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Blending of raw and treated animal manures to produce organic fertilisers

Joana Rita Viana Prado

Scientific Advisors: Doctor David Paulo Fangueiro
Doctor Henrique Manuel Filipe Ribeiro
Doctor Paula Maria da Luz Figueiredo de Alvarenga

THESIS PRESENTED TO OBTAIN THE DOCTOR DEGREE (PhD) IN
AGRICULTURE ENGINEERING

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Jury

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Abstract

Manures have an imbalance N:P ratio relative to crop needs, which prompt farmers to discard their application as fertilizer. In fact, this rendered agriculture to became dependent on the use of mineral fertilizers (MF). This thesis aims to produce a manure-based fertilizer (MBF) with a specific N:P ratio (1:1, 2:1 and 0.5:1) for basal fertilization, commonly found in MFs, under two scenarios: i) on-farm, where farmers will produce on their own the MBF, by mixing one manure with small amount of MFs and ii) central-solution, where the MBF is produced by mixing manures with other manures. First, for each scenario, an aerobic incubation was performed to estimate the N mineralization and nitrification rates, and the emission of greenhouse gases (GHG), moreover, the leaching experiment evaluated the nitrate and phosphorus potential leaching. Secondly, the agronomic efficiency of the preselected MBFs was ascertained in a pot experiment, comparing their performance with MFs. The results demonstrated that producing MBFs from raw materials can be challenging. From the MBF tested poultry manure (PoM) with pig slurry (PiS) (1:1), PiS mixed with its liquid fraction (LIQ) (2:1) and PoM with superphosphate (SP) (0.5:1) stimulated N mineralization and mitigated nitrate leaching. Even though PoM+SP is a P-enriched MBF, this enrichment did not increase the P leaching, instead enhanced P availability to values like MF. Still, the results demonstrated that it is necessary to rethink the MBFs because i) only PoM+PiS and PoM+SP obtained equal yields to MF and ii) the emissions of GHG from MBFs were considerable higher than MF but in the majority lower than the manure of origin. The production of MBF needs to be readjusted, however, the results showed promising outcomes, which suggest that with the proper alterations the adoption of MBF will potentially substitute MF in basal fertilization.

Keywords: Organic fertilizer; N dynamics; greenhouse gases emissions; leaching; agronomic efficiency

Resumo

Os efluentes pecuários têm um rácio N:P desequilibrado face às necessidades das culturas, sendo umas das razões que leva os agricultores a descartem-nos como fertilizantes tornando a agricultura dependente da utilização de fertilizantes minerais (MF). O intuito desta tese, foi produzir *manure-based fertilizer* (MBF) com um rácio específico de N:P (1:1, 2:1 and 0.5:1) para fertilização de fundo, como encontrado nos MFs, em dois cenários: i) *on-farm*, onde os agricultores produzem os seus próprios MBFs, misturando um efluente com diminutas quantidades de MFs e ii) *central-solution*, onde os MBFs serão produzidos misturando efluentes entre si. Primeiramente, para cada cenário, foi realizada uma incubação aeróbia estimando-se a taxa de mineralização e nitrificação, assim como, as emissões de gases com efeito de estufa (GHG) e, um ensaio de lixiviação aferindo-se o potencial de lixiviação de nitrato e fósforo. Posteriormente, o valor agronómico dos MBFs pré-selecionados foi determinado num ensaio em vasos, comparando-se a sua performance com a dos MFs. Produzir MBFs a partir de efluentes em bruto é desafiante. Dos MBFs testados, estrume de aves (PoM) com chorume de porco (PiS) (1:1), PiS com a sua fração líquida (LIQ) (2:1) e PoM com superfosfato (0.5:1) estimularam a mineralização de N e mitigaram a lixiviação de nitrato. Apesar de PoM+SP ser um MBF enriquecido em P, não aumentou a sua lixiviação, mas melhorou a sua disponibilidade igualando os MFs. Contudo, os resultados indicam que será necessário repensar os MBFs porque i) apenas PoM+PiS e PoM+SP obtiveram produções equivalentes aos MFs e ii) as emissões de GHG dos MBFs foram consideravelmente superiores às dos MFs, mas inferiores aos efluentes de origem. A produção de MBF deverá ser reajustada, todavia, os resultados demonstram premissas interessantes, sugerindo que com as necessárias alterações os MBFs terão o potential de substituir MF na fertilização de fundo.

Palavras-chave: fertilizante orgânico; dinâmicos de N; emissões gases de efeito de estufa; lixiviação; valor agronómico

Resumo alargado

A agricultura tornou-se dependente do uso de fertilizantes minerais (MF) para garantir a subsistência da população mundial. Por conseguinte, os solos foram sendo explorados com o intuito de alcançar o seu máximo de produção, o que culminou na sua deterioração. Com a crescente necessidade de produção de alimentos, a pecuária e a agricultura intensificaram a sua produção, mas distanciaram-se geograficamente. Concludentemente, a pecuária cresceu em áreas de menor exploração agrícola, dando lugar a que outros produtos inerentes ao setor pecuário, como os efluentes pecuários (EP), outrora importantes na agricultura, começassem a ser acumulados nas explorações pelo seu desuso. A heterogeneidade dos efluentes, assim como o erróneo rácio de azoto (N): fósforo (P) face às necessidades das culturas, levou a que os EPs fossem apenas vistos como corretivos orgânicos. Este desequilíbrio no rácio N:P levou à aplicação de P em excesso, originando problemas ambientais como a eutrofização. Todavia, os EPs são importantes para colmatar o défice de matéria orgânica no solo, cuja origem adveio da sobre utilização do solo e da aplicação excessiva de MFs.

Concomitantemente, a procura por uma agricultura mais sustentável, que contribui para a economia circular, começa a emergir pela Europa com o contexto de *bio-based fertilizer*. Esta tese é realizada no âmbito do projeto europeu que tem por base a reestruturação da agricultura a fim de a tornar mais sustentável, colmatando os ciclos de carbono (C), N e P, reconectando o fluxo de nutrientes e os pilares do setor agroalimentar através de diversas formas de processamento. Neste trabalho, é compreendida a criação de *manure-based fertilizer* (MBF), com rácio de N:P equivalentes aos usualmente encontrados nos MFs, 1:1, 2:1 e 0.5:1. A implementação destes materiais, além de permitir o reaproveitamento de nutrientes para nutrir as culturas, permitirá igualmente repor os níveis de C orgânico no solo, mitigando a pegada carbónica da agricultura. A produção de MBF foi faseada em dois cenários: i) *on-farm*: elaboração de MBFs na ótica do agricultor, onde dentro da própria exploração o agricultor produz os seus próprios MBFs, misturando EPs com pequenas quantidades de MFs e ii) *central-solution*: preparação de MBFs contemplando a existência de uma central de processamento capaz de gerir vários EPs, cuja produção de MBFs terá por base a mistura de EPs. Numa primeira etapa, foi necessário perscrutar quais os rácios de N:P dos EPs, disponíveis na

realidade portuguesa, e depreender se haverá a necessidade de realizar tratamentos aos chorumes para se atingir os rácios pretendidos.

Com base no exposto, foram analisados sete EPs, dois chorumes de vaca (CaS), com diferentes intensidades de produção, chorume de porco com produção em sistema misto (PSM), chorume de porco numa exploração com a fase de engorda (PSF), estrume de vaca (CaM), estrume de cabra (GoM) e estrume de aves (PoM). Constatou-se que o CaS, PSM e PoM já continham o desejado rácio 1:1. Contudo, através dos EPs, na sua forma fresca, não foi possível alcançar os restantes rácios. Por conseguinte, foi analisado qual a influência de tratamentos como a separação sólido-líquido, acidificação e a combinação de ambos no rácio N:P dos EPs. Determinando-se que i) a fração sólida (SOL) de PSF, pela migração de P para SOL aquando da realização da separação sólida-líquida, detinha o rácio 0.5:1, e que ii) a fração líquida dos chorumes (LIQ) poderá ser utilizada para produzir MBF enriquecidos em N.

A metodologia aplicada para averiguar o potencial dos MBFs, permitiu interpretar o ciclo de N e P após a aplicação de MBFs ao solo. Os ensaios realizados nos dois cenários, recaem sobre algumas das temáticas inerentes à utilização de efluentes pecuários na agricultura. Grande parte do N nos EPs encontra-se em moléculas orgânicas, pelo que, contrariamente aos MFs, o N não estará todo disponível para assimilação, sendo necessário quantificar as taxas de mineralização e nitrificação para melhor informar os agricultores da disponibilidade de N. A aplicação de EPs pode emitir uma grande quantidade de gases com efeito de estufa (GHG), ou pode estimular a lixiviação de nitratos (NO_3^-) ou P. Deste modo, foram realizados três ensaios, em paralelo, em solo arenoso, para aferir qual a dinâmica de mineralização e nitrificação de N (incubação aeróbia), qual a influência destes materiais nas emissões de GHGs, com subsequente quantificação das perdas de N e C (ensaio de medição de GHG) e o que sucede ao potencial de lixiviação dos nutrientes (ensaio de lixiviação).

No primeiro cenário, *on-farm*, adicionou-se diminutas quantidades de MF a cada EPs para se atingir os desejados rácios 2:1 e 0.5:1. CaS, PSF e PoM já detinham o rácio 1:1 e o PSF-SOL 0.5:1. Para se atingir o rácio 2:1, foram criados dois MBFs por cada EPs, ao qual se adicionou ureia (U) ou sulfato de amónio (AS). Para o rácio 0.5:1 incorporou-se a CaS e ao PoM superfosfato (SP) ou ácido fosfórico (PA). O rácio 1:1 como utiliza EPs na sua forma original, foi considerado como o controlo para os três ensaios. No rácio 1:1, as maiores taxas de mineralização foram obtidas com a aplicação de chorumes, onde ~35% do N orgânico veiculado foi mineralizado. Este aumento na disponibilidade de N não fomentou a lixiviação de nitrato (NO_3^-), sugerindo a aplicação destes dois materiais para culturas com maiores necessidades azotadas, mesmo que culturas de outono/inverno, dado o menor risco de lixiviação. A aplicação de PoM além de baixas taxas de mineralização, que poderia indiciar o seu uso para culturas de inverno, exponenciou os impactes ambientais tanto pelo incremento das emissões de GHG, como pelo elevado potencial de lixiviação do pouco N que vai sendo nitrificado, descartando este EP. Todavia, a produção de MBF com PoM com U ou AS, estimulou a taxa de mineralização e de nitrificação face ao PoM e mitigou para metade as emissões de óxido nitroso e dióxido de carbono e a lixiviação de NO_3^-. O rácio 2:1, surge, também, pela necessidade de colmatar o desequilíbrio nutricional do EPs, nomeadamente a concentração de P, pelo que o facto de estes MBFs terem diminuído o potencial de lixiviação de P face aos EPs de origem foi imprescindível, cuja menor redução se observou com PSF+U (52%). Mesmo para solos pobres em P, não se pretende que o potencial de lixiviação de P seja colossal, especialmente em solos arenosos, com baixa capacidade de adsorção. Todavia, PSF-SOL, PoM+PA e CaS+PA aumentaram o potencial de lixiviação de P, o que os poderá indicar para uma cultura com maiores necessidades de P, mas, também, os propicia para solos com baixa concentração de P extraível. Contudo, PoM+SP, apesar do enriquecimento em P, o potencial de lixiviação de P não foi estimulado comparativamente a PoM, e ainda mitigou a lixiviação de NO_3^- para quase metade, assim como as emissões gasosas, indiciando este MBF para uma prática mais sustentável para um solo pobre em P.

Os ensaios da *central-solution*, compara os MBFs de cada rácio, obtidos pela mistura de EPs, com um MF. Os MBFs testados nesta fase foram, rácio 1:1: PoM+CaS, PoM+PiS e MF 10:10:10, rácio 2:1 PiS+PiS-LIQ, CaS+CaS-LIQ e MF 13:06:18 e rácio 0.5:1: CaM+PoM, CaM+PiS e MF 07:14:14. Constatou-se que PoM+PiS, PoM+CaS e PiS+PiS-LIQ obtiveram taxas de mineralização similares ou superiores ao análogo MF. No caso do POUL+PSF, assim como dos MBFs de 0.5:1, o risco de lixiviação de NO_3^- foi diminuto, sendo reduzido para metade face aos MFs no caso do CAT+PSF. Adicionalmente, o risco de lixiviação de P foi apaziguado comparando com os valores de MFs, sugerindo que a aplicação de MBFs será mais vantajoso. Contudo, a veiculação de MBFs denegriu a qualidade ambiental, devido à sua rica composição em material orgânico que acaba também por estimular a atividade microbiana, estimulando as emissões. Este último fator pode vir a implicar a aplicação de alguns tratamentos para amenizar as emissões.

Na última etapa, foi avaliado o valor agronómico dos MBFs da *central-solution* mais PoM+U e PoM+SP, num ensaio em vasos perfazendo a fertilização de fundo da aveia. Nesta fase, aferiu-se a necessidade de aprimorar algumas características, a fim de assegurar uma produção similar à dos MFs. De facto, este feito, é determinante para qualquer agricultor, e só foi alcançado com PoM+SP (0.5:1) e PoM+PiS (1:1). Ainda assim, a assimilação de nutrientes aquando da aplicação de MFs ou PiS+PiS-LIQ (2:1), foi idêntica. Não obstante, a aplicação de MBFs não suscitou qualquer problema de salinidade e assegurou o pH do solo, contrariamente ao observado com MFs.

Existe um potencial de substituição de MF por MBFs em fertilização de fundo, mas será necessário repensar em alguns pontos, como acidificar os MBF para mitigar a emissão de GHGs. Antes de extrapolar a aplicação de MBFs para a realidade, é imperativo aprofundar este conceito, assim como, os MBFs analisados.

Symbols and abbreviations

+	Mix with
A-LIQ	Liquid Fraction of the acidified Slurry
ANNM	Apparent net N mineralization
AS	Ammonia sulphate
A-SLU	Acidified Slurry
A-SOL	Solid fraction of the acidified slurry
CaM	Cattle manure
CaS	Cattle slurry
CaS-	Cattle slurry from a less intensive farm
CaS+	Cattle slurry from a more intensive farm
$\mathbf{Ca_{Total}}$	Total calcium
$\mathbf{CO_2}$ **eq**	CO_2 equivalent
DM	Dry matter
EP	Efluente pecuário
GC	Gas chromatography
GHG	Greenhouse gases
GoM	Goat manure
GWP	Global warming potential
$\mathbf{K_{Total}}$	Total potassium expressed as K_2O
LIQ	Slurry liquid fraction
MBF	Manure-based fertilizer
MF	Mineral fertilizer
$\mathbf{Mg_{Total}}$	Total magnesium
min N	Mineral N
n.a.	Not applicable
N:P	Available N : total P as (P_2O_5)
$\mathbf{N_{av}}$	Estimated available nitrogen
Nit	Nitrification rate
NNM	Net N mineralization
$\mathbf{N_{Total}}$	Total nitrogen
PA	Phosphoric acid
PiS	Pig slurry
PiSF	Pig slurry from a fattening farm
PiSM	Pig slurry with all stages of pig production
PM	Potential of mineralization
PoM	Poultry manure
$\mathbf{P_{Total}}$	Total phosphorus expressed as P_2O_5
SLU	Non treated slurry
SOL	Slurry solid fraction
SP	Superphosphate

S_{Total}	Total sulphur
TOC	Total organic carbon
U	Urea
WHC	Water holding capacity

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Dissemination of results

2019 **Poster:** Prado, J., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2019) Blending of raw and treated animal manure to produce bio-based fertilizer. ManuResource, Hasselt, Belgium.

2021 **e-Poster:** Prado, J., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2021) A step towards the production of manure-based fertilizers. International Conference Agri-Food Ecosystem, Santarém/online, Portugal.

Oral presentation: Prado, J., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2021/2022) Production of bio-based fertilizers by blending animal manures and slurries: puzzling the effects of slurry treatment and animal species on their nutrients content. ManuResource, ‘s Hertogenbosch , Netherland.

Oral presentation: Prado, J., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2021/2022) The effect of tailoring N:P ratios mixing manures with mineral fertilizers in their mineral N leaching potential. ManuResource, ‘s Hertogenbosch, Netherland.

2022 **Article:** Prado, J., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2022). 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. 337, 130369. doi:10.1016/j.jclepro.2022.130369

Poster: Prado, J., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2022). A step towards the production of manure-based fertilizers. Encontro Ciência, Lisboa, Portugal.

Article: Alvarenga, P., Prado, J., Esteves, C., Silva, A.A., Fangueiro, D. (2022). Projeto nutri2cycle soluções para a circularidade de nutrientes e carbono na europa. AGROTEC 43.

2023 **Article:** Prado, J.; Fangueiro, D.; Alvarenga, P.; Ribeiro, H (2023). Assessment of the Agronomic Value of Manure-Based Fertilizers. Agronomy, 13, 140. https://doi.org/10.3390/agronomy13010140

Article: Prado, J., Alvarenga, P., Ribeiro, H., Fangueiro, D. (2023). Nutrient Potential Leachability in a Sandy Soil Amended with Manure-Based Fertilisers. Agronomy 13, 990. https://doi.org/10.3390/agronomy13040990.

Article (submitted): Prado, J., Meers, E., Ribeiro, H., Alvarenga, P., Fangueiro, D. (2023). The effect of manure-based fertilizers on nitrogen mineralization and greenhouse gases emissions after soil application. Heliyon doi:Submitted

Thesis Structure

The present thesis aggregates the results of this study, to potentially produce manure-based fertilizer with known N:P ratios, that are commonly utilized by farmers which resort to mineral fertilizers. Accordingly, the thesis was organized to address the manufacturing of these possible manure-based fertilizers in two phases: i) testing two hypothetical scenarios to produce them, with three sub-stages each (three difference experiments) and ii) a final stage of determining their agronomic value. The description of each chapter is expounded further down and is synthesised in Figure I.

Chapter I - Introduction: This chapter culminates the state of the art regarding agriculture challenges, the dependency on MF, benefits and disadvantages of manure utilization in agriculture. In a summary, it aims to introduce the necessity of creating manure-based fertilizers with specific N:P ratios. In this topic, it is also introduced the aims of this thesis and the points that need to be answered to determine the prospect of creating these organic fertilizers.

Chapter II - A step towards the production of manure-based fertilizers: disclosing the effects of animal species and slurry treatment on their nutrients content and availability: A detailed assessment of manures availability was performed, according to the Portuguese reality. Hence, the determination of N:P ratio and potential N availability was surveyed in the raw manures. The effect of low technology treatments to slurry, on the nutrients ratios and N availability was also ascertained. To summarize the results obtained and transpose them to reality, the practicality of applying either the raw manure or the sub-products obtained with the slurries treatments was determined. These results were published in the Journal of Cleaner Production.

Chapter III - The effect of manure-based fertilizers on nitrogen mineralization and greenhouse gases emissions after soil application: In this chapter, the first stage of producing manure-based fertilizer was explored, with more focus on N mineralization, to understand the alteration on N releasing rates and interaction with the soil through an aerobic incubation, and on the consequences on greenhouse gases emissions, since those mixtures can foment gaseous emissions. In this first stage to obtain the intended N:P ratios, small amounts of MF were added to the chosen raw manures.

Chapter IV - Nutrients' potential leachability in a sandy soil amended with manure-based fertilizers: This chapter is the follow-up to the first stage of manure-based fertilizer production. The nutrients potential leaching was quantified to knowledge the pollution potential of those fertilizers, but also to have an idea of nutrients potential release to the crops. The results from this chapter and chapter III facilitate the inference of manure-based fertilizer manufacturing by mixing manure with mineral fertilizers.

Chapter V - Manure-based fertilizers production: nitrogen and phosphorus dynamic: In this chapter, the second hypothetical scenario was tested, where the manures were blended with other manures to obtain the desired N:P ratios. In this section, similarly to the two above chapters, these possible fertilizers were tested to knowledge the new N dynamic (anaerobic incubation), the potential alteration of gaseous emissions (GHG emissions experiment) and the potential nutrients leached (leaching experiment).

Chapter VI - Assessment of the agronomic value of manure-based fertilizers: This chapter terminates the appraisal of the agronomic value of all the possible mixtures studied in chapter V and the considered best blends in chapters III and IV. The promising fertilizers were utilized in basal fertilization to nurture oat for three months. The yield production, N and P exportation and the apparent recovery were inferred to conclude the feasibility of those MBFs.

Chapter VII – Conclusions and final considerations: The final conclusions link all the results and survey the three years of experiments to summarize the deductions. The future perspectives of this line of work are also analysed.

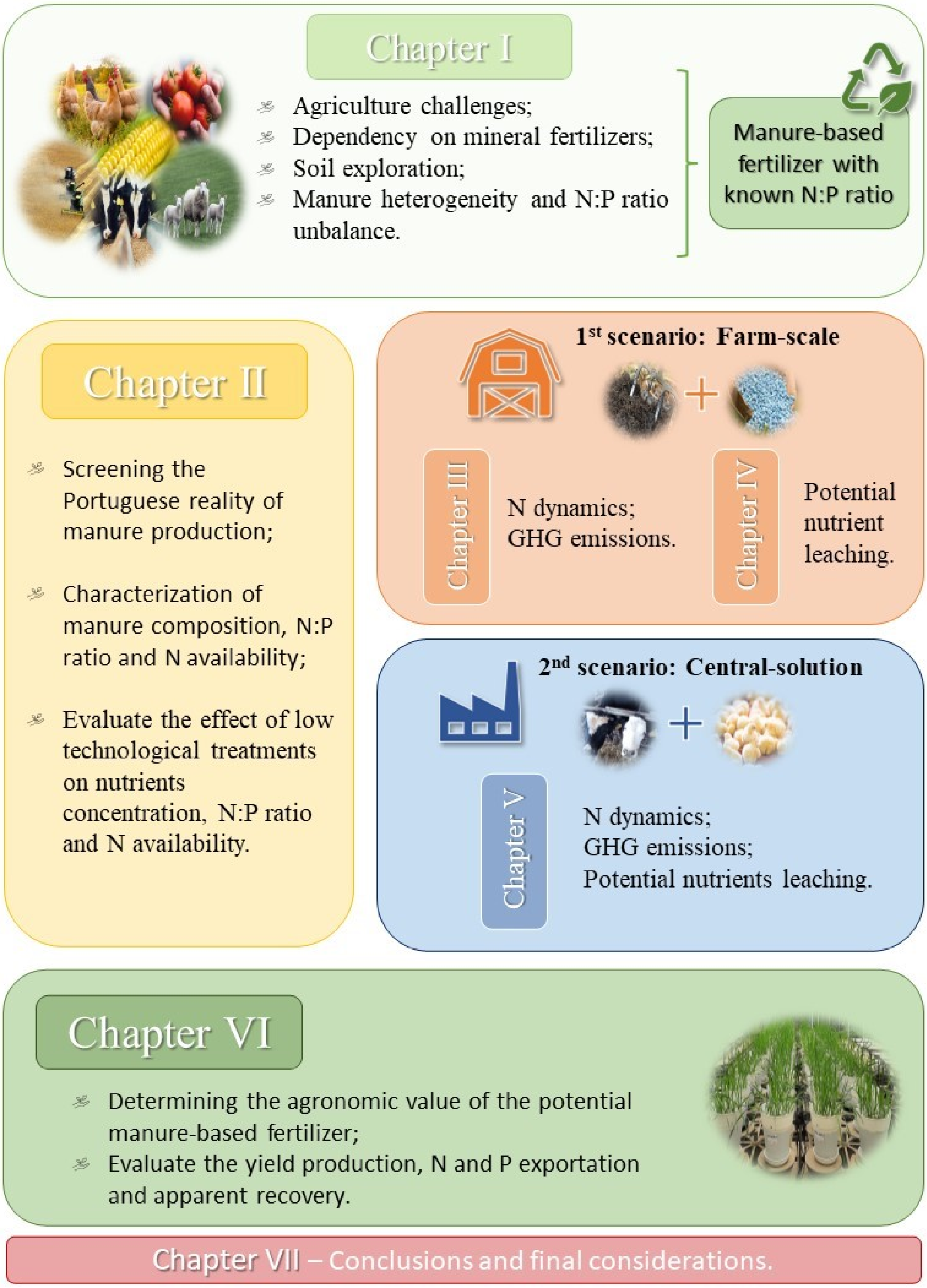

Figure I: Graphical structure of thesis organization.

Chapter I Introduction

1.1. Agriculture challenges

Crop production and agriculture’s challenges, which the sector needs to face to guarantee crop productivity and feed the population worldwide, have been the aim of many studies (Fróna et al., 2019). One of these challenges is the fact that, by 2050, the population is expected to reach 9.7 billion increasing the food demand (Green, 2019; UN, 2013). Second, the size of the urbanized area is expected to increase in the next ten years, reducing the arable land for agriculture (D’Amour et al., 2017). Third, the western diet, which considers high meat content, dairy products and eggs consumption, is now widespread in the world, leading to high pressure on the livestock sector (Fróna et al., 2019). The proportion of land used for agricultural activities depends, also, on the evolution of eating habits, which can be altered and, consequently, reduce the exponential urge of yield production.

Agriculture has many obstacles to overcome in order to comply with both people's and the environment's demands. To achieve this balanced conundrum, adjustments need to be considered and performed to the current agricultural practices. Restoring soil health, altering crop, water, and nutrient management, be conscious of environmental problems can become the first steps to achieve the desired balance (Loboguerrero et al., 2019). Conserving and ameliorating soil health is imperative to accomplish better yield and preserve biodiversity. However, the dependency and improper use of several inputs, e.g. pesticides, fertilizers, irrigation, etc, impacted negatively soil fertility and are antagonistic to a sustainable agriculture (Dar and Bhat, 2020). Nowadays, these inputs continue to be essential to increase crops’ productivity and feed the population worldwide, especially fertilizers (Pahalvi et al., 2022).

Additionally, agriculture is severely affected by fossil fuel prices, to acquire mineral fertilizers (MF), machinery, seeds, and crop protection products, which will be the main justification for the increase in farmers operating costs (EC, 2011). This dependency impacts both food prices and food security (Popp et al., 2014). Therefore, research for a more sustainable and green agriculture has been implemented, with special emphasis on diminishing food losses (Clark and Tilman, 2017).

