, promoting soil microbial abundance and activity and altering the microbial community composition and diversity. According to previous studies, we expected Raw to increase soil microbial activity and decrease the microbial community diversity more than Lagoon.

6.4. Materials and Methods

6.4.1. Field experiment and sample collection

The experimental field was conducted at the Centro Regional Sur (CRS) Experimental Station of the Faculty of Agronomy in Canelones, Uruguay (34°36' 47.83" S and 56° 12' 54.00" W). The climate is humid subtropical, with an average mean temperature of 17 °C (minimum: 11 °C, maximum: 23 °C) and light frosts between May and September. Mean annual precipitation is 1200 mm, evenly distributed throughout the year with significant variation between years [24]. The soil is an Argiudol, 370 g kg^{-1} of clay, 380 g kg^{-1} of silt, and 250 g kg^{-1} of sand, with no history of FDE application. The main soil chemical characteristics of the topsoil (0–15 cm) were: pH 6.4 (in H_2O), organic carbon (OC) 24.5 g kg^{-1}, available P 22.4 mg kg^{-1}, exchangeable K^+ 0.6 cmolc kg^{-1} and Na^+ 0.3 cmolc kg^{-1}.

The experimental field sowed with *Festuca arundinacea* cv. Rizomat was divided into plots of 3 m x 4 m with a 0.5 m wide buffer strip between plots, and each treatment was replicated three times in a complete randomized block design. Treatments were applied as four N-based applications to the pasture soil (September 6, 2019; November 11, 2019; May 5, 2020 and August 18, 2020) at an equivalent rate of 50 kg N ha^{-1} consisting of (i) raw dairy effluent (Raw), (ii) dairy effluent stabilized by two lagoon systems (Lagoon), (iii) synthetic granulated N fertilizer (Urea), and (iv) a non-amended soil (Control). Treatments were manually applied in September 2019, and again, after three fescue forage cuts, watering cans were used to ensure the effluent could not flow out of the plot area. The N-application rate was selected according to agronomical recommendations, 50 kg N ha^{-1} for fertilization after dairy cattle mowing.

The FDEs were collected from CRS's dairy farm from the Faculty of Agronomy before each application, and the total N content was determined for both effluents at each sampling date. The Raw dairy effluent was flushed from the barn floor and sampled fresh from a holding tank after solid sedimentation. The Raw applied in the experiment presented an average pH of 7.1, 4.0% of total solids, 696 mg L^{-1} of total carbon, 492 mg L^{-1} of total N, and 232 mg L^{-1} of ammoniacal N. The Lagoon dairy effluent was pumped from four points of a facultative lagoon. Lagoon had an average pH of 7.9, 2.6% of total solids, 405 mg L^{-1} of total carbon, 105 mg L^{-1} of total N, and 45 mg L^{-1} of total ammoniacal N. More information on effluent composition, measurement, and sampling can be found in Illarze et al. [7].

For microbial and chemical analysis, a composite soil sample (a mixture of 10 random points in each plot) of the topsoil (0-15 cm) from the triplicate plots was taken. Soil samples were collected following 45 days of the final treatment application in September 2020, one year after the first application of treatments. The soil samples were stored at -20 °C until DNA extraction and at 4°C for <1 week before soil chemical properties and microbial activities analyses.

6.4.2. Soil chemical analysis

The soil's mineral N (NO_3^-–N and NH_4^+–N) was extracted with a 2 M KCl solution and colorimetrically analyzed to determine NH_4^+–N according to Rhine et al. [25] and NO_3^-–N according to Doane and Horwáth [26]. Soil pH was measured in distilled water with a 1:2 soil: water ratio. Soil OC was quantified by the Walkley-Black wet combustion method [27], and P was determined by the Bray 1 method [28]. The total exchangeable bases, including Ca^{2+}, Mg^{2+}, K^+, and Na^+, were extracted with ammonium acetate buffered at pH 7 [29]. The K^+ and Na^+ were determined by atomic emission spectrometry, and Ca^{2+} and Mg^{2+} by absorption spectrometry. Soil moisture was determined by weighing the soil and drying it at 105°C until a constant weight was reached.

6.4.3. Soil enzyme activities and basal respiration

Dehydrogenase activity was determined according to von Mersi and Schinner [30]. Briefly, [2-(p-iodophenyl)-3-(p-nitrophenyl)- 5-phenyl tetrazolium chloride] solution (INT) was used as a substrate and incubated for 2 h at 40 °C. The reduced iodonitrotetrazolium formazan (INTF) was then extracted and photometrically measured at 464 nm.

The alkaline-phosphatase enzyme was determined following the method of Tabatabai [31] with the following modifications suggested by Margesin [32]: $CaCl_2$ was omitted from the sample and NaOH was added after filtration before measuring absorbance with a spectrophotometer. The results are expressed as mg p-nitrophenol g^{-1} h^{-1} at 37°C.

Urease activity was determined by a modified method, according to Kandeler [33]. Five grams of fresh soil were incubated with 2.5 ml of urea solution at 37 °C for 2 h, and the released NH_4^+ was extracted with 2 M KCl and determined in the same way as soil extracts for chemical analysis.

The soil basal respiration was measured by titration according to Öhlinger [34] with slight modifications. 15 g of fresh soil were incubated in a closed vessel for 5 days at 25 °C in the dark. The CO_2 was trapped in NaOH solution (0.25 M) and titrated against 0.1 HCl after freshly prepared saturated $BaCl_2$ (0.5 M) was added. The respiration rate was expressed as mg of CO_2 mg^{-1} dry soil d^{-1}.

6.4.4. Nitrogen potential activities

Potential nitrification activity (PNA) was assessed using a microscale method based on ISO 15685 [35]. Briefly, 2.5 g of fresh soil was placed in a 50 mL flask, and a solution with 300 mM KH_2PO_4, 700 mM K_2HPO_4, 10 mM sodium chlorate, and 1.5 mM $(NH_4)_2SO_4$ was added to create a 10 mL slurry. The reaction was incubated at 25 °C and shaken at 175 rpm. After 24 h, 1 mL samples were collected, added to 1 mL 2 M KCl, briefly vortexed, and centrifuged to separate soil particles. The resulting supernatant was removed, and the NO_2^-–N concentration was determined spectrophotometrically at 540 nm using the Griess–Ilosvay reagent.

According to Kandeler [36], potentially mineralizable nitrogen (PMN) was determined using the anaerobic method. Fresh soil (5 g) was incubated under 15 mL water for 1 week at 40 °C. At the end of the incubation, NH_4^+-N content was determined by colorimetric analysis, according to Rhine et al. [25].

6.4.5. Microbial community physiological profiling

Community-level physiological profile (CLPP) of soil microbial populations was performed by the BIOLOG EcoPlates™ (Biolog®, Hayward, CA, USA). These 96-well plates contain 31 carbon sources, one blank (no substrate) as control, and tetrazolium violet as a redox dye in triplicates. Microbial use of these substrates is reflected by color change in each of the wells, as the redox dye is reduced to tetrazolium violet. The assay was performed as previously described by Illarze et al. [13]. Color development was recorded as

the absorbance (Abs) of each well every 24 h for 120 h at 590 nm, and data recorded at the end of the exponential phase (72 h) were used to assess microbial functional diversity and perform statistical analyses. Potential catabolic microbial activity in each microplate/time point was expressed using average well color development (AWCD) in Eq. (1) below:

$$AWCD = \frac{\sum R-C}{31} \quad (1)$$

where C is the Abs_{590} of control wells, R is the Abs_{590} of each response well, and 31 is the number of carbon substrates in the plate. The AWCD was graphed and the area under the curve corresponded to the activity per unit time and the slope to the rate of substrate use [37].

The substrates were subdivided into six groups: carbohydrates, carboxylic acids, amines, amino acids, phenolic compounds, and polymers, according to Insam [38]. Substrate richness, or the number of substrates catabolized by each microbial community, was determined as the number of wells with Abs > 0.5 as the Shannon-Weaver index. The Shannon–Weaver diversity index (H') was used to estimate the catabolic diversity of microbial communities Eq. (2):

$$H' = \sum Pi \; x \; InPi \quad (2)$$

where Pi is the relative color development of each well over the total Abs of the plate.

6.4.6. Soil DNA extraction, qPCR, and 16S rRNA gene and ITS region amplicon sequencing

DNA was extracted using the PowerSoil® DNA Isolation kit (Qiagen®) using 0.25 g (fresh weight, FW) of soil. DNA was quantified (ng μL^{-1}) and absorbance ratios (260/280 nm and 260/230 nm) were measured using a NanoDrop™ 1000 Thermo Scientific spectrophotometer.

To determine if the abundance of bacteria and fungi changed with FDE application, quantitative real-time PCR (qPCR) was conducted. The copy number of total bacteria was estimated with forward primer 515F (5′-GTGC CAGCMGCCGCGGTAA-3′) and the reverse primer 806R (5′-G GACTACHVGGGTWTCTAAT-3′) with 300-bp in length [39]. The thermal cycle consists of an initial incubation of 5 min at 95 °C, followed by 30 cycles of 30 sec at 95 °C, 45 sec at the annealing temperature of 55 °C and 50 sec of extension at 72 °C. For total fungi abundance determination, the primer ITS1F (5′-CTTGGTCATTTAGAGGAGTAA-3′) [40] as forward and primer ITS2 (5′-GCTGCGTTCTTCATCGATGC-3′) [41] as reverse were used with around 250-400-bp in length. The initial incubation was followed by 35 cycles of 10 sec at 95 °C, 15 sec at the annealing temperature of 54 °C, and 10 sec of extension at 72 °C.

For bacteria community composition determination, the variable V3-V4 region of the 16S rRNA gene was amplified with forward primer 341F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer 805R (5′-GACTACHVGGGTATCTAATCC-3′) [39]. For fungi, primers ITS3 (5′-GCATCGATGAAGAACGCAGC-3′) [42] as forward and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) as reverse [43] were used to amplify the internal transcribed spacer region (ITS). Amplicon library preparation was performed using the DNA extracted from each soil sample and then sequenced by Illumina MiSeq platform (Illumina, San Diego, CA) at Macrogen Inc. Company, South Korea. The generated data were processed, demultiplexed, and quality control using DADA2 pipeline [44] with the "consensus" method to remove any remaining chimeric and low-quality sequences. The taxonomic identification (with 99% of similarity) was performed using SILVA database (v. 138) [45] for bacterial and unite (v. 8.2) [46] for fungal profiles. The taxonomic matrices generated from the amplicon sequence variants (ASV) were summarized at the Genus taxonomic level and used for further statistical analysis. Low-occurring microbes (presence in less than 5 samples) were aggregated into a single variable named 'others.' The filtered ASV table was

used to determine the Shannon-Weaver diversity index (Eq. 2) for each community (function diversity) and to estimate community richness the Chao1 richness index was calculated following Eq. (3):

$$Chao1 = Sobs + \frac{F1(F1-1)}{2(F2+1)} \quad (3)$$

where *F*1 and *F*2 are the count of singletons and doubletons, respectively, and S*obs* is the number of observed species.

6.4.7. Statistical analysis

One-way ANOVA was used to test differences between treatments on the individual variables of soil chemical and microbiological parameters, followed by least significant differences (LSD) at the level $p \leq 0.05$ as a post-hoc test for multiple comparisons.

For the Beta-diversity calculations, the entire filtered ASV table was used and normalized using the function cumNorm from the R package metagenomeSeq (v.1.12) [47]. We used a cumulative-sum scaling (CSS) method, which calculates the scaling factors equal to the sum of counts up to a particular quantile to normalize the read counts in order to avoid the biases generated with current sequencing technologies due to uneven sequencing depth [48]. Soil microbial diversity indexes and community patterns of the bacterial and fungal communities were assessed using the Bray-Curtis dissimilarity distance, represented by non-metric multidimensional scaling (NMDS). The statistical significance of the clustering patterns in the ordination plots was subsequently evaluated by PERMANOVA (function "adonis2") with Vegan package in R [49].

Generalized Joint Attribute Modeling (GJAM) package in R (v.4.1.2) [50] was performed to explore the impact of N-based applications with FDE to a fescue pasture on microbial community composition, abundance, activity, and

soil fertility properties simultaneously. This joint model approach allowed us to include variables of different types and to analyze them jointly, thus revealing the regression coefficients of the effects of different N-based applications in the relative abundance of taxa within the soil microbiome, as constrained by the compositionality [51] and the microbial activity and soil factors as continuous values [52]. For GJAM model diagnosis, the Markov chain Monte Carlo (MCMC) method was used to determine when the estimated coefficients reached a stable value (after 2000 simulations with a burn-in of 500). The regression coefficients obtained, integrating all variable groups, were built to disentangle the distinction between treatments on principal component analysis (PCA), and sensitivity analysis was performed to identify the responses to the applications on different groups of variables in a hierarchical manner. Finally, the set of regression coefficients that constitute the microbiome profile was highlighted using a heatmap. All statistical analyses were conducted in R Studio, running R version 4.02 and the ggplot2 package for data visualization [53].

6.5. Results

6.5.1. Soil chemical analysis

The application of FDE significantly increased ($p < 0.05$) soil EC, organic C and K^+ content (Table 1). On the other hand, Lagoon DE had a significant impact on soil fertility by increasing soil exchangeable cations (Ca^{2+}, Mg^{2+}, K^+, and Na^+), and P content relative to Urea and Raw treatments.

Table 1. Soil physicochemical properties of fescue plots with applications of Raw or Lagoon Dairy Effluents, Urea fertilization, or non-amended Control (mean n = 3 ± S.E). Different letters in each column indicate significant differences between treatments by LSD test at $p \leq 0.05$.

Treatment	pH	EC (µS m^{-1})	OC%	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})	P-Bray1 (mg kg^{-1})	Ca^{2+} (cmolc kg^{-1})	Mg^{2+} (cmolc kg^{-1})	K^+ (cmolc kg^{-1})	Na^+ (cmolc kg^{-1})
Control	5.9± 0.1	56.3± 1.2 **a**	2.5± 0.1 **a**	8.3± 3.6	5.3± 0.22	18.0± 1.2 **b**	7.8± 0.2 **a**	4.6± 0.3 **bc**	0.8± 0.0 **a**	0.3± 0.0 **a**
Urea	5.7± 0.2	47.8± 2.2 **a**	2.6±0.2 **a**	5.4± 1.3	4.8± 0.2	9.0± 1.2 **a**	7.5± 0.5 **a**	3.6± 0.3 **a**	**0.7± 0.1 a**	0.3± 0.1 **a**
Raw	5.9± 0.3	75.1 ± 0.9 **b**	2.9± 0.2 **b**	3.7± 0.5	5.1± 0.3	17.3± 3.8 **b**	7.9± 0.1 **a**	4.1± 0.1 **ab**	1.0± 0.0 **b**	0.4± 0.0 **a**
Lagoon	6.0± 0.1	78.1 ± 6.3 **b**	2.7± 0.2 **b**	4.5± 0.9	4.8± 0.1	27.7± 2.7 **c**	8.3± 0.4 **b**	5.1± 0.3 **c**	1.5± 0.2 **c**	0.6± 0.0 **b**

6.5.2. Soil microbial activity

In general, FDE soil applications increased some microbial activities (Fig. 1), while Raw FDE enhanced dehydrogenase activity (23%) and respiration rate (14%) relative to control soil. Lagoon FDE triggered higher PNA, but both FDE had higher PNA than Urea. Both FDE significantly enhanced soil PMN compared to the control treatment (20%), but neither alkaline phosphatase nor urease enzyme activity was affected by FDE amendments (Fig. 1).

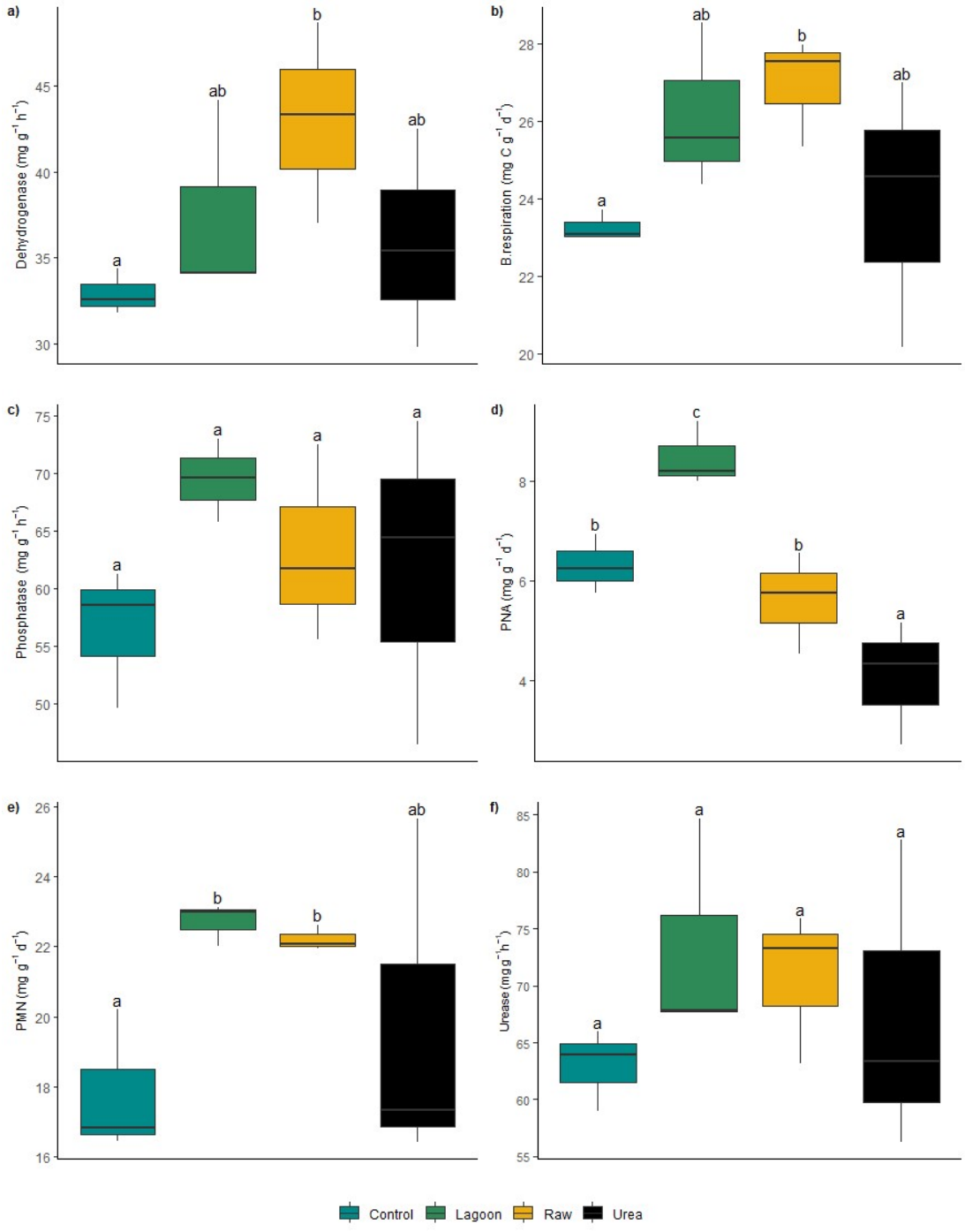

Fig. 1. Microbial enzymatic activity (a) dehydrogenase, (c) alkaline phosphatase, (f) urease, (b) basal respiration, (d) potential nitrification activity (PNA), and (e) potentially mineralizable N (PMN) of soil with the application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization or non-amended Control. Different letters denote significant differences between treatments by LSD test at $p \leq 0.05$ (mean n = 3 ± S.E).

6.5.3. Microbial community physiological profiling

The Community-level physiological profile (CLPP) of soil microbial populations revealed that the highest average well color development (AWCD) values were associated with the soils with the application of N (FDE or Urea) compared to the untreated Control, indicating a higher microbial activity (Fig. 2). Raw DE-amended soil had initially faster-metabolizing microbial communities than the control (slope of AWCD curve till 72hs, Suppl. Table 1). However, there were no significant differences between the microbial diversity indices of C substrates utilization of FDE-amended soil and soil with Urea fertilization and unamended Control (Suppl. Table S1).

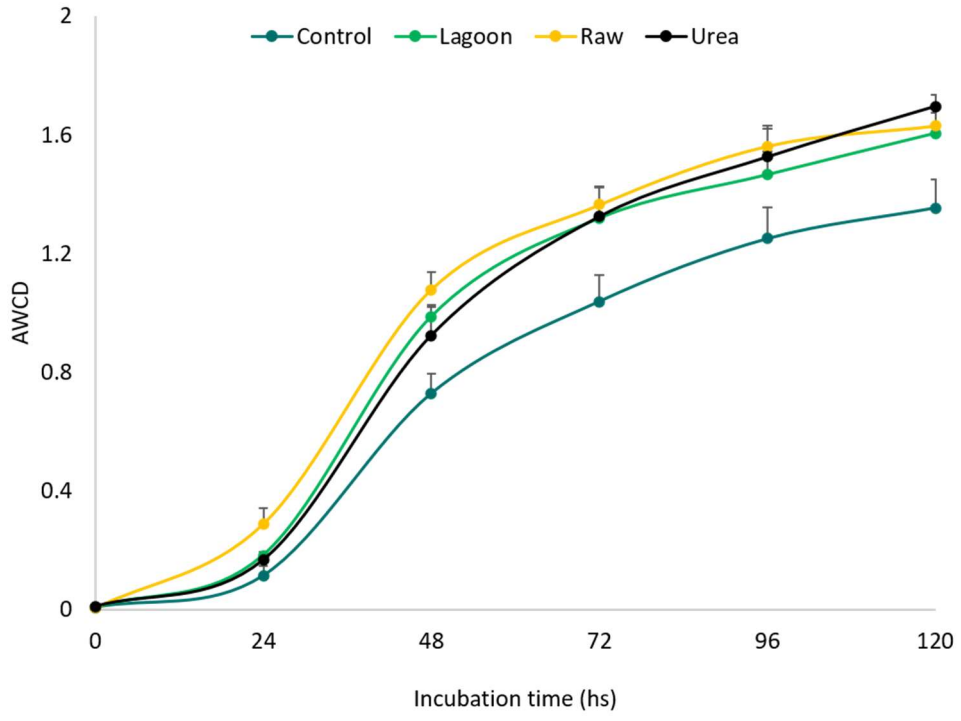

Fig.2. Average well color development (AWCD) in Biolog EcoPlates inoculated with soils with the application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization or non-amended Control (mean n = 3 ± S.E).

To gain a better understanding of microorganisms' metabolism in response to FDE applications, we characterized microbial community function based on groups of carbon sources utilization patterns (Fig. 3). In general, regardless the treatment applied to the soil, the microbial community actively utilized carbohydrates (D-mannitol, D-cellobiose, N-acetyl-D-glucosamine), some amino acids (L-asparagine, L-arginine, L-serine), 4-hydroxybenzoic acids, and γ-hydroxybutyric acid. In contrast, the microbial community was unable to catabolize 2-hydroxybenzoic acid, α-ketobutyric acid, L-treonine, i-erythritol, glycyl-L-glutamic acid, D, L-α-glycerol phosphate. Among FDE amendments, the microbial community of soil with the application of Raw had around 50% greater glycogen and α-cyclodextrin (polymers) utilization, while with Lagoon, around 10% amino acids (L-asparagine, L-arginine) and 20% γ-hydroxybutyric acid greater metabolizing capacity.

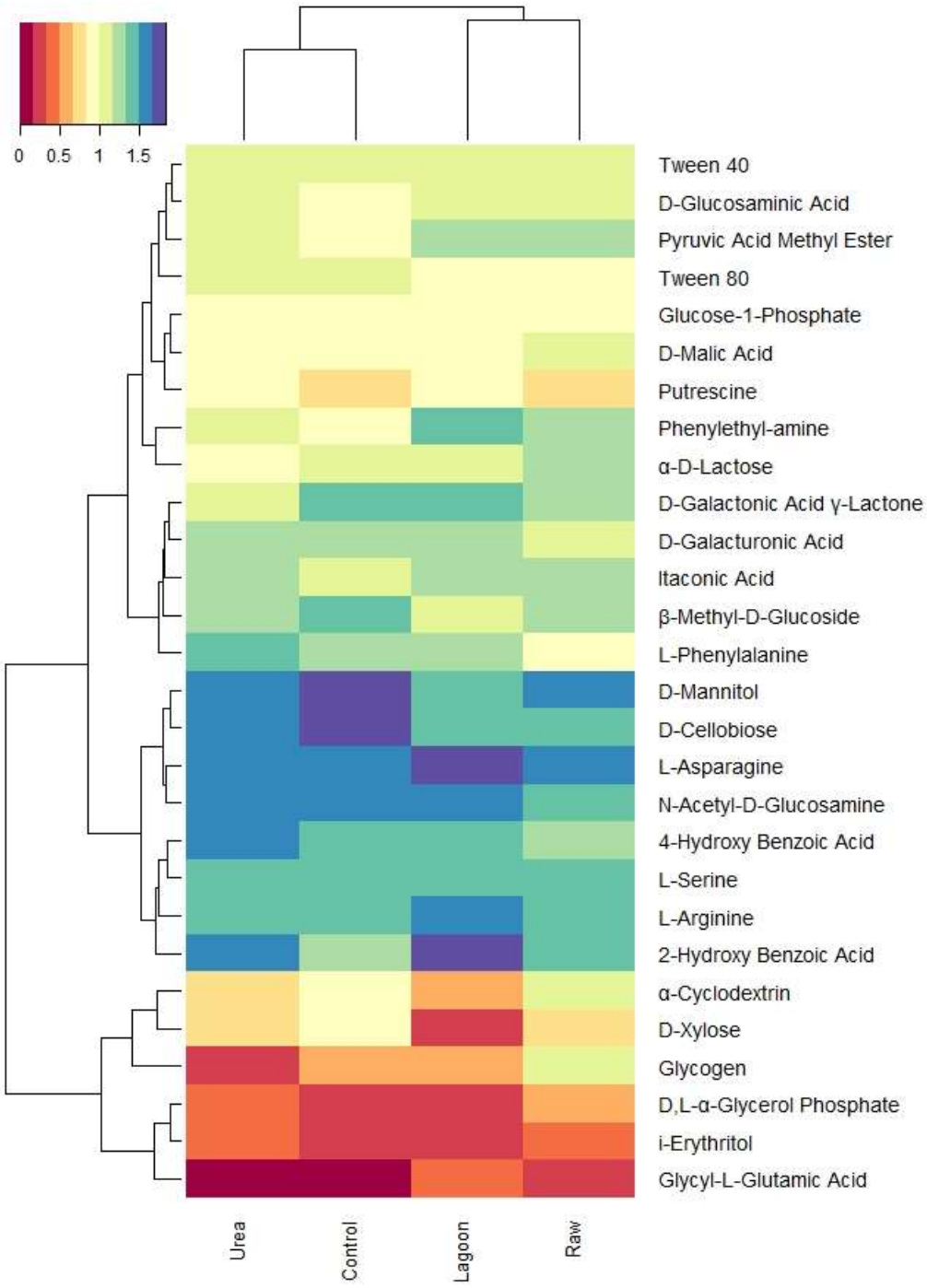

Fig. 3. Heatmap clustering of catabolic profiles of microbial communities from the soil with the application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization or non-amended Control. The color scale of the heatmap reflects the utilization of the 31 carbon substrates (values=Abs_{590}) present in the Biolog Ecoplate after 72 h of incubation (mean n=3).

6.5.4. Effect of dairy effluents on soil bacterial and fungal microbial community abundance, composition, and diversity

Applying FDE to the soil did not impact the abundance of bacteria or fungi or their ratio as revealed by qPCR analyses (Fig. 4).

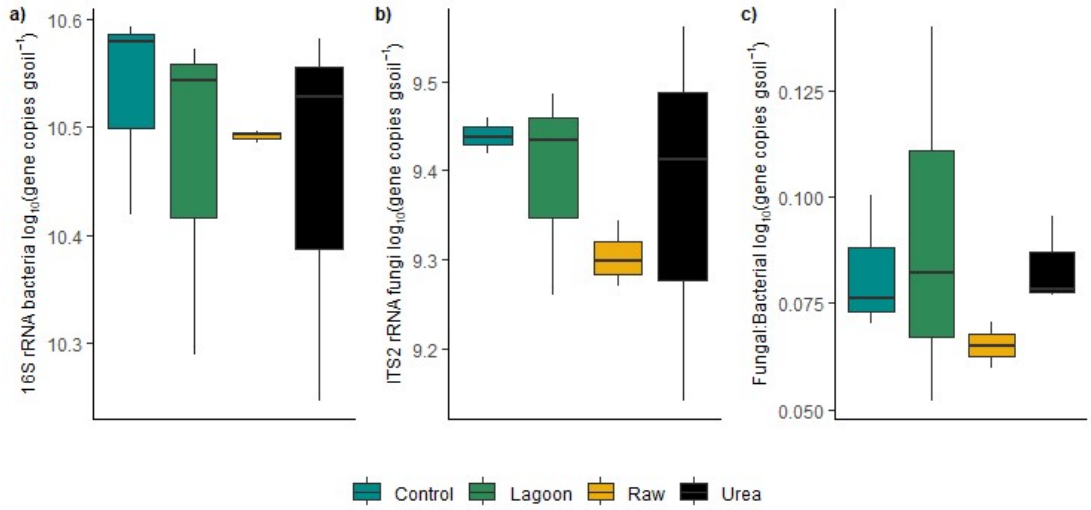

Fig. 4. Total bacterial and fungal populations (a-b), and gene abundance ratio c) of soil with application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization or non-amended Control. There were no significant differences between treatments for each parameter evaluated by LSD test at $p \leq 0.05$ (mean n = 3 ± S.E).

The microbial richness assessed by Chao1 index and evenness by Shannon index revealed that none of the diversity indexes showed significant differences between treatments ($p > 0.05$) for bacteria (Fig. S1a, and b) or fungi (Fig. S1 c,d).

Bacterial communities were dominated by the phyla Actinobacteriota (23.6%, relative abundance, on average), Proteobacteria (20.5%), Acidobacteria (15.6%), Verrucomicrobiota (8.4%), Chloroflexi (7.6%), and Firmicutes (7.3%) (Fig. S2). Almost all of the fungal species belonged to the phyla Ascomycota (92.4%), followed by Basidiomycota (2.8%) (Fig. S3). Beta-diversity analysis computed on CCS transformed dataset using Bray-Curtis distance indicated a marginal shift in bacterial community

composition (PERMANOVA test, F= 1.15, p = 0.1) and not in fungal community composition (PERMANOVA test, F= 1.08, p = 0.2), but both not significant, in response to the different N amendments type (Fig. 5).

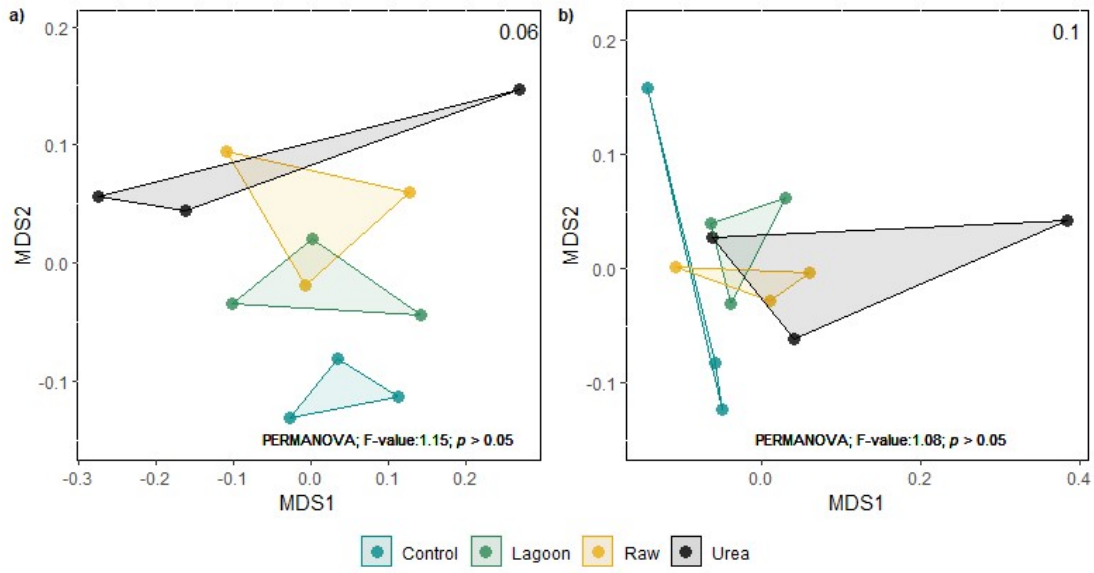

Fig. 5. Non-metric multidimensional scaling (NMDS) ordination of bacterial (a) and fungal (b) community composition of soil with the application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization or a non-amended Control.