The present era is, also, marked by climate change, and its impacts are already noticed in crops yields, e.g., maize and wheat yields declined by 3.8% and 5.5% respectively, and are expected to worsen due to increasing temperatures that surpass critical physiological thresholds (Diaz and Moore, 2017). Nevertheless, agriculture is also responsible for part of the climate changes, predominantly with gaseous emissions derived from enteric fermentation, manure management operations, MF application to soil, and biomass combustion (Fróna et al., 2019). The growing necessity for increasing yield production to feed the growing population will increase the impacts of agriculture on the environment if no actions are taken. As considered by the cited authors, one of the main causes of the emissions is conventional agricultural practices, which may contaminate the ecosystem by polluting water and soil. To overcome this subject, the European Union (EU) created a common agricultural policy (CAP), in order for the sector to be able to respond scientifically and technically to the demands of the current challenges (Eurostat, 2020a). The mentioned report aimed to emphasise the role of farms and farmers in mitigating climate change, by creating rural areas that preserve the rural landscape, the environment and guarantees food supply. CAP considers the dynamic of several factors, such as economic, environmental and socioeconomic challenges, which keep altering and may difficult the achievement of a sustainable agriculture.

One other consequence of climate change, with an impact on agriculture, is the alteration of spatiotemporal temperature and precipitation patterns, which have culminated in water shortage and increased evaporative rate (Flörke et al., 2018). Water is a parameter essential for crops and can assist in predicting yield productivity (Lowry et al., 2019). The Mediterranean region is extremely exposed to drought and this is expected to exacerbate in the future (Gu et al., 2020). Droughts in the Mediterranean tend also to occur not only during the dry season but also during the wet season, which has consequences on water resources, water reserve in dams and water supply (Tramblay et al., 2020). Nonetheless, the urban water demand will increase by 80% by 2050, competing with agricultural needs (Flörke et al., 2018). Hence, solutions to overcome this problem are crucial, since due to climate change droughts are becoming more frequent and are expanding geographically worldwide, increasing the necessity of irrigation.

Agriculture also faces an urge to mitigate its global gases emissions. Countries' contribution varies significantly, for instance, China, India, Brazil and USA contribute with 39% of the emissions of methane (CH_4) and nitrous oxide (N_2O) (Loboguerrero et al., 2019). The achievement of worldwide food supply and security needs to be projected in coherence with the mitigation of gaseous emissions, like greenhouse gaseous (GHG) emissions, as referred by the cited authors. The GHG and ammonia (NH_3) emissions have consequences for both humans and the environment. They have a direct impact on air quality, due to their contribution to tropospheric ozone formation, with non-methane volatile organic compounds, nitrogen oxides, carbon monoxide and CH_4 (Eurostat, 2020b; Hassouna et al., 2017a). The European Green Deal was created in the EU, which aims i) to turn Europe into the world's first climate-neutral continent, by transforming the economy through a clean energy, sustainable industry and a circular economy, and ii) to instigate sustainable development, structured around Sustainable Development Goals. The European Green Deal suggest building and renovating the whole food chain, with programs like the one entitled "From Farm to Fork", which aims to make food systems fair, healthy and environmentally friendly. In the present EU report, (Eurostat, 2020b), from 1990 until 2018, the GHG emissions were mitigated by 21%, indicating a good strategy to achieve the further goals of decreasing the emissions by 40% by 2030. When specifying the Portuguese case of GHG emissions, agriculture, forestry and fishing activities emerge as the fourth sector to contribute to them (Figure 1.1). It is essential for farmers to alter the current practices, in order to ameliorate the ecosystems health, compared to the deterioration observed.

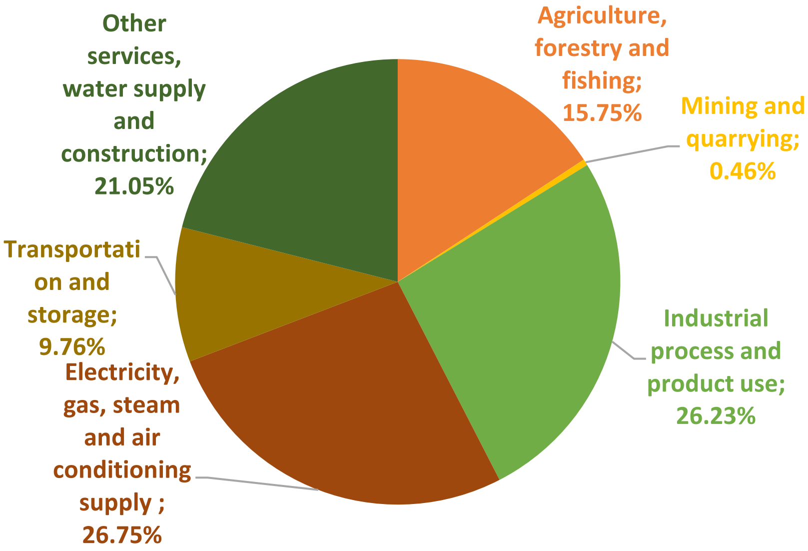

Figure 1.1: The different sectors contribution of GHG emissions in Portugal, adapted from Eurostat (2020).

Several targets for 2030 have been set to accomplish sustainability, within the EU's Farm to Fork Strategy, which aims to accelerate the transition to a sustainable food system, and pledges to act on the reduction of both MFs and nutrient losses by at least 20% and 50%, respectively (Eurostat, 2020a). Nonetheless, in the same report, the consumption of nitrogen (N) and phosphorus (P) in MFs remained high, reaching the maximum consumption of 11.8 million tonnes in 2017 (Figure 1.2). The consumption of N and P as MFs increased by 6.9% and 21.9%, respectively, in the last 10 years, demonstrating the dependency of the EU on MFs use in agriculture (Luo et al., 2021).

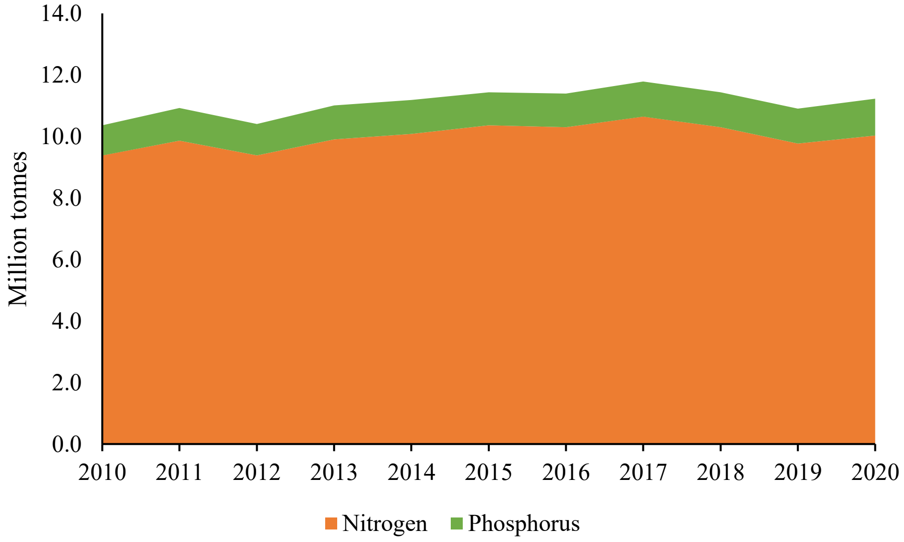

Figure 1.2: The nitrogen and phosphorus mineral fertilizer consumption in the European Union between the years of 2010 and 2020, adapted from Eurostat (2022).

The crescent consumption of MF could be attributed to the demand for higher crop production. However, other factors should be considered, for instance, the balance of N and P, which represents the difference between the nutrient applied and the amount consumed. Not all nutrients applied are absorbed by the crops and this fact can generate an excessive nutrient concentration in the soil, which can become unsafe. The latest indicator, for the EU-27 in 2013-2015, disclosed a decrease in the N balance, compared to the period of 2004-2006, representing a decrease in the use of MF. In spite of the decline in the balance of N-fertilizer consumption to 47 kg N ha^{-1} $year^{-1}$, the N applied to the soil was superior to the amount of N consume, which continues to imply a surplus of N from 2013 to 2015. The P balance from 2013 to 2015 was 1.2 kg P ha^{-1} $year^{-1}$, which also implicates a surplus of P application to soil. The persistent surplus trend observed in past years potentiates i) environmental problems, e.g. P runoff and P and nitrates (NO_3^-) leaching, which can lead to the contamination of surface and underground water, and ii) impairs achieving a sustainable agriculture as a result of soil degradation or deterioration of soil's fertility (Eurostat, 2020a; Vance et al., 2021). The use of MFs in agriculture has grown, since they vehiculate nutrients already in assimilable forms (mineral forms), fomenting higher yield and, in part, preventing soil nutrients exhaustion (Rütting et al., 2018). However, their use in agriculture also presented several inconveniences, especially when improperly used. For example, the sole application of MFs after many years can deteriorate soil's health, since they only vehiculate nutrients and do not restore soil organic matter content, which can impair carbon (C) sequestration, can disturb the soil fauna (e.g., earthworms), can stimulate nutrients losses and, even when applied properly, they represent a costly material (Pahalvi et al., 2022). Some of the general characteristics of using MFs, which depend substantially on the application technique, are synthesized in Table 1.1.

Table 1.1: Synthesis of mineral fertilizer (MF) benefits, risks and limitations according to Pahalvi et al. (2022).

Benefits	**Risks**	**Limitations**
✓ Crop development becomes more accurate.	✗ Deteriorate soil's health.	⊗ High costs
✓ Stimulate higher yields and healthier crops.	✗ Disturb some elements in soil properties.	⊗ Vulnerable areas
✓ Faster crop growth.	✗ Stimulates NO_3^- leaching.	
✓ Vehiculate nutrients quicker to the soil (mineral forms).	✗ Destroy fauna that contributes to soil fertility, e.g. earthworms.	
✓ Present nutrients in an adequate balance for crop needs.	✗ Even applied properly, MF is a part of farmers' costs.	
✓ Improve the crops' resistance to adversities, due to improvement of crop's quality.	✗ Soil overly explored will reduce the organic matter content, reduce C sequestration, and contribute to soil compaction.	

Modern society become dependent on the production of N MFs, synthesized via the Haber-Bosch process, very energy-demanding (Luo et al., 2021; Sigurnjak et al., 2019). As referred, the misuse of N MF is most likely to contribute to environmental problems, but the N deficiency will limit crop development (Rütting et al., 2018). These authors, referred that, researchers are addressing the topic of soil fertility vs. N management, improving the N use efficiency (NUE) while reducing the N losses. The NUE is diminished when the fertilization plan is done incorrectly, attending to crop and soil type. N has a reactive nature, hence N can easily be transferred out of the agricultural ecosystem

(Carswell et al., 2019). The combined application of organic and inorganic fertilizers, or the adoption of better soil and crop management, might reduce the N accumulation in soil, avoiding N losses and increasing the NUE (Rütting et al., 2018).

Phosphorus is, also, essential to guarantee appropriate crop growth, and it is originally obtained from non-renewable phosphate rock mines, which are concentrated in regions with geopolitical problems (Akram et al., 2019; Nesme et al., 2018). China, USA, Morocco and Russia are contributing to about 75% of the world's total P production, indicating that areas like the EU are dependent on P importation (Kataki et al., 2016). The current agronomic practices are leading to the collapse of the global P reserve, which has a finite capacity. The development of P recovery strategies is imperative.

Concerning the Portuguese case, it is one of the EU-27countries with the lower application of N and P fertilizers, with an apparent consumption in 2020 of 31 kg ha^{-1} of agriculture area, half the average value in the EU-27 (INE, 2022). This consumption was the fourth lowest in the past 25 years in Portugal.

Two other subjects that impacted the use of MF were the Covid-19 pandemic and the war in Ukraine. These culminated in higher prices for MF, either by the necessity of closing the borders or due to the sanctions imposed on Russia. Russia is one of the main areas that produce P fertilizers. The production of N fertilizer relies on greater inputs of energy (oil, natural gas), and Russia is one of the main responsible for exporting energy into the EU, therefore the energy costs are augmenting within the EU (Eurostat, 2020a). In this context, the MF production costs increased exponentially, as well as the costs associated with their transport and application, raising farmers' expenses. Consequently, and considering all the negative impacts of MF application, it is imperative that other approaches are considered and adopted, such as nutrient recovery or recycling.

Agriculture consists of an ecosystem, it is expected to have fluxes of nutrients between the different compartments, as well as several inputs (e.g., fertilizers, soil organic amendments, irrigation water) and outputs (e.g., crops productions, gases emissions, leaching). The challenges agriculture is facing are the consequence of current practices, but also of climate changes, which resulted in nutrients losses. A step to achieve a green agriculture, more safe and sustainable is to close the nutrients loops, mitigate nutrients losses, improve crops quality, and promote a healthy and richer agroecosystem (Mažeika et al., 2021).

1.2 Nitrogen cycle

To close the nutrients' cycles, it is imperative to understand them properly and acknowledge their key points. Nitrogen is an essential nutrient on Earth and is important to perform environment redox chemistry, but it is also fundamental to life's biomolecules (Zhang et al., 2020). Nitrogen is an element that composes deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) molecules and it is essential for the formation of amino acids to produce all the proteins and enzymes and for the formation of ribulose-1,5-bisfosfato carboxilase oxigenase (RuBisCo) (de Bang et al., 2021). The biggest N reserve is in the form of dinitrogen gas, which is inert for the organism to use directly, only a small group of bacteria and archaea can fixate N (Kuypers et al., 2018; Zhang et al., 2020). Nonetheless, the majority of the organism used N in the form of ammonium (NH_4^+) or nitrate (NO_3^-) (Kuypers et al., 2018). Therefore, the microorganism involved in the N cycle catalyses a diversity of enzymes to produce N molecules in assimilable forms. For instance, these enzymes can mineralize N organic molecules into all sorts of N molecules, including NH_4^+ or NO_3^-, so that organisms can utilize N (Soler-Jofra et al., 2021).

Comprehending the N transformation is crucial for N management and reducing the N losses during crop production (Ju and Zhang, 2017). The assimilation of mineral N is more common in the form of NH_4^+ or NO_3^- in plants, phytoplankton, fungi and microbes. The NH_4^+ absorption requires the incorporation of this ion into amino acid, while the NO_3^- assimilation is more metabolically costly, since the N needs to be oxidase firstly into ammonia by both nitrate and nitrite reductases (Zhang et al., 2020). Crops tend to absorb preferentially NO_3^- and NH_4^+, which depends on several factors, e.g., plant type, environmental conditions and the ratio of NH_4^+/NO_3^- (Xu et al., 2019). Apart from anoxic conditions, NH_4^+ is rarely accumulated in the system. For the NH_4^+ and NO_3^- to be assimilable by crops, it is necessary to occur mineralization, in which the organic N molecules are converted into N mineral forms. One other step in the N cycle is nitrification, where NH_4^+ is oxidized by oxygen into NO_3^-. When oxygen is scarce, N_2O is formed by denitrification, using NO_3^- as a terminal electron acceptor for respiration (Stein, 2019). The NH_4^+ and NO_3^- ions can also be consumed in biochemical processes, since N drive most of the biological transformations (Zhang et al., 2020). As mentioned by the cited authors, these transformations are the culmination of microbes' activities, which need to support their growth and fulfil the energy requirements, the N cycle is often referred to as the "microbial N cycle".

In any nutrient cycle, there are inputs, such as fertilizers or plants residues, and outputs, considered crops uptake, gases emissions, leaching, runoff and erosion. A schematic representation of the N cycle can be observed in Figure 1.3.

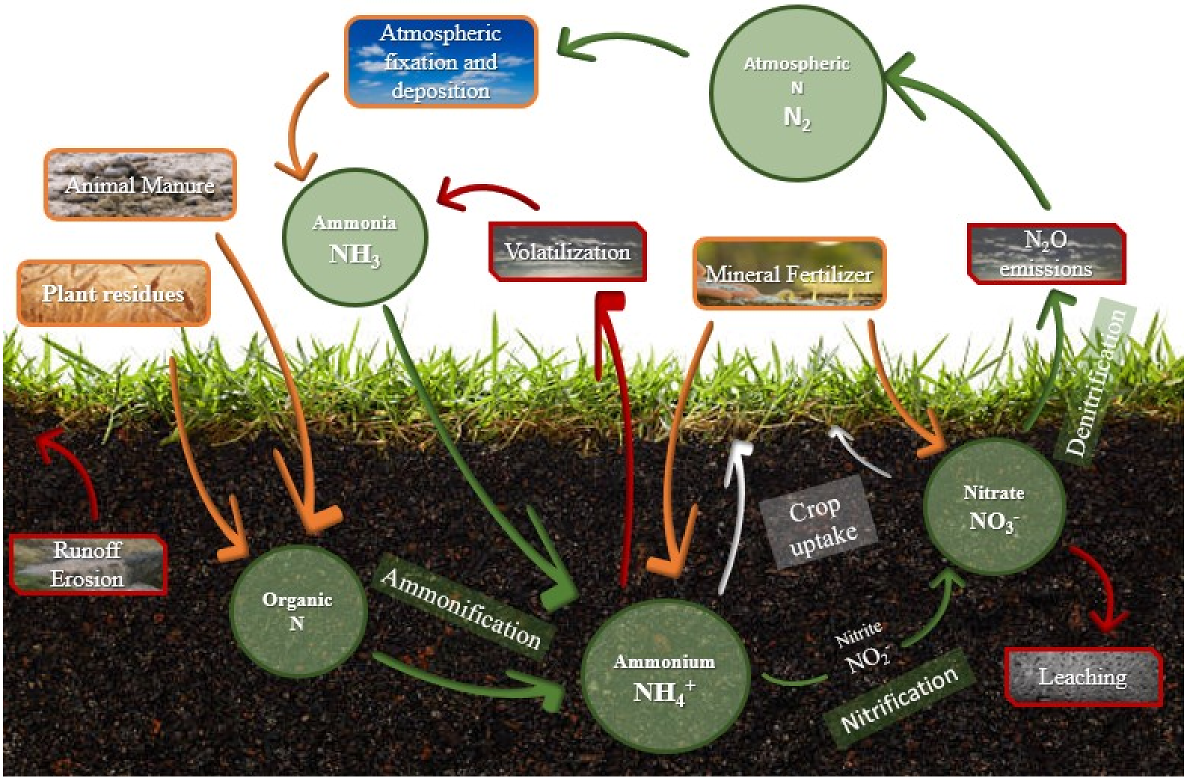

Figure 1.3: Representation of N cycle, adapted from Shields (2020).

The N outputs can represent N losses, and lead to environmental problems. One of these losses occurs as NH_3 volatilization, which contributes to the formation of aerosols, causing acid rain (Fangueiro et al., 2018). Denitrification leads to N_2O formation, contributing to GHG emissions (Petersen, 2018). The N_2O emissions, besides being a gas with a higher impact, with a global warming potential 265-times superior to carbon dioxide (CO_2), contribute to acid rain, the formation of tropospheric ozone and the degradation of stratospheric ozone (Hassouna et al., 2017b; IPCC, 2016). Soil's colloids have a negative net charge but may have become positive in Tropical and sub-tropical areas (Xu et al., 2016). When soil colloids are charged negatively, they can adsorb cations.

Therefore, since NO_3^- is an anion, it is repelled and can not be absorbed by the soils' colloids – mineral or organic. NO_3^- is considerably soluble in water, and therefore, prone to leaching, which represents N losses from the agricultural soils with consequences to the surface and groundwater quality (Padilla et al., 2018). The NO_3^- leaching and runoff are one of the biggest sources of groundwater pollution and it is favoured in sandy soils due to the lack of organic matter and clay content (Ju and Zhang, 2017).

1.3 Phosphorus cycle

Phosphorus is one of the macronutrients essential for crops' growth, especially to transfer biochemical energy, in the form of ATP, for metabolic reactions and is also necessary to form DNA and RNA formation (de Bang et al., 2021). Phosphorous is also fundamental for the creation of radicular nodules, and the absence of this element can prevent this symbiosis, limit the number of nodules, or even the N fixation by the legumes (Júnior et al., 2017).

The P cycle is considered "tight" since the P transference between the different cycle components occurs after longer periods (Yuan et al., 2018). The primary intervenient in the P cycle are bacteria, fungi and plants, that conveyed mineral P from fertilizers or other P sources (Sohrt et al., 2017). The fraction of P available for crops is diminutive, normally under 5% of the soil total P and the majority of P is associated with primary minerals, secondary minerals or organic compounds (Hou et al., 2018a). Crops can only assimilate free orthophosphate ions, hydrogen phosphate (HPO_4^-) and dihydrogen phosphate ($H_2PO_4^-$) (Cerozi and Fitzsimmons, 2016). The availability of these ions in the soil depends on soil pH, while the $H_2PO_4^-$ has a range between 2.1 to 7, the HPO_4^- is favoured when the soil pH is above 7.2 (Cerozi and Fitzsimmons, 2016).

The bioavailability is affected by organic P molecules, since i) they compete with HPO$_4^-$ for adsorption onto positive surfaces, which depends on pH, and ii) this organic P can be absorbed by metal ions, such as iron and aluminium, forming complexes, affecting the global dissolution and precipitation equilibrium (Sohrt et al., 2017). Additionally, the enhancement in the P cycle can stimulate C and N cycles, because it alters the C:P and N:P ratios of the ecosystem, modifying its equilibrium (Yuan et al., 2018). A summary of the P cycle can be found in Figure 1.4.

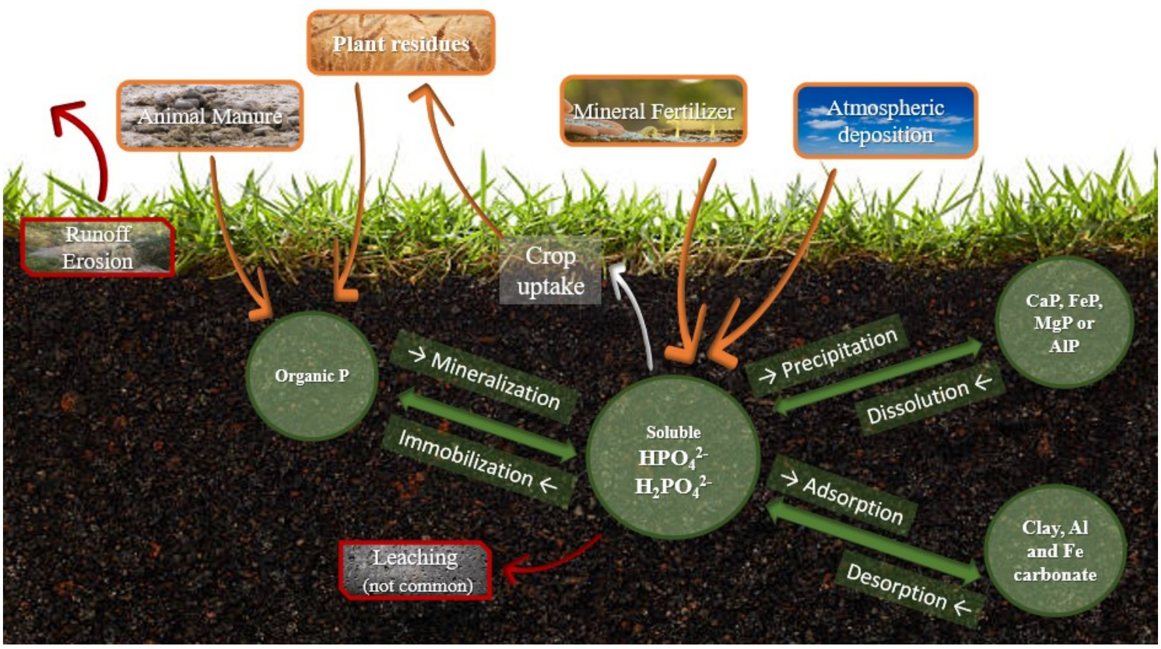

Figure 1.4: Representation of P cycle, adapted from Shields (2020).

The P cycle gained input that exponential the outputs in the past decades, e.g. the escalated use of P-MF in agriculture and the subsequent losses to surface and underground water. All of these features combined, compromise the P natural resources and the water quality, due to the potential leaching associated with P (Nesme et al., 2018). The changes referred to by the authors, were attributed to alterations in the trade of agricultural products, with greater importance after the pre-industrial era. The dependency on the P-MF affected the P movement, which, combined with inadequate MF application, and with other factors, such as manure management, propitiated P losses. Also, the livestock sector represents a P input, which increased two times in the last 50 years (Yuan et al., 2018). Nonetheless, the P contained in livestock products (eggs, milk, meat) triplicated, which could represent a decrease in the losses in the P cycle (Chen and Graedel, 2016). When the input of P is through manure, an increase in the soil P reserve can be observed, but this usually occurs after years of manure application or after the application of massive amounts of manure (Van der Bom et al., 2017).

1.4 Manure management

1.4.1 Livestock's Portuguese reality

Animal production, and the tendencies of the livestock market, can be used to calculate the quantities of manure produced and to predict future trends. The consumption of meat and milk products will be kept constant, which subsequently, the generation of manure. Even during a pandemic, in Portugal, meat production in 2021 grew by 1% compared to the homologous year (INE, 2022).

From 2009 to 2019, the number of animal farms in Portugal decreased by 28%. Still, the size of the farm increased, raising the productivity up to 9% in 2019 compared to 2009. If the number of farms decreases, the manures production could be compromised, but as seen, the farm size increased, reason why the manure production is expected to continue. For instance, from 2009 to 2019, the number of cattle farms decreased by 27.8%, but the number of animals within the exploration increased by 53.2% (INE, 2021). The animal distribution, and therefore the manure production, differ geographically. While the beef cattle farms are concentrated in Alentejo, with 2/3 in extensive production, the dairy cow farms are mainly in Douro and Minho and Açores areas, corresponding to 34% and 39% of the dairy cows in Portugal, respectively. Still, to note, the number of dairy cows decreased by 12% compared to 2009. The swine production has 88% of the total farms in Ribatejo and West. The majority of the farmers with goat and sheep production, small farmers, sold the animals, which was more notoriety on goat production. However, the number of animals per farm increased (INE, 2021).