6.5.5. Data integration of soil nutrient content and microbial community composition, abundance, and activity

Generalized joint attribute model (GJAM) was performed to explore the impact of the application of FDE to a fescue pasture on microbial community structure, abundance, activity, and soil fertility properties. A global principal component analysis (PCA) integrating the regression coefficients from all variables of GJAM indicated a clear segregation of the treatments in the first two axes (96% of the overall variation explained, Fig. S4). The N-based amended soils, urea, or FDE amendments, clearly segregated from the unamended Control. Regarding distance from the Control, Raw had a higher proximity than Lagoon applications. Sensitivity analysis showed the relative impacts of each treatment on the different groups of dependent variables

hierarchically (Fig. 6). Differences in regression coefficients point to soil nutrient status and microbial activity as the main factors explaining the differential effect of treatments (Fig. 6). In addition, among these groups of variables, the sensitivity values were highest in the Lagoon and Urea treatments. The microbial community composition or abundance (bacteria and fungi) had a lower level of sensitivity among treatments (Fig. 6).

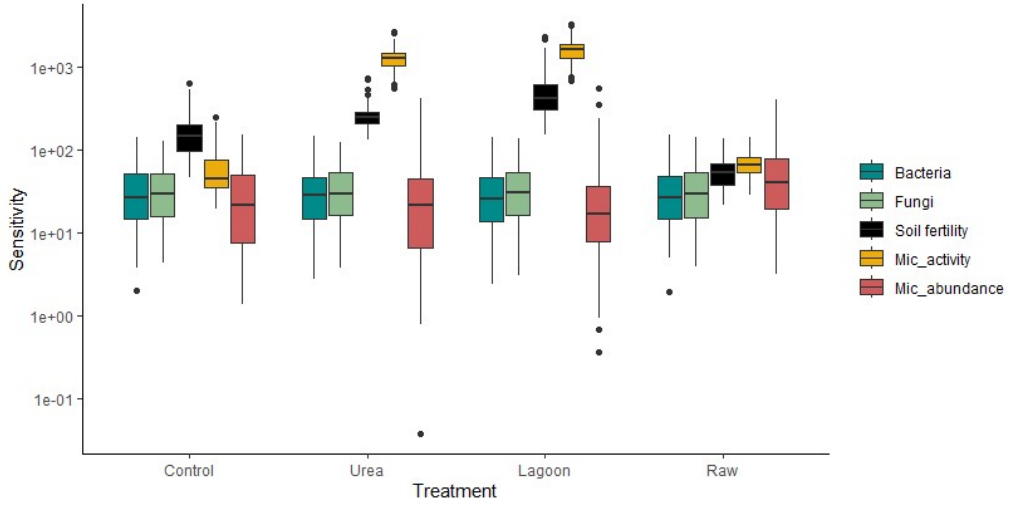

Fig. 6. Sensitivity analysis of regression coefficients of bacterial and fungal community profiles (ASVs), abundance, and microbial activity together with soil nutrient content, according to the soil application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization, and non-amended Control.

We further assessed which soil chemical or microbial parameters were responsible for the differences among treatments based on regression coefficients (Fig. 7). The relative abundances of bacterial and fungal communities (calculated using the regression coefficients from GJAM) changed (positive and negative shifts) according to the treatment (Fig. S5, S6) but were no significant.

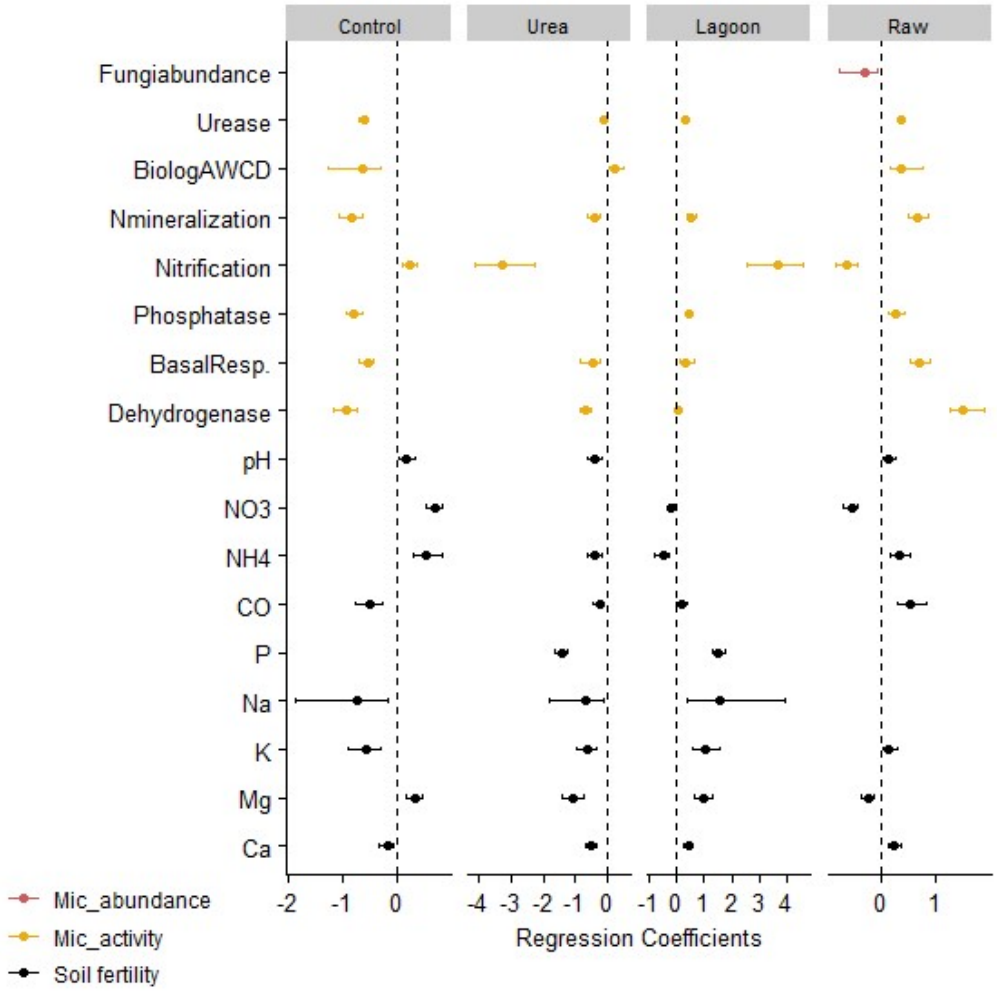

Fig. 7. Regression coefficients of only the significant shifts of response variables: abundance of total bacteria and fungi, microbial activities, and soil nutrient content, according to the soil application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization, or non-amended Control. The black dashed lines correspond to a regression coefficient of 0. Values less than zero indicate a decrease in the variable due to the treatment, and values greater than zero indicate an increase. The whiskers indicate 95% confidence intervals.

The most remarkable differences in regression coefficients for soil fertility were between the Lagoon and Control and Urea treatments. Applying Lagoon increased the soil P content and exchangeable cations K^{+}, Na^{+}, Mg^{2+}, and Ca^{2+}. Similarly, Raw application to the soil exhibited higher shifts in K^{+} and Ca^{2+} content and presented the greatest change in OC.

The contrasting effects of the control and FDE treatments on soil microbial activities were observed. The regression coefficients for these microbial parameters increased in Lagoon and, most notably, in Raw-amended soils (Fig. 7). Interestingly, Raw and Urea negatively changed potential nitrification activity, while Lagoon FDE positively shifted it respect to the Control. The abundance of bacteria was not affected by treatments, but fungal abundance decreased in response to Raw applications.

6.6. Discussion

6.6.1. FDE soil application increases soil microbial activity and nutrient availability

Our study revealed that N-based applications of FDE strongly affected soil fertility. Lagoon type had the greatest impact, consistent with the results of the regression coefficients from GJAM (Fig. 7). In accordance to our hypothesis there were more nutrients in general in FDE-treated soils, enhancing macronutrient availability (P, K^+, Na^+, Mg^{2+} and Ca^{2+}) (Table 1, Fig. 7). This higher nutrient availability in Lagoon stabilization-system than in Raw is somehow expected due to the hydrolysis and mineralization of organic forms during biodegradation, resulting in a higher concentration of plant-available nutrients [54]. The nutrient content of these FDE was variable with the season [7] but when N-based applied was higher for Lagoon's (Table 1). The increase in soil OC content with FDE application (Table 1 and Fig. 7) may contribute to long-term soil fertility and functionality since soil OM sustains physic-chemical and biological dimensions of soil fertility [55].

Microorganisms constitute a central part of the soil's total living biomass and play a key role in soil functioning [5]. Microbial activity was the group characteristic most strongly affected by the dairy effluents; it increased in Lagoon and, most notably, in Raw, relative to the Control and Urea application (Fig. 1 and 7). This finding agrees with our previous results that FDE

applications enhanced soil microbial activity and that Raw increased soil OC [13]. In that study, the same FDE were evaluated in a pot trial following a similar experimental design as in this field experiment, but with agricultural soil with higher basal nutrient content, especially higher N and P.

As soil enzymes play a vital role in breaking down and transforming OM releasing mineral compounds [56], they contribute significantly to the cycling of nutrients. In this context, the enzymatic activities serve as reliable indicators of OM decomposition, with higher levels observed with Raw, enhancing soil basal respiration. Besides, Luo et al. [57], in a meta-analysis, concluded that organic amendments increase enzymatic activity compared to mineral-only fertilization. Our results (Table 1, Fig.7) support that a major driver of soil microbial activity may be the availability of organic C and the different nutrients released from the organic amendment [58,59].

To better understand the diversity of carbon sources utilized by soil microbiota, we used Biolog CLPP. Remarkably, our study showed that the microbial functional diversity did not differ among N-treatments, synthetic fertilizer or FDE (Fig. 2 and 3). Thus, FDE, both Lagoon and Raw, had no influence in the short term on the functional diversity of the microbial communities able to grow in the Biolog assay. These communities have been mainly identified as culturable, fast-growing proteobacteria [60]. Soil microbial potential functionality differs from what has been reported for other sources of organic residues. For instance, cattle manure harbors a variety of nutrients and organic carbon fractions that, when amended into the soil, causes changes by enhancing microbial functional diversity and microbial activity [17,61–65]. However, those studies have considered the application of solid manures, either incorporated or surface applied to the soil, and most of them included long-term manure amendment. Han et al. [21] recently reported that solid manure exerted a higher effect on soil fertility and changed the microbial community relative to liquid dairy manure. Our study evaluated liquid dairy effluents that were surface-absorbed by the soil and applied during an annual forage production. In the pot experiment using a different soil and the same FDE as in this work, Illarze et al.

[13] found that soil with FDE application presented higher catabolic activity than Urea.

On the other hand, a greater rate of C substrate utilization (catabolic capability) by the microbial community in N-amended soils than in the Control (Fig. 2) indicated that the nutrient release following FDE amendment may had increased the soil total N content, which in turn improves the microbial catabolic capacity. Thus, the N availability seemed to be a limiting factor for microbial growth and may have contributed to enhancing microbial community catabolic capacity [66–68].

Microbial substrate use preference indicated a greater metabolism of some polymer compounds (α-Cyclodextrin and Glycogen) with the application of Raw than that of Lagoon-amended soil (Fig. 3). Manure amendments contain polymeric compounds, which depend on the microbial production of extracellular enzymes to break them down. The microbial community's capacity to mineralize specific C substrates may reflect differences in the type and availability of C sources present in the amendment [13,69–71]. It was previously suggested that the microbial communities less able to use polymers were less exposed to these compounds in their environment [71]. Our result suggests that the microbial community from Raw amended soil quickly adapted to the use of polymer compounds and this offers an opportunity for reducing nutrient pollution when Raw FDE is land applied. This difference suggests an influence of the type of FDE on the functional microbial community that grows in Biolog plates, adapting to the carbon sources present.

Our hypothesis that Raw induced highest microbial activity was confirmed, although Lagoon FDE seemed to affect soil's physico-chemical properties more deeply.

6.6.2. Linking the soil microbiome with soil properties and microbial functioning as affected by FDE applications

Studies have shown that using FDE for pasture fertilization determines changes in soil nutrient availability and cycling [2]; however, the impacts on microbial community composition and functioning need to be further disentangled [5].

Understanding the changes in soil microbial community composition in agroecological ecosystems and their response to soil management is challenging [72], and getting insight into the relationships and interactions between microorganisms and their environments is crucial in microbial ecology [73]. However, the enormous amount of information generated by high throughput sequencing deals with data analysis that might generate insufficient or misleading biological inferences due to the high dimensionality of sequence data [52]. To circumvent this, in this study, we integrated with GJAM data from soil fertility and microbial community composition, abundance, and activity when different forms of FDE were applied to a pasture. Using this model, we intended to understand better how different types of N-based fertilization shift soil factors that likewise modulate the microbial community.

GJAM revealed differences between the type of N amendment and the Control in changing the soil nutrient levels together with the bacterial and fungal profiles and microbial activities. Sensitivity analysis points to the soil nutrient status and the microbial activity as the main drivers of these differences (Fig. 6). Interestingly, the responses of the bacterial and fungal profiles were similar among the treatments (Fig. 6) and no significant shift in microbial ASVs were accounted (Fig.7). Moreover, no significant differences in the richness and diversity of the microbial communities (Fig. S1) or in the patterns of beta diversity (Fig. 5) were found. Coelho et al. [74], using digestate or undigestate cattle slurry in similar field conditions and duration of the current study but with less N input, found no response of either bacterial or fungal community or abundance. They argued that one season of repeated digestate applications is not enough to detect changes at the soil microbial community level. However, Lui and Haynes [3] found that neither the short-term nor the long-term FDE irrigation to a pasture affects the bacterial

community structure or the catabolic capability of the microbial community. Similarly, Shi et al [75] reported that the soil microbial community was not significantly influenced by dairy manure addition, but a liquid formulation reduced fungal abundance, while both amendments increased some soil enzymatic activities. Those findings and our results suggest that changing community composition may be more complex than increasing the overall activity of soil microorganisms with FDE amendments. Nevertheless, most DNA material in the soil is from non-living or dormant microorganisms that become active under favorable environmental conditions [76,77], and our results based on single-point observations could not have captured the transitory changes of soil microbial diversity following FDE applications [15,78,79].

When differences in microbial diversity are found, it is still unclear whether these effects are caused mainly by the survival of microbes introduced with manure or by the activation of the soil-borne microbiome [79]. Our previous work revealed that Lagoon-treated FDE shifted the bacterial community from that of the untreated one, and the Raw FDE possessed a higher risk of pathogen dissemination [7]. Based on that, we hypothesized that the type of FDE, when applied to the soil, could differentially impact the soil microbial diversity. The results of the current work led us to reject this hypothesis.

However, the long-term impact of FDE applications on soil microbial processes and bacterial and fungal communities deserves further investigation to achieve sustainable management of nutrient cycling and soil biodiversity.

6.7. Conclusions

This short-term field's results showed that repeated application of FDE, either Raw or Lagoon, to a pasture positively impacts soil fertility and microbial activity. In contrast, the microbial community structure and abundance remain undisturbed. The integration data model (GJAM) showed that Lagoon had the most potent effect on soil fertility, and Raw's main effect was enhancing soil microbial activity. These findings suggest that the different types of dairy

effluent when recycled as fertilizer, may impact the soil ecosystem differently. Long-term trials should be considered to disentangle their effect on soil microbial diversity and function. Moreover, our results are promising for recycling dairy effluents to a pasture increasing soil fertility and microbial activity without decreasing soil microbial community diversity, thus improving the dairy sector's environmental performance.

6.8. Supplementary material

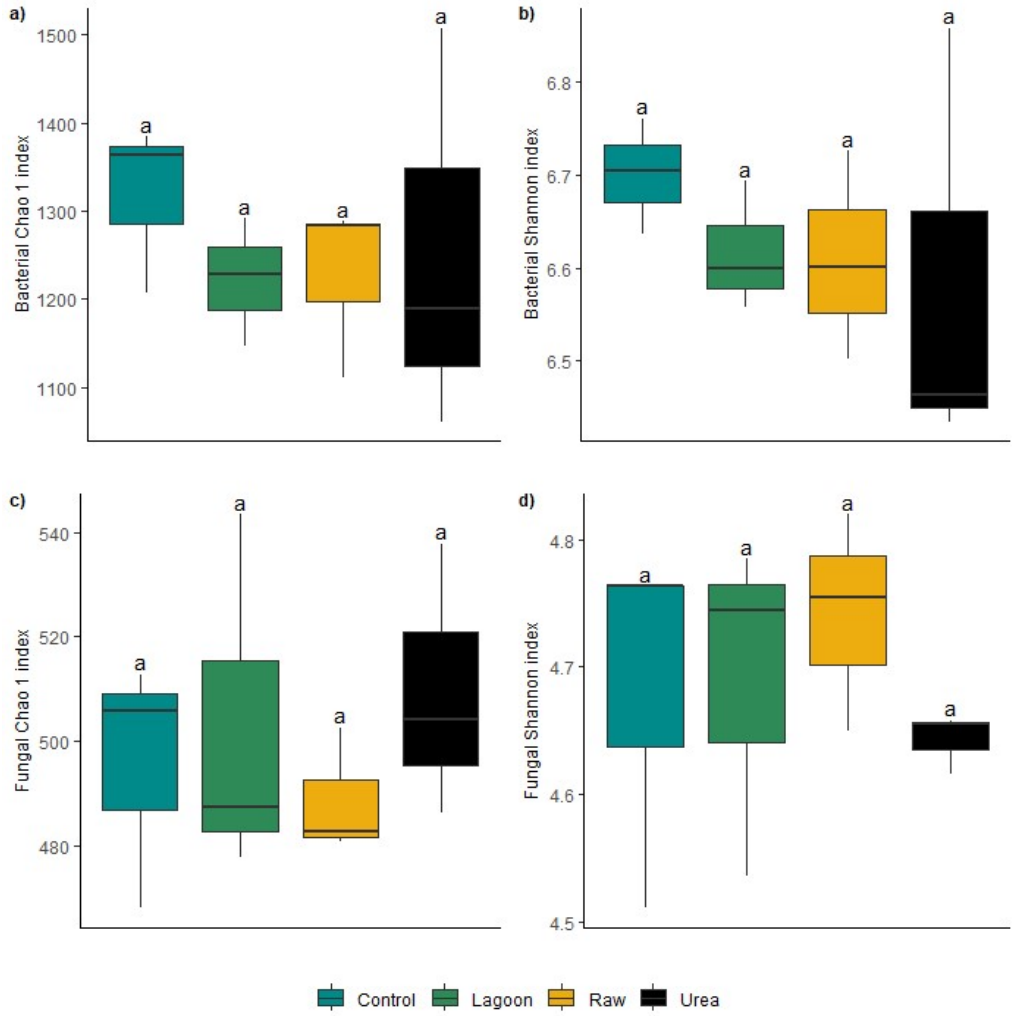

Fig. S1. Bacterial (a,b) and fungal (c,d) ASV richness (Chao1 index) and evenness (Shannon index) of soil with the application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization or a non-amended Control.

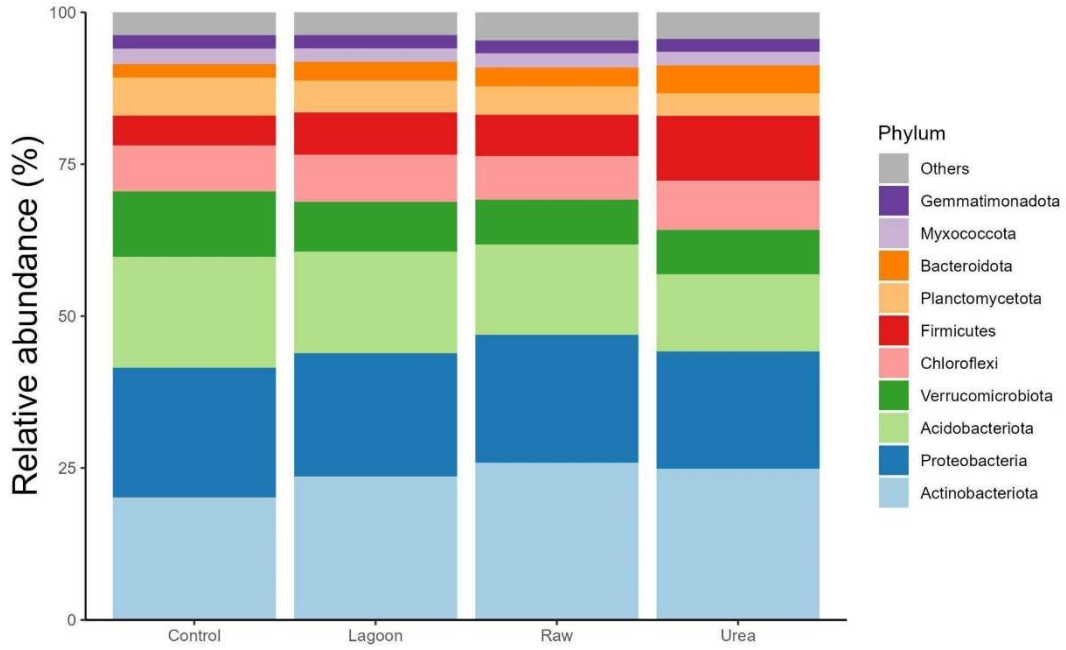

Fig. S2. Relative abundance of bacteria

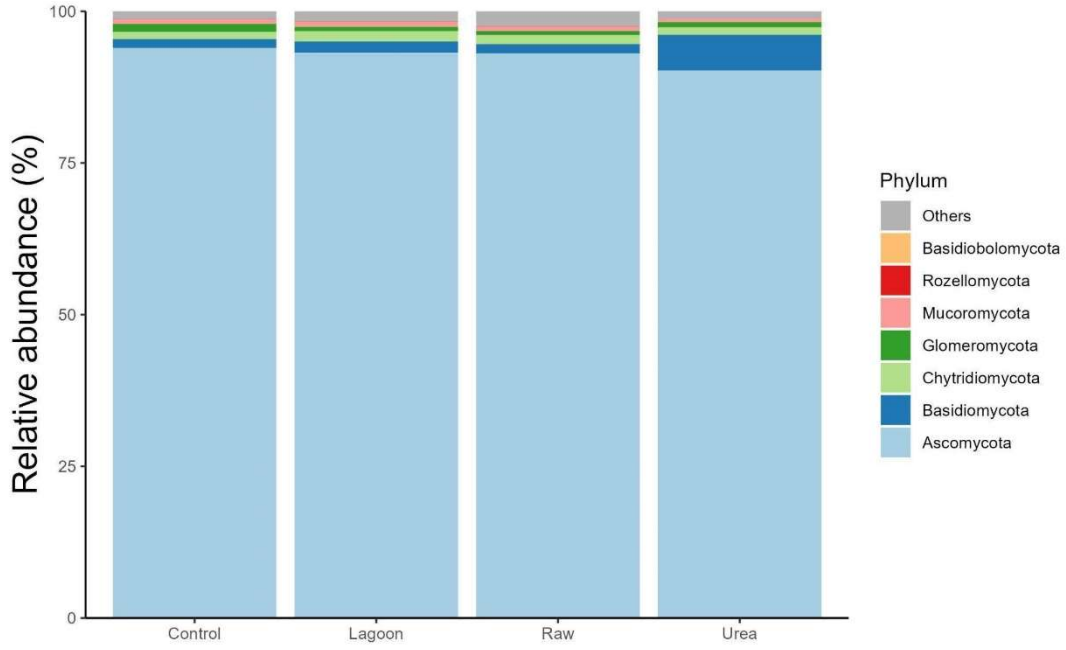

Fig. S3. Relative abundance of fungi

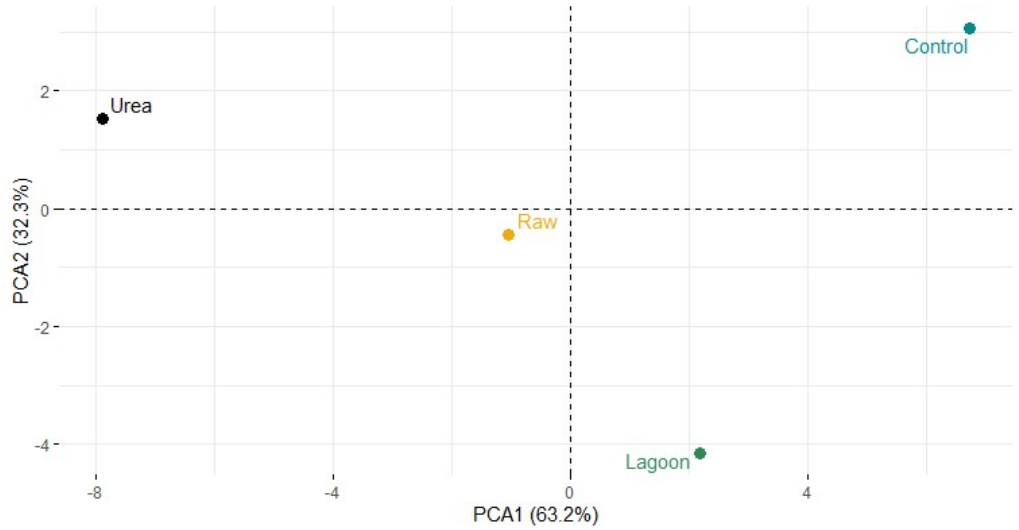

Fig. S4. Principal component analysis (PCA) of regression coefficients of bacterial and fungal community profiles (ASVs), abundance, and microbial activity together with soil nutrient content, according to the soil application of Raw dairy effluent (Raw), Lagoon dairy effluent (Lagoon), Urea fertilization, and non-amended Control.

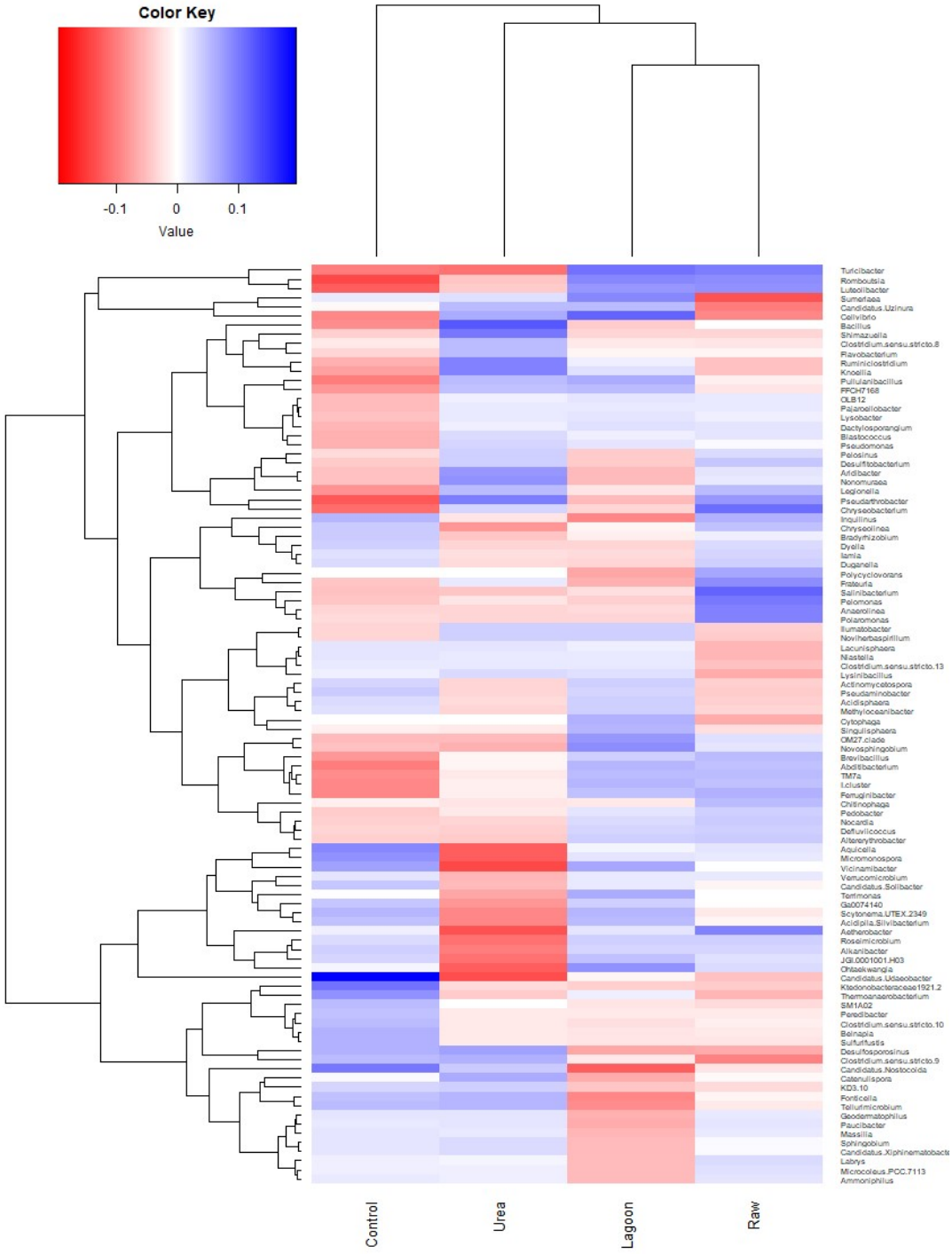

Fig. S4. Heatmap of regression coefficients from Bacterial amplicon sequence variants (ASVs) of soil with the application of Lagoon or Raw dairy effluents, Urea fertilization, or non-amended Control. The shifts in regression coefficients are blue for positive and red for negative shifts. No significant ASV differences between communities were detected among treatments.

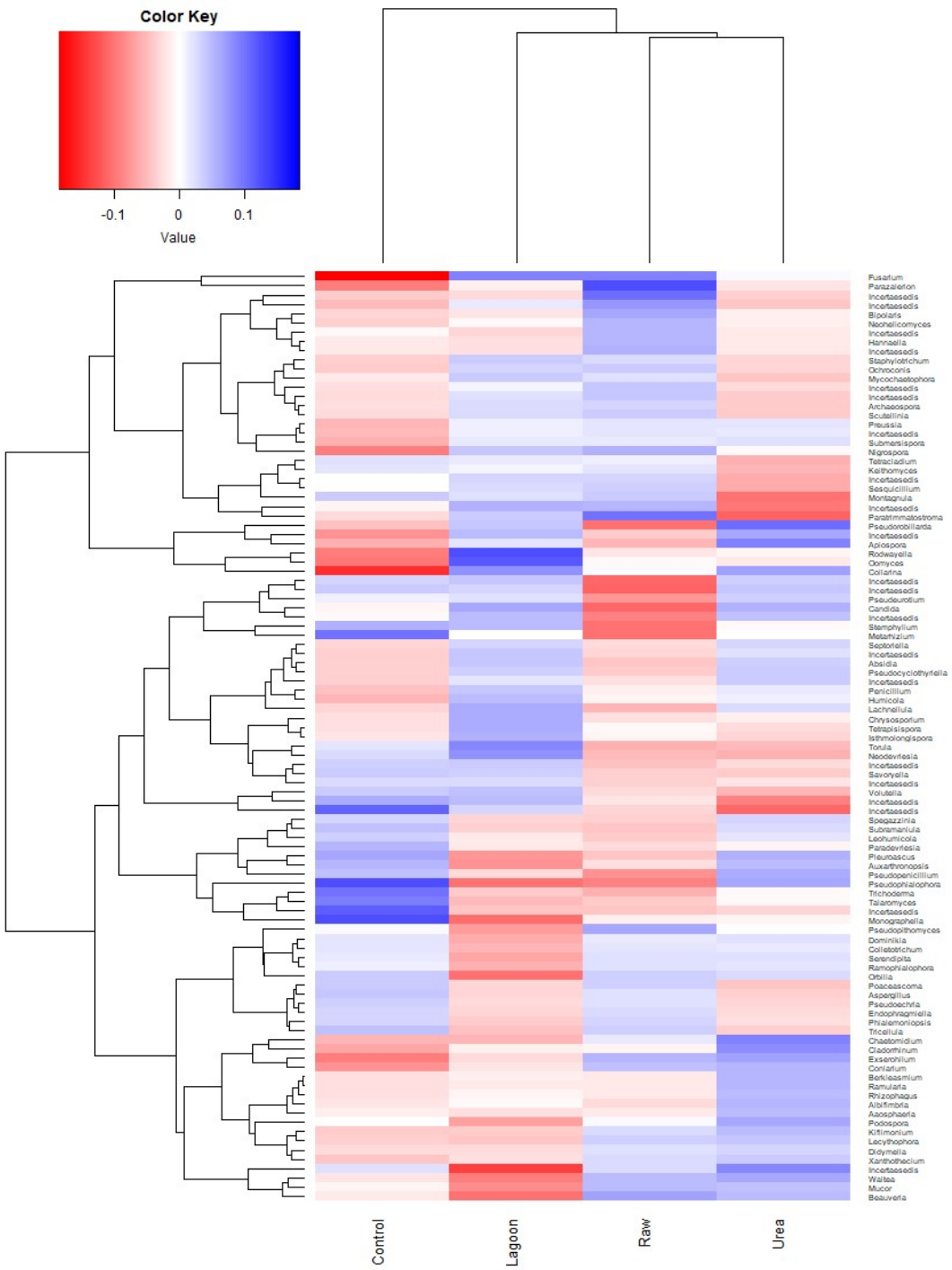

Fig. S5. Heatmap of regression coefficients from Fungal amplicon sequence variants (ASVs) of soil with the application of Lagoon or Raw dairy effluents, Urea fertilization, or non-amended Control. The shifts in regression coefficients are blue for positive and red for negative shifts. No significant ASV differences between communities were detected among treatments.

Table S1. Catabolic diversity indexes **(**Substrate richness (SR), Shannon diversity index (H′), and Shannon's evenness (E)) and Slope of AWCD curve of the soil microbial community after incubation for 72 h in Biolog EcoPlates with the application of Raw DE or Lagoon DE, urea, and non-amended control (mean n = 3 ± S.E). Significant differences between treatments were accounted for Slope of the AWCD curve indicated by different letters ($p < 0.05$).

Treatments	SR	H′	E	Slope
Control	24.67± 0.33	3.17± 0.02	0.96± 0.00	0.36± 0.03 a
Lagoon DE	25.67± 0.88	3.20± 0.03	0.95± 0.01	0.49± 0.02 ab
Raw DE	27.33± 0.67	3.27± 0.02	0.96± 0.00	0.54± 0.03 b
Urea	25.67± 1.45	3.19± 0.06	0.95± 0.00	0.46± 0.05 ab

6.9. References

[1] N.S. Bolan, S. Laurenson, H. Lowe, J. Luo, P.H. Hill, J. Sukias, M. Lakes, D.W. Limited, P. North, N. Zealand, N. Zealand, N. Zealand, Treatment of farm effluents in New Zealand's dairy operations, (2009) 120–134.

[2] R.M. Hawke, S.A. Summers, Effects of land application of farm dairy effluent on soil properties: A literature review, New Zeal. J. Agric. Res. 49 (2006) 307–320. https://doi.org/10.1080/00288233.2006.9513721.

[3] Y.Y. Liu, R.J. Haynes, Influence of land application of dairy factory effluent on soil nutrient status and the size, activity, composition and catabolic capability of the soil microbial community, Appl. Soil Ecol. 48 (2011) 133–141. https://doi.org/10.1016/j.apsoil.2011.03.014.

[4] R. Thangarajan, N.S. Bolan, G. Tian, R. Naidu, A. Kunhikrishnan, Role of organic amendment application on greenhouse gas emission from soil, Sci. Total Environ. 465 (2013) 72–96. https://doi.org/10.1016/J.SCITOTENV.2013.01.031.

[5] J. Urra, I. Alkorta, C. Garbisu, Potential benefits and risks for soil health derived from the use of organic amendments in agriculture, Agronomy 9 (2019) 1–23. https://doi.org/10.3390/agronomy9090542.

[6] J.M. Irazoqui, M.F. Eberhardt, M.M. Adjad, A.F. Amadio, Identification of key microorganisms in facultative stabilization ponds from dairy industries, using metagenomics, PeerJ 10 (2022) 1–18. https://doi.org/10.7717/peerj.12772.

[7] G. Illarze, A. del Pino, P. Irisarri, Differences in Bacterial Communities and Pathogen Indicators of Raw and Lagoon-Stabilized Farm Dairy Effluents, Microorg. 2024, Vol. 12, Page 305 12 (2024) 305. https://doi.org/10.3390/MICROORGANISMS12020305.

[8] J. Clemens, M. Trimborn, P. Weiland, B. Amon, Mitigation of greenhouse gas emissions by anaerobic digestion of cattle slurry, Agric. Ecosyst. Environ. 112 (2006) 171–177. https://doi.org/10.1016/j.agee.2005.08.016.

[9] A. Johansen, M.S. Carter, E.S. Jensen, H. Hauggard-Nielsen, P. Ambus, Effects of digestate from anaerobically digested cattle slurry and plant materials on soil microbial community and emission of CO2 and N2O, Appl. Soil Ecol. 63 (2013) 36–44. https://doi.org/10.1016/j.apsoil.2012.09.003.

[10] G. Ernst, A. Müller, H. Göhler, C. Emmerling, C and N turnover of fermented residues from biogas plants in soil in the presence of three different earthworm species (Lumbricus terrestris, Aporrectodea longa, Aporrectodea caliginosa), Soil Biol. Biochem. 40 (2008) 1413–1420. https://doi.org/10.1016/j.soilbio.2007.12.026.

[11] K.R. Neufeld, S.J. Grayston, S. Bittman, M. Krzic, D.E. Hunt, S.M. Smukler, Long-term alternative dairy manure management approaches enhance microbial biomass and activity in perennial forage grass, Biol. Fertil. Soils 53 (2017) 613–626. https://doi.org/10.1007/s00374-017-1204-2.

[12] J. Li, J. Luo, Y. Shi, D. Houlbrooke, L. Wang, S. Lindsey, Y. Li, Nitrogen gaseous emissions from farm effluent application to pastures and mitigation measures to reduce the emissions: A review, New Zeal. J. Agric. Res. 58 (2015) 339–353. https://doi.org/10.1080/00288233.2015.1028651.

[13] G. Illarze, A. del Pino, A. Rodríguez-Blanco, P. Irisarri, Application of Dairy Effluents to Pastures Affects Soil Nitrogen Dynamics and Microbial Activity, Agronomy 13 (2023) 470. https://doi.org/10.3390/agronomy13020470.

[14] C.A. Rotz, Management to reduce nitrogen losses in animal pro- duction, J. Anim. Sci. 82 (2004) E119–E137.

[15] A.K.A. Suleiman, R. Gonzatto, C. Aita, M. Lupatini, R.J.S. Jacques, E.E. Kuramae, Z.I. Antoniolli, L.F.W. Roesch, Temporal variability of soil microbial communities after application of dicyandiamide-treated swine slurry and mineral fertilizers, Soil Biol. Biochem. 97 (2016) 71–82. https://doi.org/10.1016/j.soilbio.2016.03.002.

[16] V. Chaudhry, A. Rehman, A. Mishra, P.S. Chauhan, C.S. Nautiyal, Changes in Bacterial Community Structure of Agricultural Land Due to Long-Term Organic and Chemical Amendments, Microb. Ecol. 64 (2012) 450–460. https://doi.org/10.1007/s00248-012-0025-y.

[17] A.C. Hamm, M. Tenuta, D.O. Krause, K.H. Ominski, V.L. Tkachuk, D.N. Flaten, Bacterial communities of an agricultural soil amended with solid pig and dairy manures, and urea fertilizer, Appl. Soil Ecol. 103 (2016) 61–71. https://doi.org/10.1016/j.apsoil.2016.02.015.

[18] M.D. Stocker, Y.A. Pachepsky, R.L. Hill, D.R. Shelton, Depth-dependent survival of Escherichia coli and enterococci in soil after manure application and simulated rainfall, Appl. Environ. Microbiol. 81 (2015) 4801–4808. https://doi.org/10.1128/AEM.00705-15.

[19] S. Zhang, L. Sun, Y. Wang, K. Fan, Q. Xu, Y. Li, Q. Ma, J. Wang, W. Ren, Z. Ding, Cow manure application effectively regulates the soil bacterial community in tea plantation, BMC Microbiol. 20 (2020) 1–11. https://doi.org/10.1186/s12866-020-01871-y.

[20] E. Lopatto, J. Choi, A. Colina, L. Ma, A. Howe, S. Hinsa-Leasure, Characterizing the soil microbiome and quantifying antibiotic resistance gene dynamics in agricultural soil following swine CAFO manure application, PLoS One 14 (2019). https://doi.org/10.1371/journal.pone.0220770.

[21] Z. Han, W. He, H. Shi, C. Wang, C. Liu, Y. Feng, Effects of the Integrated Use of Dairy Cow Manure on Soil Properties and Biological Fertility, Sustain. 15 (2023). https://doi.org/10.3390/su151511693.

[22] M.A. Herrero, J.C.P. Palhares, F.J. Salazar, V. Charlón, M.P. Tieri, A.M. Pereyra, Dairy Manure Management Perceptions and Needs in South American Countries, Front. Sustain. Food Syst. 2 (2018) 1–15. https://doi.org/10.3389/fsufs.2018.00022.

[23] S. Gutiérrez, N. Cabrera, A. Benítez, E. Melani, Reducing variability in estimating wastewater composition in dairy farms during milking operations, Biosyst. Eng. 103 (2009) 497–503. https://doi.org/10.1016/j.biosystemseng.2009.06.004.

[24] J.P. Castaño, A. Giménez, M. Ceroni, J. Furest, R. Aunchayna, Caracterización agroclimática del uruguay 1980-2009. Serie Técnica N° 193, INIA, 2011.

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

[26] T.A. Doane, W.R. Horwáth, Spectrophotometric determination of nitrate with a single reagent, Anal. Lett. 36 (2003) 2713–2722. https://doi.org/10.1081/AL-120024647.

[27] D.W. Nelson, L.E. Sommers, Total Carbon, Organic Carbon, and Organic Matter, in: D.L. Sparks (Ed.), Methods Soil Anal. Part 3 Chem. Methods, Soil Science Society of America and American Society of Agronomy, Madison, WI USA, 1996: pp. 961–1010.

[28] R.H. Bray, L.T. Kurtz, Determination of total, organic, and available forms of Phosphorus in soils, Soil Sci. 59 (1945) 39–46. https://journals.lww.com/soilsci/Citation/1945/01000/Determination_of_Total,_Organic,_and_Available.6.aspx.

[29] G. Thomas, Exchangeable cations, in: A.L. Page, R.H. Miller, D.. Keeney (Eds.), Methods Soil Anal. Part 2, American Society of Agronomy, Madison, WI USA, 1982: pp. 159–165.

[30] W. von Mersi, F. Schinner, An improved and accurate method for determining the dehydrogenase activity of soils with iodonitrotetrazolium chloride, Biol. Fertil. Soils 11 (1991) 216–220. https://doi.org/10.1007/BF00335770.

[31] M.A. Tabatabai, Soil enzymes, in: A.L. Page, R.H. Miller, D.R. Keeney (Eds.), Methods Soil Anal. Part2, Chem. Microbiol. Prop., 2nd ed., American Society of Agronomy, Madison, WI USA, 1982: pp. 903–947.

[32] R. Margesin, Acid and Alkaline Phosphomonoesterase Activity with the Substrate p-Nitrophenyl Phosphate, in: F. Schinner, R. Ohlinger, R. Margesin, E. Kandeler (Eds.), Methods Soil Biol., Springer, Berlin, Heidelberg, 1996: pp. 2013–2016.

[33] E. Kandeler, Urease Activity by Colorimetric Technique, in: F. Schinner, R. Ohlinger, R. Margesin, E. Kandeler (Eds.), Methods Soil Biol., Springer, Berlin, Heidelberg, 1996: pp. 171–174.

[34] R. Öhlinger, Soil respiration by tritation, in: F. Schinner, R. Öhlinger, E. Kandeler, R. Margesin (Eds.), Methodsinsoil Biol., Springer Berlin Heidelberg, New York, 1996: pp. 95–98.

[35] H. Hoffmann, M. Schloter, B.M. Wilke, Microscale-scale measurement of potential nitrification rates of soil aggregates, Biol. Fertil. Soils 44 (2007) 411–413. https://doi.org/10.1007/s00374-007-0227-5.

[36] E. Kandeler, Nitrogen mineralization, in: F. Schinner, R. Ohlinger, R. Margesin, E. Kandeler (Eds.), Methods Soil Biol., Springer, Berlin, Heidelberg, 1996: pp. 135–143.

[37] J.L. Garland, A.L. Mills, Classification and characterization of heterotrophic microbial communities on the basis of patterns of community-level sole-carbon-source utilization, Appl. Environ. Microbiol. 57 (1991) 2351–2359. https://doi.org/10.1128/aem.57.8.2351-2359.1991.

[38] H. Insam, D. Parkinson, K.H. Domsch, Influence of macroclimate on soil microbial

biomass, Soil Biol. Biochem. 21 (1989) 211–221. https://doi.org/10.1016/0038-0717(89)90097-7.

[39] J.G. Caporaso, C.L. Lauber, W.A. Walters, D. Berg-Lyons, C.A. Lozupone, P.J. Turnbaugh, N. Fierer, R. Knight, Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 4516–4522. https://doi.org/10.1073/pnas.1000080107.

[40] M. Gardes, T. Bruns, ITS primers with enhanced specificity for basidiomycetes--application to the identification of mycorrhizae and rusts, Mol. Ecol. 2 (1993) 113–8. https://doi.org/10.1111/j.1365-294x.1993.tb00005.x. PMID: 8180733.

[41] T.J. White, T. Bruns, S. Lee, J. Taylor, Amplification and Direct Sequencing of Fungal Ribosomal Rna Genes for Phylogenetics, PCR Protoc. (1990) 315–322. https://doi.org/10.1016/b978-0-12-372180-8.50042-1.

[42] S.N. Leaw, H.C. Chang, H.F. Sun, R. Barton, J.P. Bouchara, T.C. Chang, Identification of medically important yeast species by sequence analysis of the internal transcribed spacer regions, J. Clin. Microbiol. 44 (2006) 693–699. https://doi.org/10.1128/JCM.44.3.693-699.2006.

[43] A.B. Siddique, M. Unterseher, A cost-effective and efficient strategy for Illumina sequencing of fungal communities: A case study of beech endophytes identified elevation as main explanatory factor for diversity and community composition, Fungal Ecol. 20 (2016) 175–185. https://doi.org/10.1016/J.FUNECO.2015.12.009.

[44] B.J. Callahan, P.J. McMurdie, M.J. Rosen, A.W. Han, A.J.A. Johnson, S.P. Holmes, Dada2, Nat. Methods 13 (2016) 581–583. https://doi.org/10.1038/nmeth.3869.

[45] C. Quast, E. Pruesse, P. Yilmaz, J. Gerken, T. Schweer, P. Yarza, J. Peplies, F.O. Glöckner, The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools, Nucleic Acids Res. 41 (2013) 590–596. https://doi.org/10.1093/nar/gks1219.

[46] U. Kõljalg, R.H. Nilsson, K. Abarenkov, L. Tedersoo, A.F.S. Taylor, M. Bahram, S.T. Bates, T.D. Bruns, J. Bengtsson-Palme, T.M. Callaghan, B. Douglas, T. Drenkhan, U. Eberhardt, M. Dueñas, T. Grebenc, G.W. Griffith, M. Hartmann, P.M. Kirk, P. Kohout, E. Larsson, B.D. Lindahl, R. Lücking, M.P. Martín, P.B. Matheny, N.H. Nguyen, T. Niskanen, J. Oja, K.G. Peay, U. Peintner, M. Peterson, K. Põldmaa, L. Saag, I. Saar, A. Schüßler, J.A. Scott, C. Senés, M.E. Smith, A. Suija, D.L. Taylor, M.T. Telleria, M. Weiss, K.H. Larsson, Towards a unified paradigm for sequence-based identification of fungi, Mol. Ecol. 22 (2013) 5271–5277. https://doi.org/10.1111/mec.12481.

[47] J. N. Paulson, H. Talukder, M. Pop, H. C. Bravo, metagenomeSeq: Statistical analysis for sparse high-throughput sequencing. Bioconductor package: 1.16. 0 (2016). http://cbcb.umd.edu/software/metagenomeSeq.

[48] J. N. Paulson, O. C. Stine, H. C. Bravo, M. Pop, Differential abundance analysis for microbial marker-gene surveys, Nat. Methods 10 (2013) 1200–1202.

[49] J. Oksanen, Vegan: ecological diversity, R Proj. 368 (2022) 1–11. https://cran.r-project.org/package=vegan.

[50] J.S. Clark, D. Nemergut, B. Seyednasrollah, P.J. Turner, S. Zhang, Generalized joint attribute modeling for biodiversity analysis: Median-zero, multivariate, multifarious data, Ecol. Monogr. 87 (2017) 34–56. https://doi.org/10.1002/ecm.1241.

[51] G.B. Gloor, J.M. Macklaim, V. Pawlowsky-Glahn, J.J. Egozcue, Microbiome datasets are compositional: And this is not optional, Front. Microbiol. 8 (2017) 1–6.

https://doi.org/10.3389/fmicb.2017.02224.

[52] M.F.A. Leite, E.E. Kuramae, You must choose, but choose wisely: Model-based approaches for microbial community analysis, Soil Biol. Biochem. 151 (2020). https://doi.org/10.1016/j.soilbio.2020.108042.

[53] R Core Team, R: A Language and Environment for Statistical Computing., R Foundation for Statistical Computing, 2019., Viena, Austria, 2019.

[54] J.J. Walsh, D.L. Jones, G. Edwards-Jones, A.P. Williams, Replacing inorganic fertilizer with anaerobic digestate may maintain agricultural productivity at less environmental cost, J. Plant Nutr. Soil Sci. 175 (2012) 840–845. https://doi.org/10.1002/jpln.201200214.

[55] R. Hijbeek, M.K. van Ittersum, H.F.M. ten Berge, G. Gort, H. Spiegel, A.P. Whitmore, Do organic inputs matter – a meta-analysis of additional yield effects for arable crops in Europe, Plant Soil 411 (2017) 293–303. https://doi.org/10.1007/s11104-016-3031-x.

[56] S. Lladó, P. Baldrian, Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments, PLoS One 12 (2017) e0171638. https://doi.org/10.1371/journal.pone.0171638.

[57] G. Luo, L. Li, V.P. Friman, J. Guo, S. Guo, Q. Shen, N. Ling, Organic amendments increase crop yields by improving microbe-mediated soil functioning of agroecosystems: A meta-analysis, Soil Biol. Biochem. 124 (2018) 105–115. https://doi.org/10.1016/J.SOILBIO.2018.06.002.

[58] J.W. Bossolani, M.F.A. Leite, L. Momesso, H. ten Berge, J. Bloem, E.E. Kuramae, Nitrogen input on organic amendments alters the pattern of soil–microbe-plant co-dependence, Sci. Total Environ. 890 (2023). https://doi.org/10.1016/j.scitotenv.2023.164347.

[59] X. Yang, Q. He, F. Guo, X. Sun, J. Zhang, Y. Chen, Impacts of carbon-based nanomaterials on nutrient removal in constructed wetlands: Microbial community structure, enzyme activities, and metabolism process, J. Hazard. Mater. 401 (2021) 123270. https://doi.org/10.1016/j.jhazmat.2020.123270.

[60] G. Garau, P. Castaldi, L. Santona, P. Deiana, P. Melis, Influence of red mud, zeolite and lime on heavy metal immobilization, culturable heterotrophic microbial populations and enzyme activities in a contaminated soil, Geoderma 142 (2007) 47–57. https://doi.org/10.1016/J.GEODERMA.2007.07.011.

[61] R. Albiach, R. Canet, F. Pomares, F. Ingelmo, Microbial biomass content and enzymatic activities after the application of organic amendments to a horticultural soil, Bioresour. Technol. 75 (2000) 43–48. https://doi.org/10.1016/S0960-8524(00)00030-4.

[62] G. Bonanomi, F. De Filippis, M. Zotti, M. Idbella, Repeated applications of organic amendments promote beneficial microbiota, improve soil fertility and increase crop yield, Appl. Soil Ecol. 156 (2020) 103714. https://doi.org/10.1016/j.apsoil.2020.103714.

[63] A. Heijboer, Disentangling microbial decomposition networks : linking detritus-based soil microbial food webs to ecosystem processes, Wageningen University, 2018. https://edepot.wur.nl/442514.

[64] C. Saison, V. Degrange, R. Oliver, P. Millard, C. Commeaux, D. Montange, X. Le Roux, Alteration and resilience of the soil microbial community following compost amendment: Effects of compost level and compost-borne microbial community, Environ. Microbiol. 8 (2006) 247–257. https://doi.org/10.1111/j.1462-2920.2005.00892.x.

[65] E. Stokstad, Organic farms reap many benefits., Science 80 (2002) 1589. https://doi.org/DOI: 10.1126/science.296.5573.1589a.

[66] W.E.I. Dan, Y. Qian, Z. Jun-zheng, W. Shuang, C. Xue-li, Z. Xi-lin, Bacterial Community Structure and Diversity in a Black Soil as Affected by Long-Term Fertilization, Pedosph. An Int. J. 18 (2008) 582–592. https://doi.org/10.1016/S1002-0160(08)60052-1.

[67] E. Nivelle, J. Verzeaux, H. Habbib, Y. Kuzyakov, G. Decocq, D. Roger, J. Lacoux, J. Duclercq, F. Spicher, J.E. Nava-Saucedo, M. Catterou, F. Dubois, T. Tetu, Functional response of soil microbial communities to tillage, cover crops and nitrogen fertilization, Appl. Soil Ecol. 108 (2016) 147–155. https://doi.org/10.1016/j.apsoil.2016.08.004.

[68] S. Bei, X. Li, T.W. Kuyper, D.R. Chadwick, J. Zhang, Nitrogen availability mediates the priming effect of soil organic matter by preferentially altering the straw carbon-assimilating microbial community, Sci. Total Environ. 815 (2022) 152882. https://doi.org/10.1016/J.SCITOTENV.2021.152882.

[69] N.C. Banning, B.M. Lalor, W.R. Cookson, A.H. Grigg, D. V. Murphy, Analysis of soil microbial community level physiological profiles in native and post-mining rehabilitation forest: Which substrates discriminate? Appl. Soil Ecol. 56 (2012) 27–34. https://doi.org/10.1016/J.APSOIL.2012.01.009.

[70] A.S. Grandy, J.C. Neff, Molecular C dynamics downstream: The biochemical decomposition sequence and its impact on soil organic matter structure and function, Sci. Total Environ. 404 (2008) 297–307. https://doi.org/10.1016/j.scitotenv.2007.11.013.

[71] N. Nunan, T.Z. Lerch, V. Pouteau, P. Mora, F. Changey, T. Kätterer, S. Giusti-Miller, A.M. Herrmann, Metabolising old soil carbon: Simply a matter of simple organic matter? Soil Biol. Biochem. 88 (2015) 128–136. https://doi.org/10.1016/j.soilbio.2015.05.018.

[72] M.F.A. Leite, S.W.E.B. van den Broek, E.E. Kuramae, Current Challenges and Pitfalls in Soil Metagenomics, Microorganisms 10 (2022) 1–8. https://doi.org/10.3390/microorganisms10101900.

[73] J.I. Prosser, Putting science back into microbial ecology: A question of approach, Philos. Trans. R. Soc. B Biol. Sci. 375 (2020). https://doi.org/10.1098/rstb.2019.0240.

[74] J.J. Coelho, A. Hennessy, I. Casey, C. Roberto, S. Bragança, T. Woodcock, N. Kennedy, Biofertilisation with anaerobic digestates: A field study of effects on soil microbial abundance and diversity, Appl. Soil Ecol. (2019) 103403. https://doi.org/10.1016/j.apsoil.2019.103403.

[75] Y. Shi, N. Ziadi, C. Hamel, S. Bittman, D. Hunt, R. Lalande, Soil microbial biomass, activity, and community composition as affected by dairy manure slurry applications in grassland production, Appl. Soil Ecol. 125 (2017) 97–107. https://doi.org/10.1016/j.apsoil.2017.12.022.

[76] M. Kinnunen, A. Dechesne, C. Proctor, F. Hammes, D. Johnson, M. Quintela-Baluja, D. Graham, D. Daffonchio, S. Fodelianakis, N. Hahn, N. Boon, B.F. Smets, A conceptual framework for invasion in microbial communities, ISME J. 10 (2016) 2773–2779. https://doi.org/10.1038/ismej.2016.75.

[77] M. Lupatini, A.K.A. Suleiman, R.J.S. Jacques, L.N. Lemos, V.S. Pylro, J.A. Van Veen, E.E. Kuramae, L.F.W. Roesch, Moisture Is More Important than Temperature for Assembly of Both Potentially Active and Whole Prokaryotic Communities in Subtropical Grassland, Microb. Ecol. 77 (2019) 460–470. https://doi.org/10.1007/s00248-018-1310-1.

[78] J.M. Sayre, D. Wang, J.Y. Lin, R.E. Danielson, K.M. Scow, J.L. Mazza Rodrigues, Repeated manure inputs to a forage production soil increase microbial biomass and diversity and select for lower abundance genera, Agric. Ecosyst. Environ. 354 (2023) 108567. https://doi.org/10.1016/j.agee.2023.108567.

[79] M. V. Semenov, G.S. Krasnov, V.M. Semenov, N. Ksenofontova, N.B. Zinyakova, A.H.C. van Bruggen, Does fresh farmyard manure introduce surviving microbes into soil or activate soil-borne microbiota? J. Environ. Manage. 294 (2021) 113018. https://doi.org/10.1016/j.jenvman.2021.113018.

7. Farm dairy effluents as an alternative for pasture fertilization and greenhouse gas emissions

Illarze, G. [1], Gonnet, R[1], Del Pino, A. [2], and Irisarri, P. [1]

[1] Laboratorio de Microbiología, Departamento de Biología Vegetal, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

[2] Departamento de Suelos y Aguas, Facultad de Agronomía, Facultad de Agronomía, Universidad de la República, Montevideo 12900, Uruguay

7.1. Resumen

La aplicación al suelo de efluentes de tambo (DE) como fertilizante se recomienda para la producción de pasturas, pero enfrenta desafíos como aumentar el rendimiento, prevenir las pérdidas de nutrientes y mitigar las emisiones de gases de efecto invernadero (GEI). Esta investigación estudió el efecto de la aplicación basada en N de DE sin tratar (Crudo) o estabilizado en lagunas (Laguna) (a una tasa anual de 200 kg N ha^{-1} $año^{-1}$ dividida en cuatro aplicaciones) en comparación con la fertilización con urea a escala de campo en festuca alta (*Festuca arundinacea* Schreb.), sobre el rendimiento, la absorción de nutrientes y la dinámica del N del suelo y las emisiones de GEI durante un año. Se incluyó un control sin aplicación de N. El rendimiento de festuca fue mayor con urea y no difirió entre los tipos de efluente siendo mayor que el control. La absorción de macronutrientes por la pastura siguió patrones similares. La emisión de N_2O fue similar entre los tratamientos de fertilización (Urea y DE). Estos resultados son promisorios para el reciclaje de los DE en el predio permitiendo sustituir el fertilizante sintético con alrededor del 60 % de efectividad sin aumentar la emisión de GEI.

Palabras clave: efluentes líquidos de tambo, rendimiento de pasturas, absorción de nutrientes, emisiones de gases de efecto invernadero, ciclo de N en el suelo

7.2. Summary

Land application of farm dairy effluent (DE) is recommended as fertilizer for pasture production but faces challenges such as increasing yield, preventing nutrient losses and mitigating greenhouse gas (GHG) emissions. This research studied the effect of N-based application of untreated (Raw) or lagoon-estabilized (Lagoon) DE (200 kg N ha^{-1} yr^{-1}, divided into 4 applications) compared to urea fertilization at field scale on tall fescue (*Festuca arundinacea* Schreb.) over yield, nutrient content, and on soil's N dynamics and GHG emissions over one year. A control (no N applied) was included. The fescue yield was greater with Urea and did not differ between the types of effluent, being greater than the Control. The absorption of macronutrients by the pasture followed similar patterns. The N_2O emission was similar for Urea and DE. These results are promising for the recycling of DE on the farm, allowing the replacement of synthetic fertilizer with around 60% effectiveness without increasing GHG emissions.

Keywords: liquid farm dairy effluent, pasture yield, nutrient content, Greenhouse gas emissions, soil N cycling

7.3. Introduction

Uruguay is the 7th largest exporter of dairy products in the world, and despite having a population of 3.5 million people, only 5% of its territory produces enough milk for more than 18 million people (INALE 2023). Milk production from a predominantly pasture-based system is one of the top ten agricultural commodities in the country (Fariña & Chilibroste, 2019). A circular economy proposes that nutrients can be recovered after feeding animals using water and nutrients, including nitrogen (N) from urine and manure from cleaning the dairy parlor (Pascucci, 2020).

Generally, dairy effluent (DE) is land applied by tanks or irrigation pumps throughout the year (Salazar et al., 2010), directly after cleaning the milking parlor (Raw) or from the lagoons storage system (INALE, 2020). While Raw effluents generally have higher OM contents, effluents stored for some time have undergone stabilization processes. During storage, the mineralization of the most labile fractions of organic substances occurs, releasing inorganic forms of the nutrients and decreasing carbon (C) content and C:N ratio (Sukias et al., 2001).

DE can provide essential nutrients (macro and micro) other than N, but these nutrient ratios differ from those that plants need. Thus, as N rate increases, it might accumulate other nutrients in soil, e.g. P (Eghball & Power, 1999), or imbalance plant nutrient uptake (N. S. Bolan et al., 2009). Besides, the low readily N availability from DE often makes DE less efficient as a source of N for plants than mineral fertilizers and leads to higher loss of N through nitrous oxide (N_2O) emission following land application (Rita Bhandral et al., 2007; Schröder, 2005).

Milk production contributes 20% to total greenhouse gas emissions (GHG) from livestock worldwide (N. Zhang et al., 2017). The impact of DE and manure applications upon gas emissions from the soil is of concern for agronomic, ecological and environmental reasons (Nartey et al., 2021). Optimization of soil N fertilization and manure management can significantly mitigate GHG emissions from this sector, in particular N_2O and methane (CH_4) emissions (Van Doorslaer et al., 2015).

The N_2O from soil is primarily produced by the microbial nitrification and denitrification processes (Braker & Conrad, 2011). Nitrification mostly occurs under aerobic conditions, where ammonium (NH_4^+) is transformed into nitrate (NO_3^-) and denitrification is an anaerobic process that requires labile C to reduce NO_3^- into N_2 via N_2O. Two main factors influencing soil microbial nitrification and denitrification are soil temperature and moisture.

Ammonification processes depend on effluent or manure characteristics (pH, NH_4^+, NO_3^- and labile organic C) and the soil and climatic conditions (precipitation, temperature, soil texture, moisture, pH and available C) (Amon

et al., 2006; N. Bolan et al., 2004; J. Zhang et al., 2019; T. Zhang et al., 2018). These factors are spatially and temporally variable and influence the magnitude of the resulting GHG fluxes.

Land applications of DE to grasslands can significantly increase GHG emissions, to those from synthetic fertilization (Barton & Schipper, 2001; Lombardi et al., 2022; Van der Weerden et al., 2016). Several studies highlighted that N_2O emissions are a function of N input rates, application methods and the chemical form of N applied (fertilizer type) (Albanito et al., 2017). Manures contain readily metabolized C compounds and NH_4^+, which is readily converted to NO_3^-, which may stimulate microbial activity and create anaerobic conditions for denitrification (Dell et al., 2011; R. James Stevens & Laughlin, 2001; Wulf et al., 2002). Emission peaks typically comprise a considerable portion of annual emissions: up to 30% within a few weeks of manure application (Chadwick et al., 2011).

Since much of the nutrients are readily available in lagoon-stored DE rather than raw ones when used as N-base fertilizers, the nutritional value of both DE types is expected to differ. Moreover, both effluents (lagoon or raw) may alter the soil's N and C cycle processes and differentially modulate the GHG fluxes. However, local research only considered soil properties or crop yield improvements but has not assessed the impact on GHG emissions. A previous study of DE application to a fescue pasture in a pot experiment found that the effluent treated in stabilization lagoons substituted urea as an N source without increasing N_2O emissions and enhancing soil biological activity (Illarze et al., 2023).

The present study aimed to evaluate in the field, the GHG emissions, forage production and chemical composition of a fescue pasture (*Festuca arundinacea* Schreb.) with the application of either raw or lagoon-treated DE compared to urea at a normalized yearly N application rate of 200 kg N ha^{-1}. Besides, this trial investigates the soil's mineral N content, potentially N mineralization and nitrification activities, and their relationship with N_2O emissions.

This study's goal was to evaluate the feasibility of using farm dairy effluents as a nutrient source for a dairy pasture production system while minimizing GHG emissions. It is worth knowing what happens when differently managed DE is applied to pastures for Uruguayan soils, type of animal feeding and effluent management.