The referred information has consequences on manure production and, therefore, on the viability of manure as a solution for farmers to adopt as an alternative fertilizer. The majority of the farms are specialized in animal production, and only a small fraction of agricultural explorations combine crop and animal production (~12% of the total agricultural explorations) (INE, 2021). The reduction of the farms that combine crop and animal production, from 2009 to 2019, was higher than the specialized farms, reaching a decrease of 32.3%. Within the Portuguese reality, the manure is essentially used on-farm, therefore, the reduction in the combined farms decreases the land available and increases the surplus of manure. According to Nunes et al. (2022), in 2022, the percentage of the agricultural area which received manures in mainland Portugal was under 20%, indicating that the area covered represents a small part of the agricultural area.

The geographic distribution of manure production in Portugal is represented in Figure 1.5. As observed, the majority of cattle manure is produced in Entre o Douro e Minho, because most dairies are located in this area, as referred before, while Alentejo and Ribatejo and Oeste present a great number of pig farms (Nunes et al., 2022). The distribution of manure and slurry production also depends on the animal species (Figure 1.6). As expected, poultry production is mostly associated with solid manure, while pig farms are mainly associated with slurry production.

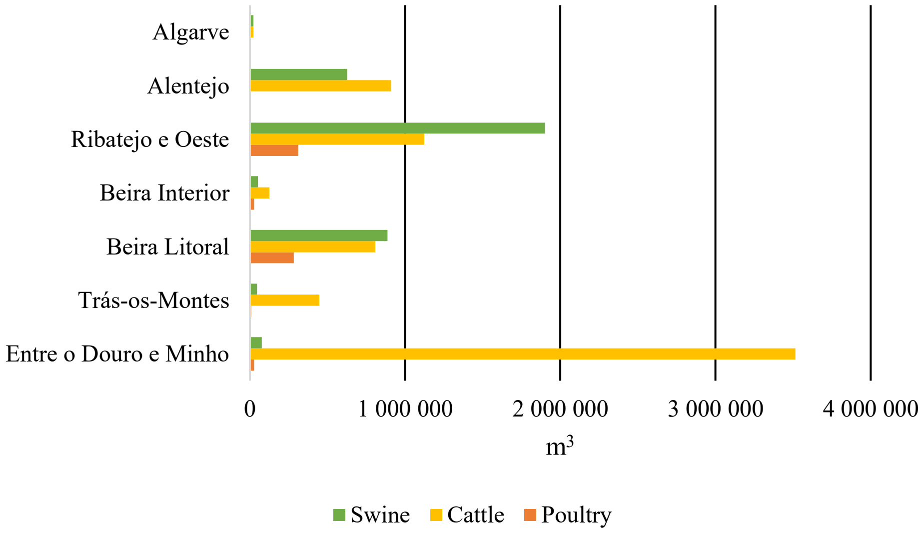

Figure 1.5: The distribution of manures (solid and slurry) of swine, bovine and poultry animals, in Portugal according to Nunes et al. (2022).

In fact, it is important to acknowledge the existence of two types of manures: i) solid manure, a mixture of faeces and urine with a material of plant origin (straws, weeds), which can also include the solid fraction of slurry, that does not present any liquid runoff and ii) slurry, a mix of animal faeces, urine and washing water, that can contain waste from animal feed or bed or runoff from silos (Ambiente e Ação Climática e Agricultura, 2022). Also, according to Portuguese law, manure is considered solid manure when the dry matter content is equal to, or above, 20%, while slurries have a dry matter content under 20% (MADRP, 2018). Solid manures are more concentrated and richer in organic C, important to restore soil organic C and ameliorate C sequestration. The slurry is a diluted material, rich in water content. This factor is important when dry season and water shortages are becoming more frequent and other water resources become essential. Nonetheless, this dilution of nutrients' characteristic of the slurries, increases the amount of slurry needed to suppress crops' needs and, in some cases, may turn its application, with that purpose, impracticable (Sigurnjak et al., 2017c). Also, the manure composition varies significantly with species, feed habits, storage and many other factors (He et al., 2016; Miller et al., 2018).

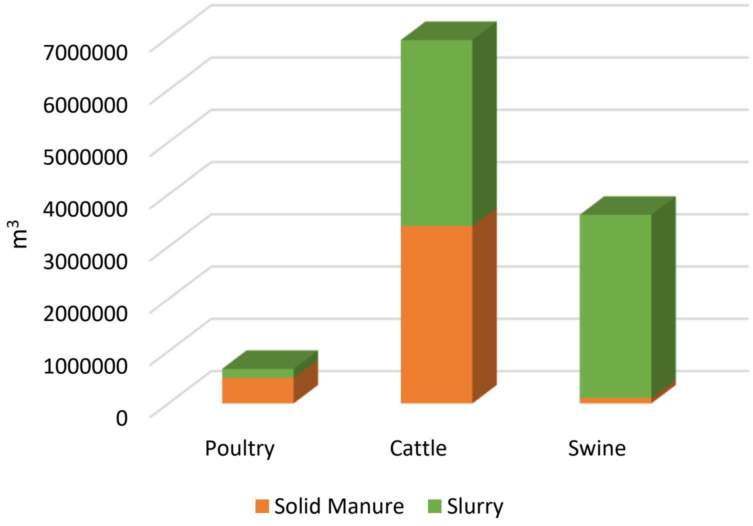

Figure 1.6: The distribution of solid manure and slurry production, according to the animal species (Nunes et al., 2022).

1.4.2 Manure use in agriculture

Agriculture includes the livestock sector, which is responsible for the production of meat, fish, milk and derived products (Fróna et al., 2019). To achieve a production sufficient to feed the world, the animal sector intensified and specialized its activity, by selecting the animal species that better suited the feeding necessities and increasing the size of farms and stables animals (Eurostat, 2018). Consequently, a massive quantity of manure is produced and accumulated on livestock farms. More than 1.4 billion tonnes of manure were produced from 2016 through 2019, in the European Union and the United Kingdom (Köninger et al., 2021). The application of manure in agriculture is an ancestral technique, adopted to fertilize agricultural soils – in this period, the manure was, in fact, the only fertilizer used (Hills et al., 2021). During the XX century, in virtue of farmers' preference for MF, livestock and agriculture started to grow into two different sectors. This indicated that while some areas focused more on agricultural production, others explored the animal sector. Hence, the separation between agricultural and livestock areas started to emerge, increasing the nutrient losses and the production costs to farmers (Wang et al., 2018). This had repercussions on manure management, since it is inconceivable to transport manure for high distances, especially slurry (higher water concentration), due to the costs and logistics associated (Silva et al., 2022b). Therefore, the dissociation of both sectors, difficult the reuse of a valuable resource and the closure of the nutrients' cycles.

The production of manure is inevitable when considering animal production. In 2014, N input into the soil was divided between the MF and manure application (Eurostat, 2020a). Indeed, in the last 10 years, manure regain importance as a fertilizer, mainly due to the production cost associated with MF (Fangueiro et al., 2018). The application of manure to the soil will decrease the surplus of those organic materials, since more manure will be considered and applied as fertilizer. Manure plays an important role in the ecosystem and can be a solution to recycle nutrients. The animal distribution is concentrated in Denmark, Netherlands, Northern Germany and Western France, representing a third of the animals in the EU and United Kingdom (Fangueiro et al., 2021). Therefore, the manure production for some countries differs from others, in the quantity and in the animal species which produced the manure (Figure 1.7).

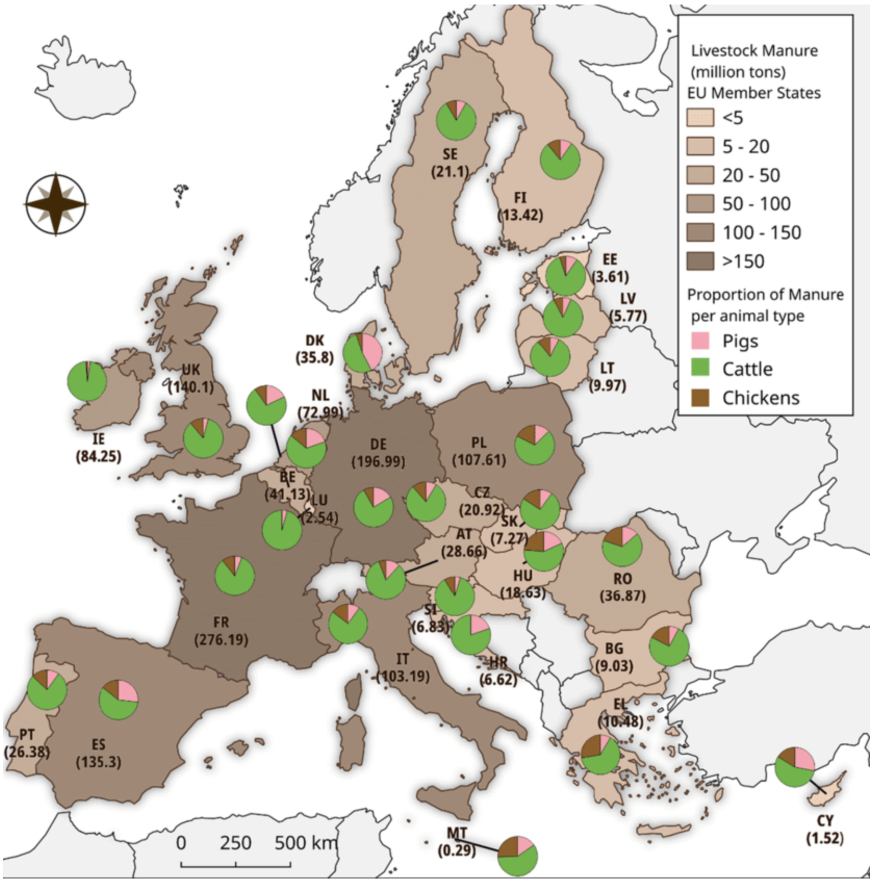

Figure 1.7: The manure distribution in the EU and United Kingdom, during 2016-2019, according to the animal species and country (Wang et al., 2018).

1.4.3 Benefits of manure application to the soil

The application of manure is a resourceful form to supress crops' needs. Also, the benefit of applying manure depends on the manure origin, soil type and handling (Li and Li, 2014). Manure is a source of both N and P, and it vehiculates other macro and micronutrients, equally essential for a healthy crop and better yields (de Bang et al., 2021; Prado et al., 2022). If within each country, the manures were applied to agricultural soils, their dependency on MFs would decrease, and the necessity of exploring non-renewable resources to produce MF would be reduced, as well as the MF importation. Of course, this strategy would also reduce the farmers' costs, since they would not need to purchase the same amount of MFs.

To achieve the intensification of agricultural practices needed to survey the food demand worldwide, factors, such as soil fertility, were neglected and culminated in the soil organic matter (SOM) depletion (Pardo et al., 2017). This raised the susceptibility to other problems, like soil erosion, very common in Mediterranean soils (Sakadevan and Nguyen, 2017). The European soils have low (<2%) or very low (<1%) contents of SOM (Hinsinger, 2014). SOM is a precursor of soil stability, and the increase in C and N organic molecules enhanced its strength (Sakadevan and Nguyen, 2017). The application of organic materials, such as manures, would help to restore SOM, due to the addition of organic C. The presence of organic C in organic materials, contrary to MFs, helps to retrieve C back to the soil, restore soil C storage, and improve C sequestration, which has diminished over the past years due to soil over-exploration (Rayne and Aula, 2020). Manure application can reduce soil compaction, increasing soil porosity, which would enhance aerobic conditions and decrease some of the GHG emissions (CH_4 and N_2O). Another important feature brought by the SOM increase is the formation of stable aggregates, which can increase the soil water holding capacity since it ameliorates the soil' structure and the resistance to droughts (Hoover et al., 2019; Melo et al., 2019). Considering the current challenge of climate change and water shortage, improving soil water holding capacity will reduce water losses and, perhaps, reduce the amount of irrigation needed.

The continuous application of manure modifies the soil's microbial community, increasing the number of organisms and therefore the soil respiration rate (Segat et al., 2019). Microorganisms perform vital functions in the soil, therefore, by enhancing their activity and diversity, the soil regains its ability to degrade and transform chemicals. Nutrients present in manure perform a fundamental role in the nutrition of soil's microorganisms, promoting biodiversity (Hoover et al., 2019). In a pasture, after 13 years of study, the application of both poultry and cattle manure enhanced the soil microbial biodiversity and potentialize the enhancement of soil health (Yang et al., 2019). The fauna biodiversity of soil fertilized with manure increased to double compared to the soils fertilized with MFs, which increased the number of earthworm species and the number of the individual (Feledyn-Szewczyk et al., 2019; Köninger et al., 2021).

Summarizing, the application of manure as a fertilizer can be beneficial and address some of the problematics that agricultural and livestock production presently faces: i) reduces the accumulation of manure, and decreases their impact on the environment; ii) reduces the necessity of applying MF, which is important to reduce farmers costs; and iii) ensures that the nutrients' cycles are closed, by retrieving them back to the soil (Kalappanavar and Gali, 2018; Malomo et al., 2018; Melo et al., 2019).

1.4.4 Risks and limitations of using manure as a fertilizer

One major drawback of using manure as a fertilizer is that it started to be applied to the soil to address the surplus of manure, in areas where the land available for its use was insufficient, and without considering the need to limit this practice (Dumont et al., 2019). Usually, manure is considered an organic amendment and, when applied to soil, farmers account for the organic matter vehiculated and not for the nutrients which, by this means, may be applied in excess (Horta et al., 2018). According to the authors, this was a consequence of manure being mostly applied on the farm of origin, leading to the geographical concentration of manure application. Being a source of both macro and micronutrients, the amount of zinc and copper conveyed through manure can be toxic to soil and crops. Therefore, the quantity of heavy metals vehiculated through animal manures application to soil is now restricted by law (Fangueiro et al., 2021; Segat et al., 2019). Also, manure can be a source of pathogenic agents. Hence, when applying fresh manure, especially in horticulture, it may lead to food contamination (Alegbeleye et al., 2018), becoming imperative to perform hygienization prior to soil application (Rodrigues et al., 2021).

The livestock sector is responsible for 14.5% of all anthropogenic GHG emissions worldwide (Kipling et al., 2019a). To better understand GHG emissions, it is important to divide manure management into three stages: i) housing, ii) storage and iii) soil application. In the housing stage, CO_2 emissions are associated with animal breathing, but also with the hydrolysis of urea into NH_3 and CO_2 and with organic matter the anaerobic fermentation (Philippe and Nicks, 2015). As referred by the authors, CO_2 emissions are majorly observed in solid manure, due to the enhancement of the organic matter degradation rate. Chadwick et al. (2011), detected N_2O emissions during animal housing, due to the presence of bedding material (straws). The cited author attributed this to the lower nitrification rate and anaerobic conditions, as a result of the accumulation of faeces and urine. Nonetheless, Sakadevan and Nguyen (2017) also referred that the N_2O occurs during the storage of manure, due to nitrification and denitrification processes. However, these emissions can be negligible, if no surface area is considered, since only with the formation of crust it is possible to occur nitrification and onward denitrification

(Chadwick et al., 2011). The anaerobic condition associated with the storage phase, foments the CH_4 emissions, due to the organic matter degradation in the absence of oxygen molecules.

The gases emissions during the phase of soil application are affected by several factors, e.g., temperature, frequency of irrigation/precipitation, technique for manure application, etc (De Rosa et al., 2018). For instance, the CH_4 emissions can be attributed to enteric fermentation, anaerobic decomposition of organic matter, manure deposited on fields, or liquid manure management systems (lagoons) (Sakadevan and Nguyen, 2017). The N_2O emissions required a symbiosis of aerobic and anaerobic areas, which are fomented in soil, especially after solid manure application, compared to the slurry application (Philippe and Nicks, 2015). Several studies have indicated that NH_3 emissions have a great impact on air quality. The emission of NH_3 is a consequence of N presence in manure, mainly as NH_4^+ which can be volatilized as NH_3 (Fangueiro et al., 2018; Silva et al., 2022a). The NH_3 emissions have a tendency to occur during storage, as a result of the ratio of the higher ammonium:ammonia on the manure surface (Prado et al., 2020).

The instability of the organic materials applied to soil propitiates environmental problems. Nitrate leaching is associated with manure application (Cameira et al., 2019). Thereby, it is framed within the EU Nitrates Directive 91/676, especially for the considered vulnerable areas, where it dictates the amount of N that can be applied through animal manures, 170 kg N ha^{-1} (EC, European Council, 1991). Some techniques of manure application were designed to mitigate the referred impacts, like slurry injection, which minimizes NH_3 emission, manure incorporation into the soil, which reduces GHG emissions, or band application, which can be used for instance to mitigate the NO_3^- leaching (Cameira et al., 2019; Kipling et al., 2019b).

The nutrients in slurries are diluted, presenting low concentrations, and their N:P ratio is inadequate for the crops' necessities (Lesschen et al., 2020). Additionally, the fertilization recommendations are based on N crops requirements, which culminated in the overapplication of P to soil (Sigurnjak et al., 2017a). The surplus application of P to the soil, and the build-up in P reserves, emerged as a new reality of P leaching, leading to problems of eutrophication, especially in the north of Europe (Vance et al., 2021). Addressing the imbalance of N:P ratios in manure, considering crops' nutrients requests, would enhance the mitigation of these impacts.

Compared to MF, the precision of N management with manure is not so well defined, and it is difficult to predict the N dynamic in soil (Rütting et al., 2018). The N present in manure is mainly in organic molecules and the mineral N is, predominantly, as NH_4^+ ion (Cameira et al., 2019). To turn the N into organic molecules assimilable by the crop, these molecules need to be mineralized, which is not an instantaneous process. Hence, part of those valuable nutrients won't be immediately available, contrary to MF, which only has nutrients in mineral forms. Acknowledging the period in which N is still in organic molecules, and how long until they start to be mineralized, becomes essential for a farmer to design the fertilization plan, including the manures as organic fertilizers.

One other drawback is that these materials are denoted as smelly. The acceptance of local communities to its application is not always easy, since the smell can be strong for a couple of days (Åkerman et al., 2020). The farms started to grow closer to the cities, due to the rural exodus, and the necessity of people to work on the farms, which intensified the aversion to manure smell. Additionally, due to the rural exodus, cities started to grow, expanding to areas that used to be countryside, decreasing the agriculture surface.

Even if manures are a resource that justifies their application in agriculture, a pragmatic strategic plan is crucial, to ensure their safe use and a genuine option to consider when as a fertilizer, and as an option to achieve a sustainable agriculture.

1.4.5 Slurry treatments

To guarantee an adequate and safe application of manure, several low-technologic treatments are implemented either to address environmental issues or nutrients concentrations (Lesschen et al., 2020). In fact, these treatments are mainly used on slurries, and their adoption usually modifies their nutrient ratio, when compared to the slurry of origin. By performing these treatments a material derived from the slurry, with a distinct N:P ratio, is obtained (Lesschen et al., 2020).

To avoid the over-application of manures in one area, it can be necessary to transport them to a different region. However, in the case of slurries, due to their high water content, it is expensive and can increase nutrients losses, diminishing slurry agronomic value (Gómez-Muñoz et al., 2016). The solid-liquid separation is a technique that can be used to concentrate nutrients in a solid fraction (SOL) (Fournel et al., 2019). Subsequently, from manure solid-liquid separation, two fractions are obtained: i) SOL, higher dry matter content and P concentrated material; and ii) liquid fraction (LIQ), rich in N and potassium (K) (Egene et al., 2021). This technique allows the valorization of the two fractions in a different manner: the LIQ may be used in the farm of origin, and the SOL can be transported more easily than the raw slurry to areas where P-fertilizer is required. This solution has a major benefit, the avoidance of the over-application of P to the soil in the farm of origin (Sigurnjak et al., 2017a). However, by performing the slurry solid-liquid separation, one problem of manure management is solved, but, during this operation, the NH_3 and GHG emissions are stimulated. Moreover, after the application of SOL or LIQ to the soil, these gases are equally emitted, when compared to fresh manure (Fangueiro et al., 2017).

A solution that has been considered to mitigate both NH_3 and GHG emissions, is slurry acidification (Sokolov et al., 2020). This treatment is not optimal to reduce the nutrients losses by gases emissions, as it also diminishes the NO_3^- leached and improves P solubility (Cameira et al., 2019; Fangueiro et al., 2017; Regueiro et al., 2016b). By acidifying the slurry to pH 3 to 5, sanitization is also accomplished, allowing its use in horticulture (Rodrigues et al., 2021). Despite these benefits, the use of acidified slurries could be a concern, since, for instance, its use in Portuguese soils could be a concern, as most Portuguese soils are acidic (Gomes et al., 2019). Nonetheless, in long-term experiments, the soil pH did not suffer any drastic changes after manure or acidified slurry application (Loide et al., 2020). Acidification is conducted with strong acids, such as sulphuric acid, but not all farmers are willing to handle these reactants or acquire equipment that mixes slurry and the acid automatically (Fangueiro et al., 2011). Therefore, new solutions are being proposed to replace those acids, by utilizing sub-products of the agri-food industry to perform bio-acidification, which is equally effective in reducing GHG emissions (Prado et al., 2020).

Some treatments can be performed on manure or slurries at the farm scale, for instance, solid-liquid separation, composting or acidification, while others, such as anaerobic digestion, have a higher impact on slurry characteristics, but are better performed at industrial scales (Hou et al., 2018b). For instance, anaerobic digestion takes advantage of the fact that manure produces a higher quantity of CH_4 and combines the production of a more stable material, the digestate, with the production of biogas, which can be utilized in energy production (Huygens et al., 2020).

Nonetheless, farmers still have some reluctancy in using some of these techniques (separation and/or acidification) due to numerous factors, that differ depending on the European region (Fangueiro et al., 2011). The next table synthesises the three previous sub-chapters, concerning the manures benefits, risks and limitations (Table 1.2).

Table 1.2: Summary of benefits, risks and limitations of manure use.

Benefits	**Risks**	**Limitations**
✓ Stimulates microorganism activity and improves soil health.	⊗ Presence of pathogenic agents.	⊗ It is a heterogeneous material.
✓ Vehiculates organic C, and restores soil organic matter.	⊗ Contain seeds of weeds.	⊗ Imbalance of N:P ratios, compared to crops' needs.
✓ Improves the soil water holding capacity and the resistance to droughts.	⊗ Stimulates NO_3^- and P leaching.	⊗ Low concentrations of nutrients.
✓ Vehiculate several nutrients with one application.	⊗ GHGs and NH_3 emissions.	⊗ Necessity of transport manure to nutrient-deficient areas.
✓ Contribute to circular economy, closing nutrients loops.		⊗ Necessity of performing treatments to reduce the risks.
✓ It can be a cost-free material or have low costs.		⊗ Vulnerable areas, which restrict their use.
		⊗ A large fraction of nutrients in organic molecules, are not immediately available.

1.5 Manure-based fertilizers

Nutrient recycling is a step forward in achieving sustainable agriculture, however, few mechanisms exist to address the surplus of nutrients in manure and the transport to nutrient-deficient areas (Spiegal et al., 2020). Nutrient management at the farm scale needs to be improved, to offer the world more sustainable opportunities without compromising food production. The European Commission (EC), in 2015, proposed a detailed plan to alter the EC Fertilizer Regulation through a circular economy, which would recognise organic and waste-based fertilizers as an EU-wide market trade for those products (Case and Jensen, 2019). This plan includes a minimum target for the recycling and reuse of organic waste, e.g., municipal waste and manures. Concomitantly, the production of manure-based fertilizers (MBFs) appears as a solution to overcome the surplus of manure, recycle nutrients from a valuable resource, and respond to some of the limitations referred to earlier (Table 1.2). Also, the RENURE criteria (Recovery Nitrogen from Manure) was created to facilitate the integration of new fertilizers, based on the concepted of organic materials recuperation. This standard implies the establishment of a new class of fertilizers, which implies the N recovery from manures in mineral forms (Huygens et al., 2020). With the RENURE criteria, the legal framework of these new fertilizers will be similar to those applied to MF, e.g., the legal framework of the NO_3^- leaching after MBFs application would be limited with the same constraints as the MFs. In order to achieve these criteria, manures needed to be transformed and respect several characteristics such as N mineral:$N_{Total} > 90\%$, total organic carbon: $N_{Total} \leq 3$ and their soil application should be similar to the MFs. Summarily, MBFs are an optimized strategy to recover N within the circular economy perspective. It would close the nutrients cycle while mitigating the environmental impacts of the current agriculture practices (Vico et al., 2020).

The farm-scale implementation of MBFs production is still far. There are still several gaps in the knowledge, such as the characterization and performance of those products, also essential to comply with the complexity of the legal aspect (Sigurnjak et al., 2019).

Where comes the necessity of creating bio-based fertilizers or, in this case, manure-based fertilizers? The treatments described earlier, solid-liquid separation and acidification, focused on manures' specific problems, but do not address the farmers' major drawback of using manure as fertilizer, which is their heterogeneity and the imbalance of N:P ratios for crops' necessities. Designing MBFs with specific N:P ratios would increase the manure acceptance by farmers, enhancing the manure value. In fact, this could also reduce farmers' production costs, by reducing their dependency on MFs, a feature that they should be aware of.

The unsustainability currently observed within the agriculture activity could be partially solved with MBFs production and application. Their use should ensure good yields, at least equal to MFs application, restore SOM, and ameliorate soil health (Tur-Cardona et al., 2018). Insofar, the knowledge of manure, its benefits, and its disadvantages, is well studied, but little is known about this innovative concept. The different studies concerning MBFs were conducted according to each country's reality. Luo et al. (2021), studied the application of liquid fraction of digested pig slurry, while Sigurnjak et al. (2019) used ammonium sulfate and ammonium nitrate end-products of (stripping) scrubbing technology from different facilities and Egene et al., (2021) used the solid fraction of digested pig slurry as a possible substitute of MF. Several solutions are being proposed with different approaches, and in either of the cases, the authors demonstrated the benefits of conceptualizing MBFs from manure.