7.4. Materials and methods

7.4.1. Study site

The study was conducted at the experimental farm of Centro Regional Sur (CRS) of the Faculty of Agronomy in Canelones, Uruguay (34°36' 47.83" S and 56° 12' 54.00" W). The climate is humid subtropical, with an average mean temperature of 17 °C (mean monthly minimum: 11 °C, maximum: 23 °C) and light frosts between May and September. Mean annual precipitation is 1200 mm, evenly distributed throughout the year but significantly varied between years (Castaño et al., 2011). Precipitation and temperature from the experimental site along the evaluated period are presented in Figure 1. Weather data was taken from the INIA Las Brujas meteorological station. We installed temperature data loggers (ThermaData® stainless steel USB temp data logger) in the study plots to obtain real-time soil temperature data (placed at 15 cm soil depth) when measuring GHG fluxes. The soil is an Argiudol, 370 g kg^{-1} of clay, 380 g kg^{-1} of silt and 250 g kg^{-1} of sand, with no history of DE application. The main soil chemical characteristics of the topsoil (0–15 cm) at the beginning of the experiment were: pH 6.4 (in H_2O), OC 24.5 g kg^{-1} (Walkley-Black method), available P 22.4 mg kg^{-1} (Bray 1 method), exchangeable K^+ 0.6 $cmol_c$ kg^{-1} and Na^+ 0.3 $cmol_c$ kg^{-1} (ammonium acetate).

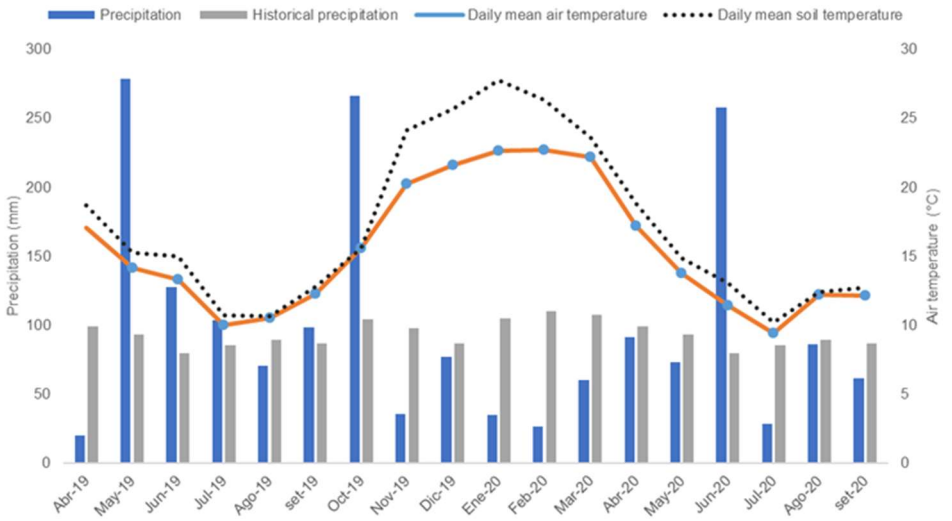

Figure 1. Precipitation, air, and soil temperature from the field experiment at Centro Regional Sur, Faculty of Agronomy.

7.4.2. Experimental design and setup

Festuca arundinacea cv. Rizomat was sown (22 kg seeds ha $^{-1}$) on 12 April 2019 with a row distance of 17 cm. Fertilization at seeding was 5.7 kg ha $^{-1}$ N and 32.4 kg ha $^{-1}$ P. We established a complete randomized block experiment with three replicates and a plot size of 4 m x 3 m with a 0.5 m wide buffer between plots. The N fertilizer treatments included four applications to pasture soil at an equivalent rate of 50 kg N ha $^{-1}$ (based on measured total Kjeldahl N) consisting of (i) untreated dairy effluent (Raw), (ii) dairy effluent from a secondary facultative lagoon (Lagoon), (iii) synthetic granulated N fertilizer (Urea) and (iv) non-amendment control (Control). Treatments were applied by hand using watering cans to ensure the effluent could not flow out of the plot area. Dates of application are provided in Table 1 and Figure 2. Fertilization/harvesting cycles of 45 days, simulating the typical management of fescue forage cuts, were tried, except for summertime, when there was no growth of the pasture due to the drought period (Figure 2). The fescue forage was mowed at 5 cm height.

Table 1. Dates of pasture harvest and N fertilization applications and mm ha^{-1} of raw dairy effluent or lagoon dairy effluent applied to the pasture plots.

Pasture harvest	N fertilization application	mm ha^{-1} applied Raw - Lagoon
04-Nov 2019	5-Sep 2019	12,5 - 37.5
-May 2020	7-Nov 2019	9.3 - 52.6
17-Aug 2020	11-May 2020	9.3 - 56.8
24-Sep 2020	18-Aug 2020	17.8 - 21.6

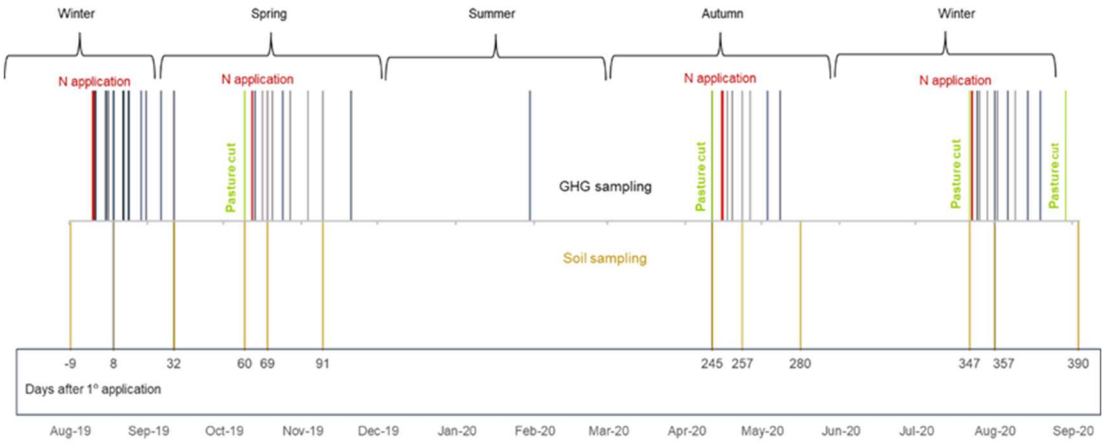

Figure 2. Timeline of the measurements performed in the experiment through seasons. The greenhouse gas emissions (GHG), soil sampling dates, and pasture fertilization and cut management are identified by bars for 390 days, from August 2019 to September 2020.

7.4.3. Farm Dairy Effluent collection and characterization

DEs were collected from the dairy farm of CRS where the study took place, collected the day before each application, the first one after 5 months of seeding. The farm site's effluent management system comprises a conventional two-stage stabilization lagoon system (an anaerobic and a facultative lagoon operated in series) with a solid trap for effluent pretreatment. The system received the

effluent generated by flushing the milking parlor and holding yard and chemically cleaning the milking machinery with sodium hypochlorite. The Raw DE was collected immediately after the cleaning from a holding tank (for solid trap) into a 2 L sterile container. The liquid portion undergoes gravimetric flushing into the two stabilization lagoons operated in series, with a hydraulic retention time of 119 days. The collection and physicochemical characterization of DE was previously described in Illarze et al. 2024. Although variable in composition along the year, dairy effluents had a total solids content lower than 2% and suspended solids were lesser than 1%. The actual amounts of nutrients added in the experiment, adjusted according to the volume of DE applied, are shown in Table 1.

Table 2. Average dairy effluent nutrients applied to the soil according to the month of application.

kg ha^{-1}	September		November		May		August	
	Raw	Lagoon	Raw	Lagoon	Raw	Lagoon	Raw	Lagoon
Org C	64	135	60	218	68	156	158	124
Org N	73	18.8	19.2	29.5	43.2	29.0	46.6	38.4
Total P	2.0	14.3	7.8	18.9	9.4	13.6	3.4	5.6
K^{+}	90.3	56.3	40.5	82.1	21.1	127.2	12.3	19.9
Mg^{++}	12.5	11.6	4.3	13.7	6.8	21.6	1.5	3.7
Ca^{++}	24.6	18.0	7.1	24.7	8.1	18.2	2.7	5.0

7.4.4. Gas sampling and analysis

Soil N_2O, CH_4, and CO_2 fluxes were measured from August 2019 to September 2020. The sampling was performed immediately after N applications. Afterward, samplings were carried out at intervals depending on the fertilization date or rainfall occurrence until 22-32 days after N-applications (Figure 2).

Gas sampling was conducted using the closed chamber technique, where chambers had steel bases 40 cm in diameter and 20 cm in height that were left in place and inserted 7 cm into the soil. The lid was fitted with a sampling port with a three-way valve and placed on top of the box at the beginning of each gas

sampling day when the effective height of each chamber was recorded. Headspace gas samples were obtained with airtight 20-ml propylene syringes and were immediately transferred to pre-evacuated 12-ml glass Exetainer® vials (Labco Ltd., Buckinghamshire, UK). The sampling times were 0 min, 20 min and 40 min. Chamber temperature and headspace height were recorded and used to calculate gas flux rates from the soil surface to the chamber atmosphere assuming a linear increase in gas concentration over time as described previously (Tarlera et al., 2016). Measurements were generally taken between 10:00 and 14:00 h as suggested by Van der Weerden et al. (2013). The flux values were then converted to daily GHG emissions (g ha $^{-1}$ d $^{-1}$), assuming that the measurements were representative of average daily flux. N_2O, CH_4 and CO_2 were analyzed on a GC-FID-mECD 7890 Agilent gas chromatograph with a HayeSep Q 80/100 mesh 1/8 column.

Cumulative GHG emissions for 21 days after N-applications were calculated as the mean flux values of the two dates multiplied by the number of days between these dates (Illarze et al., 2023). The global warming potential (GWP) for the 21 days considered was calculated in terms of CO_2 equivalents (CO_2 eq) over a 100-year time horizon using a radiative forcing potential of 273 for N_2O and 28 for CH_4 (Forster et al., 2021).

7.4.5. Soil sampling and analysis

Ten random soil core samples from 0 to 15 cm soil layer were collected from each replicated plot, homogenized by hand, composited and mixed thoroughly. Fresh soils were used for moisture content, microbial activities (nitrifying and N mineralization potentials) and the soil's mineral N (NO_3^-–N and NH_4^+–N). Soil mineral N was extracted with a 2 M KCl solution and colorimetric analysis was used to determine NH_4^+–N according to Rhine et al. (1998) and NO_3^-–N according to Mulvaney (1996). N mineralization (sum N_min) was estimated by the sum of NO_3^-–N and NH_4^+–N content plus the total N content in the aboveground biomass of fescue plants as per Arló et al. (2022).

7.4.6. Soil potential microbial activities

Assays for potential nitrification activity (PNA) were carried out using a microscale method based on ISO 15685 (Hoffmann et al., 2007). Briefly, 2.5 g of fresh soil was placed in a 50 ml flask, and a solution with 300 mM, 700 mM K_2HPO_4, 10 mM sodium chlorate and 1.5 mM $(NH_4)_2SO_4$ was added to create a slurry with exact volume of 10 ml. The reaction was incubated at 25 °C and shaken at 175 rpm. At 24 h, 1.0 ml samples were collected and added to 31.0 ml 2.0 M KCl, briefly vortexed and centrifuged to separate soil particles. The resulting supernatant was removed and NO_2^- concentration was determined spectrophotometrically at 540 nm.

Potentially mineralizable nitrogen (PMN) was analyzed using the anaerobic method (Kandeler, 1996). The soil was made into a slurry and incubated at 40 °C and accumulation of NH_4^+-N was determined after one week according to previously described for soil NH_4^+-N content.

7.4.7. Pasture yield and nutrient content analysis

The aboveground biomass obtained at each forage cut was dried at 65 °C for 48–72 h (until a constant weight was reached) and ground to pass through a 0.5 mm mesh. After that, the total N content was determined in forage biomass by the Kjeldahl method. P, K, Ca and Mg were extracted with dilute HCl (20%) in samples calcined at 550 °C for 5 h. The molybdate blue method was used to determine the total P with ascorbic acid (Murphy & Riley, 1962). The Ca and Mg elements were determined by atomic absorption spectrometry, and K by atomic emission spectrometry (Isaac & Kerber, 1971). N and other nutrient uptake was calculated as the sum of the total content in the forage biomass of each cut. Nitrogen fertilizer replacement value (NFRV %) was calculated as N-use efficiency (NUE) effluent/NUE synthetic fertilizer x 100 (Baral et al., 2017).

NUE was calculated as the subtraction of N uptake of the Control from those of each treatment divided by the applied N.

7.4.8. Statistical analyses

Data analysis was performed using Infostat® (v. 2020), a statistical software linked to the R programming environment (Di Rienzo et al., 2017). Normality of the residuals was verified by the Shapiro–Wilk test, and homogeneity of residuals variance by Levene's test. N_2O fluxes were cube root transformed prior to fitting models and assumed to follow a normal distribution according to (Albanito et al., 2017). Daily GHG fluxes were analyzed using a generalized linear mixed model (lmer package) with the treatments and sampling occasions as fixed factors, while each field plot was considered as a random factor. Several models were assessed and the most appropriate fit was selected according to the lowest Akaike information criterion. The other variables were analyzed with ANOVA and differences were determined using the LSD Fisher test at $p \leq 0.05$ level. Pearson's r was used to test for correlations between the fluxes of N_2O and the soil N variables (NH_4^+–N, NO_3^-–N, inorganic nitrogen (IN, NO_3^-–N and NH_4^+–N), PMN and PNA.

7.5. Results

7.5.1. Pasture Yield and Nutrient Uptake

Table 3 provides information on yield production and nutrient uptake for the four forage harvests. The growing conditions at the first pasture cut at the beginning of spring were the most favorable of the seasons. Low summer rainfall and cool temperatures reduced forage biomass in the rest of the seasons (Figure 1, Table 3). Although variable, results showed that the Control produced less yield than the N-treatments, at least for the two last application dates. Applying both types of DE produced a similar forage yield and Urea had the highest yield

but was significantly different from DE treatments at the first and fourth harvests (Table 3). Annual forage yield for Urea treatment accounted for 6553 kg ha^{-1}, whereas for both DE treatments was around 4990 kg ha^{-1}, producing 63% higher biomass than unamended Control.

Similar trends occurred when comparing crop nutrient uptake in the fescue (Table 3). In general, the three N-amendments increased the macronutrient uptake by pasture compared to the Control treatment. The increase in pasture P and K uptake was more pronounced and there was also a tendency to increase Ca and Mg in the N-treatments but these were variable among pasture cuts. In general, the nutrient uptake was similar among the DE types and lower from Urea at the last cut.

Urea-treated plots accumulated the most plant N, ranging from 18 to 37 kg N ha^{-1}. Lagoon DE increased the plant N uptake as Urea did at the first, second and fourth harvests, whereas Raw DE increased it similarly to Urea at the second and third harvests. Both types of DE did not significantly differ in plant N uptake. Nitrogen fertilizer replacement value (NFRV) for Raw DE averaged for the four harvests 65% and for Lagoon DE averaged 57%.

Table 3. Effect of application of synthetic fertilizer (Urea), Raw Dairy Effluent (Raw), Lagoon Dairy Effluent (Lagoon) or non-amendment (Control) on pasture yield and nutrient uptake across 4 consecutive harvestings between November 2019−September 2020. Values indicated in brackets are standard deviations (n = 4).

Treatment	**Harvest number**	**Yield (kg ha $^{-1}$)**	**Macronutrient uptake (kg ha $^{-1}$)**				
			N	P	K	Mg	Ca
Control	1	1341 (390) a	13.3 (3.4) a	3.2 (0.7) a	16.0 (4.0) a	2.5 (0.7) a	4.3 (1.3) a
Lagoon		2265 (354) b	22.0 (2.1) bc	5.0 (0.7) bc	29.0 (4.2) b	3.8 (0.6) a	6.5 (0.8) a
Raw		1964 (250) ab	17.5 (3.0) ab	4.2 (0.5) b	26.2 (4.7) ab	3.7 (0.6) a	6.8 (1.6) a
Urea		3012 (664) c	25.7 (5.9) c	5.5 (1.0) c	37.2 (10.1) b	5.8 (1.5) b	12.4 (3.8) b
Control	2	1085 (129) a	17.0 (1.5) a	1.6 (0.2) a	15.3 (1.3) a	3.5 (0.5) a	3.6 (0.5) a
Lagoon		1333 (123) ab	24.2 (2.5) ab	2.6 (0.2) b	21.9 (1.9) b	4.2 (0.5) a	5.1 (0.5) b
Raw		1451 (25) b	29.9 (1.3) b	2.7 (0.3) b	26.6 (2.0) b	4.5 (0.2) a	5.0 (0.3) b
Urea		1349 (69) ab	28.0 (3.1) b	2.4 (0.1) b	23.8 (1.8) b	4.3 (0.3) a	4.8 (0.2) ab
Control	3	458 (57) a	10.4 (1.7) a	1.1 (0.1) a	7.3 (1.4) a	1.1 (0.2) a	1.6 (0.2) a
Lagoon		917 (107) b	22.9 (1.9) b	2.4 (0.3) b	17.2 (2.0) b	1.9 (0.2) ab	3.0 (0.4) ab
Raw		1129 (161) bc	28.8 (3.9) bc	2.4 (0.4) b	21.7 (3.5) b	2.6 (0.3) bc	3.9 (0.5) bc
Urea		1451 (151) c	37.4 (5.2) c	2.8 (0.3) b	25.6 (2.5) b	3.3 (0.3) c	4.7 (0.4) c
Control	4	177 (30) a	3.4 (0.2) a	0.4 (0.0) a	2.9 (0.1) a	0.3 (0.0) a	0.5 (0.0) a
Lagoon		485 (65) b	10.7 (0.1) bc	1.2 (0.0) b	10.2 (0.7) b	1.0 (0.0) b	1.2 (0.1) b
Raw		441 (62) b	9.4 (0.2) b	0.9 (0.1) b	8.7 (0.2) b	0.9 (0.0) b	1.2 (0.1) b
Urea		741 (39) c	18.1 (0.3) c	1.5 (0.0) c	17.4 (1.5) c	1.6 (0.0) c	2.0 (0.2) c

7.5.2. Soil N dynamics and GHG fluxes

Potential N mineralization increased in both DE treatments after a month of each application (Figure 3a), except for the first application date. Lagoon DE treatment had the highest PMN at these sampling dates and remained significantly higher than the Control at the end of the experiment (Figure 3a). Potential nitrification activity was higher for Lagoon in most of the sampling dates, as well (Figure 3b). Both DE types tended to increase PNA relative to Urea fertilization (Figure 3b). Meanwhile, soil NH_4^+–N content was only higher

for the Urea treatment in some sampling dates (Figure 3c). In comparison, soil NO_3^-–N content was higher for Raw only after the 3rd fertilization (Figure 3d).

When considering the soil N mineralization as the total N content in the pasture biomass plus the mineral N in the soil, Lagoon DE treatment increased the sum N_min as did the Urea treatment only at the first application (spring season), on average 47.7 kg ha^{-1} vs 34.7 kg ha^{-1} for Raw DE and Control. However, the three N amendments increased the N mineralization compared to the Control afterward. At the third and fourth applications, Urea had a significantly higher sum N_min than the rest of the treatments, producing 57.5 and 29.1 kg ha^{-1}, respectively, whereas both DE amendments produced a similar increase in the sum N_min, 40 and 21 kg ha^{-1}, respectively.

The N_2O fluxes measured after N-treatment applications and in the Control are presented in Figure 4. The first application resulted in large N_2O peaks 8 days after Lagoon and Raw applications. Only after the application in November (spring), the DE daily fluxes exceeded 30 g N-N_2O ha^{-1}, the largest N_2O fluxes resulting from Raw DE. N_2O peaked up to 4 days after the application of Lagoon DE and up to 6 days with the application of Raw DE. In the autumn, the third application did not result in distinct N_2O peaks and similar low fluxes were observed from all treatments. After the last application and irrespective of the sampling day, Lagoon DE increased N_2O emissions concerning the Control without differences with Urea or Raw DE application.

Methane fluxes from Control and N-fertilized treatments were not significantly different after the four applications (Figure 5). In general, the CO_2 fluxes increased after the N-treatment applications (Figure 6), irrespective of the type of N source, compared to the Control. Only after the second application in spring, both types of DE significantly increased CO_2 fluxes compared to Urea fertilization or Control treatment up to 4 days of application ($p < 0.001$), remaining higher in the rest of the days.

The cumulative N-N_2O emissions for 21 days after each treatment application are shown in Table 4. For the November application date (2nd) only Raw DE cumulative N_2O fluxes were higher than Control's. However, in the last

application, only Lagoon DE N-N_2O cumulative emissions were higher than those from the Control.

Non-significant differences among the cumulative C-CH_4 fluxes were detected after the treatments' first and second application (Table 4). Lagoon DE application resulted in higher cumulative CH_4 fluxes than Urea after the third application; for the last applications, both DEs did it.

Cumulative C-CO_2 fluxes from DE and Urea fertilized plots were not significantly different and, in general, were larger than the Control (Table 4).

Regarding total CO_2-equivalents, for the second and third application dates Lagoon DE showed higher CO_2-equivalent emissions than the Control, while, at the end of the experiment, both types of DE had similar CO_2-equivalent emissions. Overall, there were no significant differences in total CO_2 equivalents caused by the use of N-fertilizers (DEs or Urea).

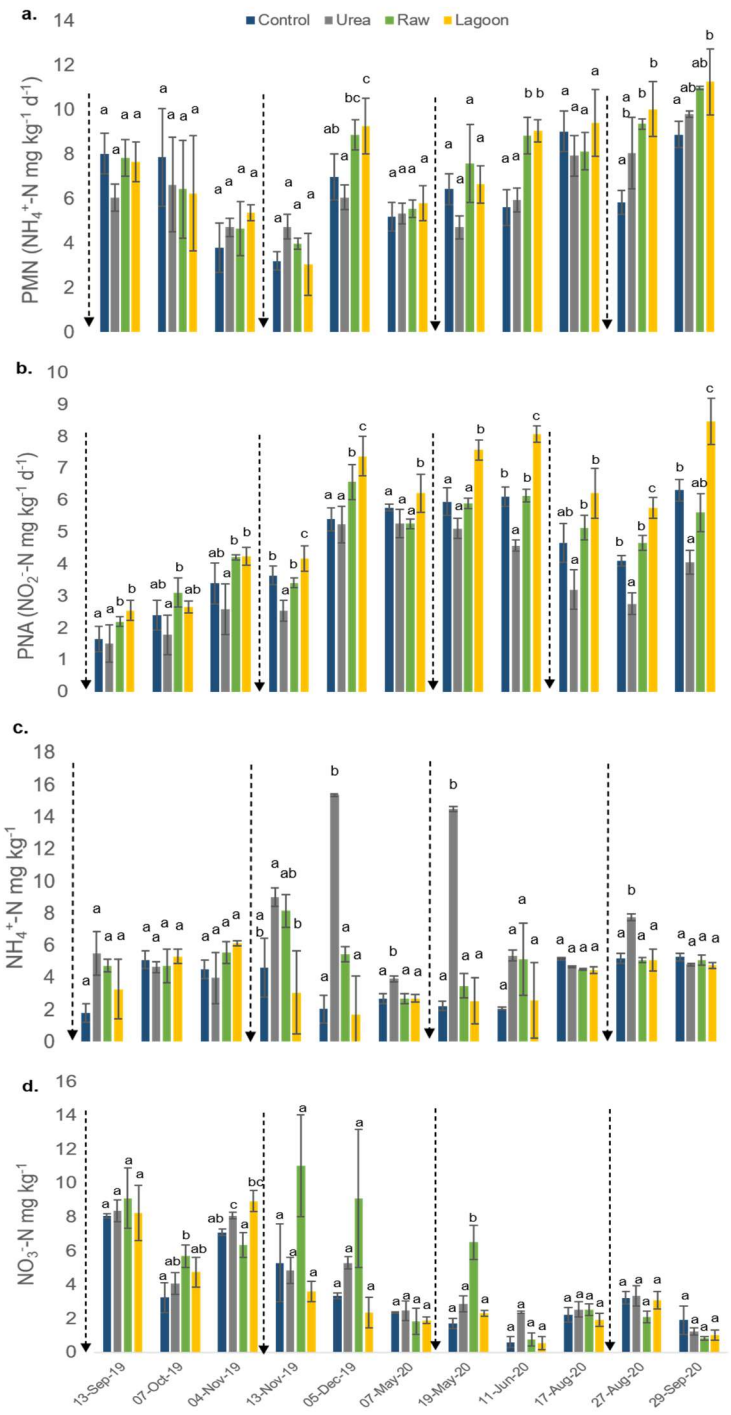

Figure 3. Soil (a) Potentially mineralizable N, (b) Potential Nitrification Activity (c) NH_4^+ and (d) NO_3^-, in the top 15-cm soil layer after application of synthetic fertilizer (Urea), Raw Dairy Effluent (Raw), Lagoon Dairy Effluent (Lagoon) or non-amendment (Control) to a fescue pasture. N sources were applied in spring-summer 2019 and autumn-winter 2020. Arrows indicate the time of N applications. Vertical bars indicate mean±SE(n=3). Mean values followed by different lower letters between each treatment within each sampling time represent significant differences between treatments ($p < 0.05$).

Table 4. Mean cumulative fluxes of N_2O, CH_4 and CO_2 of the N application of Raw or Lagoon dairy effluents, urea or non-amended control to fescue pasture. Cumulative production after 21 days of each application date is presented (mean n=3± S.E). Different letters besides the numbers indicate significant differences at $p < 0.05$ for each application date.

Application date	Treatment	N-N_2O	C-CH_4	C-CO_2	CO_2-C eq.
		---------- kg ha^{-1} ----------			
Sep 5th 2019					
	Control	0.03 (0.01) a	0.18 (0.06) a	803.06 (23.85) a	815.37 (21.93) a
	Urea	0.02 (0.01) a	0.08 (0.02) a	893.18 (28.86) a	899.93 (26.68) a
	Lagoon	0.04 (0.01) a	0.13 (0.03) a	936.00 (124.74) a	951.87 (122.91) a
	Raw	0.04 (0.01) a	0.1 (0.04) a	969.03 (42.31) a	983.77 (43.33) a
Nov 7th 2019					
	Control	0.04 (0.00) a	0.10 (0.02) a	521.03 (16.31) a	534.84 (16.48) a
	Urea	0.09 (0.00) ab	0.15 (0.07) a	555.03 (104.58) ab	574.78 (99.96) ab
	Lagoon	0.11 (0.02) ab	0.16 (0.09) a	903.10 (159.79) b	937.07 (160.88) b
	Raw	0.2 (0.05) b	0.06 (0.02) a	850.75 (86.10) ab	899.02 (86.60) ab
May 12th 2020 *					
	Control	0.09 (0.00) a	0.07 (0.01) c	160.71 (42.07) a	187.53 (42.48) a
	Urea	0.11 (0.03) a	0.01 (0.00) a	346.33 (32.53) b	375.69 (33.54) b
	Lagoon	0.09 (0.01) a	0.04 (0.01) bc	376.15 (32.27) b	401.96 (35.83) b
	Raw	0.08 (0.04) a	0.01 (0.00) ab	310.00(68.03) b	324.32 (71.12) ab
Aug 18th 2020					
	Control	0.03 (0.01) a	0.04 (0.02) ab	588.70 (77.06) a	597.52 (74.12) a
	Urea	0.08 (0.04) ab	0.02 (0.01) a	797.75 (20.45) b	817.99 (27.11) b
	Lagoon	0.11 (0.05) b	0.1 (0.00) b	940.87 (55.10) b	972.54 (66.03) b
	Raw	0.08 (0.02) ab	0.1 (0.03) b	899.11 (55.52) b	924.57 (53.55) b

* CO_2 cumulative fluxes measured up to 11 days of treatment application

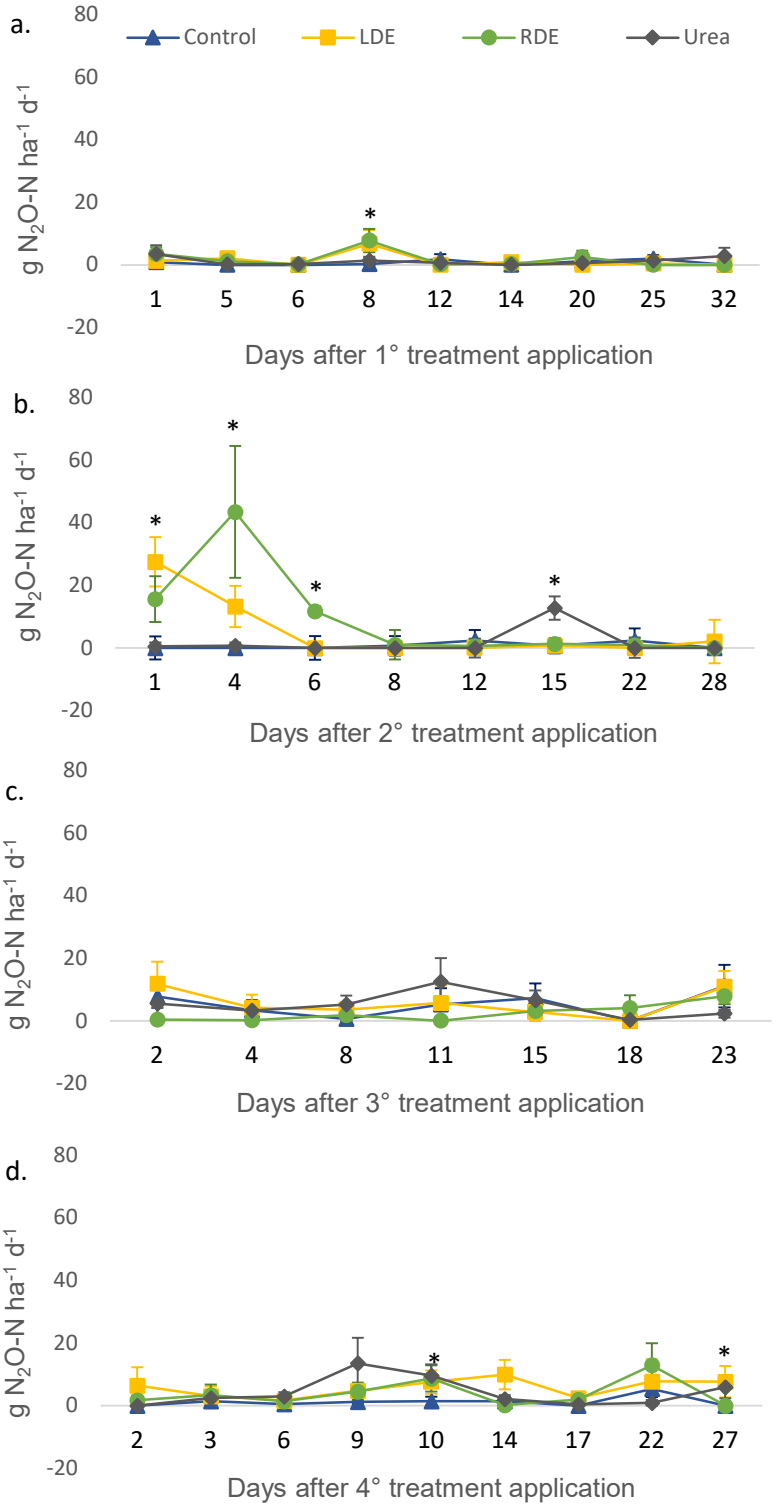

Figure 4. Temporal pattern of the N_2O fluxes during the periods measured between N applications (a) 5th September 2019, (b) 7th November 2019, (c) 12th May 2020 and (d) 29th September 2020. Data represent the mean value of three replicates and standard error. Treatments: non-amended Control; Urea synthetic fertilizer; Raw Dairy Effluent; Lagoon Dairy Effluent.

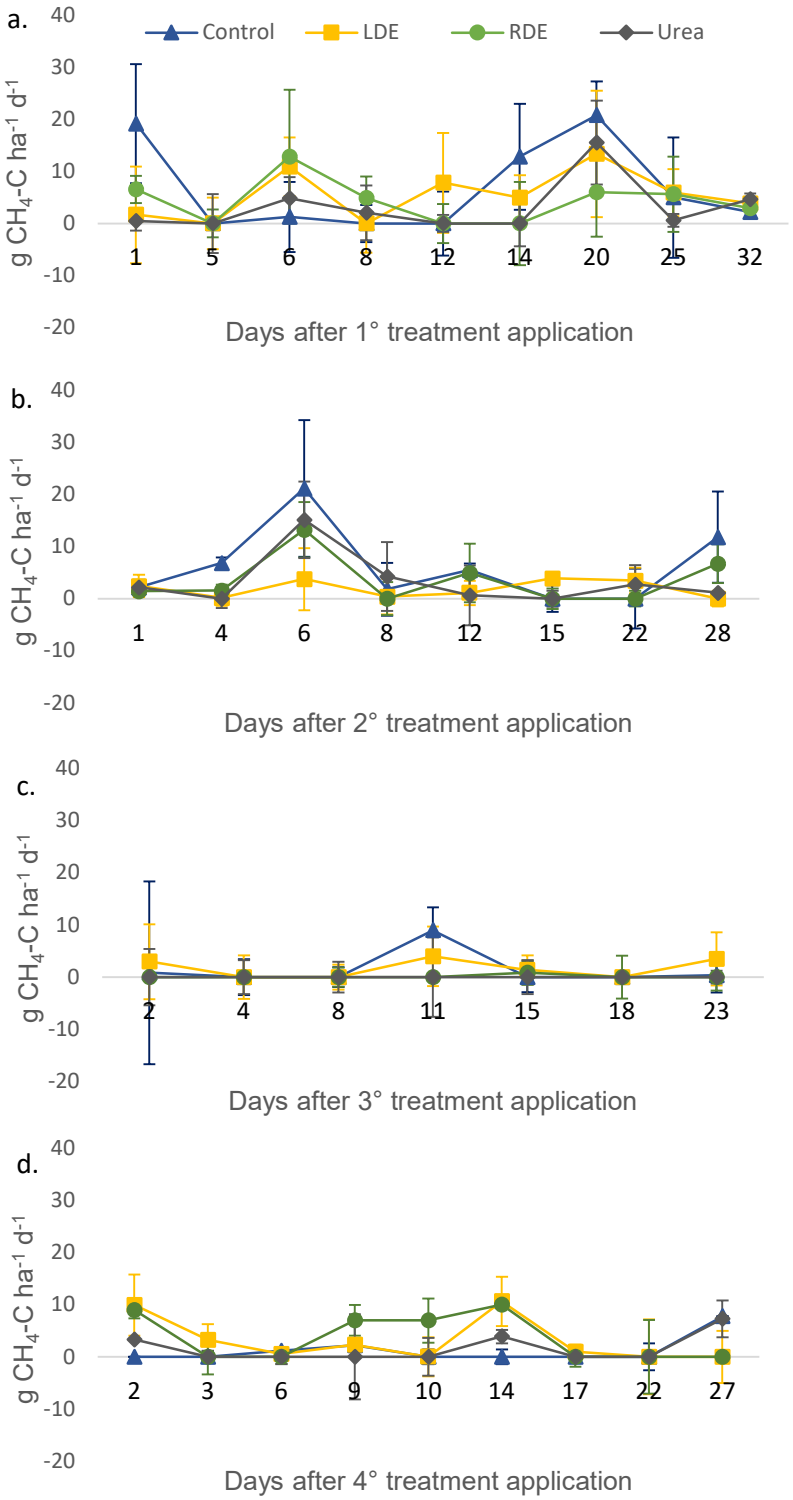

Figure 5. Temporal pattern of the CH_4 fluxes during the periods measured between N applications (a) 5th September 2019, (b) 7th November 2019, (c) 12th May 2020 and (d) 29th September 2020. Data represent the mean value of three replicates and standard error. Treatments: non-amended Control; Urea synthetic fertilizer; Raw Dairy Effluent; Lagoon Dairy Effluent.

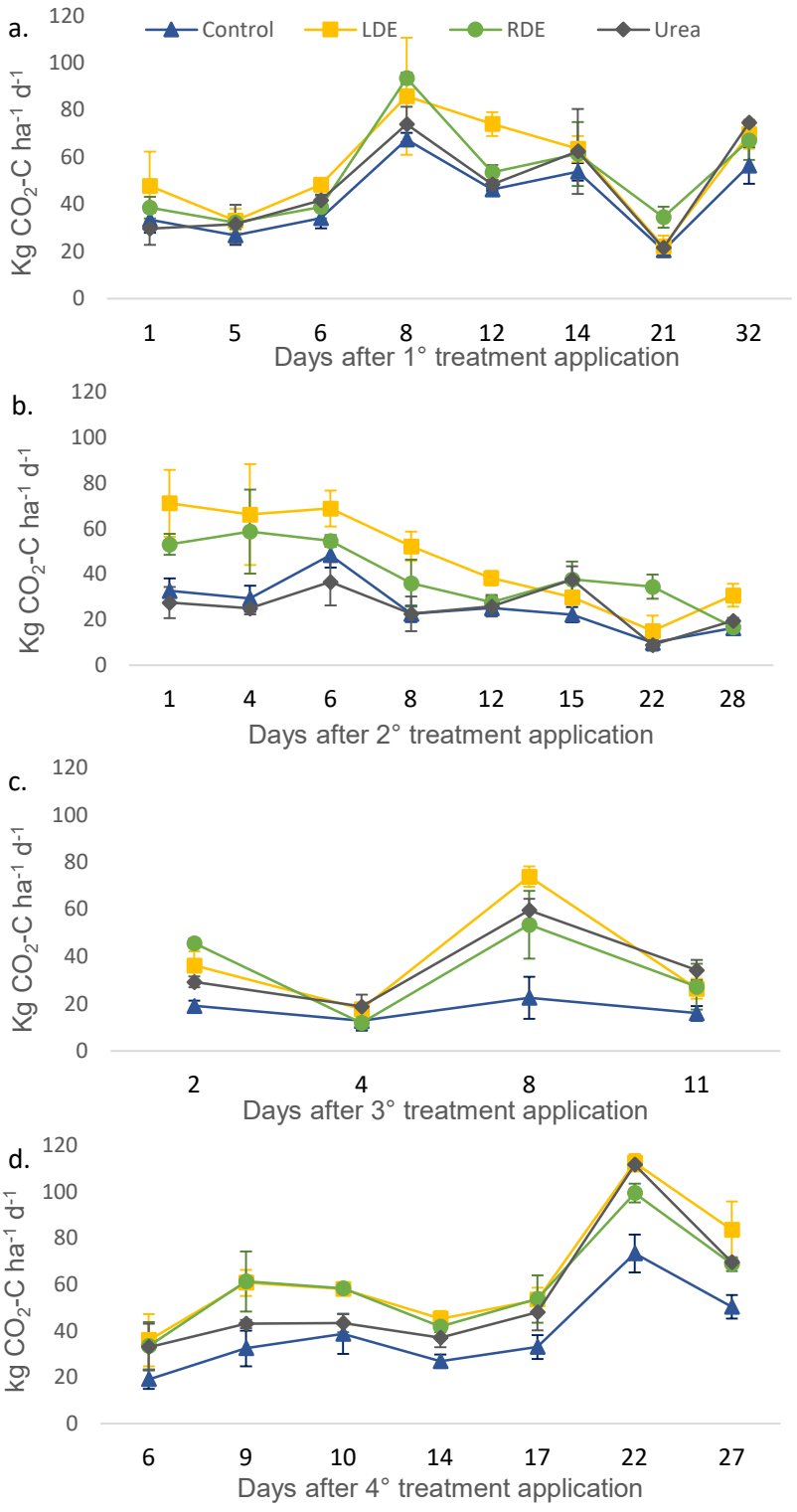

Figure 6. Temporal pattern of the CO_2 fluxes during the periods measured between N applications (a) 5th September 2019, (b) 7th November 2019, (c) 12th May 2020 and (d) 29th September 2020. Data represent the mean value of three replicates and standard error. Treatments: non-amended Control; Urea synthetic fertilizer; Raw Dairy Effluent; Lagoon Dairy Effluent.

The N_2O fluxes were positively correlated with soil PMN in Urea treatment and with NO_3^--N and IN concentrations in Lagoon treatment (Table 5). Interestingly, in both DE treatments, PMN positively correlated with PNA (0.33 and 0.57 for Raw and Lagoon, respectively, $p < 0.05$, data not shown in table).

Table 5. Pearson correlations between N_2O and soil NH_4^+ - N, NO_3^- -N, inorganic nitrogen (IN, NH_4^+ - N + NO_3^- -N), potentially mineralizable N (PMN) and potential nitrification activity (PNA).

	Treatment	NH_4^+–N	NO_3^-–N	IN	PNA	PMN
	Control	-0.24	-0.33	-0.38	0.25	-0.15
	Urea	0.03	-0.28	-0.07	0.003	0.35*
N_2O	Lagoon	0.32	0.39*	0.44*	-0.02	0.01
	Raw	-0.07	-0.06	-0.05	-0.18	-0.31

* Correlation is significant at $p < 0.05$

7.6. Discussion

7.6.1. Dairy effluents as biofertilizers

Applying DE to the soil generally increases plant yield (Hawke & Summers, 2006). Still, its reutilization as a pasture N-supplier is challenging because of its variable composition, complicating the prediction of its agronomic value. Stabilizing DE in lagoon systems enables the turnover of most organic forms, increasing the concentration of NH_4^+-N and leading to lower C concentrations and C:N ratios (Bolan et al., 2009). Due to the larger inorganic N concentrations, it is expected Lagoon DE supplies more plant-available N and increases the yield more than untreated DE.

In the current study, synthetic fertilizer and DE applications significantly increased forage yield and N uptake compared with the unfertilized Control (Table 3). It is worth noting that being mainly a source of organic N, Raw DE showed similar performance in maintaining pasture production and N uptake

compared to Lagoon DE (Table 3). These results and the similar N mineralization (sum N_min) of both types of DE suggested that the higher organic N input in Raw DE (Table 2) should have been mineralized throughout the seasons.

Fescue yield was higher for the Urea fertilizer than for DE types. Even though, when considering the yield per cut and the variability of the DE nutrient inputs with the seasons (Table 2, 3), in some cuts the biomass obtained with Urea fertilization was not different from those of at least one type of DE. The seasonality of the effect of the DE application emphasizes the need to take into account the nutrient composition of the effluents before their application to pastures to substitute fertilizer. In this sense, Pagliari et al. (2020) recommend testing DE for nutrient availability as close to field application as possible.

However, it is clear that while N from Urea is available for plant uptake almost immediately, less plant availability of the N added as DE (with about 60% NFRV from Urea) may explain the differences in pasture production between N-treatments. This was also supported by the higher soil NH_4^+ pool in the Urea-fertilized plots relative to DE (Figure 3c). However, tall fescue N-uptake was only lower for Raw DE and similar to the Control at the first forage cut (Table 3). A meta-analysis showed that concentrations of total ammoniacal nitrogen in New Zealand dairy shed manure vary markedly from 36 to 1400 mg/l, potentially reflecting seasonal changes in pasture quality and farm management practices (Clough et al., 2018).

In our study, the N-treatments (DE or Urea) increased the pasture P and K uptake relative to un-amended Control and tended to increase Ca and Mg (Table 3). It was previously reported that excessive K loading from the effluent application induces the leaching of Ca and Mg ions in soil due to competition for adsorption sites in favor of K ions (N. S. Bolan et al., 2004). This high concentration of K in soil solution induces its luxury uptake, leading to decreased uptake of other cations, such as Ca and Mg, to maintain charge balance in plants. A study on the land application of two types of DE demonstrated that both plant biomass and soil K levels increased under effluent treatments, as the amount of

K applied in DE exceeded tall fescue maintenance requirements at rates above 100 kg N/ha/year (Roach et al., 2001). In this trial, the P and cations loaded from Lagoon DE almost double the amounts of Raw DE (Table 2). In accordance, our previous work in this same trial showed an increase in soil K content after the four applications of both DE types, more pronounced in Lagoon DE plots (Illarze et al. 2024 under review). However, the pasture macronutrient uptake seemed not to be altered by the excessive cation inputs provided by DE, at least in the short term (Table 3), and this may not be a problem considering the high basal cation content of this soil.

7.6.2. Effect of dairy effluent applications on GHG emissions and N cycling

Repeated applications of dairy manures change the soil's C and N cycles due to the introduction of substrate for soil-borne microbes such as mineral N and easily mineralizable N and C, enhancing soil N_2O, CH_4 and CO_2 fluxes immediately after application (Severin et al., 2015; Viguria et al., 2015). These C and N inputs increase microbial activity which, combined with high moisture and low gas diffusivity, creates anaerobic conditions near subsurface irrigation lines, raising soil CH_4 and N_2O fluxes (Saggar et al., 2004). Petersen (2018) states that liquid DE will partly infiltrate the soil in response to water potential gradients. Therefore, it creates a gradient of most labile C and N shortly after application. For these reasons, it was expected DE enhanced GHG emissions more than Urea despite applying the same amount of N. However, in our trial, higher N_2O and CO_2 peak fluxes than Urea fertilization only occurred within 1-4 days after the second application of DE (Figure 4b-6b) in the spring season. Nevertheless, when considering cumulative emissions of N_2O, CH_4 and CO_2, no significant differences were observed between the types of N fertilizer. Li et al. (2015) reported that applying DE during spring results in higher N_2O emissions due to readily available soil N. In this season, the increasing temperatures promote higher rates of mineralization at the time of actively growing pasture.

N_2O emissions are smallest when soils are dry (Laubach et al., 2015). This could explain the low GHG fluxes during the summer period and through the successive pasture cuts/treatment applications, where an exceptional drought period occurred with reduced rainfall in comparison with the last 10-year mean which was especially important during summer and autumn (Figure 1) (Gomes et al., 2021). Luo et al. (2008) also found that strategic application of DE to pastures under dry soil-moisture status could reduce N_2O emissions by up to 96%. Louro et al. (2016) hypothesized that the low N_2O emissions under this dry soil condition are due to a reduction in nitrification activity.

Our previous work (Illarze et al., 2023) showed DE applications promote a greater microbial activity, revealed by enzyme activities and soil basal respiration than Urea or un-amended Control. In the present study, both DE increased potentially mineralizable N at some sampling dates and presented higher potential nitrification activity than Urea or un-amended plots (Figure 3). However, these could not explain greater cumulative GHG fluxes after DE applications relative to Urea fertilization (Table 4).

Storage conditions and DE composition importantly define C and N transformations and the resulting GHG emissions (Petersen, 2018). The DE composition was variable for the four applications although Raw DE always presented higher organic C and N content than Lagoon DE. However, a higher volume of Lagoon DE than Raw DE was applied to reach the same N content (Table 1), so the organic N per unit area was higher for Lagoon DE for the summer application (Table 2). Even though, no differences in cumulative N_2O emissions were found between both DE among applications.

Wulf et al. (2002) reported similar N_2O emissions between anaerobically digested and raw manure when applied to arable soils but higher emissions with the digested manure when applied to grassland soils. Bhandral et al. (2007) compared the N_2O emissions produced in a pasture irrigated with influent and effluent from an aerobic lagoon during the dry and wet seasons. The soil irrigated with treated effluent emitted the highest N_2O fluxes in autumn, despite the untreated effluent having a higher content of total N and available organic C.

The authors suggest that the variation in the C/N ratio and the availability of easily assimilable C and N in each effluent affect both denitrification rates and the N_2O: (N_2O+N_2) ratio. Thus, the fresh effluent, with a higher C/N ratio, may have slowed the release of N through mineralization and, due to its higher C content, favored complete denitrification to N_2.

7.7. Conclusions

In conclusion, our study showed the feasibility of using untreated or lagoon-treated dairy effluents to substitute or partially substitute Urea fertilization for pasture performance without increasing GHG emissions when applied in dry weather conditions.

Additional research should be performed to understand the long-term effect of dairy effluent applications to find optimal nutrient use efficiency related to synthetic fertilizer while lowering environmental impacts from dairy pastures in Uruguay.

7.8. References

Albanito, F., Lebender, U., Cornulier, T., Sapkota, T. B., Brentrup, F., Stirling, C., & Hillier, J. (2017). Direct nitrous oxide emissions from tropical and sub-tropical agricultural systems - A review and modelling of emission factors. *Scientific Reports*, *7*(September 2016), 1-12. https://doi.org/10.1038/srep44235

Alegrette, M. J. (2020). *Determinación de las propiedades químicas y físicas del suelo, ante la aplicación de efluentes de tambo con diferentes sistemas de gestión de efluentes*. Facultad de Agronomía, UDELAR.

Alegria Terrazas, R., Giles, C., Paterson, E., Robertson-Albertyn, S., Cesco, S., Mimmo, T., Pii, Y., & Bulgarelli, D. (2016). Plant-microbiota interactions as a driver of the mineral Turnover in the Rhizosphere. En *Advances in Applied Microbiology* (Vol. 95). Elsevier Ltd. https://doi.org/10.1016/bs.aambs.2016.03.001

Amon, B., Kryvoruchko, V., Amon, T., & Zechmeister-Boltenstern, S. (2006). Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agriculture, Ecosystems & Environment*, *112*(2-3), 153-162. https://doi.org/10.1016/J.AGEE.2005.08.030

Angers, D. A., Chantigny, M. H., MacDonald, J. D., Rochette, P., & Côté, D. (2010). Differential retention of carbon, nitrogen and phosphorus in grassland soil profiles with long-term manure application. *Nutrient Cycling in Agroecosystems*, *86*(2), 225-229.

https://doi.org/10.1007/s10705-009-9286-3

Arlo, L., Beretta, A., Szogi, A. A., & del Pino, A. (2022). Biomass production, metal and nutrient content in sorghum plants grown on soils amended with sewage sludge. *Heliyon*, *8*(1), e08658. https://doi.org/10.1016/j.heliyon.2021.e08658

Arocena, R., Chalar, G., Perdomo, C., Fabián, D., Pacheco, J., González, M., Olivero, V., Silva, M., García, P., & Etchevarne, V. (2013). Impacto de la producción lechera en la calidad de los cuerpos de agua. *Acta VII Congreso de Medio Ambiente AUGM*, 1-38. sedici.unlp.edu.ar

Arocena, Rafael, Chalar, G., & Pacheco, J. P. (2018). Agriculture and elevation are the main factors for Pampasic stream habitat and water quality. *Environmental Monitoring and Assessment*, *190*(4). https://doi.org/10.1007/s10661-018-6622-6

Avery, L. M., Hill, P., Killham, K., & Jones, D. L. (2004). Escherichia coli O157 survival following the surface and sub-surface application of human pathogen contaminated organic waste to soil. *Soil Biology and Biochemistry*, *36*(12), 2101-2103. https://doi.org/10.1016/j.soilbio.2004.05.018

Baral, K. R., Labouriau, R., Olesen, J. E., & Petersen, S. O. (2017). Agriculture, Ecosystems and Environment Nitrous oxide emissions and nitrogen use ef fi ciency of manure and digestates applied to spring barley. *«Agriculture, Ecosystems and Environment»*, *239*, 188-198. https://doi.org/10.1016/j.agee.2017.01.012

Barnett, J. W., Kerridge, G. J., & Russell, J. M. (1994). Effluent treatment systems for dairy industry. *Australian Biotechnology*, *4*(1), 26-30.

Barreto Wagner, Dogliotti, S., & Perdomo, C. (2017). Surface Water Quality of Intensive Farming Areas Within the Santa Lucia River Basin of Uruguay. *Air, Soil and Water Research*, *10*(0). https://doi.org/10.1177/1178622117715446

Barton, L., & Schipper, L. A. (2001). Regulation of Nitrous Oxide Emissions from Soils Irrigated with Dairy Farm Effluent. *Journal of Environmental Quality*, *30*(6), 1881-1887. https://doi.org/10.2134/jeq2001.1881

Bhandral, R., Bolan, N. S., & Saggar, S. (2010). Nitrous oxide emission from farm dairy effluent application in grazed grassland. *Revista de la Ciencia del Suelo y Nutricion Vegetal*, *10*(1), 22-34. https://doi.org/10.4067/S0718-27912010000100003

Bhandral, R., Bolan, N. S., Saggar, S., & Hedley, M. J. (2007). Nitrogen transformation and nitrous oxide emissions from various types of farm effluents. *Nutrient Cycling in Agroecosystems*, *79*, 193-208. https://doi.org/https://doi.org/10.1007/s10705-007-9107-5

Bhandral, Rita, Bolan, N. S., Saggar, S., & Hedley, M. J. (2007). Nitrogen transformation and nitrous oxide emissions from various types of farm effluents. *Nutrient Cycling in Agroecosystems*, *79*(2), 193-208. https://doi.org/10.1007/s10705-007-9107-5

Bhandral, Rita, Saggar, S., Bolan, N. S., & Hedley, M. (2004). Nitrous oxide emissions from farm effluents. *3rd Australian New Zealand Soils Conference, 5-9 December 2004, University of Sydney, Australia*, *December*.

Bhogal, A., Nicholson, F. A., Rollett, A., Taylor, M., Litterick, A., Whittingham, M. J., & Williams, J. R. (2018). Improvements in the Quality of Agricultural Soils Following Organic Material Additions Depend on Both the Quantity and Quality of the Materials Applied. *Frontiers in Sustainable Food Systems*, *2*(9), 1-13. https://doi.org/10.3389/fsufs.2018.00009

Bianco, A. (2016). *Efluentes en la producción lechera*. VIII Encuentro de la SUCS. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://www.sucs.org.uy/pdf/Encuentro20

16/Bianco.pdf

Black, Z., Balta, I., Black, L., Naughton, P. J., Dooley, J. S. G., & Corcionivoschi, N. (2021). The Fate of Foodborne Pathogens in Manure Treated Soil. *Frontiers in Microbiology*, *12*(December). https://doi.org/10.3389/fmicb.2021.781357

Bolan, N. S., Horne, D. J., & Currie, L. D. (2004). Growth and chemical composition of legume - based pasture irrigated with dairy farm effluent. *New Zealand Journal of Agricultural Research*, *47*(1), 85-93. https://doi.org/10.1080/00288233.2004.9513574

Bolan, N. S., Laurenson, S., Lowe, H., Luo, J., Hill, P. H., & Sukias, J. (2009). Treatment of farm effluents in New Zealand's dairy operations. *New Zealand Land Treatment Collective: Proceedings for the 2009 Annual Conference*, 120-134.

Bolan, N., Saggar, S., Luo, J., Bhandral, R., & Singh, J. (2004). Gaseous emissions of nitrogen from grazed pastures: processes, measurements and modelling, environmental implications, and mitigation. *Advances in agronomy*, *78*(37), 120. https://books.google.com.uy/books?hl=es&lr=&id=vnVywNimRQ4C&oi=fnd&pg=PA37&dq=Bolan+NS,+Saggar+S,+Luo+J,+Bhandral+R,+Singh+J+(2004)+Gaseous+emis-+sions+of+nitrogen+from+grazed+pastures:+processes,+measurements+and+modelling,+environmental+implications,+an

Braker, G., & Conrad, R. (2011). Diversity, structure, and size of N2O-producing microbial communities in soils-what matters for their functioning? En *Advances in Applied Microbiology* (Vol. 75). https://doi.org/10.1016/B978-0-12-387046-9.00002-5

Cameron, K. C., Di, H. J., & Moir, J. L. (2013). *Nitrogen losses from the soil / plant system : a review*. *162*, 145-173. https://doi.org/10.1111/aab.12014

Cameron, K. C., Di, H. J., Reijnen, B. P. A., Li, Z., Russell, J. M., Barnett, J. W., Di, H. J., Reijnen, B. P. A., Li, Z., Russell, J. M., Barnett, J. W., & Li, Z. (2002). *Fate of nitrogen in dairy factory effluent irrigated onto land*. *8233*(April). https://doi.org/10.1080/00288233.2002.9513511

Caradonia, F., Ronga, D., Catellani, M., Azevedo Giaretta, C. V., Alegria Terrazas, R., Robertson-Albertyn, S., Francia, E., & Bulgarelli, D. (2019). Nitrogen Fertilizers Shape the Composition and Predicted Functions of the Microbiota of Field-Grown Tomato Plants. *Phytobiomes*, *3*(4), 315-325. https://doi.org/10.1094/PBIOMES-06-19-0028-R

Casanova, O., Durán, A., Mello, R., & del Pino, A. (2007). Manejo de los efluentes de tambo. *CANGUE*, *29*(Nota técnica), 94-96.

Castagna, A., Matonte, F., Mauttone, A., Rodríguez-Gallego, L., & Blumetto, O. (2024). Land use planning to minimize the export of phosphorus: An optimization model for dairy production at a catchment area scale. *Land Use Policy*, *138*(February 2023). https://doi.org/10.1016/j.landusepol.2023.107023

Castaño, J. P., Giménez, A., Ceroni, M., Furest, J., & Aunchayna, R. (2011). *Caracterización agroclimática del uruguay 1980-2009. Serie Técnica N° 193*. INIA.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B., & Misselbrook, T. (2011). Manure management: Implications for greenhouse gas emissions. *Animal Feed Science and Technology*, *166-167*, 514-531. https://doi.org/10.1016/j.anifeedsci.2011.04.036

Chalar, G., Delbenea, L., González-Bergonzonia, I., & Arocena, R. (2013). Fish assemblage changes along a trophic gradient induced by agricultural activities (Santa Lucía, Uruguay). *Ecological Indicators*, *24*, 582-588.

Charlón, V., Serrano, P., García, K., & Taverna, M. (2009). Gestión de los efluentes originados

en las instalaciones de ordeño. *CREA: Producción de Leche - Edición 2009*.

Charlón, Verónica. (2007). Residuos en las instalaciones de ordeño. *Idia XXI : revista de información sobre investigación y desarrollo agropecuario*, *7*(9), 80-85. http://ceiba.agro.uba.ar/cgi-bin/koha/opac-detail.pl?biblionumber=27685

Chilibroste, P. (2021). A major challenge for the Uruguayan dairy industry sustainable growth. *Agrociencia Uruguay*, *25*(2), 25-27. https://doi.org/10.31285/agro.25.970

Clough, T. J., Balaine, N., Cameron, K. C., Petersen, S. O., & Sommer, S. G. (2018). Effects of dairy shed effluent dry matter content on ammonia and nitrous oxide emissions from a pasture soil. *Journal of Agricultural Science*, *156*(9), 1070-1078. https://doi.org/10.1017/S0021859618001028

Conrad, R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). *Microbiological Reviews*, *60*(4), 609-640. https://doi.org/10.1128/mr.60.4.609-640.1996

Coppola, N., Freire, B., Umpiérrez, A., Cordeiro, N. F., Ávila, P., Trenchi, G., Castro, G., Casaux, M. L., Fraga, M., Zunino, P., Bado, I., & Vignoli, R. (2020). Transferable Resistance to Highest Priority Critically Important Antibiotics for Human Health in Escherichia coli Strains Obtained From Livestock Feces in Uruguay. *Frontiers in Veterinary Science*, *7*(November). https://doi.org/10.3389/fvets.2020.588919

Correa, C., Rezzano, N., & García, F. (2016). *Manual para la gestión ambiental de tambos*. Montevideo, Julio 2016. https://www.gub.uy/ministerio-ambiente/comunicacion/publicaciones/manual-para-gestion-ambiental-tambos

Crane, S. R., Moore, J. A., Grisher, M. E., & Miner, J. R. (1983). Bacterial pollution from agricultural sources - a review. *Transactions - American Society of Agricultural Engineers*, *26*(3), 858-866. https://doi.org/10.13031/2013.34036

Dallenne, C., da Costa, A., Decré, D., Favier, C., & Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, *65*(3), 490-495. https://doi.org/10.1093/jac/dkp498

De Los Santos, R. I., Zunino, P. M., Gil, A. D., Laport, A., & Hirigoyen, D. J. (2017). Antibiotic resistance of Staphylococcus aureus associated with subclinical and clinical mastitis in Uruguay during an eight-year period. *Austral Journal of Veterinary Sciences*, *49*(3), 191-194. https://doi.org/10.4067/S0719-81322017000300191

del Pino, A., Casanova, O., Barbazán, M., Mancassola, V., Arló, L., Borzacconi, L., & Passeggi, M. (2014). Agronomic Use of Slurry from Anaerobic Digestion of Agroindustrial Residues: Effects on Crop and Soil. *Journal of Sustainable Bioenergy Systems*, *4*(1), 10. https://doi.org/10.4236/jsbs.2014.41009

Dell, C. J., Meisinger, J. J., & Beegle, D. B. (2011). Subsurface Application of Manures Slurries for Conservation Tillage and Pasture Soils and Their Impact on the Nitrogen Balance. *Journal of Environmental Quality*, *40*(2), 352-361. https://doi.org/10.2134/jeq2010.0069

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., González, L., Tablada, M., & Robledo, Y. C. (2017). *InfoStat versión 2020* (N.º 2017). Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

DIEA. (2021). *Anuario Estadístico Agropecuario*. Montevideo. MGAP. 2021. https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/comunicacion/publicaciones/anuario-estadistico-agropecuario-2021

DIEA. (2023). *Anuario Estadístico Agropecuario 2023*. Montevideo. MGAP. 2023. https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/comunicacion/publicaciones/anuario-estadistico-agropecuario-2023

Dungan, R. S., McKinney, C. W., Acosta-Martinez, V., & Leytem, A. B. (2022). Response of soil health indicators to long-term dairy manure in a semiarid irrigated cropping system. *Soil Science Society of America Journal*, *86*(6), 1597-1610. https://doi.org/10.1002/saj2.20462

Dungan, R. S., Mckinney, C. W., & Leytem, A. B. (2018). Tracking antibiotic resistance genes in soil irrigated with dairy wastewater. *Science of the Total Environment*, *635*, 1477-1483. https://doi.org/10.1016/j.scitotenv.2018.04.020

Eghball, B., & Power, J. F. (1999). Phosphorus- and Nitrogen-Based Manure and Compost Applications Corn Production and Soil Phosphorus. *Soil Science Society of America Journal*, *63*(4), 895-901. https://doi.org/10.2136/sssaj1999.634895x

Elliott, L. F., & Ellis, J. R. (1977). Bacterial and Viral Pathogens Associated with Land Application of Organic Wastes. *Journal of Environmental Quality*, *6*(3), 245-251. https://doi.org/10.2134/jeq1977.00472425000600030002x

Fariña, S. R., & Chilibroste, P. (2019). Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. *Agricultural Systems*, *176*, 102631. https://doi.org/10.1016/j.agsy.2019.05.001

Foote, K. J., Joy, M. K., & Death, R. G. (2015). New Zealand Dairy Farming: Milking Our Environment for All Its Worth. *Environmental Management*, *56*(3), 709-720. https://doi.org/10.1007/s00267-015-0517-x

Forster, P., Storelvmo, T., Armour, K., Collins, W., Dufresne, J.-L., Frame, D., Lunt, D. J., Mauritsen, T., Palmer, M. D., Watanabe, M., Wild, M., & Zhang, H. (2021). The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity. En V. Masson-Delmotte, P. Zhai, A. Pirani, V. S.L. ConMasson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, … B. Zhounors (Eds.), *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 923–1054). Cambridge University Press. https://doi.org/http://dx.doi.org/10.1017/9781009157896.009

Franz, E., Semenov, A. V., & Van Bruggen, A. H. C. (2008). Modelling the contamination of lettuce with Escherichia coli O157:H7 from manure-amended soil and the effect of intervention strategies. *Journal of Applied Microbiology*, *105*(5), 1569-1584. https://doi.org/10.1111/j.1365-2672.2008.03915.x

Gerba, C. P., & Smith, J. E. (2005). Sources of Pathogenic Microorganisms and Their Fate during Land Application of Wastes. *Journal of Environmental Quality*, *34*(1), 42-48. https://doi.org/10.2134/jeq2005.0042a

Ghezzehei, T. A., Sarkhot, D. V., & Berhe, A. A. (2014). Biochar can be used to capture essential nutrients from dairy wastewater and improve soil physico-chemical properties. *Solid Earth*, *5*(2), 953-962. https://doi.org/10.5194/se-5-953-2014

Gianneechini, R. E., Concha, C., Delucci, I., Gil, J., Salvarrey, L., & Rivero, R. (2014). Mastitis bovina, reconocimiento de los patógenos y su resistencia antimicrobiana en la Cuenca Lechera del Sur de Uruguay. *Veterinaria*, *50*(196), 4-32.

Gianneechini, R. E., Concha, C., & Franklin, A. (2002). Antimicrobial Susceptibility of Udder Pathogens Isolated from Dairy Herds in the West Littoral Region of Uruguay. *Acta*

veterinaria scandinavica, *43*(1), 31-41.

Gianneechini, R. E., Concha, C., Riveroj, R., Gil, J., Moreno-Lopez, J., Rivero, R., Gil, J., & Moreno-Lopez, J. (2005). Monitoreo de la resistencia de Staphylococcus aureus aislados en rodeos lecheros de la cuenca lechera tradicional de Uruguay. *XXXIII jornadas Uruguayas de Buiatría*, *63*, 205-207.