To better understand how these materials would perform once applied to the soil as a fertilizer, numerous characteristics should be considered. The base of these fertilizers is manure, which is an organic material, with a major part of the nutrients in organic molecules. As referred previously, mineralization is the process by which these molecules are converted into inorganic forms, assimilable by crops. This process is not an immediate process and needs to be understood in order to use them properly (Dalias and Christou, 2020). Plants can only absorb nutrients in mineral forms, therefore, when suggesting a MBF, farmers need to have a good prediction of when, in their fertilization calendar, will the nutrients in MBFs be available, i.e., will appear in mineral forms. This need to be studied carefully to inform farmers properly of what to count when applying MBFs to a specific crop and soil, and to avoid nutrient deficiency when the crop is growing.

The impact of manure relative to gaseous emissions might still occur when producing these fertilizers. Hence, this must also be considered when producing possible solutions that can either mitigate or worsen the environmental impact of manure, as well as potential nutrients leaching. Consequently, a detailed study of MBFs is required before scaling-up the production of these potential fertilizers.

The subject of closing the nutrients cycles is being addressed from several fronts in the European project Nutry2Cycle - Transition towards a more carbon and nutrient-efficient agriculture in Europe, in which this PhD work is included (Figure 1.8).

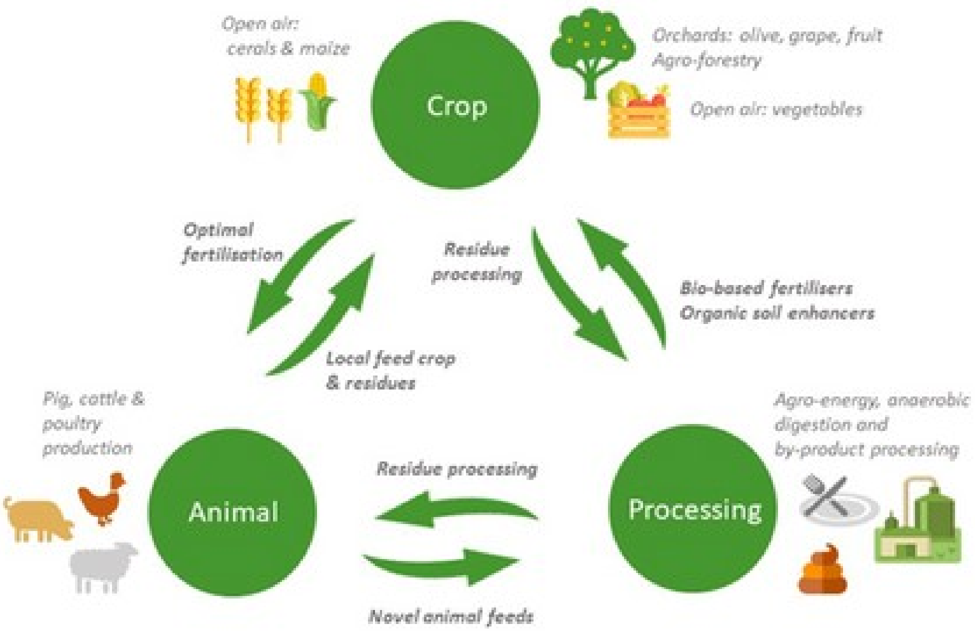

Figure 1.8: The pictogram of the aim of the Nutri2cycle.

1.6. Context and objectives

This thesis emerges within the project Nutri2Cycle, where the main goal is to create more efficient and sustainable farm practices, to boost nutrient recovery and recycling in the different European regions by strengthening the link between animal and crop production. The specific aim of this thesis is to design potential MBFs, with raw or treated manures, with three known N:P ratios equal to 1:1, 2:1 and the 0.5:1, commonly used by farmers with MFs. Therefore, it is expected that the use of MBFs will decrease the necessity of using MFs, and it is also expected to decrease the carbon footprint from fertilizers production and the nutrients losses associated with manure management. This solution will promote circular economy, by using an available resource in many animal farms, restoring the nutrients in the soil, and closing nutrient loops. It will enhance the fertilizer value of manures, by improving their usability, offering a new format for manure application, more adequate and easy handling form for farmers, and reducing, at the same time, the environmental problems associated with their use.

In this context, the first stage of screening the manures/slurries from the Portuguese reality was accomplished. The main objectives for this step were: i) to characterize solid manures and slurries from different species (total macro and micronutrients concentration) and compare their N:P ratios with those required by target MFs; ii) to characterize the N and P availability of the raw manures and slurries; iii) to evaluate the ability of low technological-demanding treatments (e.g., solid-liquid separation and acidification, by their own or combined) to increase the concentration of some nutrients in their different fractions (solid or liquid), changing their N:P ratios to values closer to commercially available MFs; and iv) to evaluate how the N and P availability are affected by those treatments.

After this extensive characterization of manures, two scenarios were considered to design MBFs with the three specific N:P ratio, and test their application: i) on-farm, where the desired N:P ratios and nutrients availability would be obtained by mixing raw slurry/manure from one animal species with MF; and ii) central-solution, where the over-production of raw manure/slurry from different animal species can be exported by livestock farmers, broadening the range of possibilities, to mix the different manures between them at the processing center. Both scenarios were first tested in incubation experiments, with the application of the manure blends to the soil, to evaluate N dynamics in soil, N and C losses through greenhouse gaseous emissions, and nutrients potential leaching. These first stages of scooping each scenario had the intention of better understand the nutrients cycle, with more emphasis on the N and P cycle, through the mineralization rate, but also recognising the potential losses through GHG emissions and nutrients losses. These aspects are essential to assemble a proper description of each MBF, and to know what to expect, which MBFs had potential, which aspects need improvement and which MBFs should be discarded.

Considering the results obtained in the two scenarios, several of the possible MBFs solutions, those delivering the most interesting MBFs, were tested in pot experiments. The aim of the last stage was to assess their agronomic value.

To better understand the present results, several questions were made to idealize the objectives and guide the studies:

1. Is it possible to obtain MFBs with the desired N:P ratios just by using raw manures? If not, can it be solved by treating slurry before making the MBF?
2. Is the application of MBFs at farm scale a viable option? Can the farmer obtain the three N:P ratios with each manure?
3. What are the nutrients dynamics after the MBF application to the soil?
4. Can the design of MBFs close the N and P loops? Or does their application foment nutrients losses, through gaseous emissions or potential leaching?
5. Do the MBFs have an agronomic efficiency at least similar to MFs?
6. Is it possible to partially substitute MF in basal fertilization with MBF?

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Chapter II A step towards the production of manure-based fertilizers: disclosing the effects of animal species and slurry treatment on their nutrients content and availability

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Abstract

In a context of climate change and circular economy, it is fundamental to produce animal manure-based fertilizers attractive to farmers who are reluctant to use heterogeneous materials, poorly characterized and with unbalanced nutrient contents, namely N:P ratios different from plant requirements. Hence, the aim of the present study was to assess the potential of different treated and non-treated animal manures to produce tailor-made manure-based fertilizers with known N:P ratios. Seven manures were considered: cattle, caprine and poultry solid manures, two contrasting cattle slurries and two contrasting swine slurries. Slurries were further treated by solid-liquid separation, acidification, and acidification followed by solid-liquid separation. Raw and treated manures were fully characterized to assess the effect of manure type and treatment on their nutrient contents and N:P ratios, to incorporate them in manure-based fertilizers with selected N:P ratio. The present results were essential to support the manure-based fertilizer production and identify which materials could be considered: it appears clearly that the pig slurry with all stages of production and the correspondent liquid fraction and cannot be used due to their low nutrients content. It was shown that the solid fractions rich in P and with a N_T: NH_4^+-N ratio close to 1 are an excellent material to prepare manure-based fertilizers. Acidification, combined with solid-liquid separation, produce a liquid fraction rich in both N and P and with a N_T:P_T ratio close to 1, with the additional benefit of a slow N-release material. Some of the manures or sub-products obtained after treatment provided the usual N:P ratio required by farmers and could be use directly as substitutes of mineral fertilizers. Nevertheless, the blending of some of these materials has a stronger potential to the production of specific manure-based fertilizer.

Keywords: animal manures; nutrient recycling; nutrients ratio; slurry acidification; slurry solid-liquid separation; animal manures blending

2.1 Introduction

Food security for a population in constant growth, with 9.7 billion expected by 2050 (United Nation, 2019), will only be possible with a high degree of specialization and intensification of agriculture and livestock production (Green, 2019). A highly productive agriculture relies on high rates of NPK inputs, mostly from chemical and mineral fertilizers produced from fossil fuels or other non-renewable sources, like phosphate rock (Akram et al., 2019; Eurostat, 2016). On the other hand, livestock production generates a massive amount of organic residues, with 1.4 billion tonnes of animal manure produced per year in 2014, only at the European Union (EU) level (EC, 2014). In fact, manure management became a serious issue in some EU regions, with the intensification and geographical concentration of livestock farms, causing overload production in some areas where the soil available for manure application, at farm scale, might not be enough. It is crucial to develop sustainable nutrient management strategies, not only to ensure food security but, also, to adopt more eco-friendly practices in agricultural systems (Dumont et al., 2019).

The use of manure as a source of macro and micronutrients in agriculture is an ancient practice that can help to solve this nutrients management conundrum: increasing use of fertilizers originated from non-renewable sources in one side and overload production of animal manures on the other (Malomo et al., 2018; Melo et al., 2019). However, manures need to be handled responsibly, i.e., apply the adequate quantity, based on crop nutrient needs, guarantee good storage condition, and perform the correct practices when applying manure. The use of animal manure as fertilizer has the potential to reduce i) the environmental impact of the livestock sector, ii) the fertilizing costs to farmers and iii) the dependence on mineral and synthetic fertilizers (He et al., 2016), advantages that could boost this practice to regain the importance that once had. Other important advantages, relative to conventional mineral fertilizers, are the additional supply of micronutrients, important for some crops, and organic matter, with the ability to improve soil health. The manure application to soil in the EU is strictly regulated, considering the environmental risks associated with nitrate diffuse pollution (EC, European Council, 1991), and farmers prefer to use mineral fertilizers, not only due to logistic barriers and costs related to raw manure use (e.g., transport, storage, treatment, application), but also because they are reluctant to use materials with a wide range of physical and chemical characteristics and with unbalanced nutrients content (Fangueiro et al., 2018).

The major differences between manures and mineral fertilizers are related to the nutrient's concentration, the nutrients ratios (N:P) and their availability for crops after soil application. Nutrients concentration in manure are much lower than in mineral fertilizers, manures composition vary widely (with animal species, handing practice, bed's composition, storage conditions), their N:P ratios are not similar to the crops needs (Fangueiro et al., 2015b; Webb et al., 2010), and a large amount of N and P in manure exists in a non-readily available form for plants (organic fraction). The manure application rate is usually based on the crop's N demand, often resulting in an over-application of P and other elements, with a negative impact on the environment, for instance the accumulation of copper (Cu) and zinc (Zn) in soil top layer that can become toxic to the crops (Popovic et al., 2012).

Some treatments (e.g., slurry acidification, ammonia stripping, solid-liquid separation) for animal slurries deliver materials that, directly or combined with others, have the potential to increase their value and acceptance. The solid-liquid separation is a technology that produces a solid fraction with higher dry matter content and richer in nutrients, especially P and N (Hjorth et al., 2010), enabling their transport over longer distances. Another interesting treatment is slurry acidification, a solution that mitigate NH_3 emissions and solubilize P and some micro-nutrients (Fangueiro et al., 2015b; ten Hoeve et al., 2016), resulting in a material with a higher value as fertilizer. The animal slurry acidification performed prior to the solid-liquid separation, is also an interesting solution, able to minimize NH_3 emissions during the solid-liquid separation and to increase the P content of the liquid fraction (Cocolo et al., 2016; Regueiro et al., 2016a).

These treatments, that partially increase the nutrients concentrations and plant availability, do not solve the farmers' major problem: there is still a gap between farmers demands in terms of N:P ratio of mineral fertilizers and that provided by available organic fertilizers. The new approach proposed in this study will allow to obtain new organic fertilizers with a known N:P ratio close to what is commonly used by farmers.

Hence, the main objective of the present study was to assess the potential of different treated and non-treated animal manures to produce tailor-made manure-based fertilizers, with selected N:P ratios. For this, manures and slurries from different species were characterized (total macro and micronutrients concentration) and their N:P ratios compared with those commonly found in mineral fertilizers; secondly, some treatments (solid-liquid separation and acidification, by their own or combined) were tested to assess their ability to increase the concentration of some nutrients in their different fractions (solid or liquid), changing their N:P ratios to values closer to commercially available mineral fertilizers. This information will support next stages of the study, where the selected materials will be used in the manure-based fertilizers production, with a specific N:P ratio, and tested to replace, partially or totally, mineral fertilizers in basal fertilization.

2.2 Material and Methods

2.2.1 Manure sampling

Seven manures, representative of the main Portuguese livestock production systems, were collected in commercial farms: cattle solid manure (CaM) and cattle slurry (CaS+) were collected in an intensive dairy farm (high milk production per cow, milking three times a day, high inputs of concentrated feed) located at Benavente, Portugal. A second cattle slurry (CaS-) was collected in a less intensive dairy farm (lower milk production and concentrated feed inputs) at Palmela, Portugal. The goat manure (GoM) was sampled in an intensive goat farm (indoor production), located at Benavente, Portugal. Two different pig slurries were obtained: one from a farm with all stages of pig production (PiSM), located at Águas de Moura, Portugal, and the other from a pig fattening farm (PiSF), located at Montijo, Portugal. The poultry manure (PoM) was sampled in a commercial farm dedicated to the production of poultry meat, Herdade Daroeira, at Alvalade-Sado, Portugal.

The raw samples were collected in the main solid manure or slurry store and transported in polyethylene recipients to the lab where they were stored at 4ºC before utilization.

2.2.2 Manure treatments

The raw slurries were submitted to three different treatments solid-liquid separation, acidification and combined acidification and solid-liquid separation, leading to five derived materials for each slurry.

Slurry acidification was performed by the addition of concentrated sulphuric acid (H_2SO_4, 98% w/w), to reach a final pH of 5.5 as described in Fangueiro et al. (2009). The amount of acid needed to obtain the target pH varied between 3 to 9 mL kg^{-1} of slurry.

Slurry solid-liquid separation was performed through centrifugation at 4000 rpm for seven minutes in a laboratory centrifuge (5804, Eppendorf, Germany) using 50 mL vials. The 4000 rpm speed is the value established in the lab-scale centrifuge that better simulates the separation performed in the large scale centrifuges used in commercial farms.

The third treatment consisted of a combination of the two previously described treatments: acidification to pH 5.5 followed by the solid-liquid separation by centrifugation at 4000 rpm for seven minutes (Fangueiro et al., 2009).

A total of six different materials were then considered for each slurry: i) non-treated slurry (SLU), ii) liquid fraction of SLU (LIQ), iii) solid fraction of SLU (SOL), iv) acidified slurry (A-SLU), v) liquid fraction of A-SLU (A-LIQ), and vi) solid fraction A-SLU (A-SOL).

2.2.3 Manure physicochemical characterization

All the solid manures, slurries, and the derived materials obtained from the slurry treatment were analysed in triplicate for dry matter content (DM), pH, total organic carbon (TOC), total nitrogen (N_{Total}), ammonium nitrogen (NH_4^+-N), total phosphorus (P_{Total}, expressed as P_2O_5), total K (K_{Total}, expressed as K_2O), and total Ca, Mg and S, as well as for the micronutrients (Fe, Cu, Zn, Mn and B) and Na.

The DM content was determined by drying fresh samples for 24 h at 105ºC in a drying oven (Heraeus Function Line, Thermo Fisher Scientific, USA). The pH of solid manures, SOL and A-SOL were determined in a sample to deionized water suspension (1:10, m/v), after 1 h of occasional agitation, while the pH of SLU, LIQ, A-SLU and A-LIQ was measured directly (Orion 3 Star, Thermo Fisher Scientific, USA). The OM content was determined by calcination of the dried sample for 3 h at 550ºC in a calcination furnace (B180, Naberttherm, Germany) (Clesceri et al., 1989), allowing the total organic carbon (TOC) assessment by dividing the OM content by 1.8 (Sigurnjak et al., 2017). The Total N and NH_4^+-N were measured by the Kjeldahl method (Horneck and Miller, 1998), using the complete procedure (digestion, distillation and titration) for the Total N, and the last two steps for the NH_4^+-N determination.

Total P, K, Ca, Mg, S, Na and micronutrients were measured after the digestion of an oven-dried sample (± 0.2-0.3 g), with 9 mL nitric acid and 3 mL hydrogen peroxide, at 100ºC, in a block digestion system (Digipress MS, SCP Science, Canada). The elements concentrations were determined in the suspensions, using an inductively coupled plasma optical emission spectrometer (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, America).

2.2.4 Potential N mineralization assessment

An anaerobic incubation, based on the method described by Fangueiro et al. (2008), was performed to assess the potential N mineralization (PM) of each manure, slurry or derived materials obtained by treatment. A sample amount, equivalent to 0.02 g of total N, was added to 10 g of field-moist soil in a 60 mL syringe, and the total water content was corrected to 25 mL in the soil+manure mixture. The soil used here as inoculum was a sandy soil with an OM content of 7.4 g kg^{-1} soil. Eight replicates per sample were assembled, and a treatment containing only soil was used as control. Four replicates were prepared in syringes to be incubated for 7 days at 40ºC, while the other half were immediately extracted, after the injection of 25 mL of 4 M KCl to have a final KCl concentration of 2 M and 1:5 (m:V) extraction ratio. The samples were shaken for 1 h, followed by centrifugation at 4000 rpm (Eppendorf 5804), and 10 mL sub-samples of the supernatant were collected and stored at 4ºC before the analysis of the NH_4^+-N content by segmented flow autoanalyzer SAN plus (San Plus System, Skalar, Nederland) with a modified Berthelot method (Krorn, 1956). The same extraction procedure was used after the 7-days incubation period.

To calculate the PM, the following equation was used (Fangueiro et al., 2008):

$$PM = \{[NH_4^+\text{-}N]_{post\text{-}incub.}(\text{sample}) - [NH_4^+\text{-}N]_{pre\text{-}incub.}(\text{sample})\} - \{[NH_4^+\text{-}N]_{post\text{-}incub.}(\text{soil}) - [NH_4^+\text{-}N]_{pre\text{-}incub.}(\text{soil})\}$$

All samples were analysed in triplicate and the PM values were expressed as a percentage of the total N mineralized, considering the total N which was used in the incubated sample.

2.2.5 Statistical analysis of data

Data was analysed by one way ANOVA to evaluate the isolated effect of the manure/slurry species or treatment on the nutrient concentrations. Data were also analysed by two-way ANOVA to assess the effect of the interaction specie x treatment. To define the statistical significance of the mean, a Tukey's test was performed with a 95% degree of confidence ($\alpha = 0.05$), using Statistix 7.

2.3 Results and Discussion

Two scenarios were considered to prepare tailored manure-based fertilizers: i) at a farm level, where the desired N:P ratios and nutrients availability would be obtained by mixing raw or treated manure/slurry from a single animal species, with mineral fertilizers supplementation when needed; and ii) at a centralized plant receiving raw manure/slurry from different animal species, broadening the range of possibilities to blend materials, to obtain the desired manure-based fertilizers.

2.3.1 Effects of the animal species on the nutrients content and availability

The different animal species considered in the present study produced manure with very distinct chemical and physical characteristics, due not only to their different metabolism and age but also to the type of feeding (Trabue et al., 2019) and bedding material used (Miller et al., 2018). The farm practices, namely manure management techniques, have a marked effect on manure composition that can explain the differences between the results obtained here (Table 2.1) and those reported by other authors.

Table 2.1: Physicochemical characteristics of the raw manures from the animal species considered in the study, all expressed in fresh matter basis (mean value, n=3). Values in the same column followed by the same letter are not statistically different (Tukey HSD test, $P > 0.05$).

	DM g kg^{-1}	**pH**	**TOC** g kg^{-1}	**N$_{\text{Total}}$** g N kg^{-1}	**NH$_4^+$-N** g N kg^{-1}	**PM** % of total N	**P$_{\text{Total}}$** g P$_2$O$_5$ kg^{-1}	**K$_{\text{Total}}$** g K$_2$O kg^{-1}	**Ca$_{\text{Total}}$** g kg^{-1}	**Mg$_{\text{Total}}$** g kg^{-1}	**S$_{\text{Total}}$** g kg^{-1}
CaS+	71.64^{f}	8.55^{b}	24.24^{f}	3.10^{c}	1.70^{c}	13.41^{b}	1.32^{d}	4.98^{b}	1.08^{de}	0.62^{d}	0.01^{cd}
CaS-	97.78^{e}	7.13^{e}	40.09^{e}	3.53^{c}	1.65^{b}	11.81^{bc}	1.62^{d}	3.21^{bc}	1.37^{de}	0.57^{d}	1.37^{a}
PiSM	22.85^{g}	7.19^{e}	10.03^{g}	1.31^{d}	0.74^{e}	12.75^{bc}	0.68^{e}	0.59^{d}	0.54^{e}	0.27^{e}	0.00^{d}
PiSF	124.12^{d}	6.69^{f}	55.22^{d}	6.16^{b}	3.71^{a}	7.99^{d}	3.74^{b}	4.71^{b}	2.61^{c}	1.27^{c}	0.96^{b}
CaM	176.79^{c}	7.77^{d}	85.43^{c}	3.14^{c}	0.44^{f}	11.07^{c}	3.54^{b}	1.88^{cd}	2.02^{cd}	1.38^{c}	0.01^{cd}
GoM	244.00^{b}	8.09^{c}	112.18^{b}	5.06^{b}	0.90^{d}	8.02^{d}	2.66^{c}	0.82^{cd}	7.84^{b}	2.01^{b}	0.01^{cd}
PoM	739.88^{a}	9.04^{a}	342.32^{a}	21.41^{a}	3.14^{b}	22.56^{a}	11.71^{a}	19.71^{a}	11.79^{a}	4.80^{a}	0.10^{c}

CaS+: Intensive cattle slurry; CaS- : Cattle slurry; PiSM: Pig slurry with all stages of pig production; PiSF: Pig slurry from a fattening farm; CaM: Cattle manure; GoM: Goat manure; PoM: Poultry manure. DM: Dry matter; TOC: Total organic carbon; N$_{\text{Total}}$: Total nitrogen; NH$_4^+$-N: Ammoniacal nitrogen; PM: Potential of mineralization; P$_{\text{Total}}$: Total phosphorus expressed as P$_2$O$_5$; K$_{\text{Total}}$: Total potassium expressed as K$_2$O; Ca$_{\text{Total}}$: Total calcium; Mg$_{\text{Total}}$: Total magnesium; S$_{\text{Total}}$: Total sulphur.

Slurries presented lower dry matter content than the solid manures and significant differences were also observed among the slurries and solid manures considered (Table 2.1). The slurries DM ranged between ~20 g kg^{-1}, for PiSM, and ~120 g kg^{-1} for PiSF. The differences were even higher when comparing slurry and solid manure, for instance, ~740 g kg^{-1} for PoM. That is one of the main constraints of using slurry as fertilizer, when comparing with solid manure: the lower DM content of slurry (Table 2.1) implies a higher dilution of nutrients and the handling of a significantly higher amount of slurry to provide the same amount of nutrients. This point is the main limitation for a massive use of slurry as organic fertilizers in arable crops, since transport and soil application of manure will be much more expensive compared to mineral fertilizer. On the opposite, the higher DM of the solid manure can affect strongly soil application with potential clogging of the equipment and lead to a heterogeneous application. A tailor-made fertilizer would bring the benefit of using both solid manure and slurries to reach a compromise between a material extremely diluted or too solid, being one of the aspects to take into account when planning to produce a manure-base fertilizer.

More than a nutrient supplier, solid manures are an excellent source of carbon (C). The solid manures presented the higher organic C concentration (in a fresh material basis), with ~ 342 g kg^{-1}, 112 g kg^{-1} and ~ 85 g kg^{-1}, for PoM, GoM, and CaM, respectively (Table 2.1). Slurries also provide interesting amounts of C to the soil, but with lower concentrations than manures and with significant differences between products, for instance, 10.03 and 55.22 g C kg^{-1} in PiSM and PiSF, respectively. To provide the same amount of C to the soil, the quantity of PiSM needed is five times higher than the PiSF. It is well known that there is a positive correlation between the C content of organic amendments and their capacity to restore the soil organic matter (SOM) (FAO, 2019), indicating that the use of manures as organic fertilizers may be a good option to improve soil's health. The Mediterranean soils have either a low (<2%) or very low (<1%) SOM content, a fact that needs to be counterbalanced by the application of exogenous sources of OM to soil (Hinsinger, 2014).

Besides the rich C content of solid manures, these materials had also higher or similar N, P and K content relative to slurries, but lower than mineral fertilizers, which might limit their use as a nutrient supplier and highlight them instead as interesting C providers (i.e., organic amendments), becoming essential to produce a manure-based fertilizer with a higher nutrient concentration and known N:P ratio. Some nutrients in solid manures are mostly in forms not readily available for plants, which might be seen as a drawback, if an immediate plant availability is foreseen, or as an important advantage, if a slower nutrient release is expected (as occurs in the slow-releasing mineral fertilizers). Both goat and cattle manure had higher total N content but lower NH_4^+-N content than both cattle slurries and pig fattening slurry (Table 2.1), resulting in a lower N availability to the crops immediately after soil application (low NH_4^+: total N ratio), but providing a longer lasting N supply to the crops, extended over time. From the three solid manures, poultry manure appears as the most interesting when an extra source of N is needed since it has the highest DM, total N and NH_4^+-N content (Table 2.1).

The values of pH ranged from 6.69 to 9.04, with significant differences among animal species with the lower value for the pig slurry from the fattening farm and the higher for the poultry manure. The higher pH of cattle slurry (intensive farm), goat and poultry manure can be utilized to correct or increase the pH in acidic soils (Cai et al., 2018). Nonetheless, a higher pH in manure, especially with a pH between 7 and 10, increase the risk of ammonia emissions (Ndegwa et al., 2008), so the referred materials should be blended with materials with lower pH or even treated by acidification (Fangueiro et al., 2015).

The values of potential N mineralization (PM) indicated that poultry manure is the material with the higher mineralization rate (PM ~23% of total N), which suggests that, besides the higher content of immediately available N (NH_4^+-N), a significant part of the organic N should be quickly mineralized, allowing a significant N uptake by the plants (Table 2.1). The same cannot be said relatively to cattle and goat manures, which had lower PM values, ~11% and 8% of the total N, respectively, in the same range of the slurries' PM values, indicating that the organic N from these two manures will be slowly mineralized. The low PM values of these manures might be a consequence of their richness in recalcitrant materials, like straws and parings, elements rich in fibres, leading to higher C:N ratios and, consequently, to less labile nutrients (Vahdat et al., 2011). Accordingly, the slurries with more plant residues (e.g., bedding materials), namely cattle slurries, should have presented the lower PMs. No major differences were observed for the PM of the slurries, except for the PiSF, the slurry with the lower PM value and the higher total N content. PiSF presented a PM value corresponding to ~ 8% of the total N applied, identical to GoM, which may be attributed to the bigger particles size existing in PiSF, compared to the other three slurries. Fangueiro et al. (2012) reported similar results with different slurries and attributed it to the fact that bigger particle size in slurry turned the total N less soluble and, therefore, the N mineralization was slowed down, a feature that can be considered important in a manure-based fertilizer able to slowly supply N to the plant.

The poultry manure presented a P concentration ~ three, ~four and 10 times higher than GoM, CaM and PiSF, and both cattle slurry and PiSM, respectively (Table 2.1). This can be an important material to add to a blend for a crop with higher P needs. The P content of PoM (~12 g P kg^{-1}) is coherent with the value of 13 g P kg^{-1} reported by Adekiya et al. (2019), similar to the results of Shah et al. (2016), which reported values of P_2O_5 between 14.1 to 19 g kg^{-1} DM in a solid cattle manure, values higher than those observed here in fresh manures, perhaps due to the higher maturity and DM content of the manure referred by these authors. The slurry from the pig fattening farm, had the higher total P content (~5.5 times higher than the value of the other pig slurry, and almost the double P of the cattle slurries). On the opposite side, PiSM presented the lower P concentration followed by both cattle slurries. This aspect is important when a blend richer in N is needed or when it is necessary to apply a poor-P material to a soil with a high P concentration.

Regarding total K, it is noteworthy the high content present in PoM and in the slurries CaS+ and PiSF, indicating their ability to provide this essential macronutrient to the crop (Table 2.1). The differences observed within each species, which are very marked in the case of total K concentrations in PiSM and PiSF, might be attributed to distinct animal dieting programs. It is still to refer that the poultry manure appears also as an optimal material to supply both Ca and Mg while, in the case of slurries, the best option to provide Ca and Mg is PiSF. CaS- is as well an excellent source of S, a nutrient essential for many crops and that starts to be in deficit in some European soils (Pötzsch et al., 2019; Scherer, 2009).

2.3.2 Effects of slurry treatment on nutrients content and N availability

One of the hypotheses considered to obtain manure-based materials with more interesting fertilizer characteristics was to submit the raw materials to pre-treatments, to deliver new products with a composition more adjusted to the nutritional needs of the main crops, namely a higher concentration of some specific elements or higher nutrients availability. The application of these pre-treatments is very important for slurries, which have lower DM content, in this case CaS+, CaS-, PiSM and PiSF. The treatments which were applied, i) solid-liquid separation, ii) acidification, and iii) acidification followed by solid-liquid separation, were chosen considering their common use at farm scale (separation) (Dennehy et al., 2017), and their potential to minimize ammonia emissions (acidification) (Fangueiro et al., 2015b).

2.3.2.1 Solid-liquid separation

The solid-liquid separation was first introduced to lower the costs and efficiency of slurry management, allowing the transport of a concentrated solid fraction from regions with overproduction of manure to other regions with nutrient deficiency (Fangueiro et al., 2011). Sommer et al. (2015) also emphasized that, since manure application rates are based firstly on N, the over-application of P can be avoided with this strategy. The liquid fraction is, usually, richer in N and K, which becomes an optimal solution to their application directly at the farm, while the solid fraction, with a higher content of organic matter and P, can be exported to a different area. This technique only modifies the nutrients ratios within each phase, solid or liquid, it does not completely remove P from the liquid fraction, only allows the reduction of P concentration in this fraction.

The impact of the separation on the solid and the liquid fractions, in terms of their physicochemical characteristics, were similar for all slurries (Table 2.2). Solid-liquid separation led to solid fractions (SOL) with pH values higher than the respective liquid fractions (LIQ) (Table 2.2), in agreement with other studies (Fournel et al. 2019; Gómez-Muñoz et al., 2016). In terms of total N, the LIQ fractions presented lower concentrations, comparatively to SLU and SOL, due to the removal of a significant part of the organic matter. However, the concentration of NH_4^+-N did not differ significantly between the fractions and the untreated materials, in the case of the cattle slurries, while for the pig slurries SOL presented a higher concentration of NH_4^+-N. But it is to note that ~72% of the total N was in the form of NH_4^+-N, in the case of the LIQ of CaS+, CaS- and PiSF, and ~83% for PiSM, against ~40% in the SOL of CaS+, CaS- and PiSF and ~22% in the SOL of the PiSF. This can be an important factor to consider when designing a manure-based fertilizer with higher quantities of available N (higher N:P ratio). Similar results were reported by Fangueiro et al. (2009), who obtained a liquid fraction from pig slurry poor in organic N. No significant differences were found between PM values of solid and liquid fractions obtained from cattle slurry but, when considering pig slurry, significantly higher values of PM were found in the SOL than in LIQ fractions (Table 2.2). These results are in contrast with those reported by Regueiro et al. (2016a), who did not observe significant differences in PM values between fractions derived from pig slurry. The reason for such difference is not clear and might be related to the particle size or C speciation existing between pig and cattle slurry since the SOL and LIQ fractions derived from pig and cattle slurry had a similar C:N ratio.

The use of solid-liquid separation resulted in solid fractions not only richer in organic C content, but also richer in P, as already referred by Sommer et al. (2017). All solid fractions, independently of being obtained from cattle or pig slurries, presented a higher total P content. However, for pig slurries, the P concentrations obtained here were slightly lower than the values presented by Regueiro et al., (2016c) in a study to evaluate the behaviour of nutrient distribution with the treatments applied. The solid fractions obtained in this study were an optimal solution to supply larger amounts of P and, also, to provide Ca and Mg and, thereby, obtain a manure-based fertilizer with a lower N:P ratio.

When considering the possibility of using a subproduct of the solid-liquid separation to produce manure-based fertilizers, SOL is the best option to simultaneously provide P and C to the soil. Also, solid fractions derived from PiSM and PiSF presented the higher PM, an important factor when the intention is to obtain a blend rich in readily available N. However, the high Cu and Zn content of these fractions might be problematic (Table S2.5 from supplementary material). Popovic et al. (2012), have already alerted for the enrichment of the solid fractions with Cu and Zn, and the concomitant risk of soil toxicity and impaired crop productivity. The liquid fractions, with lower N content than the solid fractions, have a higher proportion of available N, which might be a good option to enrich some blends in available N (higher N:P ratio).

Table 2.2: Physicochemical characteristics of the materials obtained from the treated slurries, expressed in fresh matter basis (mean value, n=3). Values in the same column followed by the same letter are not statistically different (Tukey HSD test, $P > 0.05$).

		DM	**pH**	**TOC**	**NTotal**	**NH_4^+-N**	**PM**	**PTotal**	**KTotal**	**CaTotal**	**MgTotal**	**STotal**
		g kg^{-1}		**g kg^{-1}**	**g N kg^{-1}**	**g N kg^{-1}**	**% of total N**	**g P_2O_5 kg^{-1}**	**g K_2O kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**
CaS+	**SLU**	71.64^{i}	8.55^{bc}	24.24^{i}	3.10^{hi}	1.70^{e}	13.41^{efg}	1.32^{h}	4.98^{a}	1.08^{fg}	0.62^{fg}	0.01^{j}
	LIQ	37.54^{jkl}	7.45^{e}	12.50^{k}	2.70^{ij}	1.97^{de}	11.66^{g}	0.43^{ij}	1.81^{cde}	0.64^{hi}	0.37^{hi}	0.21^{ij}
	SOL	159.96^{d}	8.84^{a}	54.85^{ef}	4.70^{f}	1.90^{de}	12.08^{fg}	4.02^{c}	1.79^{e}	2.59^{c}	1.62^{b}	0.61^{hi}
	A-SLU	76.71^{i}	4.97^{q}	25.21^{i}	3.15^{hi}	1.85^{de}	7.05^{hi}	1.48^{gh}	1.89^{cde}	1.30^{fg}	0.69^{efg}	4.14^{d}
	A-LIQ	45.61^{j}	5.04^{pq}	12.53^{k}	2.89^{i}	1.86^{de}	5.11^{lj}	1.46^{gh}	1.89^{cde}	1.04^{fg}	0.70^{ef}	3.98^{de}
	A-SOL	137.49^{ef}	5.10^{op}	49.46^{fg}	4.16^{g}	1.76^{de}	14.66^{def}	1.36^{h}	1.48^{f}	1.24^{fg}	0.57^{fgh}	3.67^{a}
CaS-	**SLU**	97.78^{h}	7.13^{f}	40.09^{h}	3.53^{h}	1.65^{e}	11.81^{g}	1.62^{fgh}	3.21^{b}	1.37^{ef}	0.56^{fgh}	1.37^{fg}
	LIQ	25.92^{klm}	8.53^{v}	8.89^{k}	2.32^{j}	1.66^{e}	18.97^{bc}	0.53^{ij}	0.91^{g}	0.57^{i}	0.25^{ij}	0.17^{ij}
	SOL	150.48^{de}	8.82^{a}	56.67^{e}	4.73^{f}	1.87^{de}	17.41^{cd}	3.12^{d}	0.86^{gh}	1.93^{d}	0.82^{e}	0.66^{hi}
	A-SLU	99.98^{h}	5.81^{j}	37.83^{h}	3.45^{h}	1.72^{de}	7.19^{hi}	1.85^{fg}	0.87^{gh}	1.24^{fg}	0.51^{gh}	3.93^{de}
	A-LIQ	39.54^{jk}	5.92^{i}	12.50^{k}	2.70^{ij}	1.74^{de}	3.88^{j}	1.71^{fgh}	0.95^{g}	0.97^{gh}	0.53^{fgh}	3.50^{e}
	A-SOL	192.62^{c}	6.45^{h}	76.76^{c}	4.79^{f}	1.76^{de}	4.66^{ij}	2.37^{e}	0.96^{g}	1.92^{d}	0.65^{efg}	4.95^{c}
PiSM	**SLU**	22.85^{lmn}	7.19^{f}	10.03^{k}	1.31^{k}	0.74^{g}	12.76^{fg}	0.68^{ij}	0.59^{hi}	0.54^{i}	0.27^{ij}	0.00^{j}
	LIQ	4.50^{o}	7.14^{f}	1.49^{m}	0.80^{l}	0.66^{g}	3.36^{j}	0.26^{j}	0.26^{j}	0.10^{j}	0.10^{j}	0.03^{j}
	SOL	156.95^{d}	7.82^{d}	68.98^{d}	6.35^{cd}	1.37^{f}	27.54^{a}	4.62^{b}	0.43^{ij}	4.18^{b}	1.74^{b}	0.80^{h}
	A-SLU	19.20^{mno}	5.19^{no}	7.46^{kl}	1.09^{kl}	0.74^{g}	-3.13^{m}	0.79^{i}	0.26^{j}	0.54^{i}	0.25^{ij}	0.93^{gh}
	A-LIQ	9.00^{no}	5.67^{k}	2.62^{lm}	0.80^{l}	0.71^{g}	-8.39^{n}	0.71^{ij}	0.29^{ij}	0.45^{ij}	0.21^{ij}	0.93^{gh}
	A-SOL	134.59^{f}	5.16^{no}	63.98^{d}	4.51^{fg}	1.18^{f}	7.37^{hi}	1.99^{ef}	0.33^{ij}	1.73^{de}	1.05^{d}	1.63^{f}
PiSF	**SLU**	124.12^{fg}	6.69^{g}	55.22^{e}	6.16^{cd}	3.71^{c}	7.99^{h}	3.74^{c}	4.71^{a}	2.61^{c}	1.27^{c}	0.96^{gh}
	LIQ	46.18^{j}	7.48^{e}	18.01^{j}	5.51^{e}	4.06^{ab}	15.59^{de}	0.78^{i}	1.76^{ef}	1.18^{fg}	0.26^{ij}	0.49^{hij}
	SOL	232.04^{a}	8.62^{b}	107.63^{a}	9.87^{a}	4.26^{a}	20.44^{b}	11.03^{a}	2.09^{cd}	5.86^{a}	3.44^{a}	1.44^{fg}
	A-SLU	117.63^{g}	5.21^{n}	48.95^{g}	6.58^{c}	4.20^{a}	4.72^{lj}	4.08^{c}	2.03^{cde}	2.80^{c}	1.37^{c}	5.49^{b}
	A-LIQ	62.62^{i}	5.41^{l}	20.57^{ij}	6.07^{d}	4.24^{a}	0.69^{l}	4.16^{bc}	2.10^{c}	1.84^{d}	1.36^{c}	4.69^{c}
	A-SOL	210.59^{b}	5.31^{m}	98.15^{b}	7.77^{b}	3.92^{bc}	6.30^{hij}	3.88^{c}	1.80^{de}	4.08^{b}	1.27^{c}	6.15^{a}

CaS+: Intensive cattle slurry; CaS-: Cattle slurry; PiSM: Pig slurry with all stages of pig production; PiSF: Pig slurry from fattening farm. SLU: Non-treated slurry; LIQ: Liquid fraction; SOL: Solid fraction; A-SLU: acidified slurry; A-LIQ: Liquid fraction of the acidified slurry; A-SOL: Solid fraction of the acidified slurry. DM: Dry matter; TOC: Total organic carbon; N_{Total}: Total nitrogen; NH_4^+-N: Ammoniacal nitrogen; PM: Potential of mineralization; P_{Total}: Total phosphorus expressed as P_2O_5; K_{Total}: Total potassium expressed as K_2O; Ca_{Total}: Total calcium; Mg_{Total}: Total magnesium; S_{Total}: Total sulphur.

Despite the benefits that may arise from the slurry solid-liquid separation, the process might lead to NH_3 emissions during the treatment and, individually, the fractions can still release, during the storage period, larger amounts of NH_3 and greenhouse gases emissions (GHG) than raw slurries, decreasing their fertilizer value (Regueiro et al., 2016c). That is why it is also important to consider the slurry acidification.

2.3.2.2 Acidification

Slurry acidification appears as a mitigation technique to reduce not only NH_3 but also GHG emissions during storage (Prado et al., 2020). It is applied at farm scale in several countries from the North and East Europe, and also started to be implemented in countries from other European regions, like in Spain (Rodhe et al., 2018). The additives used for acidification are known to have an impact on the characteristics of the acidified slurry (Regueiro et al., 2016b) but it was not possible to test several additives in the present study. Sulphuric acid was selected for being the most used and, also a source of sulphur (S). By using this technique, N losses by NH_3 volatilization are minimized and, consequently, the slurry fertilizer value, in terms of N, is increased. Additionally, P becomes more soluble, which could result in a higher availability of this element to the crop (Pedersen et al., 2017; Roboredo et al., 2012). This technique can be used to obtain a higher N:P ratio, due to the increase in N fertilize value and P solubility. Nevertheless, the safety issues related to acid handling and associated cost might be a limitation to the applicability of acidification at the farm level (Fangueiro et al., 2015b).

In terms of TOC concentration, there were no differences between acidified and raw slurries, as expected, despite the inorganic C losses through CO_2 emissions that may occur during treatment by acidification (Fangueiro et al., 2013).

The total N and NH_4^+-N contents were similar in the raw and the respective acidified slurries, as observed by other authors (Fangueiro et al., 2009; Regueiro et al., 2016a). Such results were expected since the storage time of the materials was very short, leading to residual NH_3 losses from the untreated slurries, or to no emissions at all. Previous studies indicated that slurry acidification alters the N dynamic after soil application, inhibiting or delaying the nitrification and decreasing the N mineralization (Fangueiro et al., 2017; Sigurnjak et al., 2017a). This effect was evident in the results, with a significantly lower value of PM in the A-SLU fractions, relatively to the non-treated slurries, namely in the PiSM where the PM value of the A-SLU was four times lower than in SLU (Table 2.2). As observed in the present study, Regueiro et al. (2016a) also reported that despite their similar total N and NH_4^+-N contents, the PM values of acidified materials are minor than their non-acidified counterparts.

When using an acidified slurry with other raw or treated manures to produce a manure-based fertilizer, a fraction of the N in the resulting blend will not be available immediately, acting as a slow-release N fertilizer.

As found for N, acidification did not alter the total P concentration comparing the raw slurries and their acidified equivalents. However, acidification is known to solubilize P and, thereby, may increase P availability to crops (Regueiro et al., 2016a; Roboredo et al., 2012), but this aspect was not assessed in the present experiment.

The additive used, sulphuric acid, led to an increase in the S concentration in the acidified slurries, comparatively to their respective raw slurries (Table 2.2). This can be seen as an advantage when the crops' fertilization plan demands for a S supply. However, it may be problematic when considering the hydrogen sulphate emissions since the addition of inorganic sulfur stimulates the sulfate-reducing bacteria activity (Dai and Blanes-Vidal, 2013). Nevertheless, different acidifying agents might be used as an alternative to sulfuric acid to avoid such problem (Prado et al., 2020; Regueiro et al., 2016b).

2.3.2.3 Acidification followed by solid-liquid separation

Solid-liquid separation after acidification was considered by Fangueiro et al. (2009) and Regueiro et al. (2016a) as an important strategy to mitigate the N losses from NH_3 volatilization and to increase the fertilizer value of the acidified liquid fractions (A-LIQ) by enriching them in P, Ca, Mg and S, in agreement with the results obtained in this study (Table 2.2). However, this treatment had no effect on both total and mineral N content, or the effect was very reduced. Similarly to what was observed for the acidified slurries, the PM values of the obtained fractions, A-LIQ and A-SOL, were lower than in their respective non-acidified counterparts, LIQ and SOL. Gómez-Muñoz et al. (2016) used fattening pig slurry to assess the potential of N mineralization with fresh and acidified slurry and their solid and liquid fractions, with and without acidification and observed that the solid fraction presented higher PM than the correspondent acidified solid fraction, obtained using centrifugation. Acidification with sulphuric acid turns both A-LIQ and A-SOL a source of S, relatively to their non-acidified fractions. Furthermore, the A-LIQs were richer in total P when compared to the respective liquid fractions (~3.4 times richer in cattle slurries, ~2.7 times richer in PiSM and ~5 times richer in PiSF). Accordingly, the obtained acidified solid fractions were poorer in P than the respective solid fractions (~3 times in CaS+ and PiSF, ~2.3 times in PiSM and 1.31 times in CaS). The results obtained here for total and mineral N, as well as for total P, were in agreement with those obtained by Fangueiro et al. (2017) with cattle slurry. The materials obtained by the combination of solid-liquid separation and acidification were distinct than those obtained only by solid-liquid separation, which may lead to different N:P ratios and different N and P availabilities.

2.3.3 Potential of manures and slurries to be used in manure-based fertilizers

Some of the manures considered in this study and discussed in sub-chapter 3.1 showed a strong potential to be used in manure-based fertilizers. PiSF presented the highest DM content of all the slurries, the highest concentration of mineral N of all the materials (3.71 g NH_4^+-N kg^{-1}), and the lowest PM of all slurries, with a high total N content (6.16 g kg^{-1}). This makes PiSF one of the best options to provide high inputs of available N with an extra N amount that will be slowly released to the crop (higher N:P ratio). On the other hand, PoM may supply both N and P, and it may be an option to supply N more quickly to the crop, due to the higher PM, with the benefit of adding a considerable amount of C to the soil. When considering Ca and Mg concentrations (Table 2.1), as well as Cu, Zn, and B (Supplementary Material, Table S2.4), PiSF and PoM were the slurry and the manure, respectively, with the higher concentrations for those important nutrients, meaning that, when used in a manure-based fertilizer, besides the N and P, also essential macro and micronutrient will be provided. Cattle manure presented a lower concentration of mineral N, but a higher concentration of total P, turning this material valuable to provide a manure-based fertilizer rich in P (lower N:P ratio).

Nutrient's concentrations and N availability in the manures were evaluated within each species or derived materials obtained by submitting the slurries to low-technological treatments, i.e., at a farm level scenario. Nevertheless, blends might also be prepared at manure treatment plants, receiving several types of manures, and applying distinct treatments. In this study, it was hypothesized that the nutrient ratios would be used as an indicator to prepare blends of raw or treated manures from different species at a treatment-plant level. In this sense, different N, P and C ratios were calculated for all the manures and raw/treated slurries (Figure 2.1): i) total N to total P ratio (N_T:P_T ratio), ii) NH_4^+-N to total P $_{ratio}$ (NH_4^+-N: P_T ratio), iii) total N to NH_4^+-N ratio (N_T:NH_4^+ ratio) and iv) TOC to total N ratio (C_{org}:N ratio). These indicators will allow identifying the materials with greater ability to provide organic C to soil, materials richer in N or P, with higher or lower N:P ratio, as well as those with lower or faster nutrients release. This information will support the choice of materials to be used for the preparation of manure-based fertilizer with a known ratio of N:P. In all these ratios, P was expressed as P_2O_5 as occurs in mineral fertilizers.

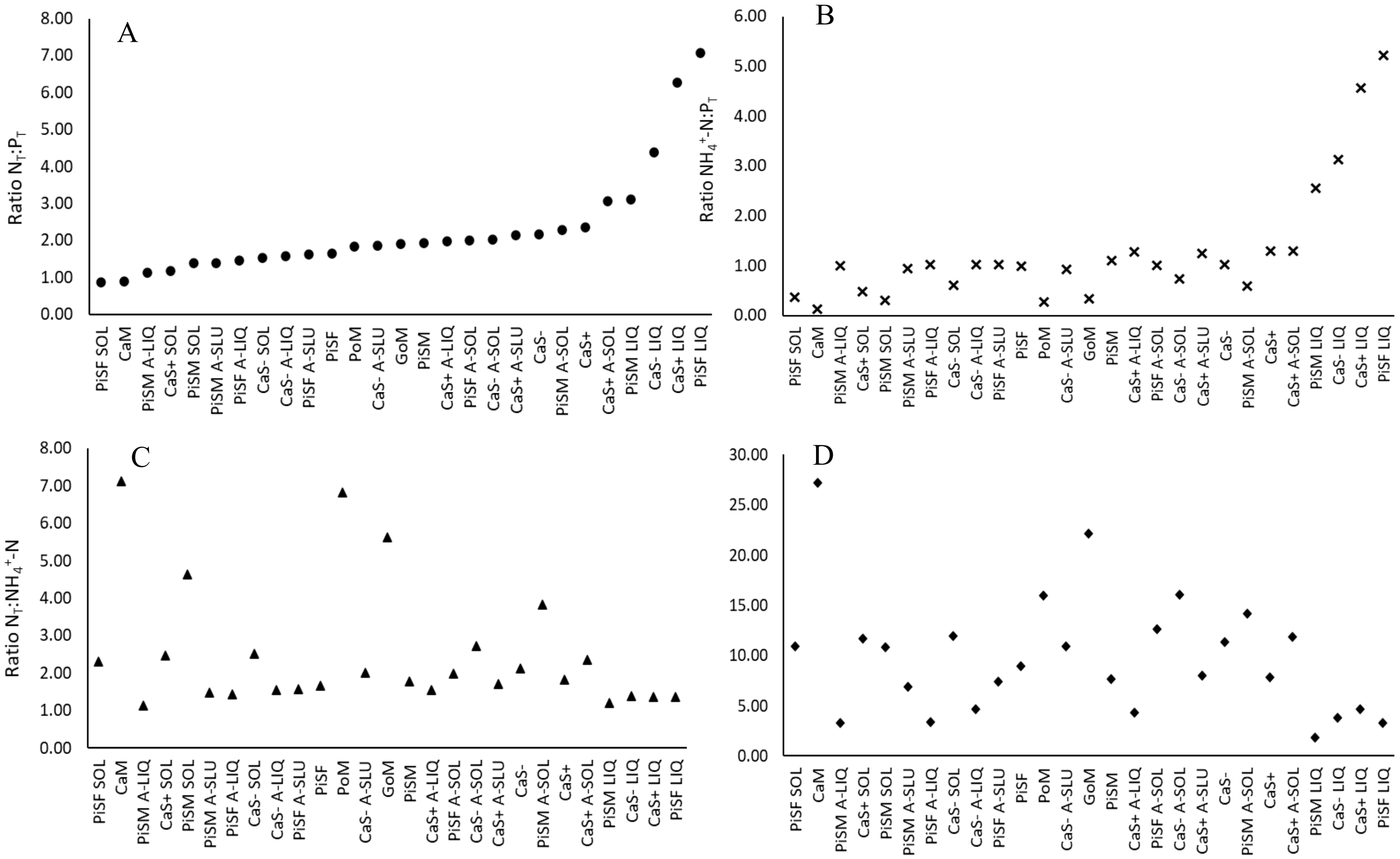

Figure 2.1: Main ratios of C, N and P in the materials considered in the study. (A) NT:PT, the ratio of total N to total P (as P_2O_5); (B) NH_4^+-N:P_T, the ratio of the NH_4^+-N to the total P (as P_2O_5); (C) N_T: NH_4^+-N, the ratio of total N to NH_4^+-N; and (D) C:N_T, the ratio of C to the total N. CaS+: Intensive Cattle Slurry; CaS- : Cattle Slurry; PiSM: Pig Slurry with all stages of pig production; PiSF: Pig Slurry from fattening farm; CaM: Cattle Manure; GoM: Goat Manure; PoM: Poultry Manure. LIQ: Liquid Fraction; SOL: Solid Fraction; ACID: Acidified Slurry; A-LIQ: Liquid Fraction of the Acidified Slurry; A-SOL: Solid Fraction of the Acidified Slurry.

The manures and raw/treated slurries were presented in Figure 2.1A in ascending order of N_T:P_T ratio. This layout emphasizes the materials which are richer in total P (lower N:P ratio), relatively to their N content (in the left side of the graph), and those which are richer in total N, relatively to their P content (in the right side of the graph), allowing their selection considering the crops demands, giving important information to be used in the preparation of the tailored made fertilizers. The same order for the materials was used in the following graphs (Figure 2.1B, C and D), to facilitate the comparison.