Gibson, C. (1995). Farm dairy effluent management. *New Zealand dairy exporter*, *72*(22-24).

Gilchrist, M. J., Greko, C., Wallinga, D. B., Beran, G. W., Riley, D. G., & Thorne, P. S. (2007). The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environmental Health Perspectives*, *115*(2), 313-316. https://doi.org/10.1289/ehp.8837

Gomes, M. S., Cavalcanti, I. F. de A., & Müller, G. V. (2021). 2019/2020 drought impacts on South America and atmospheric and oceanic influences. *Weather and Climate Extremes*, *34*, 100404. https://doi.org/10.1016/j.wace.2021.100404

Goss, M. J., Barry, D. A. J., & Rudolph, D. L. (1998). Contamination in Ontario farmstead domestic wells and its association with agriculture: 1. Results from drinking water wells. *Journal of Contaminant Hydrology*, *32*(3-4), 267-293. https://doi.org/10.1016/S0169-7722(98)00054-0

Groffman, P. M. (2012). Terrestrial denitrification: challenges and opportunities. *Ecological Processes*, *1*, 1-11. https://doi.org/10.1186/2192-1709-1-11

Gutiérrez, S., & Cabrera, N. (2012). Estimación de los parámetros nacionales y básicos para el manejo de los efluentes de tambos. Parte 1: Caracterización de la descarga. *Ingeniaría Química*, *41*, 3 - 12.

Habteselassie, M. Y., Miller, B. E., Thacker, S. G., Stark, J. M., & Norton, J. M. (2006). Soil Nitrogen and Nutrient Dynamics after Repeated Application of Treated Dairy-Waste. *Soil Science Society of America Journal*, *70*(4), 1328-1337. https://doi.org/10.2136/sssaj2005.0189

Habteselassie, M. Y., Xu, L., & Norton, J. M. (2013). Ammonia-oxidizer communities in an agricultural soil treated with contrasting nitrogen sources. *Frontiers in Microbiology*, *4*(NOV), 1-10. https://doi.org/10.3389/fmicb.2013.00326

Hagare, D., Hong, W. T., Siddiqui, Z., Natarajan, S. K., & Fyfe, J. (2022). Effect of Dairy Pond Sludge/Supernatant Application on Ryegrass Dry Matter Yield and Phosphorus Fractions in Soil. *Agriculture (Switzerland)*, *12*(3), 1-12. https://doi.org/10.3390/agriculture12030351

Hawke, R. M., & Summers, S. A. (2006). Effects of land application of farm dairy effluent on soil properties: A literature review. *New Zealand Journal of Agricultural Research*, *49*(3), 307-320. https://doi.org/10.1080/00288233.2006.9513721

He, M., Tian, G., Semenov, A. M., & van Bruggen, A. H. C. (2012). Short-term fluctuations of sugar beet damping-off by Pythium ultimum in relation to changes in bacterial communities after organic amendments to two soils. *Phytopathology*, *102*(4), 413-420. https://doi.org/10.1094/PHYTO-07-11-0189

Heatley, P. R. (1995). Dairying and the environment: managing farm dairy effluent. *Dairying and the Environment Committe of New Zeland Dairy Board*.

Heuer, H., Schmitt, H., & Smalla, K. (2011). Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology*, *14*(3), 236-243. https://doi.org/10.1016/j.mib.2011.04.009

Hoffmann, H., Schloter, M., & Wilke, B. M. (2007). Microscale-scale measurement of potential nitrification rates of soil aggregates. *Biology and Fertility of Soils*, *44*(2), 411-413. https://doi.org/10.1007/s00374-007-0227-5

Houlbrooke, D. J. (2015). *Manejo de efluentes de la lechería en Uruguay: resumen de los hallazgos y recomendaciones para futuras mejoras.* INALE. https://www.inale.org/proyectos/manejo-de-efluentes-de-la-lecheria-en-uruguay/

Houlbrooke, D. J., Horne, D. J., Hedley, M. J., Hanly, J. A., Snow, V. O., Horne, D. J., Hedley, M. J., Hanly, J. A., & Snow, V. O. (2004). *A review of literature on the land treatment of farm - dairy effluent in New Zealand and its impact on water quality*. *8233*(April). https://doi.org/10.1080/00288233.2004.9513617

Hu, R., Leytem, A. B., Moore, A. D., & Strawn, D. G. (2024). Long-term dairy manure amendment promotes legacy phosphorus buildup and mobility in calcareous soils. *Journal of Environmental Quality*, *53*(3), 365-377. https://doi.org/10.1002/jeq2.20559

IFCN. (2017). Dairy Report 2017. En *International Farm Comparison Network*.

Illarze, G., del Pino, A., Rodríguez-Blanco, A., & Irisarri, P. (2023). Application of Dairy Effluents to Pastures Affects Soil Nitrogen Dynamics and Microbial Activity. *Agronomy*, *13*(2), 470. https://doi.org/10.3390/agronomy13020470

INALE. (2020). *Cartilla sobre criterios de aplicación de efluente a terreno y su implicancia práctica en el diseño e implementación*. 2020. https://www.inale.org/informes/criterios-de-aplicacion-de-efluente-a-terreno-y-su-implicancia-practica-en-el-diseno-e-implementacion/

INALE. (2021). *Situación y perspectiva de la lechería Uruguaya*. 2021. https://www.inale.org/informes/informe-2021/

INALE. (2023). *Situación 2023 y perspectivas 2024 de la lechería uruguaya*. Instituto Nacional de la Leche. https://www.inale.org/informes/situacion-2023-y-perspectivas-2024-de-la-lecheria-uruguaya/

IPCC. (2007). *Climate change 2007: the physical science basis.* (Cambridge).

Isaac, R. A., & Kerber, J. D. (1971). Atomic absorption and flame photometry: Techniques and uses in soil, plant, and water analysis. *Instrumental Methods for Analysis of Soils and Plant Tissue*, 17-37. https://doi.org/10.2136/1971.instrumentalmethods.c2

Jamieson, R. C., Gordon, R. J., Sharples, K. E., Stratton, G. W., & Madani, A. (2002). Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Canadian Biosystems Engineering*, *44*, 1.1-1.9.

Jensen, L. S., & Sommer, S. G. (2013). Manure organic matter–characteristics and microbial transformations. En S. G. Sommer, M. L. Christensen, T. Schmidt, & L. S. Jensen (Eds.), *Animal Manure Recycling - Treatment and Management.* (pp. 67-90). John Wiley & Sons Ltd.

Jones, C. M. (2010). *Denitrification : From Genes to Ecosystems*. Swedish University of Agricultural Sciences, Uppsala.

Kandeler, E. (1996). Nitrogen mineralization. En F. Schinner, R. Ohlinger, R. Margesin, & E. Kandeler (Eds.), *Methods in soil biology* (pp. 135-143). Springer.

La Manna, A. (1995). Manejo de residuos orgánicos en Tambo. En *Boletín de Divulgación N°53* (2.ª ed.). INIA La Estanzuela.

La Manna, A., Mieres, J., Acosta, Y., & Torres, I. (2004). Utilización de Efluentes en Tambos.

Resumen de Investigación. *Resultados experimentales en lechería. La Estanzuela, INIA*, *361*, 35-44.

Laconi, A., Mughini-Gras, L., Tolosi, R., Grilli, G., Trocino, A., Carraro, L., Di Cesare, F., Cagnardi, P., & Piccirillo, A. (2021). Microbial community composition and antimicrobial resistance in agricultural soils fertilized with livestock manure from conventional farming in Northern Italy. *Science of the Total Environment*, *760*(xxxx), 143404. https://doi.org/10.1016/j.scitotenv.2020.143404

Lang, N. L., Smith, S. R., Bellett-Travers, D. M., Pike, E. B., & Rowlands, C. L. (2003). COll IN SOIL FOLLOWING THE APPLICATION OF BlOSOLlDS TO AGRICULTURAL LAND. *Water and Environment Journal*, *17*(1), 23-28.

Lau, M. M., & Ingham, S. C. (2001). Survival of faecal indicator bacteria in bovine manure incorporated into soil. *Letters in Applied Microbiology*, *33*(2), 131-136. https://doi.org/10.1046/j.1472-765X.2001.00962.x

Laubach, J., Heubeck, S., Pratt, C., Woodward, K. B., Guieysse, B., Van Der Weerden, T. J., Chung, M. L., Shilton, A. N., & Craggs, R. J. (2015). Review of greenhouse gas emissions from the storage and land application of farm dairy effluent. *New Zealand Journal of Agricultural Research*, *58*(2), 203-233. https://doi.org/10.1080/00288233.2015.1011284

Li, J., Luo, J., Shi, Y., Houlbrooke, D., Wang, L., Lindsey, S., & Li, Y. (2015). Nitrogen gaseous emissions from farm effluent application to pastures and mitigation measures to reduce the emissions: A review. *New Zealand Journal of Agricultural Research*, *58*(3), 339-353. https://doi.org/10.1080/00288233.2015.1028651

Li, J., Shi, Y., Luo, J., Zaman, M., Houlbrooke, D., Ding, W., Ledgard, S., & Ghani, A. (2014). Use of nitrogen process inhibitors for reducing gaseous nitrogen losses from land-applied farm effluents. *Biology and Fertility of Soils*, *50*(1), 133-145. https://doi.org/10.1007/s00374-013-0842-2

Liu, Y. Y., & Haynes, R. J. (2011). Influence of land application of dairy factory effluent on soil nutrient status and the size, activity, composition and catabolic capability of the soil microbial community. *Applied Soil Ecology*, *48*(2), 133-141. https://doi.org/10.1016/j.apsoil.2011.03.014

Lladó, S., & Baldrian, P. (2017). Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. *PLoS ONE*, *12*(2), e0171638. https://doi.org/10.1371/journal.pone.0171638

Lombardi, B., Orden, L., Varela, P., Garay, M., Iocoli, G. A., Montenegro, A., Sáez-Tovar, J., Bustamante, M. Á., Juliarena, M. P., & Moral, R. (2022). Is Dairy Effluent an Alternative for Maize Crop Fertigation in Semiarid Regions? An Approach to Agronomic and Environmental Effects. *Animals*, *12*(16). https://doi.org/10.3390/ani12162025

Longhurst, B., Rajendram, G., Miller, B., & Dexter, M. (2017). Nutrient Content of Liquid and Solid Effluents on Nz Dairy Cow Farms. *Science and policy: nutrient management challenges*, *30*, 1-9.

Longhurst, R. D., Roberts, A. H. C., & O'Connor, M. B. (2000). Farm dairy effluent: A review of published data on chemical and physical characteristics in New Zealand. *New Zealand Journal of Agricultural Research*, *43*(1), 7-14. https://doi.org/10.1080/00288233.2000.9513403

Lopes, A. R., Becerra-Castro, C., Vaz-Moreira, I., Silva, M. E. F., Nunes, O. C., & Manaia, C. M. (2016). Irrigation with treated wastewater: Potential impacts on microbial function

and diversity in agricultural soils. *Handbook of Environmental Chemistry*, *44*, 105-128. https://doi.org/10.1007/698_2015_346

Lu, Y., Sun, R., Zhang, C., Ding, S., Ying, M., & Shan, S. (2021). In situ analysis of antibiotic resistance genes in anaerobically digested dairy manure and its subsequent disposal facilities. *Bioresource Technology*, *333*(January). https://doi.org/10.1016/j.biortech.2021.124988

Luo, J., Saggar, S., Bhandral, R., Bolan, N., Ledgard, S., Lindsey, S., & Sun, W. (2008). Effects of irrigating dairy-grazed grassland with farm dairy effluent on nitrous oxide emissions. *Plant and Soil*, *309*(1-2), 119-130. https://doi.org/10.1007/s11104-008-9550-3

Manono, B O, Moller, H., & Morgan, R. (2016). Effects of irrigation, dairy effl uent dispersal and stocking on soil properties of the Waimate District, New Zealand. *Geoderma Regional*, *7*(1), 59-66. https://doi.org/10.1016/j.geodrs.2016.01.002

Manono, Bonface Ombasa. (2014). *Effects of irrigation, effluent dispersal and organic farming on earthworms and soil microbes in New Zealand dairy farms* (Número October) [University of Otago]. https://hdl.handle.net/10523/5097

Manshadi, F. D., Karpiscak, M., & Gerba, C. P. (2013). *Enteric bacterial contamination and survival on produce during irrigation with dairy wastewater in the fi eld*. *3*(2), 102-110. https://doi.org/10.2166/wrd.2013.161

McDowell, R. W., & Nash, D. (2012). A Review of the Cost-Effectiveness and Suitability of Mitigation Strategies to Prevent Phosphorus Loss from Dairy Farms in New Zealand and Australia. *Journal of Environment Quality*, *41*(3), 680. https://doi.org/10.2134/jeq2011.0041

McKinney, C. W., Dungan, R. S., Moore, A., & Leytem, A. B. (2018). Occurrence and abundance of antibiotic resistance genes in agricultural soil receiving dairy manure. En *FEMS Microbiology Ecology* (Vol. 94, Número 3). https://doi.org/10.1093/femsec/fiy010

Mello, R., & Bonino, C. (2010). Efecto del uso de efluentes de salas de ordeño en la producción de forraje de raigras. *Agrociencia Uruguay*, *14*(3), 129.

Mulvaney, R. L. (1996). Nitrogen—inorganic forms. En D. K. Page, AL, RH Miller (Ed.), *Methods of soil analysis. Part 3. Chemical Methods* (Número 5, pp. 1123-1184). Amer. Soc. Agron.

Murphy, J., & Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*(C), 31-36. https://doi.org/10.1016/S0003-2670(00)88444-5

MVOTMA. (2013). *Plan de acción para la protección del agua en la cuenca del Santa Lucía*. http://www.mvotma.gub.uy/ambiente/prevencion-y- control-para-el-cuidado-del-ambiente/estado-del-ambiente/plan-santa-lucia

Nartey, O. D., Liu, D., Luo, J., Lindsey, S., Di, H. J., Chen, Z., Yuan, J., He, T., & Ding, W. (2021). Optimizing the application of dairy farm effluent and manure to mitigate gas emission. *Journal of Soils and Sediments*, *21*(6), 2381-2393. https://doi.org/10.1007/s11368-021-02935-w

Neufeld, K. R., Grayston, S. J., Bittman, S., Krzic, M., Hunt, D. E., & Smukler, S. M. (2017). Long-term alternative dairy manure management approaches enhance microbial biomass and activity in perennial forage grass. *Biology and Fertility of Soils*, *53*(6), 613-626. https://doi.org/10.1007/s00374-017-1204-2

Nicholson, F. A., Groves, S. J., & Chambers, B. J. (2005). Pathogen survival during livestock

manure storage and following land application. *Bioresource Technology*, *96*(2), 135-143. https://doi.org/10.1016/j.biortech.2004.02.030

Niraula, S., Choi, Y. K., Payne, K., Muir, J. P., Kan, E., & Chang, W. S. (2021). Dairy effluent-saturated biochar alters microbial communities and enhances bermudagrass growth and soil fertility. *Agronomy*, *11*(9). https://doi.org/10.3390/agronomy11091794

Nõlvak, H., Truu, M., Kanger, K., Tampere, M., Espenberg, M., Loit, E., Raave, H., & Truu, J. (2016). Science of the Total Environment Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance and integron-integrase genes in agricultural grassland soil. *Science of the Total Environment*, *562*, 678-689. https://doi.org/10.1016/j.scitotenv.2016.04.035

Norton, J. M., & Stark, J. M. (2011). Regulation and measurement of nitrification in terrestrial systems. En M. G. Klotz (Ed.), *Methods in Enzymology* (1.ª ed., Vol. 486, pp. 343-368). Academic Press. https://doi.org/10.1016/B978-0-12-381294-0.00015-8

Oun, A., Kumar, A., Harrigan, T., Angelakis, A., & Xagoraraki, I. (2014). Effects of biosolids and manure application on microbial water quality in rural areas in the US. *Water (Switzerland)*, *6*(12), 3701-3723. https://doi.org/10.3390/w6123701

Owen, J. J., & Silver, W. L. (2015). Greenhouse gas emissions from dairy manure management: a review of field-based studies. *Global Change Biology*, *21*(2), 550-565. https://doi.org/10.1111/gcb.12687.Greenhouse

Pagliari, P. ., Wilson, M., Waldrip, H. ., & He, Z. (2020). Nitrogen and Phosphorus Characteristics of Beef and Dairy Manure. En H. Waldrip, P. Pagliari, & Z. He (Eds.), *Animal Manure: Production, Characteristics, Environmental Concerns, and Management* (John Wiley, pp. 45-62). ASA Special Publications 67.

Pandey, P., Chiu, C., Miao, M., Wang, Y., Settles, M., Del Rio, N. S., Castillo, A., Souza, A., Pereira, R., & Jeannotte, R. (2018). 16S rRNA analysis of diversity of manure microbial community in dairy farm environment. *PLoS ONE*, *13*(1), 1-18. https://doi.org/10.1371/journal.pone.0190126

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S., & Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *AMB Express*, *4*(1), 1-16. https://doi.org/10.1186/s13568-014-0051-x

Pascucci, S. (2020). *Building natural resource networks: urban agriculture and the circular economy*. *May*, 101-120. https://doi.org/10.19103/as.2019.0063.08

Patni, N. K., Toxopeus, H. R., & Jui, P. Y. (1985). Bacterial Quality of Runoff From Manured and Non-Manured Cropland. *Transactions of the American Society of Agricultural Engineers*, *28*(6), 1871-1877, 1884. https://doi.org/10.13031/2013.32534

Petersen, S. O. (2018). Greenhouse gas emissions from liquid dairy manure: Prediction and mitigation. *Journal of Dairy Science*, *101*(7), 6642-6654. https://doi.org/10.3168/jds.2017-13301

Peyton, D. P., Healy, M. G., Fleming, G. T. A., Grant, J., Wall, D., Morrison, L., Cormican, M., & Fenton, O. (2016). Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil. *Science of the Total Environment*, *541*, 218-229. https://doi.org/10.1016/j.scitotenv.2015.09.053

Philippot, L., Chenu, C., Kappler, A., Rillig, M. C., & Fierer, N. (2023). The interplay between microbial communities and soil properties. *Nature Reviews Microbiology*. https://doi.org/10.1038/s41579-023-00980-5

Ravishankara, A. R., Daniel, J. S., & Portmann, R. W. (2009). Nitrous oxide (N2O): the

dominant ozone-depleting substance emitted in the 21st century. *Science*, *326*(October), 123-125. https://doi.org/10.1126/science.1176985

Rhine, E. D., Mulvaney, R. L., Pratt, E. J., & Sims, G. K. (1998). Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. *Soil Science Society of America Journal*, *62*(2), 473. https://doi.org/10.2136/sssaj1998.03615995006200020026x

Roach, C. G., Longhurst, R. D., & Ledgard, S. F. (2001). Land application of farm dairy effluent for sustainable dairy farming. *Proceedings of the New Zealand Grassland Association*, 53-57. https://doi.org/10.33584/jnzg.2001.63.2430

Rogers, S. W., Donnelly, M., Peed, L., Kelty, C. A., Mondal, S., Zhong, Z., & Shanks, O. C. (2011). Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure-amended soils. *Applied and Environmental Microbiology*, *77*(14), 4839-4848. https://doi.org/10.1128/AEM.02427-10

Saggar, S., Bolan, N. S., Bhandral, R., Hedley, C. B., & Luo, J. (2004a). A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, *47*(4), 513-544. https://doi.org/10.1080/00288233.2004.9513618

Saggar, S., Bolan, N. S., Bhandral, R., Hedley, C. B., & Luo, J. (2004b). A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, *47*(4), 513-544. https://doi.org/10.1080/00288233.2004.9513618

Saggar, S., Jha, N., Deslippe, J., Bolan, N. S., Luo, J., Giltrap, D. L., Kim, D., Zaman, M., & Tillman, R. W. (2013). Denitrification and N2O:N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of the Total Environment, The*, *2013*(March). https://doi.org/10.1016/j.scitotenv.2012.11.050

Sahlström, L. (2003). A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology*, *87*(2), 161-166. https://doi.org/10.1016/S0960-8524(02)00168-2

Salazar, F., Herrero, M. A., Charlón, V., & La Manna, A. (2010). Slurry management in dairy grazing farms in south american countries. *Proceedings of the 14th International Conference of the FAO ESCORENA Network on the Recycling of Agricultural, Municipal and Industrial Residues in Agriculture*, 1-4. http://www.ramiran.net/ramiran2010/docs/Ramiran2010_0211_final.pdf

Saunders, O. E., Fortuna, A.-M., Harrison, J. H., Whitefield, E., Cogger, C. G., Kennedy, A. C., & Bary, A. I. (2012). Comparison of Raw Dairy Manure Slurry and Anaerobically Digested Slurry as N Sources for Grass Forage Production. *International Journal of Agronomy*, *2012*(C), 1-10. https://doi.org/10.1155/2012/101074

Sayre, J. M., Wang, D., Lin, J. Y., Danielson, R. E., Scow, K. M., & Mazza Rodrigues, J. L. (2023). Repeated manure inputs to a forage production soil increase microbial biomass and diversity and select for lower abundance genera. *Agriculture, Ecosystems and Environment*, *354*(May), 108567. https://doi.org/10.1016/j.agee.2023.108567

Schröder, J. (2005). Revisiting the agronomic benefits of manure: A correct assessment and exploitation of its fertilizer value spares the environment. *Bioresource Technology*, *96*(2), 253-261. https://doi.org/10.1016/j.biortech.2004.05.015

Semenov, M. V., Krasnov, G. S., Semenov, V. M., Ksenofontova, N., Zinyakova, N. B., & van Bruggen, A. H. C. (2021). Does fresh farmyard manure introduce surviving microbes into soil or activate soil-borne microbiota? *Journal of Environmental Management*,

294(May), 113018. https://doi.org/10.1016/j.jenvman.2021.113018

Severin, M., Fuß, R., Well, R., Garlipp, F., & Van den Weghe, H. (2015). Soil, slurry and application effects on greenhouse gas emissions. *Plant, Soil and Environment*, *61*(8), 344-351. https://doi.org/10.17221/21/2015-PSE

Shi, Y., Ziadi, N., Hamel, C., Bittman, S., Hunt, D., & Lalande, R. (2017). Soil microbial biomass, activity, and community composition as a ff ected by dairy manure slurry applications in grassland production. *Applied Soil Ecology*, *125*, 97-107. https://doi.org/10.1016/j.apsoil.2017.12.022

Smith, K. a., Ball, T., Conen, F., Dobbie, K. E., Massheder, J., & Rey, a. (2003). Exchange of greenhousegases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science*, *54*(December), 779-791. https://doi.org/10.1046/j.1365-2389.2003.00567.x

Sosa, N., Orcellet, J. M., & Gambaudo, S. (2016). *Uso agronómico de residuos orgánicos de origen animal*. https://produccion-animal.com.ar/produccion_y_manejo_pasturas/pasturas_fertilizacion/65-Uso-Agronomico-Residuos.pdf

Stevens, R. J., Laughlin, R. J., Burns, L. C., Arah, J. R. M., & Hood, R. C. (1997). Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry*, *29*(2), 139-151. https://doi.org/10.1016/S0038-0717(96)00303-3

Stevens, R. James, & Laughlin, R. J. (2001). Cattle Slurry Affects Nitrous Oxide and Dinitrogen Emissions from Fertilizer Nitrate. *Soil Science Society of America Journal*, *65*(4), 1307-1314. https://doi.org/10.2136/sssaj2001.6541307x

Sukias, J. P. S., Tanner, C. C., Davies-Colley, R. J., Nagels, J. W., & Wolters, R. (2001). Algal abundance, organic matter, and physico-chemical characteristics of dairy farm facultative ponds: Implications for treatment performance. *New Zealand Journal of Agricultural Research*, *44*(4), 279-296. https://doi.org/10.1080/00288233.2001.9513485

Suleiman, A. K. A., Gonzatto, R., Aita, C., Lupatini, M., Jacques, R. J. S., Kuramae, E. E., Antoniolli, Z. I., & Roesch, L. F. W. (2016). Temporal variability of soil microbial communities after application of dicyandiamide-treated swine slurry and mineral fertilizers. *Soil Biology and Biochemistry*, *97*, 71-82. https://doi.org/10.1016/j.soilbio.2016.03.002

Sun, L., Pope, P. B., Eijsink, V. G. H., & Schnürer, A. (2015). Characterization of microbial community structure during continuous anaerobic digestion of straw and cow manure. *Microbial Biotechnology*, *8*(5), 815-827. https://doi.org/10.1111/1751-7915.12298

Tarlera, S., Capurro, M. C., Irisarri, P., Scavino, A. F., Cantou, G., & Roel, A. (2016). Yield-scaled global warming potential of two irrigation management systems in a highly productive rice system. *Scientia Agricola*, *73*(1), 43-50. https://doi.org/10.1590/0103-9016-2015-0050

Taverna, M., Charlón, V., García, K., & Walter., E. (2007). Manejo de Efluentes de Tambos «INTA Rafaela». *Revista IDIA XXI*, *9*, 86-92.

Tayyab, U., & McLean, F. A. (2015). Phosphorus losses and on-farm mitigation options for dairy farming systems: A review. *Journal of Animal and Plant Sciences*, *25*(2), 318-327.

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P., Jackson, R. B., Janssens-Maenhout, G., Prather, M. J., Regnier, P., Pan, N., Pan, S., Peters, G. P., Shi, H., Tubiello, F. N., Zaehle, S., Zhou, F.,

… Yao, Y. (2020). A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, *586*(7828), 248-256. https://doi.org/10.1038/s41586-020-2780-0

Tiedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Biology of anaerobic microorganisms*, *717*, 179-244.

Tillman, R. W., & Surapaneni, A. (2002). Some soil-related issues in the disposal of effluent on land. *Aus- tralian Journal of Experimental Agriculture*, *42*(3), 225-235. https://doi.org/https://doi.org/10.1071/EA00133

Umpiérrez, A., Bado, I., Oliver, M., Acquistapace, S., Etcheverría, A., Padola, N. L., Vignoli, R., & Zunino, P. (2017). Zoonotic potential and antibiotic resistance of Escherichia coli in neonatal calves in Uruguay. *Microbes and Environments*, *32*(3), 275-282. https://doi.org/10.1264/jsme2.ME17046

Unc, A., & Goss, M. J. (2004). Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, *25*(1), 1-8. https://doi.org/10.1016/j.apsoil.2003.08.007

Urra, J., Alkorta, I., & Garbisu, C. (2019). Potential benefits and risks for soil health derived from the use of organic amendments in agriculture. *Agronomy*, *9*(9), 1-23. https://doi.org/10.3390/agronomy9090542

USEPA. (2015). *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2013*. https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2013

van Bruggen, A. H. C., Goss, E. M., Havelaar, A., van Diepeningen, A. D., Finckh, M. R., & Morris, J. G. (2019). One Health - Cycling of diverse microbial communities as a connecting force for soil, plant, animal, human and ecosystem health. *Science of the Total Environment*, *664*, 927-937. https://doi.org/10.1016/j.scitotenv.2019.02.091

van der Heijden, M. G. A., & Wagg, C. (2013). Soil microbial diversity and agro-ecosystem functioning. *Plant and Soil*, *363*(1-2), 1-5. https://doi.org/10.1007/s11104-012-1545-4

Van Der Weerden, T. J., Clough, T. J., & Styles, T. M. (2013). Using near-continuous measurements of N2O emission from urine-affected soil to guide manual gas sampling regimes. *New Zealand Journal of Agricultural Research*, *56*(1), 60-76. https://doi.org/10.1080/00288233.2012.747548

Van der Weerden, T. J., Cox, N., Luo, J., Di, H. J., Podolyan, A., Phillips, R. L., Saggar, S., de Klein, C. A. M., Ettema, P., & Rys, G. (2016). Refining the New Zealand nitrous oxide emission factor for urea fertiliser and farm dairy effluent. *Agriculture, Ecosystems and Environment*, *222*(May), 133-137. https://doi.org/10.1016/j.agee.2016.02.007

Van Doorslaer, B., Witzke, H.-P., Huck, I., Weiss, F., Fellmann, T., Salputra, G., Jansson, T., Drabik, D., & Leip, A. (2015). An economic assessment of GHG mitigation policy options for EU agriculture. En *JRC Science for Policy Report* (Vol. EUR27973). https://doi.org/10.2791/180800

van Groenigen, J. W., Huygens, D., Boeckx, P., Kuyper, T. W., Lubbers, I. M., Rütting, T., & Groffman, P. M. (2015). The soil N cycle: new insights and key challenges. *Soil*, *1*(1), 235-256. https://doi.org/10.5194/soil-1-235-2015

Viguria, M., Sanz-Cobeña, A., López, D. M., Arriaga, H., & Merino, P. (2015). Ammonia and greenhouse gases emission from impermeable covered storage and land application of cattle slurry to bare soil. *Agriculture, Ecosystems and Environment*, *199*, 261-271. https://doi.org/10.1016/j.agee.2014.09.016

Wallenstein, M. D., Myrold, D. D., Firestone, M., & Voytek, M. (2006). Environmental Controls on Denitrifying Communities and Denitrification Rates : Insights from

Molecular Methods. *Ecological Applications*, *16*(6), 2143-2152.

Wulf, S., Maeting, M., & Clemens, J. (2002). Application Technique and Slurry Co-Fermentation Effects on Ammonia, Nitrous Oxide, and Methane Emissions after Spreading. *Journal of Environmental Quality*, *31*(6), 1795-1801. https://doi.org/10.2134/jeq2002.1795

Zaman, M., Cameron, K. C., Di, H. J., & Inubushi, K. (2002). Changes in mineral N, microbial biomass and enzyme activities in different soil depths after surface applications of dairy shed effluent and chemical fertilizer. *Nutrient Cycling in Agroecosystems*, *63*, 275-290.

Zelenev, V. V., van Bruggen, A. H. C., Leffelaar, P. A., Bloem, J., & Semenov, A. M. (2006). Oscillating dynamics of bacterial populations and their predators in response to fresh organic matter added to soil: The simulation model «BACWAVE-WEB». *Soil Biology and Biochemistry*, *38*(7), 1690-1711. https://doi.org/10.1016/j.soilbio.2005.11.024

Zhang, J., Zhuang, M., Shan, N., Zhao, Q., Li, H., & Wang, L. (2019). Substituting organic manure for compound fertilizer increases yield and decreases NH3 and N2O emissions in an intensive vegetable production systems. *Science of the Total Environment*, *670*, 1184-1189. https://doi.org/10.1016/j.scitotenv.2019.03.191

Zhang, N., Bai, Z., Luo, J., Ledgard, S., Wu, Z., & Ma, L. (2017). Nutrient losses and greenhouse gas emissions from dairy production in China: Lessons learned from historical changes and regional differences. *Science of the Total Environment*, *598*, 1095-1105. https://doi.org/10.1016/j.scitotenv.2017.04.165

Zhang, T., Liu, H., Luo, J., Wang, H., Zhai, L., Geng, Y., Zhang, Y., Li, J., Lei, Q., Bashir, M. A., Wu, S., & Lindsey, S. (2018). Long-term manure application increased greenhouse gas emissions but had no effect on ammonia volatilization in a Northern China upland field. *Science of the Total Environment*, *633*, 230-239. https://doi.org/10.1016/j.scitotenv.2018.03.069

Zhang, Y., Hao, X., Alexander, T. ., Thomas, B. ., Shi, X., & Lupwayi, N. . (2018). Long-term and legacy effects of manure application on soil microbial community composition. *Biology and Fertility of Soils*, *54*, 269-283. https://doi.org/https://doi.org/10.1007/s00374-017-1257-2

Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and molecular biology reviews*, *61*(4), 533-616. http://mmbr.asm.org/content/61/4/533.short%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232623&tool=pmcentrez&rendertype=abstract

8. Discusión general

8.1. Aportes del presente trabajo

La intensificación de la industria lechera en los últimos treinta años ha resultado en el aumento de efluentes de tambo generados en las áreas de ordeñe. En este contexto, surge la alternativa del reciclado de estos efluentes para el riego y fertilización de pasturas y cultivos. Sin embargo, la gestión de estos implica un desafío desde el punto de vista ambiental, ya sea por el riesgo sanitario asociado a la diseminación de patógenos y ARG, ya sea por la pérdida y acumulación de nutrientes y su contribución a la emisión de GEI.

Dada la variabilidad en la composición de los efluentes de tambo, surge la necesidad de profundizar el conocimiento de sus características para mejorar el uso de los nutrientes y hacer la práctica de la aplicación de los efluentes al suelo más sustentable desde el punto de vista ambiental. En este sentido, el presente trabajo buscó contribuir al conocimiento local sobre el uso de efluentes de tambo para riego y fertilización en pasturas y sus externalidades ambientales y permitir avanzar en la valoración de estos no como un desecho, sino como un elemento útil para proveer de nutrientes a las pasturas en las condiciones climáticas y con los tipos de suelos del Uruguay.