The materials with the lower total P concentrations, like the slurry liquid fractions and the acidified solid fractions, were those with the higher N_T:P_T ratios (Figure 2.1A). This effect was more noticeable in PiSM LIQ and CaS+ LIQ. Contrariwise, the materials with the lower total N concentrations, like the CaM and the solid fraction of PiSF, were those with the lower N_T/P_T ratios. To prepare a manure-based fertilizer with materials with the higher N_T:P_T ratios, like the previously referred, it is important to mix them with contrasting materials with a lower ratio when the intention is to have a more balanced ratio, as PiSF SOL, that have a higher P concentration. When considering NH_4^+-N: P_T ratio (Figure 2.1B), all the SOLs and solid manures presented the lower ratios, indicating a higher concentration of total P. This is an important indicator since it considers the readily available N and, in the case of the slurry solid fractions and solid manures, it means that they will supply a higher amount of P for the same amount of N applied. It is also to note that the A-SLU and SLU presented an NH_4^+-N: P_T ratio close to 1, meaning that, with these materials, NH_4^+-N and total P will be supplied in similar quantities, which can be a good solution for basal applications (pre-planting or pre-sowing) but should be used with cautious afterwards, to avoid the surplus application of P.

Considering the N_T:NH_4^+ ratio (Figure 2.1C), as expected, the solid manures (CaM, GoM and PoM) that presented a higher concentration of organic N, presented the highest N_T:NH_4^+ ratio. It is also to note that, the ratios presented by the LIQ fractions were only slightly above 1, highlighting a balance between total and mineral N, which can indicate that these materials can be a good option when more readily available N is needed. Data plotted in Figure 2.1A and B can also be used to identify if the N is readily available (lower ratios) or will be slowly released in the medium or long term (higher ratios), an important information to be used in the design of crop-tailored fertilizers from the blend of raw or treated manures.

Another important aspect, that affects mainly the N availability after soil application, is the C:N_T ratio (Figure 2.1D). As expected the materials which presented the higher C:N_T ratio were the solid manures, CaM, GoM and PoM, with ratios of 27, 22 and 16, respectively. These materials were those with the higher N_T:NH_4^+-N, due to the higher content of organic materials like straws and parings (Vahdat et al., 2011), poorer in mineral N (plant available form), as a result of the presence of recalcitrant materials that may inhibit/delay the activity of bacteria responsible for N mineralization. However, this effect was reflected on PM only in the case of GoM, which presented the lower PM rates. This fact can be explained by the C:N_T ratios, which were not at a critical level that would result in a more marked effect on the mineralization.

Another challenge is to use the information provided by these ratios and design possible manure-based blendings. One specific application, regarding the centralized solution at a treatment plant, could be the blending of PiSM, which has a higher concentration of N_T, NH_4^+-N and P, with CaS+, the one richest in K. Also, both slurries have a high, or medium, PM value, reason why it would be interesting to blend them with a material with a lower PM, or higher N_T:NH_4^+ ratio, indicating a higher quantity of organic N, and a lower mineralization rate (e.g., an acidified slurry). This should guarantee a continuous N supply to the crop, besides the mineral N initially available and, also, the capacity to increase soil nutrients and the C pool, important to enhance the soil's health.

Considering the formulation of blends at the farm scale, one important material to be used should be the PiSF slurry, due to its high concentrations in both total and mineral N, and considerable high concentrations for P and K, which will be a good contender for a manure-based fertilizer with an even ratio of N:P. Moreover, the materials obtained from the application of the tested treatments to PiSF slurry were those richer in most of the parameters evaluated.

To be able to evaluate the potential of each material to be used in the production of manure-based fertilizers, some calculations were made (Table 2.3) taking as a reference the provision of 100 kg N ha^{-1} to the soil (first column), and further calculating (i) the quantity of manure or slurry (raw or treated) needed to provide the referred 100 kg N ha^{-1} (second column), (ii) the estimated available N content (N_{av}) (third column), (iii) the N_{av}:P_2O_5:K_2O ratios (fourth column), (iv) the amount of macronutrients provided to the crop by the application of that quantity of manure (P_2O_5, K_2O, Mg, Ca and S), and (v) the amount of organic C provided by that application (last column, Table 2.3). To estimate the available N content (N_{av}; i.e., mineral N + organic N that can, potentially, be mineralized), the Portuguese legislation was considered where: 50% of the total N is considered as available N in solid manures (CaM, GoM and PoM), and in solid fractions obtained by solid-liquid separation (SOL and A-SOL), while, approximately, 60% of the total N is considered available in slurries and in liquid fractions (MADRP, 2018).

Table 2.3: Quantity of manure or slurry (raw or treated) needed to provide 100 kg N ha^{-1} and estimated available N content (Nav; according to MADRP, 2018) Nav:P_2O_5:K_2O ratio, amount of macronutrients and amount of organic C provided by the application of that quantity of manure.

Specie	**Treatment**	**N_{av} applied kg ha^{-1}**	**t ha^{-1}**	**N_{av} g kg^{-1}**	**N_{av}:P_2O_5 ratio**	**N_{av}:K_2O ratio**	**Total P_2O_5 kg ha^{-1}**	**Total K_2O kg ha^{-1}**	**Total Mg kg ha^{-1}**	**Total Ca kg ha^{-1}**	**Total S kg ha^{-1}**	**TOC kg ha^{-1}**
CaS+*	**SLU**	100	53.76	1.86	1.41	0.37	70.97	267.74	33.33	58.06	0.54	1303.23
	LIQ	100	61.73	1.62	3.77	0.90	26.54	111.73	22.84	39.51	12.96	771.60
	SOL	100	35.46	2.82	2.76	1.58	36.17	63.48	57.45	91.84	21.63	1945.04
	A-SLU	100	52.91	1.89	1.28	1.00	78.31	100.00	37.04	68.78	219.05	1333.86
	A-LIQ	100	57.67	1.73	1.19	0.92	84.20	109.00	40.37	59.98	229.53	722.61
	A-SOL	100	40.06	2.50	1.84	1.69	54.49	59.29	22.84	49.68	147.04	1981.57
CaS-	**SLU**	100	47.21	2.12	1.31	0.66	76.49	151.56	26.44	93.01	64.68	1892.82
	LIQ	100	71.84	1.39	2.63	1.53	38.07	65.37	17.96	40.95	12.21	638.65
	SOL	100	35.24	2.84	0.91	3.30	109.94	30.30	28.89	68.01	23.26	1996.83
	A-SLU	100	48.31	2.07	1.12	2.38	89.37	42.03	24.64	59.90	189.86	1827.54
	A-LIQ	100	61.73	1.62	0.95	1.71	105.56	58.64	32.72	59.88	216.05	771.60
	A-SOL	100	34.79	2.87	1.21	2.99	82.46	33.40	22.62	66.81	172.23	2670.84
PiSM	**SLU**	100	127.23	0.79	1.16	1.33	86.51	75.06	34.35	68.70	0.00	1276.08
	LIQ	100	208.33	0.48	1.85	1.85	54.17	54.17	20.83	20.83	6.25	310.42
	SOL	100	26.25	3.81	2.35	8.28	42.52	12.07	45.67	109.71	21.00	1810.50
	A-SLU	100	152.91	0.65	0.83	2.52	120.80	39.76	38.23	82.57	142.20	1140.67
	A-LIQ	100	208.33	0.48	0.68	1.66	147.92	60.42	43.75	93.75	193.75	545.83
	A-SOL	100	36.95	2.71	1.36	8.20	73.54	12.20	38.80	63.93	60.24	2364.38
PiSF	**SLU**	100	27.06	3.70	0.99	0.78	101.19	127.44	34.36	70.62	25.97	1494.05
	LIQ	100	30.25	3.31	4.24	1.88	23.59	53.24	7.86	35.69	14.82	544.77
	SOL	100	16.89	5.92	0.54	2.83	186.25	35.29	58.09	98.95	24.32	1817.46
	A-SLU	100	25.33	3.95	0.97	1.94	103.34	51.42	49.90	70.92	139.06	1239.87
	A-LIQ	100	27.46	3.64	0.88	1.73	114.22	57.66	37.34	50.52	127.92	564.80
	A-SOL	100	21.45	4.66	1.20	2.59	83.23	38.61	27.24	87.52	131.92	2105.32
CaM		100	63.69	1.57	0.44	0.84	225.48	119.75	87.90	128.66	0.64	5441.40
GoM		100	39.53	2.53	0.95	3.09	105.14	32.41	79.45	309.88	0.40	4433.99
PoM		100	9.34	10.71	0.91	0.54	109.39	184.12	44.84	110.14	0.93	3197.76

CaS+: Intensive Cattle Slurry; CaS- : Cattle Slurry; PiSM: Pig Slurry with all stages of pig production; PiSF: Pig Slurry from fattening farm; CaM: Cattle Manure; GoM: Goat Manure; PoM: Poultry Manure. SLU: Non-treated Slurry; LIQ: Liquid Fraction; SOL: Solid Fraction; A-SLU: acidified Slurry; A-LIQ: Liquid Fraction of the acidified Slurry; A-SOL: Solid Fraction of the acidified Slurry; N_{av}: estimated available nitrogen.

Table 2.3 allows the discussion of the results from a “user-friendly” point of view, and the real possibility of replacing totally, or partially, the mineral fertilizers with manure-based fertilizers. The information on the N_{av}:P_2O_5 ratios allows, for instance, the selection of materials richer in available N, and poor in P, to avoid the usual surplus of P when applying animal manure to the soil (Sommer et al., 2015). This condition is fulfilled by the LIQ fractions, but with the disadvantage of increasing the quantity of material needed to provide the necessary amount of nutrients (e.g., see the amounts needed to provide 100 kg N ha^{-1}, Table 2.3), or with the application of the A-SOL fractions, more concentrated, while reducing the amount of material needed to provide the same quantity of N to the crops. In the case of raw PiSM, or their LIQ fractions, for instance, the amounts needed to supply 100 kg N ha^{-1} would be enormous (127.23 to 208.33 t ha^{-1}), hindering the use of this material as an integral substitute for mineral fertilization, or to the exportation of their nutrients content, leaving that hypothesis only to its solid fractions (SOL or A-SOL). On the opposite side, for instance, PoM only requires a small amount of material to provide a large amount of N (e.g., about 9.3 t to provide 100 kg N ha^{-1}, Table 2.3), with the bonus of providing other nutrients, except P, which may be, eventually, supplied by the use of other manure derived materials, richer in P (e.g., the solid fraction of PiSF), or supplemented with a mineral fertiliser.

One aspect that it is important to highlight, and that is obvious from Table 2.3, is that these materials can suppress the needs of the crop, partially or totally, not only regarding N, P and K, but also for the secondary macronutrients (Mg, Ca and S, Table 2.3). Another positive point, relative to the use of mineral fertilizers, is the addition of organic C to the soil. In fact, taking, for example, PoM, the referred application dose (9.3 t ha^{-1} year^{-1} to provide 100 kg N), will correspond to an input of 3197.8 kg ha^{-1} year^{-1} of exogenous C, which can be an important contribution to the increase of the soil organic carbon pool.

An adequate supply of nutrients, combined with organic matter, is crucial to ensure a high quality production in agriculture (Souri et al., 2018) and it can be beneficial towards soils health. The uncertainty of nutrients availability on a manure-based fertilizer may lead to unbalanced inputs of other elements, like P (Keskinen et al., 2020). These authors did accomplish the refining of broiler manure as a N fertilizer, allowing to adjustment of the amount of amendment required to a specific purpose, promoting the agronomic enhancement of this nutrient resource. Also, Mažeika et al. (2021) assessed the agrochemical efficiency of the granulated organic and organo-mineral fertilizers obtained from chicken manure waste, demonstrating that its application can keep a constant nutrient concentration in soil with a double benefit of minimizing the mineral fertilizer environmental impacts. It can be then stated that it is possible to produce manure-based fertilizers by concentrating nutrients and altering the N:P ratio to a more adequate and known value, one of the specifications most valued by farmers (Tur-Cardona et al., 2018), but it require the blending of manures or sub products of the treatments applied . However, more studies will be essential to understand the nutrients dynamics in soil and ensure that both scenarios proposed (farm scale or centralized level) close the nutrients loops and offers a sustainable hypothesis towards a circular economy.

2.4 Conclusion

In this study, it was possible to identify the most promising materials for the production of tailor-made organic fertilizers as well as those that do not fulfil the needed requirements, due to feasibility issues, e.g., the large quantity of PiSM or its liquid fractions (LIQ, A-SLU and A-LIQ) required to supress the crops needs. Indeed, some of these materials can individually solve some of the problems initially pointed out, namely the unbalanced nutrients ratio of manures relatively to plant requirements that usually lead to the overapplication of P. Nevertheless, these low-technological demanding techniques may not be sufficient to produce the intended manure-based fertilizer with a known N:P ratio but will surely help to cope with the reality towards a more sustainable agricultural practice. The results obtained in this study demonstrated that the blending of some of these materials has a strong potential to the production of specific manure-based fertilizer.

More studies are needed to properly assess some potential interesting blends and some questions still need to to be solved, namely the behaviour of the materials when blended to produce a possible manure-based fertilizer in both scnearios considered, which may differ from their individual performance.

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Supplementary material

Table S2.4: Micronutrients and sodium (Na) concentration of the raw manures from the animal species considered in the study (mean value, n=3). Values in the same column followed by the same letter are not statistically different (Tukey HSD test, P > 0.05).

	Fe	**Cu**	**Zn**	**Mn**	**B**	**Na**
	mg kg^{-1}	**mg kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**	**mg kg^{-1}**	**g kg^{-1}**
CaS+	206.12^{bc}	5.14^{de}	18.85^{c}	18.38^{cd}	2.19^{c}	1.38^{bc}
CaS-	174.05^{c}	3.67^{e}	21.44^{c}	67.57^{b}	2.66^{bc}	0.82^{cd}
PiSM	35.84^{d}	4.60^{e}	28.13^{c}	7.53^{d}	0.83^{c}	0.23^{d}
PiSF	237.43^{b}	19.76^{b}	83.71^{b}	67.57^{b}	6.91^{b}	1.44^{b}
CaM	150.56^{c}	7.74^{d}	31.83^{c}	34.61^{bcd}	1.64^{c}	0.46^{d}
GoM	473.23^{a}	12.72^{c}	49.46^{c}	66.97^{bc}	3.83^{bc}	0.45^{d}
PoM	408.80^{a}	59.24^{a}	265.79^{a}	314.85^{a}	30.82^{a}	4.62^{a}

CaS+: Intensive cattle slurry; CaS-: Cattle slurry; PiSM: Pig slurry with all stages of pig production; PiSF: Pig slurry from fattening farm. CaM: Cattle Manure; GoM: Goat Manure; PoM: Poultry Manure; Fe: Total iron; Cu: Total copper; Zn: Total zinc; Mn: Total manganese; B: Total Boron; Na: Total sodium.

Table S2.5: Micronutrients and Na concentration in the materials obtained from the treated slurries (mean value, n=3). Values in the same column followed by the same letter are not statistically different (Tukey HSD test, P > 0.05).

		Fe mg kg^{-1}	**Cu** mg kg^{-1}	**Zn** mg kg^{-1}	**Mn** mg kg^{-1}	**B** mg kg^{-1}	**Na** g kg^{-1}
CaS+	**SLU**	206.12^{ef}	5.14^{efg}	18.85^{jk}	18.38^{i}	2.19^{efghi}	1.38^{ef}
	LIQ	50.56^{hi}	3.37^{efg}	10.95^{klm}	9.65^{j}	1.54^{hijk}	1.51^{de}
	SOL	360.73^{b}	15.83^{cd}	39.07^{gh}	43.49^{f}	3.24^{def}	1.49^{de}
	A-SLU	131.54^{g}	5.88^{efg}	20.09^{jk}	19.05^{i}	1.86^{ghij}	1.58^{bcd}
	A-LIQ	77.69^{h}	4.06^{efg}	13.72^{jklm}	18.60^{i}	1.89^{ghij}	1.57^{cd}
	A-SOL	213.83^{ef}	7.70^{e}	27.45^{hij}	17.01^{i}	1.83^{ghij}	1.24^{f}
CaS-	**SLU**	174.05^{fg}	3.67^{efg}	21.44^{jk}	67.57^{c}	2.66^{efgh}	0.82^{g}
	LIQ	35.82^{hi}	1.52^{fg}	10.15^{klm}	8.19^{j}	1.04^{ijk}	0.75^{g}
	SOL	248.35^{de}	5.38^{efg}	36.44^{ghi}	32.10^{gh}	3.42^{de}	0.72^{g}
	A-SLU	149.80^{g}	3.89^{efg}	24.30^{ijk}	19.72^{i}	2.13^{fghi}	0.72^{g}
	A-LIQ	65.56^{h}	2.31^{efg}	16.04^{jkl}	16.97^{i}	1.63^{hijk}	0.79^{g}
	A-SOL	285.71^{cd}	6.35^{ef}	41.12^{fgh}	30.36^{h}	2.94^{defg}	0.80^{g}
PiSM	**SLU**	35.84^{hi}	4.60^{efg}	28.13^{hij}	7.53^{j}	0.83^{jk}	0.23^{h}
	LIQ	3.38^{i}	0.35^{g}	1.42^{m}	0.67^{k}	0.49^{k}	0.22^{h}
	SOL	319.99^{bc}	36.51^{b}	235.39^{b}	61.34^{d}	3.42^{de}	0.37^{h}
	A-SLU	30.60^{hi}	3.72^{efg}	23.22^{ijk}	6.63^{j}	0.70^{jk}	0.22^{bc}
	A-LIQ	6.77^{i}	0.51^{g}	2.18^{lm}	4.26^{jk}	0.63^{jk}	0.25^{h}
	A-SOL	287.25^{cd}	45.57^{a}	295.03^{a}	36.25^{g}	1.72^{ghijk}	0.31^{h}
PiSF	**SLU**	237.43^{e}	19.76^{cd}	83.71^{e}	67.57^{c}	6.91^{c}	1.44^{de}
	LIQ	48.81^{hi}	14.11^{d}	46.58^{fg}	18.47^{i}	4.13^{d}	1.47^{de}
	SOL	429.28^{a}	35.59^{b}	143.94^{c}	154.54^{a}	13.40^{a}	1.74^{ab}
	A-SLU	176.58^{fg}	21.39^{c}	75.93^{e}	65.24^{cd}	6.80^{c}	1.70^{abc}
	A-LIQ	70.59^{h}	14.37^{d}	54.32^{f}	54.11^{e}	5.76^{c}	1.75^{a}
	A-SOL	342.57^{b}	31.71^{b}	119.10^{d}	80.84^{b}	8.32^{b}	1.50^{de}

CaS+: Intensive cattle slurry; CaS-: Cattle slurry; PiSM: Pig slurry with all stages of pig production; PiSF: Pig slurry from fattening farm. SLU: Non-treated slurry; LIQ: Liquid fraction; SOL: Solid fraction; A-SLU: acidified slurry; A-LIQ: Liquid fraction of the acidified slurry; A-SOL: Solid fraction of the acidified slurry. Fe: Total iron; Cu: Total copper; Zn: Total zinc; Mn: Total manganese; B: Total Boron; Na: Total sodium.

Chapter III The effect of manure-based fertilizers on nitrogen mineralization and greenhouse gases emissions after soil application

Based on the submitted paper to Heliyon

Abstract

It is important to provide strategies that could enhance the use of manures in agriculture, recycling nutrients and organic matter. This study aimed to evaluate the use of manure-based fertilizers (MBF), with tailored N:P ratios, to values commonly used by farmers: 1:1, 2:1 and 0.5:1. These MBFs were applied to a sandy soil and the resulting nitrogen mineralization, nitrification rates, and greenhouse gases emission were measured. Raw manures (cattle slurry, pig slurry, and poultry manure) were used directly to obtain the 1:1 N:P ratio. For the 2:1 ratio, two MBFs were produced with each raw manure, plus the addition of urea or ammonium sulphate to provide additional N. To prepare the P richer fertilizer with a 0.5:1 ratio, the pig slurry solid fraction was used on its own, while the cattle slurry and poultry manure were blended with superphosphate or with phosphoric acid, to provide the additional P. In the 1:1 ratio, both slurries had higher mineralization rates (~35% of organic N applied) and lower environmental impact, compared to poultry manure. Blending poultry manure with urea, for the 2:1 ratio, improved the N mineralization rate, while decreasing the N_2O and CO_2 emissions to almost half the value observed with the raw poultry manure, enhancing its fertilizer value. The addition of superphosphate to poultry manure decreased the N_2O emissions and presented a similar nitrification rate as the raw material. The present results demonstrate that it is possible to produce MBFs with these specific N:P ratios, with this potential agronomical and environmental benefits, compared to the raw material.

Keywords:

Manure-based fertilizer; nitrogen to phosphorus ratio; nitrogen mineralization; nitrification rates; greenhouse gases emissions; global warming potential

3.1 Introduction

The link between animal and agricultural production was a common practice and the application of manure to the soil used to be the main source of nutrients for crops in the surrounding lands (Hills et al., 2021). With the specialization and separation of agriculture and animal production led to the break of nitrogen (N) and phosphorous (P) cycles, by stopping breaking of manure and forages between both types of farms. It also led to a concentration of manure in areas with high livestock farming density, leading sometimes to an overapplication of nutrients to soil (Svanbäck et al., 2019). On the contrary, areas more specialized in crop production needed to import nutrients and European agriculture depends widely on the importation of mineral fertilizers (Luo et al., 2021).

The introduction of the Circular Economy concept to the agricultural sector appears as a possibility to recover materials while closing the nutrients loops and mitigating their losses and potential pollution outcomes (Ritzén and Sandström, 2017; Vaneeckhaute et al., 2017). The establishment of bio-based fertilizer application appears as an alternative to balance the nutrients flow, while agriculture becomes more economic and eco-friendly for repurposing materials considered as a waste (Chojnacka et al., 2020; Lesschen et al., 2020). The use of manure-based fertilizers (MBFs) may not only replace mineral fertilizers but also improve nutrient use efficiency. Tur-Cardona et al. (2018) presented the production of a MBF as a process to improve manure's characteristics, while decreasing the uncertainty of the nutrients content, meliorating the safety aspects (e.g., pathogenic microorganisms, heavy metals), and altering the manure form to facilitate the handling and storage. If several of these criteria were achieved, the acceptance of farmers for new fertilizers, namely MBF, would be higher, and the concept of sustainable agriculture would be accomplished. Other factors, such as the N and P use efficiency of the MBFs are important to the validation of this practice.

Another issue related to the use of raw manures is their nutrients ratio, namely N:P ratio, that is imbalanced relative to crops necessities, and can, consequently, generate nutrient deficiency and environmental problems (Liu et al., 2019). By producing MBFs, it is possible to adjust this N:P ratio to specific values, closer to the crop demands and adjusted to specific soil deficiencies. To achieve the intended ratios, it may be necessary to supplement the animal manure with a mineral N or P source, usually from commercially available fertilizers, and/or to adopt manure processing technologies (e.g., solid-liquid separation, acidification) (Fangueiro et al., 2011), to fulfil the desired N:P ratios and available N content. One other characteristic of organic materials, such as manure or derived products, is that most of their N is in organic compounds (Whalen et al., 2019). This affects the N availability, since organic compounds still need to be mineralized in order to turn their nutrients available to the plants, meaning that the nutrients will be available more steadily, compared to mineral fertilizers, and not necessarily when needed (Bary et al., 2016; Dalias and Christou, 2020; Fangueiro et al., 2016). It is important to have a good knowledge of the N dynamic over time, namely the N mineralization and nitrification rates of the MBFs produced.

On the other hand, the solutions purposed as MBF should not lead to an increase in the N and C losses through greenhouse gases emissions (GHG), nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4), commonly associated with manure handling (Dennehy et al., 2017).

The present study emerges from the necessity of creating a MBF from the farmers' perspective while conceiving a hypothetic scenario called here "on-farm", to appraise the possible solutions to recycle nutrients from manures, producing the MBFs at the livestock farm where manure is produced while promoting a green agriculture. In this scenario, it was considered that each livestock farmer would combine their manure with a small quantity of mineral fertilizers to alter the manure N:P ratios according to their needs. Taking this into consideration, the premises were that: (1) the MBF should be produced using only one manure or slurry as the base of the fertilizer, (2) the MBF should have a well know N:P ratio, close to that commonly used in the fertilizers market (in Portugal the most commons are 1:1, 2:1 and 0.5:1), considering the fraction of available N (N_{av}), determined accordingly to the Portuguese legislation (MADRP, 2018), and (3) minimum quantities of commercially available mineral fertilizers should be used to achieve the desired N:P ratios. In agriculture, the separate application of manure and mineral fertilizer may be a practice performed by some farmers, but they are still reluctant in using manures. The combination of the two materials before soil application to obtain MBFs, with similar features as the mineral fertilizer, is a novelty. The application of MBFs can become a solution that combines the benefits of manure application, e.g. adding organic matter to the soil, stimulation of soil microbiota, vehiculation of micronutrients (Schlegel et al., 2017; Yang et al., 2019), and the benefits of mineral fertilizers, e.g. know nutrients concentrations and balance between the nutrients applied and crops necessities (Nesme et al., 2018).

Therefore, the aims of the present paper were: i) to design and evaluate possible MBFs, within the hypothetical "on-farm" scenario by the mixture of one type of manure with small amounts of mineral fertilizers, to obtain the desired N:P ratios; ii) to evaluate the N dynamics in soil amended with the designed MBFs through an aerobic incubation, and iii) to evaluate the impact of the different designed MBF on the GHG emissions following soil application.

3.2 Material and methods

3.2.1 Manure sampling and characterization

Three different manures were utilized in these experiments: i) a cattle slurry (CaS), (ii) a pig slurry from a fattening pig farm (PiS), and (iii) a poultry manure (PoM). These materials were selected considering their previous characterization (Prado et al., 2022), and their representativeness of livestock farms in Portugal. The three manures were sampled at the same farms as those reported by Prado et al. (2022) and analysed for their dry matter (DM), total organic carbon, total N (N_{Total}), ammonium N (NH_4^+-N), total P and K contents, and pH, according to the methodologies described by the same authors (Table 3.1).

Table 3.1. Physicochemical characteristics of the raw manures considered in the study, which correspond, approximately, to the 1:1 ratio, all expressed in fresh matter basis (mean value ± standard deviation, n=3), and composition of the blends designed to provide the 2:1 and 0.5:1 N:P ratios (calculated values, considering the composition and proportion of manure and mineral fertilizer).

		Blend composition		**DM**	**TOC**	**N_{Total}**	**NH_4^+-N**	**N_{av}**	**Total P**	**N:P**
		% raw manure	**% Mineral fertilizer**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g P_2O_5 kg^{-1}**	
1:1 Ratio	**PiS**	100	0	129.7±2.16	55.0±2.16	8.4±0.07	5.6±0.02	5.6±0.04	5.7±0.05	0.98
	CaS	100	0	103.9±1.31	39.5±2.16	3.5±0.01	1.5±0.05	2.1±0.01	1.9±0.05	1.11
	PoM	100	0	749.4±1.11	352.1±2.16	20.3±0.39	3.5±0.12	10.2±0.19	11.8±0.42	0.86
2:1 Ratio	**PiS+U**	98.7	1.3	126.0	55.0	14.1	11.4	11.4	5.5	2.07
	PiS+AS	97.2	2.8	125.8	55.0	13.9	11.3	11.3	5.5	2.03
	CaS+U	99.6	0.4	103.6	39.5	5.1	3.2	3.8	1.9	2.02
	CaS+AS	99.2	0.8	103.1	39.5	5.2	3.2	3.8	1.9	2.06
	PoM+U	97.1	2.9	727.5	352.1	33.1	16.5	23.3	11.5	2.03
	PoM+AS	93.8	6.3	702.5	352.1	32.2	16.1	22.6	11.1	2.04
0.5:1 Ratio	**PiS-SOL**	100	0	232.5	96.7	10.9	6.5	5.5	10.9	0.50
	CaS+SP	99.5	0.5	103.5	39.5	3.4	1.5	2.1	3.9	0.52
	CaS+PA	95.9	4.2	99.7	39.5	3.3	1.4	2.0	3.9	0.50
	PoM+SP	98.0	1.9	743.7	352.1	19.9	3.1	10.0	19.8	0.50
	PoM+PA	86.2	13.8	718.2	352.1	19.5	3.0	9.7	19.3	0.50

PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

The N:P ratios were calculated using the estimated available N (N_{av}) content and the total P content, expressed as P_2O_5, in order to obtain a value comparable with those associated with mineral fertilizers. According to Portuguese legislation, 60% of the N_{Total} content of the manure can be considered as N_{av} for animal manures with DM content < 20%, and 50% of the N_{Total}, for animal manures with DM > 20% (MADRP, 2018). When the analytically determined NH_4^+ content of the manure (or MBF) was higher than the estimated N_{av}, the measured NH_4^+ content was used as the value of N_{av}.

3.2.2 Manure-based fertilizer preparation and composition

MBFs with targeted N:P ratios of 1:1, 2:1 and 0.5:1 were prepared using the sampled manures. Considering that the raw manures already presented a N:P ratio close to 1:1 (Table 3.1), they were used without any supplementation to obtain that ratio.

In a previous study, the slurries were processed using low-technology demanding treatments (i.e., solid-liquid separation, acidification, and the combination of both; Prado et al., 2022) and the solid fraction of the pig slurry from a fattening farm already presented the intended ratio of 0.5:1, reason why this material was used in the case of PiS, without further supplementation of mineral fertilizers. Concerning the CaS and PoM, two individual MBFs were considered for each of them, to reach the intended N:P ratio of 0.5:1: one supplemented with superphosphate (SP) and a second supplemented with phosphoric acid (PA). Thus, the following MBFs were considered: cattle slurry with superphosphate (CaS+SP), cattle slurry with phosphoric acid (CaS+PA), poultry manure with superphosphate (PoM+SP) and poultry manure with phosphoric acid (PoM+PA).

To obtain the 2:1 N:P ratio, each of the three manures was blended with two alternative sources of mineral N: urea (U) or ammonium sulphate (AS) leading to the following MBFs: pig slurry with urea (PiS+U), pig slurry with ammonium sulphate (PiS+AS), cattle slurry with urea (CaS+U), cattle slurry with ammonium sulphate (CaS+AS), poultry manure with urea (PoM+U), and poultry manure with ammonium sulphate (PoM+AS).

Considering the manures composition (Table 3.1), calculations were made to determine the quantity of each material, organic (manure) and inorganic (mineral fertilizer), needed to provide the desired ratio (Table 3.1). Calculations were further made to provide the DM, total organic carbon (TOC) content, nutrients concentrations (N_{Total},

NH_4^+-N, N_{av} and P_{Total}) and nutrients ratios (N:P) obtained through each MBF (Table 3.1). This study intends to produce a material able to replace mineral fertilizers. Therefore, the P content was expressed in P_2O_5 since it is the units used in the commercial mineral fertilizers composition.

3.2.3 Aerobic incubation experiment

A destructive aerobic incubation was performed to assess N dynamics after the incorporation of the MBFs into the soil, emphasizing the N mineralization/immobilization and nitrification rates. The amount of each MBF added to the soil was calculated in order to supply the equivalent to 210 kg N ha^{-1}. However, since the present study aimed to evaluate the potential of MBFs to substitute the application of mineral fertilizer, the 210 kg N ha^{-1} accounted for the N_{av} estimated to be provided by the MBFs (MADRP, 2018). The application of each material was replicated four times and soil without treatment was used as the control. The containers were randomly disposed during the experiment.

The soil used in this experiment was a Haplic Arenosols, a sandy soil, very poor in P and K, with a pH of 5, common in Portugal. For each replicate, 1000 g of air-dried soil was weighed in plastic containers. Prior to the experiment set-up, the soil was moistened at ~30% of the soil water holding capacity (WHC) with distilled water and then pre-incubated at 20 ºC for 7 days. After this pre-incubation, the MBFs were mixed with the soil and distilled water was added to each container to reach a final moisture equivalent to 70% of the soil WHC, which was kept constant during the experiment with regular weighing. The samples were incubated for 90 days at 20 ºC, with the container not totally closed, to guarantee an aerobic environment.

The incubation mixture of each box was sampled on days 1, 3, 8, 14, 21, 28, 35, 42, 49, 63, 77 and 90, collecting a 26 g sub-sample. On each date, the concentration of mineral N (NH_4^+ and NO_3^-) was determined, using methods previously described (Fangueiro et al., 2016; Schinner et al., 2014). The mineral N ionic forms (NH_4^+ and NO_3^-) were extracted using KCl 2M (1:5 *w/v*, soil:solution ratio), after 1h of agitation and centrifugation at 4000 rpm for 7 minutes (centrifuge 5804, Eppendorf, Germany). The NH_4^+ and NO_3^- concentrations were analysed in a segmented flow autoanalyzer (San Plus System, Skalar, Nederland) with a modified Berthelot method (Krorn, 1956).

3.2.4 Greenhouse gases emission assessment

The GHG emissions from soils amended with the different MBFs considered were studied in a parallel experiment, assembled with three repetitions of each mixture (soil + MBF) per MBF, and the control (soil without treatment). Before the experiment set-up, the soil was pre-incubated at 20 ºC for 7 days as referred previously. After that, for each treatment, 0.5 kg of air-dried soil was mixed in a glass jar with the required amount of MBF and distilled water to ensure 70% of the soil WHC, which was kept constant during the experiment by regular weighing. Each treatment received an amount of MBF equivalent to 210 kg N ha^{-1}, the same application rate as the aerobic incubation experiment. The jars remained open between measurements and stored in the dark for 90 days at 20 ºC. GHG emissions rates (CH_4, CO_2 and N_2O) were measured on days 1, 3, 8, 10, 15, 17, 21, 24, 28, 35, 42, 49, 56, 66, 76, 83 and 91, after application as described by Fangueiro et al. (2015b). Briefly, at each sampling date, the jars were hermetically closed, and headspace air sampling was performed immediately (T_0), 30 minutes (T_{30}), and 60 minutes (T_{60}) after closure. The emissions rates were calculated by linear regression, through the T_0, T_{30} and T_{60}, and adjusted for the 20 ºC. The gases concentrations in the air samples were measured by gas chromatography (GC) using a GC-2014 (Shimadzu, Japan), equipped with an electron capture 63Ni to detect N_2O, a thermal conductivity detector for CO_2, and a flame ionization detector for CH_4.

Cumulative gases emission was calculated for each of the sampled gases by summing the daily emissions. In that calculation, when no daily data was available, concentrations were estimated assuming that the daily gas flux changed linearly between the nearest sampling dates. Also, the cumulative emissions of N_2O and CH_4 were converted into CO_2 equivalent (CO_2eq) to account for their contribution to the global warming potential (GWP), using the GWP convertor factor for a 100-year time horizon, which, for CH_4 is 28-times higher than for CO_2, while for N_2O is 265-times higher than for CO_2 (IPCC, 2016). Despite the contribution of CO_2 to GHG emissions, within the agriculture sector, it is estimated that its contribution to GWP is under 1%, due to the atmospheric fixation by the crop and since its emissions are well surpassed by emissions from burning fossil fuels (Maris et al., 2016; Provolo et al., 2018). Hence, the CO_2 was not accounted for in the final GWP.

3.2.5 Data treatment and statistics

The values of net N mineralization (NNM) and the apparent net N mineralization (ANNM) were calculated using the formulas proposed by Ribeiro et al. (2010). The ANNM was calculated considering the organic N applied, since the aim was to determine how much N would be conveyed from the organic fraction of MBF application. The values of ANNM presented corresponded to the end of the first, second and third months of the incubation experiment.

$$\text{NNM (mg N kg}^{-1}\text{)} = \text{min N (t)} - \text{min (N) (t}_1\text{)}$$

$$\text{ANNM (\% organic N applied)} = \frac{(\min N(t) - \min N(t1))treatment - (\min N(t) - minN\ (t1))soil)}{Organic\ N\ applied} \times 100$$

Where min N (t) represents the mineral N (NH_4^+-N + NO_3^--N) at a specific time (t = 3, 8, 14, 21, 28, 35, 42, 49, 63, 77 and 91 days), and min N (t1) represents the mineral N (NH_4^+-N + NO_3^--N) at day 1.

The net nitrification (Nit), was calculated for specific time intervals [ti; tf], using the following formula (Fangueiro et al., 2016), where i= initial and f=final time.

$$\text{Nit (mg } NO_3^-\text{-N kg}^{-1}\text{)} = NO_3^-\ (t_f) - NO_3^-\ (t_i)$$

Also, the expected nitrate concentration in the soil was estimated ($NO_{3\ es}^-$), according to the initial NH_4^+ applied via MBF. This value was compared with the actual NO_3^- observed ($NO_{3\ ob}^-$), the difference observed will be due to the mineralization (ammonification and nitrification) of the organic N applied.

Statistical treatment of the data was performed by one-way ANOVA for the N:P ratios of 1:1 and 0.5:1. For the 2:1 ratio, the two-way ANOVA was used to determine the interaction of manure x N source, since two different N sources were added to each manure. To infer the statistical significance of the means, a Tukey test was performed with $P<0.05$, having a 95% degree of confidence, using Statistix 7.

3.3 Results and discussion

3.3.1 Design of MBFs with known N:P ratio

The results obtained in this study (Table 3.1), evidenced that the manures presented, in their raw forms, N:P ratios close to 1:1. This result is in line with those previously obtained in a study on the impact of the animal species on the N:P ratio, considering a broad range of manures representative of the Portuguese animal production sector (Prado et al., 2022). Hence, these three manures can be used as possible MBFs, when the desired N:P ratio is 1:1, without further processing or supplementation to alter their composition.

Even though the N:P ratios were identical between these three manures, several differences in their composition can be observed (Table 3.1). CaS had a lower concentration of nutrients, compared to the other two manures, namely, a N_{Total} of 3.46 g N kg^{-1} against 8.41 and 20.30 g N kg^{-1}, for PiS and PoM respectively. These results are coherent with other studies, where poultry manure appeared as the material richer in N and, obviously, with the higher dry matter (Zhang et al., 2019). The lower dry matter content of ~104 and ~130 g kg^{-1} for CaS and PiS, respectively, when compared with PoM, will enable a more even application of these types of manures to the soil. Still, the lower dry matter content of the slurries implies that a larger quantity of material is needed to be applied to the soil to supply the same amount of nutrients as the solid manure, due to the dilution effect.

To obtain the MBFs with N:P ratios of 2:1 and 0.5:1, each of the manures used in the present study was enriched with small quantities of mineral fertilizers, those usually purchased by farmers, urea (U) or ammonium sulphate (AS) to supply N, and superphosphate (SP) or phosphoric acid (PA) to enrich the mixture in P. The only exception was the solid-liquid separation of PiS, which resulted in a material, PiS-SOL, 2.5 times more concentrated in the dry matter than the PiS, and, more important, 1.9 times more concentrated in P. This was expected since, during the solid-liquid separation, P remains in the solid fraction (Egene et al., 2021). Therefore, in this specific case, PiS can also provide the 0.5:1 N:P ratio, demonstrating that the slurry processing is sufficient to modify the N:P ratio to an alternative proportion. Nevertheless, the amount of mineral fertilizer needed to reach the targeted N:P ratio for each MBF was, in fact, small, reaching a maximum value of 14% of the total in the case of PoM+PA (Table 3.1). In terms of costs, the 2:1 ratio will be the more expensive to obtain in the “on-farm” scenario, since, to produce any of the MBFs, it will be necessary to use a mineral N source, while in the case of ratio 0.5:1 PiS-SOL and the case of the fertilizers with the ratio 1:1, no mineral sources were needed. Comparing both N mineral sources, the amount of mineral fertilizer added to the MBF when U was used was almost half that required with AS, for all manures. Still, the use of AS may also supply sulfur, essential for crops' healthy growth, providing a beneficial effect that can be also important for the farmer.

The main challenge when blending manures with mineral fertilizers was to obtain a homogeneous material. Indeed, it was much more difficult to obtain a homogenous blend when using a solid manure, like PoM, relative to slurries, due to the small quantities of mineral fertilizer required to achieve the desired N:P ratios. This important aspect needs to be considered when this type of solution is proposed to be applied on-farm.

3.3.2 Effects of manure-based fertilisers on N dynamics in soil

3.3.2.1 Temporal variation of NH_4^+ and NO_3^-

One of the greatest challenges in determining the fertilizing value of manures is to predict their nutrient availability, especially N, since only the mineral N fraction is directly available to the crop immediately after manure application, and it is difficult to predict the mineralization of the N organic forms (Reuland et al., 2022).

In this study, the raw manures presented a N:P ratio close to 1:1 (Table 3.1). Hence the organic N mineralization patterns obtained for the raw manures can be considered as the baseline to evaluate the effects promoted by the blending or processing of the manures to achieve the desired ratios. Also, the presence of organic N will require the mineralization of N into NH_4^+, which will be nitrified by the bacteria into NO_3^- (Sigurnjak et al., 2017). Therefore, in all the analysed ratios, during the first 14 days, NH_4^+-N concentration in the soil remained or should remain stable or even increase and, after that period, will decrease or should decrease abruptly due to the higher nitrification rates.

The NH_4^+-N content marked a clear difference between the three manures applied in this study, with CaS presenting the lower initial concentration of NH_4^+-N in the soil, ~20 mg kg^{-1} (Figure 3.1a), while PiS exhibited the higher initial concentration of both mineral N forms, over 40 mg NH_4^+-N kg^{-1} soil and ~10 mg NO_3^--N kg^{-1} soil (Figure 3.1a and b).

This indicates that, with PiS application, both N forms will be available to the crops after soil application. The lower NO_3^- concentration reached with the PoM application can be attributed to the higher C/N ratio of PoM, due to its richness in straws, resulting in lower N availability and longer periods of N immobilization or lower nitrification rates. Sigurnjak et al. (2017), demonstrated that the application of a bio-based fertilizer with a low C/organic N ratio presented a similar agronomic performance as the mineral fertilizers. Therefore, the use of slurries should promote a higher provision of mineral N to the crops, when compared to solid manures, due to a faster conversion of NH_4^+-N to NO_3^--N, which was evident in the present study, when comparing CaS with PoM (Figure 3.1a and b). However, the lower N availability of PoM can be important in situations where N leaching needs to be prevented, e.g., fertilization of winter crops.

Similar to the 1:1 ratio, the N enrichment manures had a rapid NH_4^+-N release, subsequently nitrified to NO_3^--N, with more emphasis from days 8 to 28 (Figure 3.1c and 1d). Still, to note, the final NO_3^- concentrations for PoM+U and PoM+AS in the soil, at the end of the incubation experiment, corresponded to, approximately, 70 mg NO_3^--N kg^{-1} soil (Figure 3.1d), which represents an increase of around 1.75 relative to that verified for the raw PoM (~40 mg NO_3^- kg^{-1} soil).

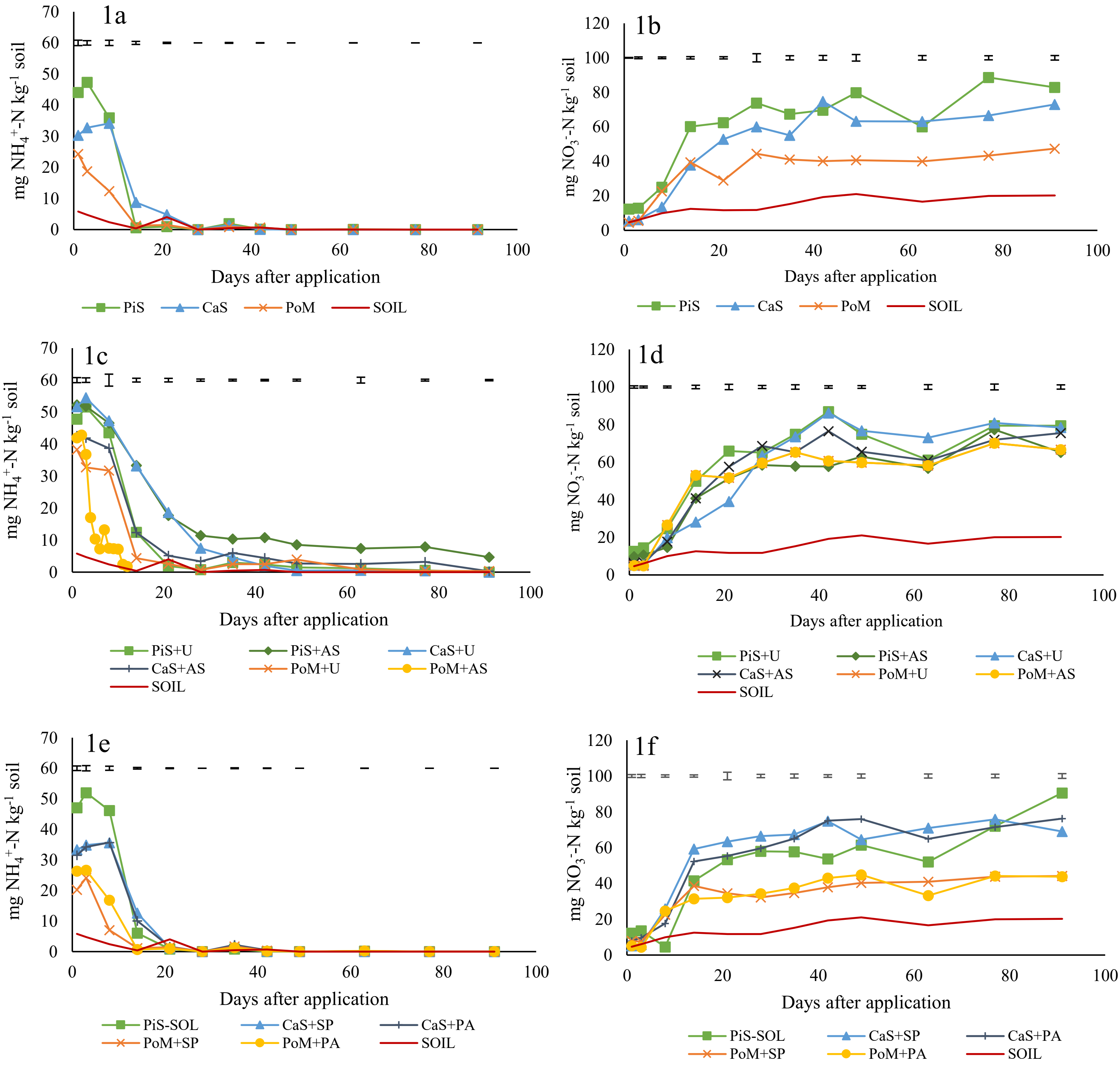

Figure 3.1: The NH_4^+ (left) and the NO_3^- (right) concentrations on the soil for each 1:1 ratio (1a and 1b), 2:1 (1c and 1d) and 0.5:1 (1e and 1f) during the incubation. Values presented are arithmetic means (n=4). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date. PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

Considering the MBFs with PoM and CaS supplemented with mineral P, to obtain the 0.5:1 N:P ratio, the alternative use of SP or PA did not induce a different performance of the materials, with similar NH_4^+-N and NO_3^--N concentrations during the incubation period (Figure 3.1e and f). However, their addition to CaS increased the initial concentration of NH_4^+-N, relative to PoM, reaching a concentration of ~30 mg kg^{-1} in the case of CaS+SP and CaS+PA after soil application, values higher than those noticed for PoM+SP and PoM+PA (Figure 3.1e), which was not the case of the raw materials (Figure 3.1a). The NH_4^+-N concentration observed after the CaS+SP and CaS+PA application to soil even increased during the first 8 days of the experiment, which was not the case with the raw CaS application. This fact may be caused by a delayed nitrification, or by an initial mineralization of organic N, promoted by the addition of the mineral P source to CaS. Nevertheless, the final NO_3^--N concentration in the CaS MBFs did not diverge, compared to raw CaS, evidencing that the overall nitrification was not affected by the mineral P addition.

Regarding the PiS-SOL addition to soil, the NH_4^+-N concentration was kept constant for the first 8 days, indicating a delayed nitrification, as already observed by other authors (Fangueiro et al., 2010). For this material, as well as for the PoM+SP and PoM +AS, the NO_3^--N concentration in the soil remained stable between days 14 and 63 and, only after that, increased more markedly. This could be the result of a higher C/N ratio compared to the CaS MBF in the 0.5:1 N:P ratio. This impaired the soil organic matter decomposition, since soils microorganism used the initial mineral N available to feed themselves and only released the surplus N to the crops (Zare and Ronaghi, 2019). This can indicate a lower potential for N losses since N will be more slowly nitrified, but with a rapid increase later, which can be interesting for the crop's late growth stage (after two months of growth), when more N may be required.

3.3.2.2 Nitrification and mineralization rates

Considering the raw materials, CaS always presented positive nitrification rates, which were higher from days 8 to 14, with a value of 24.3 mg NO_3^--N kg^{-1} soil day^{-1} (Table 3.2). PiS was the raw material that presented higher nitrification rates in some periods, from days 8 to 14 and from days 63 to 77 (35.09 and 28.63 mg NO_3^--N kg^{-1} soil day^{-1}, respectively), but with negative nitrification rates on the last 20 days, which was also evident by a decrease in the NO_3^--N concentration in that interval (Figure 3.1b). This had repercussions on the $NO_3^-{}_{ob}$, which accounts for the total mineralization, and not for that registered in specific periods, indicating a global lower N mineralization than that predicted from the manure composition. These negative values could be attributed to losses not accounted for in the incubation experiment, such as N_2O emissions or N immobilization periods. Both slurries presented similar ANNM values, indicating a quick N release into the soil (Table 3.2). The nitrification rates registered for PoM were higher in the first 14 days of the incubation, but with lower rates than those observed for the slurries with 17 mg NO_3^--N kg^{-1} soil day^{-1}. Similar results were presented by Zare and Ronaghi (2019), which alerted to the fact that poultry manure have lower N availability rates, relative to the use of pig manure, and the authors attributed it to the higher C/N ratio of poultry manure. Indeed, considering the organic N mineralized, the values corresponded to, approximately, half the mineralization values obtained for the slurries. This effect can be explained by the forms of N present in PoM, such as the quickly available N (in urea or uric acid forms) and organic composts, which can only be slowly mineralized (Bhogal et al., 2016). The higher C/N ratio of PoM is, also, a consequence of its rich content in straws, resulting in lower N availability and longer periods of N immobilization or lower mineralization values (close to zero). However, the lower N release resulted in a positive mineralization, since $NO_3^-{}_{ob}$ was higher than the $NO_3^-{}_{es}$, indicating that, even in a smaller amount, all the NH_4^+-N vehiculated by PoM was mineralized.

Table 3.2: Nitrification rates in significant time intervals during the incubation experiment, and apparent net N mineralization (ANNM) in chosen dates over the incubation time for each blend (mean values, n=4). Results for the estimated mineralization rate ($NO_3^-{}_{es}$), the NO_3^- observed at the end of the experiment ($NO_3^-{}_{ob}$), and the difference between the estimated and the observed ($\neq NO_3^-$) are also presented. Results for each ratio in each column followed by different letters differ significantly for P<0.05 (Tukey test).