La disposición final de los efluentes de tambo al suelo, ya sea crudos o tratados en sistemas de lagunas, pone de manifiesto la importancia del estudio de las comunidades microbianas, responsables de los procesos de transformación de los nutrientes. Por otro lado, dado el riesgo de la introducción de microrganismos al suelo, especialmente de los efluentes crudos, conocer las comunidades microbianas de estos efluentes y sus cambios estacionales resulta un gran aporte de este trabajo. Estos conocimientos pueden contribuir al diseño de prácticas adecuadas de manejo para su disposición final. Los resultados obtenidos de la caracterización dos años de evaluación estacional demuestran la eficiencia del tratamiento de los efluentes de tambo en un sistema de doble laguna (anaeróbica y facultativa) para la reducción de la carga orgánica y los

indicadores de patogenicidad y la alteración de la comunidad bacteriana (capítulo 3, 4, 5). Sin embargo, estos cambios tuvieron un marcado efecto estacional. En el invierno, los indicadores de eficiencia no son logrados, lo que lleva a la necesidad de profundizar el conocimiento de los procesos microbianos en estas condiciones ambientales. Al respecto, este estudio caracterizó las comunidades microbianas de los efluentes de tambo y sus cambios estacionales (capítulo 5). Estos resultados pueden contribuir a la búsqueda e identificación de los microorganismos clave implicados en la biodegradación y estabilización de los efluentes de tambo y la reducción de su impacto ambiental.

Aplicar efluentes en forma directa, luego del ordeñe, o poco estabilizados ha mostrado ser una alternativa por el bajo costo de inversión y por el mayor aporte de nutrientes al suelo (Houlbrooke, 2015), pero tiene un mayor riesgo sanitario asociado a la diseminación de patógenos (Nicholson et al., 2005). Generalmente, se recomienda cierto tiempo de espera luego de la aplicación de efluentes para el pastoreo (Correa et al., 2016). Sin embargo, las mediciones de supervivencia de patógenos en los sistemas de producción lechera en Uruguay son escasas y, dada la influencia esperada del clima y el suelo, es importante proporcionar evaluaciones locales. A través de la evaluación del número de bacterias indicadoras de patogenicidad en los efluentes de tambo y la determinación de la sobrevivencia en el suelo, este estudio contribuyó a la estimación del riesgo sanitario asociado a la aplicación de efluentes de tambo (capítulo 4, 5). Los efluentes crudos superaron hasta en dos órdenes el número de indicadores de patogenicidad respecto a cuando fueron estabilizados en la laguna. Estos indicadores aumentaron en el suelo enseguida de ser aplicados los efluentes crudos y persistieron en bajo nivel, lo que indica que se necesitan al menos 45 días para la eliminación total de las bacterias entéricas provenientes de los efluentes crudos. Por otro lado, la caracterización de la comunidad bacteriana de los efluentes permitió desentrañar que los efluentes crudos contaron con una alta proporción de géneros entéricos potencialmente patógenos y que estos desaparecen luego de que los efluentes son estabilizados en sistema de lagunas de tratamiento.

La resistencia a los antimicrobianos es una preocupación creciente para la salud humana y animal y el medioambiente. Aunque en nuestro país está prohibido el uso de antibióticos como promotores del crecimiento en el ganado y se utilizan principalmente en los establecimientos lecheros para tratar casos de mastitis (Gianneechini et al., 2005), se ha reportado un aumento en el número de bacterias multirresistentes que producen beta-lactamasas (Coppola et al., 2020; Gianneechini et al., 2002, 2005, 2014). La inducción de resistencia antimicrobiana por beta-lactámicos es un problema creciente y el mecanismo más común de resistencia (Dallenne et al., 2010). Varios estudios han demostrado que los efluentes de tambo son una fuente de ARG y pueden desempeñar un papel clave en su diseminación al suelo (Dungan et al., 2018; Lu et al., 2021; McKinney et al., 2018; Nõlvak et al., 2016). Este primer estudio exploratorio sobre la posibilidad de diseminación de ARG por la aplicación de efluentes de tambo indicó que, si bien los efluentes de tambo portaron genes de resistencia a beta-lactámicos, estos no persistieron en el suelo (capítulo 4).

Además, de las posibles implicancias sanitarias del uso de efluentes de tambo es relevante considerar que estos inputs orgánicos al suelo afectan la dinámica de los nutrientes y, por consiguiente, pueden cambiar la actividad y la diversidad funcional y estructural de la comunidad microbiana, que tienen un rol clave en la salud y productividad de los suelos. La bibliografía al respecto no es clara, en gran parte, debido a la alta variabilidad en la composición fisicoquímica de los efluentes de tambo, el tipo del suelo al que se aplican, la frecuencia y años de aplicación, entre otros. Por otro lado, se ha visto que las comunidades microbianas responden rápidamente a los cambios inducidos por las enmiendas orgánicas enseguida que son aplicadas al suelo, pero, pasado un corto tiempo, vuelven a su estado basal (Sayre et al., 2023; Suleiman et al., 2016) y además existe redundancia funcional (Lopes et al., 2016), lo que dificulta determinar en qué medida las poblaciones microbianas son impactadas. A este respecto, este estudio mostró que, luego de cuatro aplicaciones de efluentes de tambo a una pastura, no se vio afectada la abundancia y diversidad de la comunidad de hongos y bacterias del suelo, pero sí su actividad y la diversidad funcional, lo

que pone de manifiesto el rol de los microorganismos en la activa degradación de los compuestos orgánicos de los efluentes (capítulo 3, 6). Por lo tanto, la entrada de materia orgánica debido al riego con efluentes de tambo puede ser beneficiosa para el suelo al estimular el catabolismo no solo de compuestos lábiles, sino también de sustratos complejos. De todos modos, ambos tipos de efluentes mostraron distinto comportamiento en el suelo. La aplicación de efluentes crudos aportó mayor CO al suelo y esto se vio reflejado en una mayor actividad microbiana global y de mineralización de N y un cambio funcional de la capacidad catabólica microbiana hacia sustratos carbonados específicos, componentes típicos de las excretas vacunas. Por otro lado, la aplicación de los efluentes de la laguna produjo un aumento en el contenido mineral de macronutrientes del suelo y promovió la actividad potencial de nitrificación, así como también otros indicadores de actividad microbiana del suelo, aunque en menor medida respecto a la aplicación de efluentes crudos. Estos resultados confirman que un factor importante de la actividad microbiana del suelo es la disponibilidad de C y los diferentes nutrientes liberados del tipo de enmienda orgánica, en este caso, los efluentes de tambo.

Los desafíos de la reutilización de los efluentes de tambo como proveedor de N a una pastura se deben a su composición variable y a la forma en que el N esté disponible, lo que dificulta la predicción de su valor fertilizante. En general, entre el 60 y el 85 % del total del N de los efluentes de tambo está en forma orgánica y, por tanto, no está disponible inmediatamente para ser absorbido por las plantas. Por lo tanto, cuando se considera su uso como una alternativa a la fertilización con urea, los procesos microbianos de mineralización, nitrificación y desnitrificación en el suelo son claves para entender la dinámica del N, determinar su disponibilidad para el cultivo y estimar las pérdidas por la emisión de N_2O.

Este estudió abordó el ciclo del N en particular, para conocer el ciclo del N, producto del manejo y la aplicación de efluentes al suelo, y aporta al entendimiento de los patrones de emisión de N_2O de suelos con aplicación de efluentes, que resulta de interés para el desarrollo de estrategias de mitigación,

la reducción de la pérdida de nutrientes y la mejora de la eficiencia de uso del N por las plantas (capítulo 3, 7). Como se mencionó en el párrafo anterior, los efluentes crudos con mayor contenido de C y N orgánico promovieron la actividad microbiana del suelo y aumentaron el contenido de NO_3^- en el suelo; estos factores explicaron los mayores flujos de emisión de N_2O por este tipo de efluente en condiciones controladas (capítulo 3). Ambos efluentes produjeron mayores flujos de N_2O y aumentaron los procesos microbianos del ciclo del N en el suelo en relación con la fertilización sintética. Estos flujos aparecieron como pulsos enseguida que fueron aplicados los efluentes, explicados por el aumento en la humedad del suelo y la disponibilidad de C y N para la microbiota (Laubach et al., 2015), lo que sugiere que este es un momento clave en la búsqueda de estrategias de mitigación. Los resultados del experimento a campo también indicaron que hay un claro efecto de la aplicación de los efluentes de tambo sobre el aumento del ciclo del N que, sumado a los volúmenes aplicados al suelo, podrían explicar los mayores flujos de N_2O relativo a la aplicación de urea (capítulo 7). Sin embargo, en las condiciones climáticas de un año con una inusual sequía (Gomes et al., 2021) no se pudieron detectar estas diferencias entre tipos de fertilización nitrogenada. Aplicar efluentes en estas condiciones al suelo es una práctica apropiada para minimizar las pérdidas de N.

Aun cuando la aplicación de efluentes crudos puede contribuir a una mayor emisión de GEI, se debe considerar que esta podría ser significativamente menor a la producida durante la estabilización en las lagunas (Laubach et al., 2015; Owen y Silver, 2015). También se debe tener en cuenta que durante la síntesis de la urea se requiere energía fósil y, consecuentemente, se generan emisiones de GEI (Lombardi et al., 2022).

Los resultados de la evaluación en condiciones de invernáculo y a campo sobre la productividad, la absorción de N y el contenido de macronutrientes de festuca indicaron que el riego con efluentes aparece como una alternativa prometedora en la búsqueda de sustituir total o parcialmente la fertilización con urea (capítulo 3, 4, 7). En general, hubo una respuesta positiva a la fertilización con efluentes de tambo y ambos tipos de efluentes tuvieron un efecto similar

sobre el crecimiento y contenido de nutrientes de la pastura, pero los resultados fueron variables según el tipo de suelo y el momento de la aplicación. En condiciones de invernáculo, la respuesta se vio enmascarada por la alta fertilidad base del suelo (capítulo 3, 4). Sin embargo, en condiciones de campo, la pastura respondió a la adicción de N por los efluentes y, más pronunciadamente, a la fertilización con urea (capítulo 7). Los niveles de P, K y Na del suelo aumentaron en superficie por la aplicación de efluentes de la laguna e incluso se encontró esta tendencia en el suelo de alta fertilidad (capítulo 4). Pero la absorción de estos macronutrientes por la pastura no aumentó, lo que significa que se están acumulando en el suelo. Las lagunas de estabilización fueron diseñadas para reducir el porcentaje de C y N, lo que explica la disminución en la relación N:P:K de estos efluentes en relación con los efluentes crudos. Por consiguiente, basar la dosis de aplicación en el contenido de N genera un desbalance de estos otros macronutrientes en el suelo, que pueden acumularse y perderse. Estos desbalances de nutrientes en el suelo han sido reportados en Uruguay (Alegrette, 2020) y son la principal fuente de pérdida difusa de P en conjunto con la aplicación de fertilización sintética (Tayyab y McLean, 2015), con consecuencias de excesos de P hacia cursos de agua y pérdida de su calidad que ya han sido reportados en Uruguay (Arocena et al., 2018; Barreto et al., 2017). Los resultados de este trabajo confirman la necesidad de establecer planes de monitoreo del suelo cuando se considera la aplicación repetida de los efluentes de tambo a un mismo sitio.

8.2. Conclusiones y perspectivas

Este estudio consideró el uso productivo de efluentes de tambo en un ciclo de crecimiento de festuca que abarcó cuatro cortes estacionales. La aplicación repetida de efluentes de tambo, ya sea crudos o estabilizados en lagunas, afectó la fertilidad y la actividad microbiana del suelo, mientras que la estructura y la abundancia de la comunidad microbiana fueron más resilientes al cambio. Los efluentes crudos con mayor proporción de C y N orgánico tuvieron su principal efecto al mejorar la actividad microbiana y proporcionar más CO al suelo. Por su parte, los efluentes de la laguna tuvieron el efecto más marcado sobre la fertilidad del suelo. Estos hallazgos sugieren que los diferentes manejos de los efluentes de lechería, cuando se reciclan como fertilizantes, pueden afectar el ecosistema del suelo de manera diferencial y se deben considerar ensayos a largo plazo para desentrañar su efecto sobre la diversidad y la función microbiana de este.

La aplicación de efluentes de tambo crudos y tratados en lagunas no produjo diferencias significativas en el crecimiento o en la composición química de la pastura que pudieran ser motivo de preocupación y podría sustituir parcialmente la fertilización sintética. Estos resultados son prometedores para reciclar los efluentes de tambo a una pastura y, así, contribuir a mejorar la rentabilidad del sector lechero, aunque se deben considerar los compromisos con otros indicadores de sostenibilidad, como la emisión de GEI y la acumulación de nutrientes y patógenos en el suelo. En este sentido, los resultados de este trabajo indicaron que no es recomendable aplicar efluentes de tambo en invierno, dado un mayor riesgo de sobrevivencia de indicadores de patogenicidad en los efluentes. Aplicar efluentes crudos tiene mayor riesgo de diseminación de microorganismos entéricos y se debe considerar un tiempo de espera para el pastoreo en virtud de lograr la inactivación de la microbiota entérica potencialmente patógena en el suelo.

Estos efluentes también contribuyeron a una mayor emisión de GEI; aun así, su uso como fertilizante contrabalancea los efectos negativos de su

almacenamiento en las áreas de ordeñe, sumado a que promueve una mayor actividad microbiana y CO en el suelo.

Pero esta investigación fue desarrollada en un corto plazo y se justifican estudios a más largo plazo para determinar los efectos de la aplicación de los diferentes efluentes sobre las propiedades del suelo, su valor fertilizante y las externalidades ambientales, contrastando distintos tipos de suelo y la influencia de las condiciones climáticas en años sucesivos de estudio.

Finalmente, se requiere un mejor entendimiento de las comunidades microbianas de los efluentes de tambo, su rol en la estabilización y en la diseminación de bacterias y genes de resistencia a otros antibióticos de uso frecuente en el tratamiento de mastitis, para minimizar el impacto ambiental del uso de efluentes de tambo sobre el ecosistema.

9. Bibliografía

Albanito, F., Lebender, U., Cornulier, T., Sapkota, T. B., Brentrup, F., Stirling, C. y Hillier, J. (2017). Direct nitrous oxide emissions from tropical and sub-tropical agricultural systems - A review and modelling of emission factors. *Scientific Reports*, *7*(September 2016), 1-12. https://doi.org/10.1038/srep44235

Alegrette, M. J. (2020). *Determinación de las propiedades químicas y físicas del suelo, ante la aplicación de efluentes de tambo con diferentes sistemas de gestión de efluentes*. Facultad de Agronomía, Udelar.

Alegria Terrazas, R., Giles, C., Paterson, E., Robertson-Albertyn, S., Cesco, S., Mimmo, T., Pii, Y. y Bulgarelli, D. (2016). Plant-microbiota interactions as a driver of the mineral Turnover in the Rhizosphere. En *Advances in Applied Microbiology* (vol. 95). Elsevier Ltd. https://doi.org/10.1016/bs.aambs.2016.03.001

Amon, B., Kryvoruchko, V., Amon, T. y Zechmeister-Boltenstern, S. (2006). Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agriculture, Ecosystems & Environment*, *112*(2-3), 153-162. https://doi.org/10.1016/J.AGEE.2005.08.030

Angers, D. A., Chantigny, M. H., MacDonald, J. D., Rochette, P. y Côté, D. (2010). Differential retention of carbon, nitrogen and phosphorus in grassland soil profiles with long-term manure application. *Nutrient Cycling in Agroecosystems*, *86*(2), 225-229. https://doi.org/10.1007/s10705-009-9286-3

Arlo, L., Beretta, A., Szogi, A. A. y Del Pino, A. (2022). Biomass production, metal and nutrient content in sorghum plants grown on soils amended with sewage sludge. *Heliyon*, *8*(1), e08658. https://doi.org/10.1016/j.heliyon.2021.e08658

Arocena, R., Chalar, G., Perdomo, C., Fabián, D., Pacheco, J., González, M., Olivero, V., Silva, M., García, P. y Etchevarne, V. (2013). Impacto de la

producción lechera en la calidad de los cuerpos de agua. *Acta VII Congreso de Medio Ambiente AUGM*, 1-38. sedici.unlp.edu.ar

Arocena, Rafael, Chalar, G. y Pacheco, J. P. (2018). Agriculture and elevation are the main factors for Pampasic stream habitat and water quality. *Environmental Monitoring and Assessment*, *190*(4). https://doi.org/10.1007/s10661-018-6622-6

Avery, L. M., Hill, P., Killham, K. y Jones, D. L. (2004). Escherichia coli O157 survival following the surface and sub-surface application of human pathogen contaminated organic waste to soil. *Soil Biology and Biochemistry*, *36*(12), 2101-2103. https://doi.org/10.1016/j.soilbio.2004.05.018

Baral, K. R., Labouriau, R., Olesen, J. E. y Petersen, S. O. (2017). Agriculture, Ecosystems and Environment Nitrous oxide emissions and nitrogen use ef fi ciency of manure and digestates applied to spring barley. *Agriculture, Ecosystems and Environment*, *239*, 188-198. https://doi.org/10.1016/j.agee.2017.01.012

Barnett, J. W., Kerridge, G. J. y Russell, J. M. (1994). Effluent treatment systems for dairy industry. *Australian Biotechnology*, *4*(1), 26-30.

Barreto Wagner, 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*(0). https://doi.org/10.1177/1178622117715446

Barton, L. y Schipper, L. A. (2001). Regulation of Nitrous Oxide Emissions from Soils Irrigated with Dairy Farm Effluent. *Journal of Environmental Quality*, *30*(6), 1881-1887. https://doi.org/10.2134/jeq2001.1881

Bhandral, R., Bolan, N. S. y Saggar, S. (2010). Nitrous oxide emission from farm dairy effluent application in grazed grassland. *Revista de la Ciencia del Suelo y Nutrición Vegetal*, *10*(1), 22-34. https://doi.org/10.4067/S0718-27912010000100003

Bhandral, R., Bolan, N. S., Saggar, S. y Hedley, M. J. (2007). Nitrogen transformation and nitrous oxide emissions from various types of farm

effluents. *Nutrient Cycling in Agroecosystems*, *79*, 193-208. https://doi.org/https://doi.org/10.1007/s10705-007-9107-5

Bhandral, Rita, Bolan, N. S., Saggar, S. y Hedley, M. J. (2007). Nitrogen transformation and nitrous oxide emissions from various types of farm effluents. *Nutrient Cycling in Agroecosystems*, *79*(2), 193-208. https://doi.org/10.1007/s10705-007-9107-5

Bhandral, Rita, Saggar, S., Bolan, N. S. y Hedley, M. (2004). Nitrous oxide emissions from farm effluents. *3rd Australian New Zealand Soils Conference, 5-9 December 2004, University of Sydney, Australia*, *December*.

Bhogal, A., Nicholson, F. A., Rollett, A., Taylor, M., Litterick, A., Whittingham, M. J. y Williams, J. R. (2018). Improvements in the Quality of Agricultural Soils Following Organic Material Additions Depend on Both the Quantity and Quality of the Materials Applied. *Frontiers in Sustainable Food Systems*, *2*(9), 1-13. https://doi.org/10.3389/fsufs.2018.00009

Bianco, A. (2016). *Efluentes en la producción lechera*. VIII Encuentro de la SUCS. chrome-extension://efaidnbmnnnibpcajpcglclefindmkaj/http://www.sucs.org.uy/pdf/Encuentro2016/Bianco.pdf

Black, Z., Balta, I., Black, L., Naughton, P. J., Dooley, J. S. G. y Corcionivoschi, N. (2021). The Fate of Foodborne Pathogens in Manure Treated Soil. *Frontiers in Microbiology*, *12*(December). https://doi.org/10.3389/fmicb.2021.781357

Bolan, N. S., Horne, D. J. y Currie, L. D. (2004). Growth and chemical composition of legume-based pasture irrigated with dairy farm effluent. *New Zealand Journal of Agricultural Research*, *47*(1), 85-93. https://doi.org/10.1080/00288233.2004.9513574

Bolan, N. S., Laurenson, S., Lowe, H., Luo, J., Hill, P. H. y Sukias, J. (2009). Treatment of farm effluents in New Zealand's dairy operations. *New Zealand Land Treatment Collective: Proceedings for the 2009 Annual*

Conference, 120-134.

Bolan, N., Saggar, S., Luo, J., Bhandral, R. y Singh, J. (2004). Gaseous emissions of nitrogen from grazed pastures: processes, measurements and modelling, environmental implications, and mitigation. *Advances in agronomy*, *78*(37), 120. https://books.google.com.uy/books?hl=es&lr=&id=vnVywNimRQ4C&oi=fnd&pg=PA37&dq=Bolan+NS,+Saggar+S,+Luo+J,+Bhandral+R,+Singh+J+(2004)+Gaseous+emis-+sions+of+nitrogen+from+grazed+pastures:+processes,+measurements+and+modelling,+environmental+implications,+an

Braker, G. y Conrad, R. (2011). Diversity, structure, and size of N2O-producing microbial communities in soils-what matters for their functioning? En *Advances in Applied Microbiology* (vol. 75). https://doi.org/10.1016/B978-0-12-387046-9.00002-5

Cameron, K. C., Di, H. J. y Moir, J. L. (2013). Nitrogen losses from the soil / plant system: a review. *Annals of Applied Biology*, *162*(2), 145-173. https://doi.org/10.1111/aab.12014

Cameron, K. C., Di, H. J., Reijnen, B. P. A., Li, Z., Russell, J. M. y Barnett, J. (2002). Fate of nitrogen in dairy factory effluent irrigated onto land. *New Zealand Journal of Agricultural Research*, *45*(3), 207-216. https://doi.org/10.1080/00288233.2002.9513511

Caradonia, F., Ronga, D., Catellani, M., Azevedo Giaretta, C. V., Alegria Terrazas, R., Robertson-Albertyn, S., Francia, E. y Bulgarelli, D. (2019). Nitrogen Fertilizers Shape the Composition and Predicted Functions of the Microbiota of Field-Grown Tomato Plants. *Phytobiomes*, *3*(4), 315-325. https://doi.org/10.1094/PBIOMES-06-19-0028-R

Casanova, O., Durán, A., Mello, R. y Del Pino, A. (2007). Manejo de los efluentes de tambo [nota técnica]. *Cangüé*, *29*, 94-96.

Castagna, A., Matonte, F., Mauttone, A., Rodríguez-Gallego, L. y Blumetto, O. (2024). Land use planning to minimize the export of phosphorus: An optimization model for dairy production at a catchment area scale. *Land*

Use Policy, *138*(February 2023). https://doi.org/10.1016/j.landusepol.2023.107023

Castaño, J. P., Giménez, A., Ceroni, M., Furest, J. y Aunchayna, R. (2011). *Caracterización agroclimática del uruguay 1980-2009. Serie técnica n.º 193*. INIA.

Chadwick, D., Sommer, S., Thorman, R., Fangueiro, D., Cardenas, L., Amon, B. y Misselbrook, T. (2011). Manure management: Implications for greenhouse gas emissions. *Animal Feed Science and Technology*, *166-167*, 514-531. https://doi.org/10.1016/j.anifeedsci.2011.04.036

Chalar, G., Delbenea, L., González-Bergonzonia, I. y Arocena, R. (2013). Fish assemblage changes along a trophic gradient induced by agricultural activities (Santa Lucía, Uruguay). *Ecological Indicators*, *24*, 582-588.

Charlón, V., Serrano, P., García, K., y Taverna, M. (2009). Gestión de los efluentes originados en las instalaciones de ordeño. *CREA: Producción de Leche - Edición 2009*.

Charlón, V. (2007). Residuos en las instalaciones de ordeño. *Idia XXI : revista de información sobre investigación y desarrollo agropecuario*, *7*(9), 80-85. http://ceiba.agro.uba.ar/cgi-bin/koha/opac-detail.pl?biblionumber=27685

Chilibroste, P. (2021). A major challenge for the Uruguayan dairy industry sustainable growth. *Agrociencia Uruguay*, *25*(2), 25-27. https://doi.org/10.31285/agro.25.970

Clough, T. J., Balaine, N., Cameron, K. C., Petersen, S. O. y Sommer, S. G. (2018). Effects of dairy shed effluent dry matter content on ammonia and nitrous oxide emissions from a pasture soil. *Journal of Agricultural Science*, *156*(9), 1070-1078. https://doi.org/10.1017/S0021859618001028

Conrad, R. (1996). Soil microorganisms as controllers of atmospheric trace gases (H2, CO, CH4, OCS, N2O, and NO). *Microbiological Reviews*, *60*(4), 609-640. https://doi.org/10.1128/mr.60.4.609-640.1996

Coppola, N., Freire, B., Umpiérrez, A., Cordeiro, N. F., Ávila, P., Trenchi, G., Castro, G., Casaux, M. L., Fraga, M., Zunino, P., Bado, I. y Vignoli, R.

(2020). Transferable Resistance to Highest Priority Critically Important Antibiotics for Human Health in Escherichia coli Strains Obtained From Livestock Feces in Uruguay. *Frontiers in Veterinary Science*, *7*(November). https://doi.org/10.3389/fvets.2020.588919

Correa, C., Rezzano, N., y García, F. (2016). *Manual para la gestión ambiental de tambos*. Montevideo, Julio 2016. https://www.gub.uy/ministerio-ambiente/comunicacion/publicaciones/manual-para-gestion-ambiental-tambos

Crane, S. R., Moore, J. A., Grisher, M. E. y Miner, J. R. (1983). Bacterial pollution from agricultural sources - a review. *Transactions - American Society of Agricultural Engineers*, *26*(3), 858-866. https://doi.org/10.13031/2013.34036

Dallenne, C., da Costa, A., Decré, D., Favier, C. y Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, *65*(3), 490-495. https://doi.org/10.1093/jac/dkp498

De Los Santos, R. I., Zunino, P. M., Gil, A. D., Laport, A. y Hirigoyen, D. J. (2017). Antibiotic resistance of Staphylococcus aureus associated with subclinical and clinical mastitis in Uruguay during an eight-year period. *Austral Journal of Veterinary Sciences*, *49*(3), 191-194. https://doi.org/10.4067/S0719-81322017000300191

Del Pino, A., Casanova, O., Barbazán, M., Mancassola, V., Arló, L., Borzacconi, L. y Passeggi, M. (2014). Agronomic Use of Slurry from Anaerobic Digestion of Agroindustrial Residues: Effects on Crop and Soil. *Journal of Sustainable Bioenergy Systems*, *4*(1), 10. https://doi.org/10.4236/jsbs.2014.41009

Dell, C. J., Meisinger, J. J. y Beegle, D. B. (2011). Subsurface Application of Manures Slurries for Conservation Tillage and Pasture Soils and Their Impact on the Nitrogen Balance. *Journal of Environmental Quality*, *40*(2), 352-361. https://doi.org/10.2134/jeq2010.0069

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., González, L., Tablada, M. y Robledo, Y. C. (2017). *InfoStat versión 2020* (n.º 2017). Centro de Transferencia InfoStat, FCA, Universidad Nacional de Córdoba, Argentina. http://www.infostat.com.ar

DIEA. (2021). *Anuario Estadístico Agropecuario*. Montevideo. MGAP. 2021. https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/comunicacion/publicaciones/anuario-estadistico-agropecuario-2021

DIEA. (2023). *Anuario Estadístico Agropecuario 2023*. Montevideo. MGAP. 2023. https://www.gub.uy/ministerio-ganaderia-agricultura-pesca/comunicacion/publicaciones/anuario-estadistico-agropecuario-2023

Dungan, R. S., McKinney, C. W., Acosta-Martinez, V. y Leytem, A. B. (2022). Response of soil health indicators to long-term dairy manure in a semiarid irrigated cropping system. *Soil Science Society of America Journal*, *86*(6), 1597-1610. https://doi.org/10.1002/saj2.20462

Dungan, R. S., Mckinney, C. W. y Leytem, A. B. (2018). Tracking antibiotic resistance genes in soil irrigated with dairy wastewater. *Science of the Total Environment*, *635*, 1477-1483. https://doi.org/10.1016/j.scitotenv.2018.04.020

Eghball, B. y Power, J. F. (1999). Phosphorus- and Nitrogen-Based Manure and Compost Applications Corn Production and Soil Phosphorus. *Soil Science Society of America Journal*, *63*(4), 895-901. https://doi.org/10.2136/sssaj1999.634895x

Elliott, L. F. y Ellis, J. R. (1977). Bacterial and Viral Pathogens Associated with Land Application of Organic Wastes. *Journal of Environmental Quality*, *6*(3), 245-251. https://doi.org/10.2134/jeq1977.00472425000600030002x

Fariña, S. R. y Chilibroste, P. (2019). Opportunities and challenges for the growth of milk production from pasture: The case of farm systems in Uruguay. *Agricultural Systems*, *176*, 102631. https://doi.org/10.1016/j.agsy.2019.05.001

Foote, K. J., Joy, M. K. y Death, R. G. (2015). New Zealand Dairy Farming:

Milking Our Environment for All Its Worth. *Environmental Management*, *56*(3), 709-720. https://doi.org/10.1007/s00267-015-0517-x

Forster, P., Storelvmo, T., Armour, K., Collins, W., Dufresne, J.-L., Frame, D., Lunt, D. J., Mauritsen, T., Palmer, M. D., Watanabe, M., Wild, M. y Zhang, H. (2021). The Earth's Energy Budget, Climate Feedbacks, and Climate Sensitivity. En V. Masson-Delmotte, P. Zhai, A. Pirani, V. S.L. ConMasson-Delmotte, P. Zhai, A. Pirani, S. L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M. I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J. B. R. Matthews, T. K. Maycock, T. Waterfield, … B. Zhounors (eds.), *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change* (pp. 923–1054). Cambridge University Press. https://doi.org/http://dx.doi.org/10.1017/9781009157896.009

Franz, E., Semenov, A. V. y Van Bruggen, A. H. C. (2008). Modelling the contamination of lettuce with Escherichia coli O157:H7 from manure-amended soil and the effect of intervention strategies. *Journal of Applied Microbiology*, *105*(5), 1569-1584. https://doi.org/10.1111/j.1365-2672.2008.03915.x

Gerba, C. P. y Smith, J. E. (2005). Sources of Pathogenic Microorganisms and Their Fate during Land Application of Wastes. *Journal of Environmental Quality*, *34*(1), 42-48. https://doi.org/10.2134/jeq2005.0042a

Ghezzehei, T. A., Sarkhot, D. V. y Berhe, A. A. (2014). Biochar can be used to capture essential nutrients from dairy wastewater and improve soil physico-chemical properties. *Solid Earth*, *5*(2), 953-962. https://doi.org/10.5194/se-5-953-2014

Gianneechini, R. E., Concha, C., Delucci, I., Gil, J., Salvarrey, L. y Rivero, R. (2014). Mastitis bovina, reconocimiento de los patógenos y su resistencia antimicrobiana en la Cuenca Lechera del Sur de Uruguay. *Veterinaria*, *50*(196), 4-32.

Gianneechini, R. E., Concha, C. y Franklin, A. (2002). Antimicrobial

Susceptibility of Udder Pathogens Isolated from Dairy Herds in the West Littoral Region of Uruguay. *Acta Veterinaria Scandinavica*, *43*(1), 31-41.

Gianneechini, R. E., Concha, C., Riveroj, R., Gil, J., Moreno-Lopez, J., Rivero, R., Gil, J. y Moreno-Lopez, J. (2005). Monitoreo de la resistencia de Staphylococcus aureus aislados en rodeos lecheros de la cuenca lechera tradicional de Uruguay. *XXXIII Jornadas Uruguayas de Buiatría*, *63*, 205-207.

Gibson, C. (1995). Farm dairy effluent management. *New Zealand Dairy Exporter*, *72*(22-24).

Gilchrist, M. J., Greko, C., Wallinga, D. B., Beran, G. W., Riley, D. G. y Thorne, P. S. (2007). The potential role of concentrated animal feeding operations in infectious disease epidemics and antibiotic resistance. *Environmental Health Perspectives*, *115*(2), 313-316. https://doi.org/10.1289/ehp.8837

Gomes, M. S., Cavalcanti, I. F. de A. y Müller, G. V. (2021). 2019/2020 drought impacts on South America and atmospheric and oceanic influences. *Weather and Climate Extremes*, *34*, 100404. https://doi.org/10.1016/j.wace.2021.100404

Goss, M. J., Barry, D. A. J. y Rudolph, D. L. (1998). Contamination in Ontario farmstead domestic wells and its association with agriculture: 1. Results from drinking water wells. *Journal of Contaminant Hydrology*, *32*(3-4), 267-293. https://doi.org/10.1016/S0169-7722(98)00054-0

Groffman, P. M. (2012). Terrestrial denitrification: challenges and opportunities. *Ecological Processes*, *1*, 1-11. https://doi.org/10.1186/2192-1709-1-11

Gutiérrez, S. y Cabrera, N. (2012). Estimación de los parámetros nacionales y básicos para el manejo de los efluentes de tambos. Parte 1: Caracterización de la descarga. *Ingeniaría Química*, *41*, 3 - 12.

Habteselassie, M. Y., Miller, B. E., Thacker, S. G., Stark, J. M. y Norton, J. M. (2006). Soil Nitrogen and Nutrient Dynamics after Repeated Application of Treated Dairy-Waste. *Soil Science Society of America Journal*, *70*(4),

1328-1337. https://doi.org/10.2136/sssaj2005.0189

Habteselassie, M. Y., Xu, L. y Norton, J. M. (2013). Ammonia-oxidizer communities in an agricultural soil treated with contrasting nitrogen sources. *Frontiers in Microbiology*, *4*(NOV), 1-10. https://doi.org/10.3389/fmicb.2013.00326

Hagare, D., Hong, W. T., Siddiqui, Z., Natarajan, S. K. y Fyfe, J. (2022). Effect of Dairy Pond Sludge/Supernatant Application on Ryegrass Dry Matter Yield and Phosphorus Fractions in Soil. *Agriculture (Switzerland)*, *12*(3), 1-12. https://doi.org/10.3390/agriculture12030351

Hawke, R. M. y Summers, S. A. (2006). Effects of land application of farm dairy effluent on soil properties: A literature review. *New Zealand Journal of Agricultural Research*, *49*(3), 307-320. https://doi.org/10.1080/00288233.2006.9513721

He, M., Tian, G., Semenov, A. M. y van Bruggen, A. H. C. (2012). Short-term fluctuations of sugar beet damping-off by Pythium ultimum in relation to changes in bacterial communities after organic amendments to two soils. *Phytopathology*, *102*(4), 413-420. https://doi.org/10.1094/PHYTO-07-11-0189

Heatley, P. R. (1995). Dairying and the environment: managing farm dairy effluent. *Dairying and the Environment Committee of New Zealand Dairy Board*.

Heuer, H., Schmitt, H. y Smalla, K. (2011). Antibiotic resistance gene spread due to manure application on agricultural fields. *Current Opinion in Microbiology*, *14*(3), 236-243. https://doi.org/10.1016/j.mib.2011.04.009

Hoffmann, H., Schloter, M. y Wilke, B. M. (2007). Microscale-scale measurement of potential nitrification rates of soil aggregates. *Biology and Fertility of Soils*, *44*(2), 411-413. https://doi.org/10.1007/s00374-007-0227-5

Houlbrooke, D. J. (2015). *Manejo de efluentes de la lechería en Uruguay: resumen de los hallazgos y recomendaciones para futuras mejoras.* INALE. https://www.inale.org/proyectos/manejo-de-efluentes-de-la-

lecheria-en-uruguay/

Houlbrooke, D. J., Horne, D. J., Hedley, M. J., Hanly, J. A., Snow, V. O., Horne, D. J., Hedley, M. J., Hanly, J. A. y Snow, V. O. (2004). *A review of literature on the land treatment of farm -dairy effluent in New Zealand and its impact on water quality*. *8233*(April). https://doi.org/10.1080/00288233.2004.9513617

Hu, R., Leytem, A. B., Moore, A. D. y Strawn, D. G. (2024). Long-term dairy manure amendment promotes legacy phosphorus buildup and mobility in calcareous soils. *Journal of Environmental Quality*, *53*(3), 365-377. https://doi.org/10.1002/jeq2.20559

IFCN. (2017). Dairy Report 2017. En *International Farm Comparison Network*.

Illarze, G., Del Pino, A., Rodríguez-Blanco, A. e Irisarri, P. (2023). Application of Dairy Effluents to Pastures Affects Soil Nitrogen Dynamics and Microbial Activity. *Agronomy*, *13*(2), 470. https://doi.org/10.3390/agronomy13020470

INALE. (2020). *Cartilla sobre criterios de aplicación de efluente a terreno y su implicancia práctica en el diseño e implementación*. 2020. https://www.inale.org/informes/criterios-de-aplicacion-de-efluente-a-terreno-y-su-implicancia-practica-en-el-diseno-e-implementacion/

INALE. (2021). *Situación y perspectiva de la lechería Uruguaya*. 2021. https://www.inale.org/informes/informe-2021/

INALE. (2023). *Situación 2023 y perspectivas 2024 de la lechería uruguaya*. https://www.inale.org/informes/situacion-2023-y-perspectivas-2024-de-la-lecheria-uruguaya/

IPCC. (2007). *Climate change 2007: the physical science basis.* (Cambridge).

Isaac, R. A. y Kerber, J. D. (1971). Atomic absorption and flame photometry: Techniques and uses in soil, plant, and water analysis. *Instrumental Methods for Analysis of Soils and Plant Tissue*, 17-37. https://doi.org/10.2136/1971.instrumentalmethods.c2

Jamieson, R. C., Gordon, R. J., Sharples, K. E., Stratton, G. W. y Madani, A.

(2002). Movement and persistence of fecal bacteria in agricultural soils and subsurface drainage water: A review. *Canadian Biosystems Engineering*, *44*, 1.1-1.9.

Jensen, L. S. y Sommer, S. G. (2013). Manure organic matter–characteristics and microbial transformations. En S. G. Sommer, M. L. Christensen, T. Schmidt, y L. S. Jensen (eds.), *Animal Manure Recycling - Treatment and Management.* (pp. 67-90). John Wiley & Sons Ltd.

Jones, C. M. (2010). *Denitrification: From Genes to Ecosystems*. [Tesis de doctorado] Swedish University of Agricultural Sciences, Uppsala.

Kandeler, E. (1996). Nitrogen mineralization. En F. Schinner, R. Ohlinger, R. Margesin, y E. Kandeler (eds.), *Methods in soil biology* (pp. 135-143). Springer.

La Manna, A. (1995). Manejo de residuos orgánicos en Tambo. En *Boletín de Divulgación n.º 53* (2.ª ed.). INIA La Estanzuela.

La Manna, A., Mieres, J., Acosta, Y. y Torres, I. (2004). Utilización de Efluentes en Tambos. Resumen de Investigación. *Resultados experimentales en lechería. La Estanzuela, INIA*, *361*, 35-44.

Laconi, A., Mughini-Gras, L., Tolosi, R., Grilli, G., Trocino, A., Carraro, L., Di Cesare, F., Cagnardi, P. y Piccirillo, A. (2021). Microbial community composition and antimicrobial resistance in agricultural soils fertilized with livestock manure from conventional farming in Northern Italy. *Science of the Total Environment*, *760*, 143404. https://doi.org/10.1016/j.scitotenv.2020.143404

Lang, N. L., Smith, S. R., Bellett-Travers, D. M., Pike, E. B. y Rowlands, C. L. (2003). COll IN SOIL FOLLOWING THE APPLICATION OF BlOSOLlDS TO AGRICULTURAL LAND. *Water and Environment Journal*, *17*(1), 23-28.

Lau, M. M. y Ingham, S. C. (2001). Survival of faecal indicator bacteria in bovine manure incorporated into soil. *Letters in Applied Microbiology*, *33*(2), 131-136. https://doi.org/10.1046/j.1472-765X.2001.00962.x

Laubach, J., Heubeck, S., Pratt, C., Woodward, K. B., Guieysse, B., Van Der

Weerden, T. J., Chung, M. L., Shilton, A. N. y Craggs, R. J. (2015). Review of greenhouse gas emissions from the storage and land application of farm dairy effluent. *New Zealand Journal of Agricultural Research*, *58*(2), 203-233. https://doi.org/10.1080/00288233.2015.1011284

Li, J., Luo, J., Shi, Y., Houlbrooke, D., Wang, L., Lindsey, S. y Li, Y. (2015). Nitrogen gaseous emissions from farm effluent application to pastures and mitigation measures to reduce the emissions: A review. *New Zealand Journal of Agricultural Research*, *58*(3), 339-353. https://doi.org/10.1080/00288233.2015.1028651

Li, J., Shi, Y., Luo, J., Zaman, M., Houlbrooke, D., Ding, W., Ledgard, S. y Ghani, A. (2014). Use of nitrogen process inhibitors for reducing gaseous nitrogen losses from land-applied farm effluents. *Biology and Fertility of Soils*, *50*(1), 133-145. https://doi.org/10.1007/s00374-013-0842-2

Liu, Y. Y. y Haynes, R. J. (2011). Influence of land application of dairy factory effluent on soil nutrient status and the size, activity, composition and catabolic capability of the soil microbial community. *Applied Soil Ecology*, *48*(2), 133-141. https://doi.org/10.1016/j.apsoil.2011.03.014

Lladó, S. y Baldrian, P. (2017). Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. *PLoS ONE*, *12*(2), e0171638. https://doi.org/10.1371/journal.pone.0171638

Lombardi, B., Orden, L., Varela, P., Garay, M., Iocoli, G. A., Montenegro, A., Sáez-Tovar, J., Bustamante, M. Á., Juliarena, M. P. y Moral, R. (2022). Is Dairy Effluent an Alternative for Maize Crop Fertigation in Semiarid Regions? An Approach to Agronomic and Environmental Effects. *Animals*, *12*(16). https://doi.org/10.3390/ani12162025

Longhurst, B., Rajendram, G., Miller, B. y Dexter, M. (2017). Nutrient Content of Liquid and Solid Effluents on Nz Dairy Cow Farms. *Science and Policy: nutrient Management Challenges*, *30*, 1-9.

Longhurst, R. D., Roberts, A. H. C. y O'Connor, M. B. (2000). Farm dairy effluent: A review of published data on chemical and physical

characteristics in New Zealand. *New Zealand Journal of Agricultural Research*, *43*(1), 7-14. https://doi.org/10.1080/00288233.2000.9513403

Lopes, A. R., Becerra-Castro, C., Vaz-Moreira, I., Silva, M. E. F., Nunes, O. C. y Manaia, C. M. (2016). Irrigation with treated wastewater: Potential impacts on microbial function and diversity in agricultural soils. *Handbook of Environmental Chemistry*, *44*, 105-128. https://doi.org/10.1007/698_2015_346

Lu, Y., Sun, R., Zhang, C., Ding, S., Ying, M. y Shan, S. (2021). In situ analysis of antibiotic resistance genes in anaerobically digested dairy manure and its subsequent disposal facilities. *Bioresource Technology*, *333*(January). https://doi.org/10.1016/j.biortech.2021.124988

Luo, J., Saggar, S., Bhandral, R., Bolan, N., Ledgard, S., Lindsey, S. y Sun, W. (2008). Effects of irrigating dairy-grazed grassland with farm dairy effluent on nitrous oxide emissions. *Plant and Soil*, *309*(1-2), 119-130. https://doi.org/10.1007/s11104-008-9550-3

Manono, B O, Moller, H. y Morgan, R. (2016). Effects of irrigation, dairy effl uent dispersal and stocking on soil properties of the Waimate District, New Zealand. *Geoderma Regional*, *7*(1), 59-66. https://doi.org/10.1016/j.geodrs.2016.01.002

Manono, B. O. (2014). *Effects of irrigation, effluent dispersal and organic farming on earthworms and soil microbes in New Zealand dairy farms* [tesis doctoral, University of Otago]. https://hdl.handle.net/10523/5097

Manshadi, F. D., Karpiscak, M. y Gerba, C. P. (2013). Enteric bacterial contamination and survival on produce during irrigation with dairy wastewater in the field. *Journal of Water Reuse and Desalination*, *3*(2), 102-110. https://doi.org/10.2166/wrd.2013.161

McDowell, R. W. y Nash, D. (2012). A Review of the Cost-Effectiveness and Suitability of Mitigation Strategies to Prevent Phosphorus Loss from Dairy Farms in New Zealand and Australia. *Journal of Environment Quality*, *41*(3), 680. https://doi.org/10.2134/jeq2011.0041

McKinney, C. W., Dungan, R. S., Moore, A. y Leytem, A. B. (2018).

Occurrence and abundance of antibiotic resistance genes in agricultural soil receiving dairy manure. *FEMS Microbiology Ecology*, *94*(3). https://doi.org/10.1093/femsec/fiy010

Mello, R. y Bonino, C. (2010). Efecto del uso de efluentes de salas de ordeño en la producción de forraje de raigras. *Agrociencia Uruguay*, *14*(3), 129.

Mulvaney, R. L. (1996). Nitrogen—inorganic forms. En Page, A.L., Miller, R.H., Keeney, D.R. (eds.), *Methods of soil analysis. Part 3. Chemical Methods* (número 5, pp. 1123-1184). Amer. Soc. Agron.

Murphy, J. y Riley, J. P. (1962). A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, *27*(C), 31-36. https://doi.org/10.1016/S0003-2670(00)88444-5

MVOTMA. (2013). *Plan de acción para la protección del agua en la cuenca del Santa Lucía*. http://www.mvotma.gub.uy/ambiente/prevencion-y-control-para-el-cuidado-del-ambiente/estado-del-ambiente/plan-santa-lucia

Nartey, O. D., Liu, D., Luo, J., Lindsey, S., Di, H. J., Chen, Z., Yuan, J., He, T. y Ding, W. (2021). Optimizing the application of dairy farm effluent and manure to mitigate gas emission. *Journal of Soils and Sediments*, *21*(6), 2381-2393. https://doi.org/10.1007/s11368-021-02935-w

Neufeld, K. R., Grayston, S. J., Bittman, S., Krzic, M., Hunt, D. E. y Smukler, S. M. (2017). Long-term alternative dairy manure management approaches enhance microbial biomass and activity in perennial forage grass. *Biology and Fertility of Soils*, *53*(6), 613-626. https://doi.org/10.1007/s00374-017-1204-2

Nicholson, F. A., Groves, S. J. y Chambers, B. J. (2005). Pathogen survival during livestock manure storage and following land application. *Bioresource Technology*, *96*(2), 135-143. https://doi.org/10.1016/j.biortech.2004.02.030

Niraula, S., Choi, Y. K., Payne, K., Muir, J. P., Kan, E. y Chang, W. S. (2021). Dairy effluent-saturated biochar alters microbial communities and enhances bermudagrass growth and soil fertility. *Agronomy*, *11*(9).

https://doi.org/10.3390/agronomy11091794

Nõlvak, H., Truu, M., Kanger, K., Tampere, M., Espenberg, M., Loit, E., Raave, H. y Truu, J. (2016). Science of the Total Environment Inorganic and organic fertilizers impact the abundance and proportion of antibiotic resistance and integron-integrase genes in agricultural grassland soil. *Science of the Total Environment*, *562*, 678-689. https://doi.org/10.1016/j.scitotenv.2016.04.035

Norton, J. M. y Stark, J. M. (2011). Regulation and measurement of nitrification in terrestrial systems. En M. G. Klotz (ed.), *Methods in Enzymology* (1.ª ed., vol. 486, pp. 343-368). Academic Press. https://doi.org/10.1016/B978-0-12-381294-0.00015-8

Oun, A., Kumar, A., Harrigan, T., Angelakis, A. y Xagoraraki, I. (2014). Effects of biosolids and manure application on microbial water quality in rural areas in the US. *Water (Switzerland)*, *6*(12), 3701-3723. https://doi.org/10.3390/w6123701

Owen, J. J. y Silver, W. L. (2015). Greenhouse gas emissions from dairy manure management: a review of field-based studies. *Global Change Biology*, *21*(2), 550-565. https://doi.org/10.1111/gcb.12687.Greenhouse

Pagliari, P., Wilson, M., Waldrip, H. y He, Z. (2020). Nitrogen and Phosphorus Characteristics of Beef and Dairy Manure. En H. Waldrip, P. Pagliari y Z. He (eds.), *Animal Manure: Production, Characteristics, Environmental Concerns, and Management* (John Wiley, pp. 45-62). ASA Special Publications 67.

Pandey, P., Chiu, C., Miao, M., Wang, Y., Settles, M., Del Rio, N. S., Castillo, A., Souza, A., Pereira, R. y Jeannotte, R. (2018). 16S rRNA analysis of diversity of manure microbial community in dairy farm environment. *PLoS ONE*, *13*(1), 1-18. https://doi.org/10.1371/journal.pone.0190126

Pandey, P. K., Kass, P. H., Soupir, M. L., Biswas, S. y Singh, V. P. (2014). Contamination of water resources by pathogenic bacteria. *AMB Express*, *4*(1), 1-16. https://doi.org/10.1186/s13568-014-0051-x

Pascucci, S. (2020). *Building natural resource networks: urban agriculture*

and the circular economy. En Wiskerke, H. (ed), *Achieving sustainable urban agriculture* (pp. 101-120). Burleigh Dodds Science Publishing. https://doi.org/10.19103/as.2019.0063.08

Patni, N. K., Toxopeus, H. R. y Jui, P. Y. (1985). Bacterial Quality of Runoff From Manured and Non-Manured Cropland. *Transactions of the American Society of Agricultural Engineers*, *28*(6), 1871-1877, 1884. https://doi.org/10.13031/2013.32534

Petersen, S. O. (2018). Greenhouse gas emissions from liquid dairy manure: Prediction and mitigation. *Journal of Dairy Science*, *101*(7), 6642-6654. https://doi.org/10.3168/jds.2017-13301

Peyton, D. P., Healy, M. G., Fleming, G. T. A., Grant, J., Wall, D., Morrison, L., Cormican, M. y Fenton, O. (2016). Nutrient, metal and microbial loss in surface runoff following treated sludge and dairy cattle slurry application to an Irish grassland soil. *Science of the Total Environment*, *541*, 218-229. https://doi.org/10.1016/j.scitotenv.2015.09.053

Philippot, L., Chenu, C., Kappler, A., Rillig, M. C. y Fierer, N. (2023). The interplay between microbial communities and soil properties. *Nature Reviews Microbiology*. https://doi.org/10.1038/s41579-023-00980-5

Ravishankara, A. R., Daniel, J. S. y Portmann, R. W. (2009). Nitrous oxide (N2O): the dominant ozone-depleting substance emitted in the 21st century. *Science*, *326*(October), 123-125. https://doi.org/10.1126/science.1176985

Rhine, E. D., Mulvaney, R. L., Pratt, E. J. y Sims, G. K. (1998). Improving the Berthelot Reaction for Determining Ammonium in Soil Extracts and Water. *Soil Science Society of America Journal*, *62*(2), 473. https://doi.org/10.2136/sssaj1998.03615995006200020026x

Roach, C. G., Longhurst, R. D. y Ledgard, S. F. (2001). Land application of farm dairy effluent for sustainable dairy farming. *Proceedings of the New Zealand Grassland Association*, 53-57. https://doi.org/10.33584/jnzg.2001.63.2430

Rogers, S. W., Donnelly, M., Peed, L., Kelty, C. A., Mondal, S., Zhong, Z. y

Shanks, O. C. (2011). Decay of bacterial pathogens, fecal indicators, and real-time quantitative PCR genetic markers in manure-amended soils. *Applied and Environmental Microbiology*, *77*(14), 4839-4848. https://doi.org/10.1128/AEM.02427-10

Saggar, S., Bolan, N. S., Bhandral, R., Hedley, C. B. y Luo, J. (2004a). A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, *47*(4), 513-544. https://doi.org/10.1080/00288233.2004.9513618

Saggar, S., Bolan, N. S., Bhandral, R., Hedley, C. B. y Luo, J. (2004b). A review of emissions of methane, ammonia, and nitrous oxide from animal excreta deposition and farm effluent application in grazed pastures. *New Zealand Journal of Agricultural Research*, *47*(4), 513-544. https://doi.org/10.1080/00288233.2004.9513618

Saggar, S., Jha, N., Deslippe, J., Bolan, N. S., Luo, J., Giltrap, D. L., Kim, D., Zaman, M. y Tillman, R. W. (2013). Denitrification and N2O:N2 production in temperate grasslands: Processes, measurements, modelling and mitigating negative impacts. *Science of the Total Environment*, *465*, 173-195. https://doi.org/10.1016/j.scitotenv.2012.11.050

Sahlström, L. (2003). A review of survival of pathogenic bacteria in organic waste used in biogas plants. *Bioresource Technology*, *87*(2), 161-166. https://doi.org/10.1016/S0960-8524(02)00168-2

Salazar, F., Herrero, M. A., Charlón, V. y La Manna, A. (2010). Slurry management in dairy grazing farms in south american countries. *Proceedings of the 14th International Conference of the FAO ESCORENA Network on the Recycling of Agricultural, Municipal and Industrial Residues in Agriculture*, 1-4. http://www.ramiran.net/ramiran2010/docs/Ramiran2010_0211_final.pdf

Saunders, O. E., Fortuna, A.-M., Harrison, J. H., Whitefield, E., Cogger, C. G., Kennedy, A. C. y Bary, A. I. (2012). Comparison of Raw Dairy Manure Slurry and Anaerobically Digested Slurry as N Sources for Grass Forage

Production. *International Journal of Agronomy*, *2012*(C), 1-10. https://doi.org/10.1155/2012/101074

Sayre, J. M., Wang, D., Lin, J. Y., Danielson, R. E., Scow, K. M. y Mazza Rodrigues, J. L. (2023). Repeated manure inputs to a forage production soil increase microbial biomass and diversity and select for lower abundance genera. *Agriculture, Ecosystems and Environment*, *354*(May), 108567. https://doi.org/10.1016/j.agee.2023.108567

Schröder, J. (2005). Revisiting the agronomic benefits of manure: A correct assessment and exploitation of its fertilizer value spares the environment. *Bioresource Technology*, *96*(2), 253-261. https://doi.org/10.1016/j.biortech.2004.05.015

Semenov, M. V., Krasnov, G. S., Semenov, V. M., Ksenofontova, N., Zinyakova, N. B. y van Bruggen, A. H. C. (2021). Does fresh farmyard manure introduce surviving microbes into soil or activate soil-borne microbiota? *Journal of Environmental Management*, *294*(May), 113018. https://doi.org/10.1016/j.jenvman.2021.113018

Severin, M., Fuß, R., Well, R., Garlipp, F. y Van den Weghe, H. (2015). Soil, slurry and application effects on greenhouse gas emissions. *Plant, Soil and Environment*, *61*(8), 344-351. https://doi.org/10.17221/21/2015-PSE

Shi, Y., Ziadi, N., Hamel, C., Bittman, S., Hunt, D. y Lalande, R. (2017). Soil microbial biomass, activity, and community composition as a ff ected by dairy manure slurry applications in grassland production. *Applied Soil Ecology*, *125*, 97-107. https://doi.org/10.1016/j.apsoil.2017.12.022

Smith, K. a., Ball, T., Conen, F., Dobbie, K. E., Massheder, J. y Rey, A. (2003). Exchange of greenhousegases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science*, *54*(December), 779-791. https://doi.org/10.1046/j.1365-2389.2003.00567.x

Sosa, N., Orcellet, J. M. y Gambaudo, S. (2016). *Uso agronómico de residuos orgánicos de origen animal*. https://produccion-animal.com.ar/produccion_y_manejo_pasturas/pasturas_fertilizacion/65-

Uso-Agronomico-Residuos.pdf

Stevens, R. J., Laughlin, R. J., Burns, L. C., Arah, J. R. M. y Hood, R. C. (1997). Measuring the contributions of nitrification and denitrification to the flux of nitrous oxide from soil. *Soil Biology and Biochemistry*, *29*(2), 139-151. https://doi.org/10.1016/S0038-0717(96)00303-3

Stevens, R. J., y Laughlin, R. J. (2001). Cattle Slurry Affects Nitrous Oxide and Dinitrogen Emissions from Fertilizer Nitrate. *Soil Science Society of America Journal*, *65*(4), 1307-1314. https://doi.org/10.2136/sssaj2001.6541307x

Sukias, J. P. S., Tanner, C. C., Davies-Colley, R. J., Nagels, J. W. y Wolters, R. (2001). Algal abundance, organic matter, and physico-chemical characteristics of dairy farm facultative ponds: Implications for treatment performance. *New Zealand Journal of Agricultural Research*, *44*(4), 279-296. https://doi.org/10.1080/00288233.2001.9513485

Suleiman, A. K. A., Gonzatto, R., Aita, C., Lupatini, M., Jacques, R. J. S., Kuramae, E. E., Antoniolli, Z. I. y Roesch, L. F. W. (2016). Temporal variability of soil microbial communities after application of dicyandiamide-treated swine slurry and mineral fertilizers. *Soil Biology and Biochemistry*, *97*, 71-82. https://doi.org/10.1016/j.soilbio.2016.03.002

Sun, L., Pope, P. B., Eijsink, V. G. H. y Schnürer, A. (2015). Characterization of microbial community structure during continuous anaerobic digestion of straw and cow manure. *Microbial Biotechnology*, *8*(5), 815-827. https://doi.org/10.1111/1751-7915.12298

Tarlera, S., Capurro, M. C., Irisarri, P., Scavino, A. F., Cantou, G. y Roel, A. (2016). Yield-scaled global warming potential of two irrigation management systems in a highly productive rice system. *Scientia Agricola*, *73*(1), 43-50. https://doi.org/10.1590/0103-9016-2015-0050

Taverna, M., Charlón, V., García, K. y Walter., E. (2007). Manejo de Efluentes de Tambos «INTA Rafaela». *Revista IDIA XXI*, *9*, 86-92.

Tayyab, U. y McLean, F. A. (2015). Phosphorus losses and on-farm mitigation options for dairy farming systems: A review. *Journal of Animal and Plant*

Sciences, *25*(2), 318-327.

Tian, H., Xu, R., Canadell, J. G., Thompson, R. L., Winiwarter, W., Suntharalingam, P., Davidson, E. A., Ciais, P., Jackson, R. B., Janssens-Maenhout, G., Prather, M. J., Regnier, P., Pan, N., Pan, S., Peters, G. P., Shi, H., Tubiello, F. N., Zaehle, S., Zhou, F., … Yao, Y. (2020). A comprehensive quantification of global nitrous oxide sources and sinks. *Nature*, *586*(7828), 248-256. https://doi.org/10.1038/s41586-020-2780-0

Tiedje, J. M. (1988). Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *Biology of anaerobic microorganisms*, *717*, 179-244.

Tillman, R. W. y Surapaneni, A. (2002). Some soil-related issues in the disposal of effluent on land. *Australian Journal of Experimental Agriculture*, *42*(3), 225-235. https://doi.org/https://doi.org/10.1071/EA00133

Umpiérrez, A., Bado, I., Oliver, M., Acquistapace, S., Etcheverría, A., Padola, N. L., Vignoli, R. y Zunino, P. (2017). Zoonotic potential and antibiotic resistance of Escherichia coli in neonatal calves in Uruguay. *Microbes and Environments*, *32*(3), 275-282. https://doi.org/10.1264/jsme2.ME17046

Unc, A. y Goss, M. J. (2004). Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology*, *25*(1), 1-8. https://doi.org/10.1016/j.apsoil.2003.08.007

Urra, J., Alkorta, I. y Garbisu, C. (2019). Potential benefits and risks for soil health derived from the use of organic amendments in agriculture. *Agronomy*, *9*(9), 1-23. https://doi.org/10.3390/agronomy9090542

USEPA. (2015). *Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990–2013*. https://www.epa.gov/ghgemissions/inventory-us-greenhouse-gas-emissions-and-sinks-1990-2013

van Bruggen, A. H. C., Goss, E. M., Havelaar, A., van Diepeningen, A. D., Finckh, M. R. y Morris, J. G. (2019). One Health - Cycling of diverse microbial communities as a connecting force for soil, plant, animal,

human and ecosystem health. *Science of the Total Environment*, *664*, 927-937. https://doi.org/10.1016/j.scitotenv.2019.02.091

van der Heijden, M. G. A. y Wagg, C. (2013). Soil microbial diversity and agro-ecosystem functioning. *Plant and Soil*, *363*(1-2), 1-5. https://doi.org/10.1007/s11104-012-1545-4

Van Der Weerden, T. J., Clough, T. J. y Styles, T. M. (2013). Using near-continuous measurements of N2O emission from urine-affected soil to guide manual gas sampling regimes. *New Zealand Journal of Agricultural Research*, *56*(1), 60-76. https://doi.org/10.1080/00288233.2012.747548

Van der Weerden, T. J., Cox, N., Luo, J., Di, H. J., Podolyan, A., Phillips, R. L., Saggar, S., de Klein, C. A. M., Ettema, P. y Rys, G. (2016). Refining the New Zealand nitrous oxide emission factor for urea fertiliser and farm dairy effluent. *Agriculture, Ecosystems and Environment*, *222*(May), 133-137. https://doi.org/10.1016/j.agee.2016.02.007

Van Doorslaer, B., Witzke, H.-P., Huck, I., Weiss, F., Fellmann, T., Salputra, G., Jansson, T., Drabik, D. y Leip, A. (2015). An economic assessment of GHG mitigation policy options for EU agriculture. En *JRC Science for Policy Report* (vol. EUR27973). https://doi.org/10.2791/180800

van Groenigen, J. W., Huygens, D., Boeckx, P., Kuyper, T. W., Lubbers, I. M., Rütting, T. y Groffman, P. M. (2015). The soil N cycle: new insights and key challenges. *Soil*, *1*(1), 235-256. https://doi.org/10.5194/soil-1-235-2015

Viguria, M., Sanz-Cobeña, A., López, D. M., Arriaga, H. y Merino, P. (2015). Ammonia and greenhouse gases emission from impermeable covered storage and land application of cattle slurry to bare soil. *Agriculture, Ecosystems and Environment*, *199*, 261-271. https://doi.org/10.1016/j.agee.2014.09.016

Wallenstein, M. D., Myrold, D. D., Firestone, M. y Voytek, M. (2006). Environmental Controls on Denitrifying Communities and Denitrification Rates: Insights from Molecular Methods. *Ecological Applications*, *16*(6), 2143-2152.

Wulf, S., Maeting, M. y Clemens, J. (2002). Application Technique and Slurry Co-Fermentation Effects on Ammonia, Nitrous Oxide, and Methane Emissions after Spreading. *Journal of Environmental Quality*, *31*(6), 1795-1801. https://doi.org/10.2134/jeq2002.1795

Zaman, M., Cameron, K. C., Di, H. J. e Inubushi, K. (2002). Changes in mineral N, microbial biomass and enzyme activities in different soil depths after surface applications of dairy shed effluent and chemical fertilizer. *Nutrient Cycling in Agroecosystems*, *63*, 275-290.

Zelenev, V. V., van Bruggen, A. H. C., Leffelaar, P. A., Bloem, J. y Semenov, A. M. (2006). Oscillating dynamics of bacterial populations and their predators in response to fresh organic matter added to soil: The simulation model «BACWAVE-WEB». *Soil Biology and Biochemistry*, *38*(7), 1690-1711. https://doi.org/10.1016/j.soilbio.2005.11.024

Zhang, J., Zhuang, M., Shan, N., Zhao, Q., Li, H. y Wang, L. (2019). Substituting organic manure for compound fertilizer increases yield and decreases NH3 and N2O emissions in an intensive vegetable production systems. *Science of the Total Environment*, *670*, 1184-1189. https://doi.org/10.1016/j.scitotenv.2019.03.191

Zhang, N., Bai, Z., Luo, J., Ledgard, S., Wu, Z. y Ma, L. (2017). Nutrient losses and greenhouse gas emissions from dairy production in China: Lessons learned from historical changes and regional differences. *Science of the Total Environment*, *598*, 1095-1105. https://doi.org/10.1016/j.scitotenv.2017.04.165

Zhang, T., Liu, H., Luo, J., Wang, H., Zhai, L., Geng, Y., Zhang, Y., Li, J., Lei, Q., Bashir, M. A., Wu, S. y Lindsey, S. (2018). Long-term manure application increased greenhouse gas emissions but had no effect on ammonia volatilization in a Northern China upland field. *Science of the Total Environment*, *633*, 230-239. https://doi.org/10.1016/j.scitotenv.2018.03.069

Zhang, Y., Hao, X., Alexander, T., Thomas, B., Shi, X. y Lupwayi, N. (2018). Long-term and legacy effects of manure application on soil microbial

community composition. *Biology and Fertility of Soils*, *54*, 269-283. https://doi.org/https://doi.org/10.1007/s00374-017-1257-2

Zumft, W. G. (1997). Cell biology and molecular basis of denitrification. *Microbiology and molecular biology reviews*, *61*(4), 533-616. http://mmbr.asm.org/content/61/4/533.short%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=232623&tool=pmcentrez&rendertype=abstract