		Nitrification Rate					**ANNM**			**$NO_3^-{}_{es}$**	**$NO_3^-{}_{ob}$**	**$\neq NO_3^-$**
		3 to 8	**8 to 14**	**14 to 21**	**63 to 77**	**77 to 91**	**28**	**49**	**91**			
		mg NO_3^--N kg^{-1} soil					**% of organic N applied**			**mg N kg^{-1}**		
1:1 Ratio	**PiS**	12.13^{b}	35.09^{a}	2.31^{b}	28.63^{a}	-5.70^{c}	25.87^{a}	22.37^{a}	30.34^{b}	70.00^{a}	62.73^{a}	-7.27^{c}
	CaS	7.42^{c}	24.30^{b}	14.87^{a}	3.43^{b}	6.47^{a}	34.26^{a}	28.09^{a}	40.97^{a}	49.57^{b}	52.79^{b}	3.23^{b}
	PoM	17.07^{a}	16.78^{c}	-10.53^{d}	3.25^{b}	4.10^{ab}	12.07^{b}	1.45^{b}	8.92^{c}	21.66^{c}	27.19^{c}	5.53^{a}
	SOIL	3.86^{d}	2.58^{d}	-0.84^{c}	3.38^{b}	0.16^{b}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
2:1 Ratio	**PiS+U**	10.15^{c}	25.28^{ab}	16.11^{ab}	17.50^{a}	18.20^{a}	24.64^{b}	36.94^{a}	55.41^{a}	70.00^{a}	59.20^{a}	-10.80^{c}
	PiS+AS	4.03^{d}	26.12^{a}	10.65^{ab}	20.78^{a}	8.49^{b}	33.22^{ab}	< 0	< 0	70.00^{a}	45.05^{b}	-24.95^{d}
	CaS+U	14.48^{b}	8.33^{d}	10.85^{ab}	9.63^{b}	5.35^{bc}	34.31^{ab}	26.39^{b}	31.94^{b}	58.73^{b}	58.23^{a}	-0.50^{a}
	CaS+AS	11.08^{c}	23.08^{b}	16.80^{a}	11.36^{b}	14.56^{a}	60.34^{ab}	21.90^{bc}	47.59^{b}	58.91^{b}	55.26^{ab}	-3.65^{b}
	PoM+U	21.79^{a}	26.45^{a}	-1.37^{c}	12.46^{b}	8.38^{b}	31.07^{ab}	18.25^{c}	27.80^{bc}	49.51^{c}	46.44^{b}	-3.07^{b}
	PoM+AS	13.91^{b}	12.61^{c}	9.93^{b}	0.25^{c}	7.64^{b}	4.40^{a}	18.25^{c}	10.30^{a}	49.68^{c}	41.06^{c}	-8.62^{c}
	SOIL	3.86^{d}	2.58^{e}	-0.84^{c}	3.17^{c}	3.54^{c}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
0.5:1 Ratio	**PiS-SOL**	-8.96^{e}	36.95^{a}	11.74^{a}	19.59^{a}	38.69^{a}	< 0	< 0	64.19^{a}	70.00^{a}	70.39^{a}	0.39^{c}
	CaS+SP	19.99^{a}	33.38^{b}	4.21^{ab}	4.82^{c}	-1.97^{e}	39.74^{a}	23.04^{b}	31.08^{c}	49.57^{b}	48.84^{b}	-0.72^{d}
	CaS+PA	8.02^{c}	34.58^{ab}	3.20^{bc}	6.64^{c}	11.28^{b}	28.20^{b}	38.74^{a}	40.46^{b}	49.57^{b}	56.04^{b}	6.47^{a}
	PoM+SP	15.02^{b}	16.18^{c}	-4.20^{c}	2.87^{c}	3.22^{d}	3.41^{c}	0.63^{c}	5.21^{d}	21.66^{c}	23.99^{c}	2.34^{b}
	PoM+PA	20.31^{a}	6.75^{d}	0.68^{bc}	10.93^{b}	10.48^{c}	2.13^{c}	1.32^{c}	2.17^{d}	21.66^{c}	23.55^{c}	1.89^{b}
	SOIL	3.86^{d}	2.58^{e}	-0.84^{bc}	3.38^{c}	3.54^{d}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

The nitrification rates were enhanced when adding a N source, compared to the raw manures (1:1 ratio). In the case of PiS+U or PiS+AS, the value of NO_3^--N concentration in the amended soil was not different between the two, and periods of N immobilization were not observed. Hence, the ANNM of PiS+AS in the last months presented negative values, since part of organic N must have been immobilized. The addition of U might augment nitrification rates, which may increase the possibility of NO_3^--N leaching by applying PiS+U to the soil, leading to a lower N-fertilizing efficiency. The MBFs of CaS supplemented with AS or U did not differ significantly in terms of nitrification rate (Table 3.2), not even in the difference of $NO_3^-{}_{ob}$, from the organic N vehiculated. Therefore, the potential of NO_3^- leaching should not be enhanced by the N sources added. The higher initial nitrification rate (~22 mg NO_3^--N kg soil^{-1} day^{-1}) observed with PoM+U compared to PoM, indicates an enhancement of N availability, which can be positive if there is a crop to assimilate it or can represent a higher risk of NO_3^--N leaching. This indicates that the organic N was stimulated to be mineralized in the present study, with values of ANNM close to 18% of the organic N applied (Table 3.2), in line with the results from Erhunmwunse et al. (2019), who enriched poultry manure compost with N source. However, one aspect should be settled, with the 2:1 MBFs, the difference between the $NO_3^-{}_{ob}$ and the $NO_3^-{}_{es}$ was always negative, indicating that the N-enrichment led to a net N immobilization. However, to ascertain if these results are only blend-dependent, other soils should be considered to perform this incubation.

The addition of PA to PoM and CaS improved the nitrification rate (~3 times and ~2 times, respectively), for the last 30 days of the experiment, when compared to the raw materials. In this way, the addition of PA will not only improve the N availability for the last 30 days but will also be beneficial for crops by vehiculating sulphur. In the case of PoM+PA and PoM+SP, the ANNM varied between 0.63 and 5.21% of organic N applied, values lower than those observed for PoM. This may indicate that adding a P source to PoM will not stimulate the release of N, on the contrary, it can slow it down. However, if N will be continuously supplied in the presence of a crop to assimilate the N, the accumulation of NO_3^- is lower and consequently, the risk of leaching pollution is diminutive. The use of PiS-SOL might have induced an NH_4^+-N immobilization until day 8, considering the negative ANNM and nitrification rate observed. The decrease in NO_3^- concentration in soil (Figure 3.1f), may indicate the reduction in NH_4^+, the substrate responsible for N nitrification, which led to a discrepancy between the $NO_3^-{}_{ob}$ and the $NO_3^-{}_{es}$, meaning that N immobilization occurred. The mineralization occurs when the bacteria in the soil fulfil their need and start to supply N to the soil reserve. Still, when there is a shortage in soil's N reserve, the mineral N is used by soil's bacteria and immobilization occurs (Dalias and Christou, 2020). Onwards, the nitrification rates of PiS-SOL were equal, or superior to the values presented for the raw material, presenting a final NO_3^--N concentration superior to PiS. Thereby, PiS-SOL presented a slow initial N nitrification but, after day 8, kept a constant release of NO_3^--N, which increased the risk of leaching if the crop is not in an uptake stage.

More detailed information relative to net N mineralization can be consulted in the supplementary material (Table S3.5).

3.3.4 GHG emissions from manure-based fertilisers after soil application

3.3.4.1 N_2O emissions

The N_2O emissions in soils amended with raw manures (1:1 ratio) remained low over the whole experiment, except with the PoM treatment, where several peaks were observed on days 3, 15, 24, and 28 (Figure 3.2a). This manure presented the highest N_{Total} losses by gaseous emissions, relative to the other raw manures, with total losses equivalent to 0.59% of available N and 0.29% of total N (Table 3.3). Each peak of N_2O emissions observed for PoM corresponded, simultaneously, to increases in the mineralization rates (Table S3.5) and, for the first 14 days, the nitrification rate was constant (Table 3.2), which may have supplied more NO_3^- to the potential denitrification and, therefore, increased the N_2O emissions. On the contrary, PiS led to the lowest emissions of N_2O, less than half the value emitted by PoM. Fangueiro et al. (2010), also observed residuals emissions of N_2O with PiS and the authors attributed this to the fact that the main source of N_2O was denitrification, which may have been limited in the present aerobic incubation, despite the 70% WHC soil conditions that allow both nitrification and denitrification processes.

In contrast to what was observed regarding N mineralization in the raw manures, the impact of urea or ammonium sulphate addition on N_2O emissions varied significantly between manures (Figure 3.2b). The addition of U or AS to PiS, had a clear influence on the total N_2O emitted, inducing a 2-fold increase in N_2O emissions, relative to raw PiS (~0.29% and ~0.22 of available and total N, respectively, with PiS plus U or AS, against ~0.18% and ~0.11% of available and total N, respectively, with raw PiS (Table 3.3). This is in agreement with Wu et al. (2019), who showed that the combination of pig slurry with urea led to N_2O emissions similar to those observed with urea only, but doubled the emissions relative to PiS. The referred results indicated that the increase of the N_2O emissions can be attributed to the urea addition, and not to the manure itself. Nonetheless, to reduce denitrification and thereby the N_2O emission from the PiS+U treatment, the use of urease inhibitor should be considered (Park et al., 2021). In the case of the CaS, the addition of AS reduced the percentage of available N emitted, relative to raw CaS, 0.23%

against 0.26%, respectively (Table 3.3). As seen in Figure 3.2b, PoM+AS kept a higher emission of N_2O, relative to PoM+U, which might be attributed to the higher mineralization and constant nitrification rates observed in this treatment for the first 21 days of the experiment. However, relative to PoM, the addition of AS to poultry manure did not alter the total amount of N_2O emitted. Attending that the use of raw poultry manure can be, per se, an important source of N_2O emission, caution must be taken when adding an N source to PoM, to avoid an increase in N losses through N_2O emissions (Anderson et al., 2021), and AS is, apparently, a good choice.

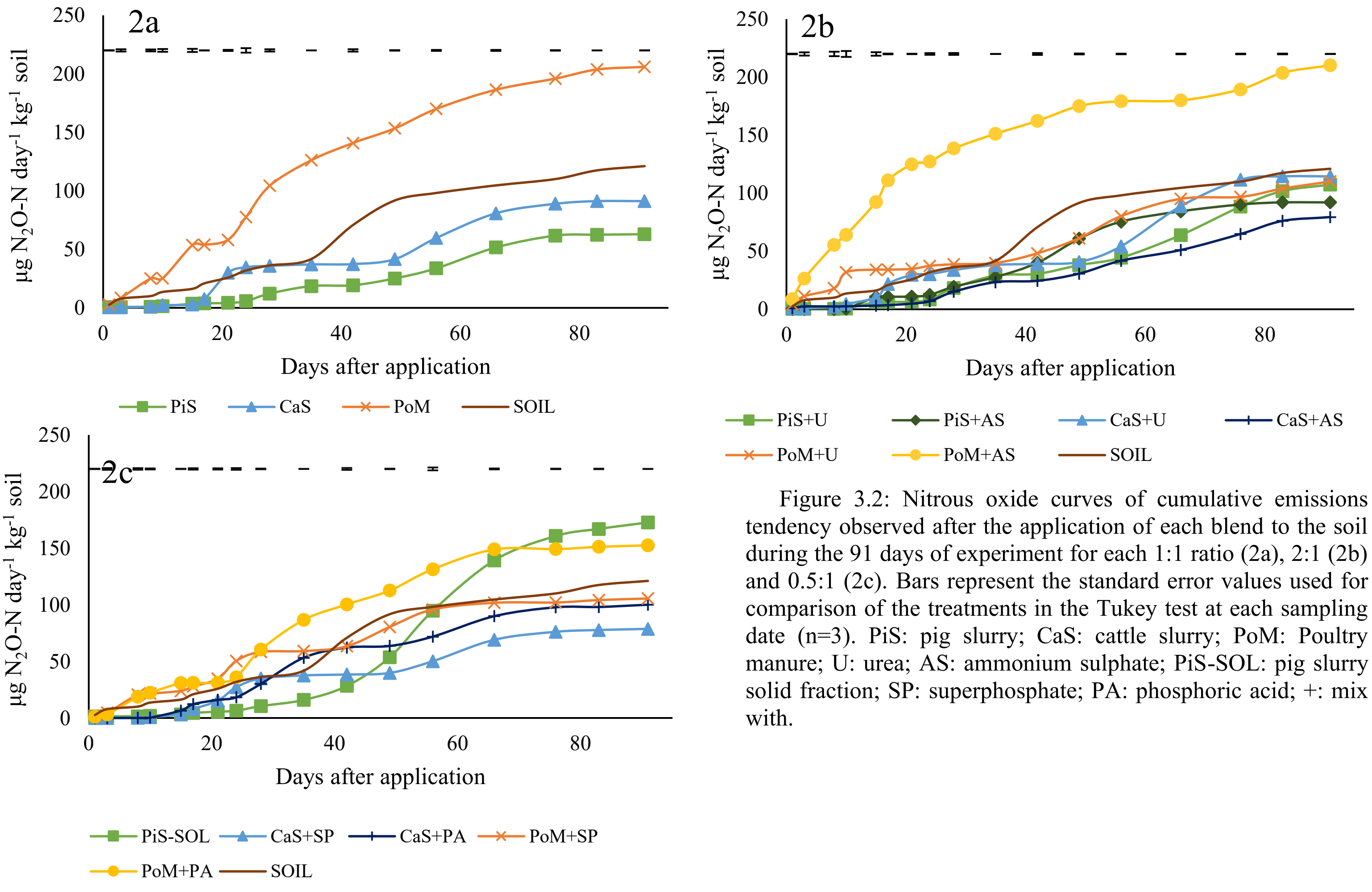

Figure 3.2: Nitrous oxide curves of cumulative emissions tendency observed after the application of each blend to the soil during the 91 days of experiment for each 1:1 ratio (2a), 2:1 (2b) and 0.5:1 (2c). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date (n=3). PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

When combining any manure with a mineral N source, it was expected to observe an increase in the N losses, namely through N_2O emissions. Nevertheless, De Rosa et al. (2018) referred that with manure incorporation into the soil, like the conditions simulated in this study, the N_2O emissions could be mitigated which explains the low values of emissions observed, compared to other studies where the conditions promoted these emissions (Wang et al., 2022; Wu et al., 2019).

Table 3.3: Total cumulative emissions of nitrous oxide (N_2O-N), carbon dioxide (CO_2-C) and methane (CH_4-C) observed after application of each blend to the soil, and the respective amount of CO_2 equivalents (CO_2eq), quantifying the global warming potential (GWP) of each blend. A percentage of the element emitted as a gas (N as N_2O, and C as CO_2 or CH_4), relative to the element applied through the manure-based fertilizer. For each ratio in each column the mean (n=3) followed by different letters differ significantly for $P<0.05$ (Tukey test).

		N_2O-N			**CO_2-C**		**CH_4-C**		**GWP**		
		mg N kg^{-1} soil	**% N_{av} applied**	**% N_t applied**	**mg C kg^{-1} soil**	**% C applied**	**mg C kg^{-1} soil**	**% C applied**	**mg CO_2eq kg^{-1} soil**	**% N_2O**	**% CH_4**
1:1 Ratio	**PiS**	0.06^{d}	0.18^{c}	0.11^{c}	17.51^{c}	3.00^{b}	0.07^{c}	0.013^{a}	55.21^{c}	94.95^{b}	5.05^{a}
	CaS	0.09^{bc}	0.26^{b}	0.16^{b}	28.70^{b}	4.92^{a}	0.09^{c}	0.014^{a}	79.09^{c}	96.00^{a}	4.00^{b}
	PoM	0.21^{a}	0.59^{a}	0.29^{a}	64.42^{a}	5.31^{a}	0.11^{b}	0.009^{b}	175.63^{a}	97.66^{a}	2.34^{c}
	SOIL	0.12^{b}	n.a.	n.a.	13.71^{c}	n.a.	0.13^{a}	n.a.	105.66^{b}	95.42^{b}	4.58^{b}
2:1 Ratio	**PiS+U**	0.11^{bc}	0.31^{bc}	0.24^{b}	12.92^{b}	5.09^{c}	0.11^{ab}	0.044^{a}	93.63^{bc}	95.55^{a}	4.45^{a}
	PiS+AS	0.09^{cd}	0.26^{cd}	0.20^{bc}	15.76^{b}	6.07^{bc}	0.09^{b}	0.036^{b}	80.24^{cd}	95.60^{a}	4.40^{a}
	CaS+U	0.11^{bc}	0.33^{b}	0.24^{b}	30.16^{a}	9.34^{a}	0.09^{b}	0.028^{c}	98.79^{bc}	96.56^{a}	3.44^{a}
	CaS+AS	0.08^{d}	0.23^{d}	0.17^{c}	16.42^{b}	5.15^{c}	0.09^{b}	0.028^{c}	69.53^{d}	95.12^{a}	4.88^{a}
	PoM+U	0.11^{bc}	0.31^{b}	0.22^{b}	33.96^{a}	6.41^{b}	0.09^{b}	0.017^{d}	95.04^{bc}	96.42^{a}	3.58^{a}
	PoM+AS	0.21^{a}	0.60^{a}	0.42^{a}	32.14^{a}	5.90^{bc}	0.13^{a}	0.023^{c}	179.84^{a}	97.36^{a}	2.64^{b}
	SOIL	0.12^{b}	n.a.	n.a.	13.71^{b}	n.a.	0.13^{a}	n.a.	105.66^{b}	85.42^{b}	4.58^{a}
0.5:1 Ratio	**PiS-SOL**	0.18^{a}	0.49^{a}	0.25^{a}	23.75^{b}	3.83^{bc}	0.03^{e}	0.005^{d}	144.94^{a}	99.24^{a}	0.76^{e}
	CaS+SP	0.08^{d}	0.22^{b}	0.13^{c}	24.86^{b}	4.24^{a}	0.11^{c}	0.018^{b}	69.48^{d}	94.26^{b}	5.74^{b}
	CaS+PA	0.10^{cd}	0.29^{b}	0.17^{bc}	25.72^{b}	4.23^{a}	0.20^{a}	0.033^{a}	90.88^{cd}	91.66^{c}	8.34^{a}
	PoM+SP	0.11^{cd}	0.30^{b}	0.15^{c}	51.35^{a}	4.15^{ab}	0.08^{d}	0.007^{c}	90.92^{cd}	96.64^{a}	3.36^{c}
	PoM+PA	0.15^{ab}	0.44^{a}	0.22^{ab}	47.94^{a}	3.78^{c}	0.09^{cd}	0.007^{c}	130.37^{ab}	97.40^{a}	2.60^{d}
	SOIL	0.12^{bc}	n.a.	n.a.	13.71^{c}	n.a.	0.13^{b}	n.a.	105.66^{bc}	95.42^{b}	4.38^{b}

PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with; n.a: not applicable.

The application of PiS-SOL to the soil increased the N_2O emissions by about 3-times, compared to the raw PiS (1:1 ratio; Table 3.3). This fact can be attributed to the higher nitrification rate observed in the soil amended with this material. To prevent the increase in N_2O emissions, pig slurry could be acidified first in order to reduce nitrification (Fangueiro et al., 2017), or treated with nitrification inhibitors (Gómez-Garrido et al., 2018). Contrariwise, the addition of a P source decreased the N_2O emissions of the MBF with PoM by almost half the value observed with the raw manure (Table 3.3). Nonetheless, the use of superphosphate reduced the percentage of N available and total N to half, compared to raw PoM, which could be attributed to the lower values of mineralization and nitrification observed in the previous chapter.

3.3.4.2 CO_2 and CH_4 emissions

As anticipated, the CH_4 emissions in all treatments were residual (Figure 3.3 and Table 3.3) since the experiment was conducted in aerobic conditions. Indeed, the emissions were so low that the highest cumulative value of CH_4 emissions was observed in the control treatment. The total CH_4 emission represented ~0.01% of the total C applied with the slurries and 0.009% with the PoM. In a similar experiment, Anderson et al. (2021) also observed residual CH_4 emissions, coherent with the results obtained in this study. In the case of using solid manures, like PoM, soil aeration was increased, enhancing the soil aerobic conditions, and minimising the risk of CH_4 emissions.

The raw PoM presented the highest CO_2 emissions of all the manures, until day 56 (Figure 3.3a). This stimulation of soil respiration was, somehow, expected, and can be attributed to the higher C concentration in poultry manure. However, considering the C applied to the soil lost as CO_2, PoM and CaS led to similar losses, ~5 % of total C applied (Table 3.3). On the other hand, PiS emitted the same amount of CO_2 as the control, ~18 mg CO_2-C kg^{-1} soil, demonstrating its potential, not only as a fertilizer but also as an organic amendment, since a large part of the C applied is kept in the soil. Fangueiro et al. (2010) referred that pig slurry application to the soil increased the soil microbial activity, which should have led to higher CO_2 emissions, but, in the present study, the soil pH of 5.8 may have inhibited its activity.

Similarly, to the 1:1 ratio, the 2:1 mixture with PoM and both N sources, presented the higher CO_2 emissions for the first three days, relatively to the other mineral N-supplemented MBFs (Figure 3.3c). It is still to refer that the addition of U or AS to PoM resulted in a 50% reduction of the CO_2 emissions (32-34 g CO_2-C kg^{-1} soil) relative to the raw PoM (~64 g CO_2-C kg^{-1} soil) (Table 3.3). However, when considering the total C applied released as CO_2, the values were similar between PoM, PoM+U, and PoM+AS. This indicates that even though the quantity of C applied with the MBFs was lower than with the PoM alone, the C was equally lost, but with a lower impact. In the specific case of CaS, the use of ammonium sulphate reduced the cumulative CO_2 emissions to almost half compared to the raw CaS. However, the percentage of C emitted considering the C applied with CaS+AS and CaS did not differ significantly, due to the quantity of CaS+AS applied to soil, which was half the value compared to CaS.

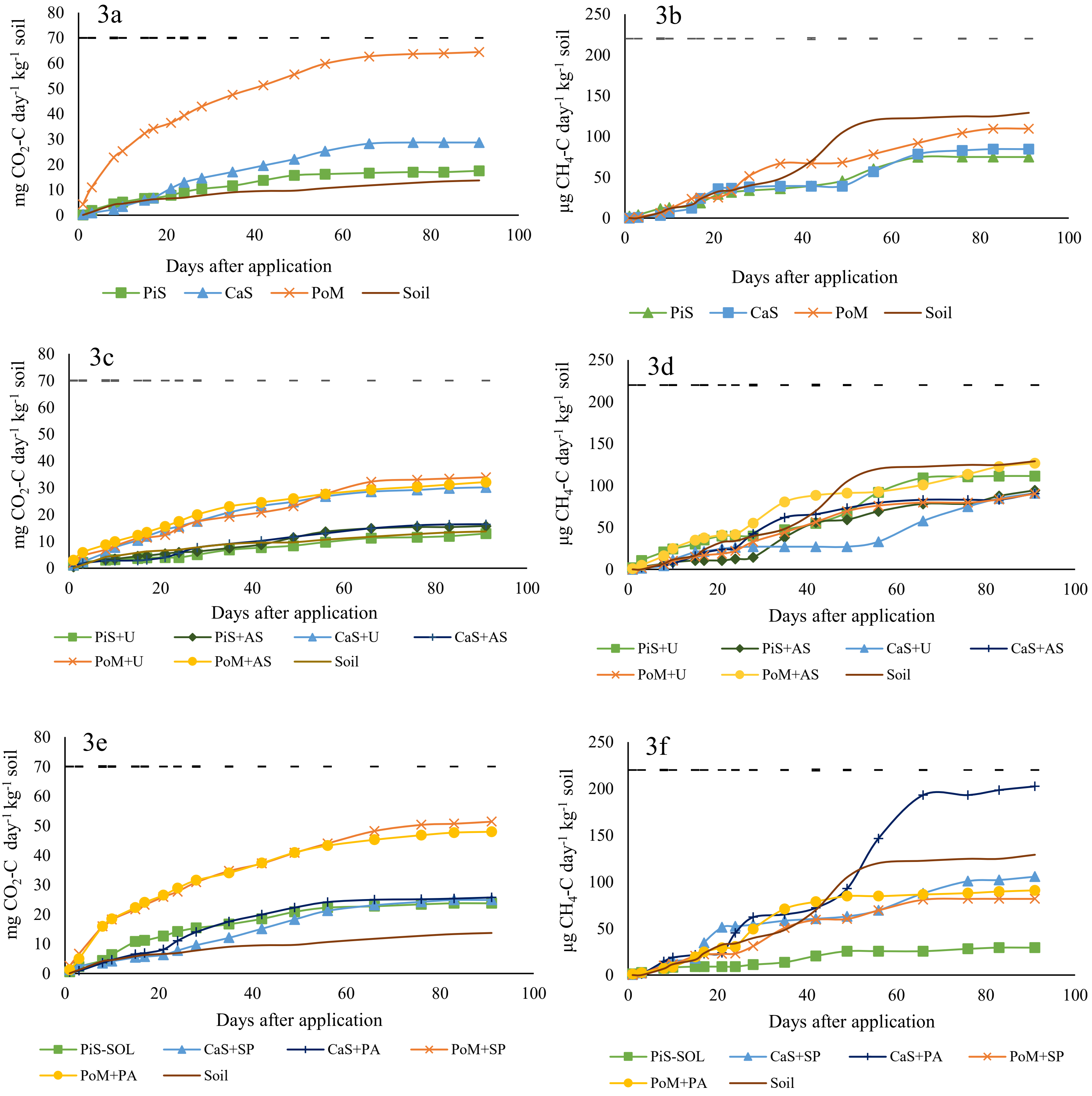

Figure 3.3: Carbon dioxide (left) and methane (right) curves of cumulative emissions tendency observed after the application of each blend to the soil during the 90 days of experiment in each 1:1 ratio (3a,3b), 2:1 (3c,3d) and 0.5:1 (3e, 3f). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date (n=3). PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

3.3.4.3 Global warming potential

The losses through GHG emissions have an environmental impact, usually quantified by the global warming potential (GWP), expressed in mg CO_2eq kg^{-1} soil (Table 3.3). Due to the very lower CH_4 emissions and the higher GWP of N_2O, the main responsible for the GWP was the N_2O emissions, reaching ~98% in the case of PoM and ~95% in the case of PiS. In fact, due to the higher N_2O emissions observed, PoM presented the higher GWP, two and three times higher than the GWP of CaS and PiS, respectively (Table 3.3), results similar to those presented by Shakoor et al. (2021).

The addition of urea to PoM reduced the N_2O emission to half the value observed with the raw PoM application, diminishing the impact on the GWP. The use of PoM+U not only enriched the MBF in N, because of its supplementation with urea but also avoided some of the N losses as N_2O emissions, which naturally occurred in the case of PoM application to soil. However, the contrary occurred when U or AS was added to PiS, with an increase in N losses, through N_2O emissions, when compared to the raw PiS. This resulted in an intensification of the GWP, almost doubling the value presented for PiS. No major alterations were found in the GWP when the mineral N was added to CaS, indicating that the formulation of MBFs with CaS in the 2:1 ratio should not induce any increase in the GWP.

The higher nitrification rate observed for PiS-SOL (Table 3.3), justified the higher N_2O emissions following its application to soil, which resulted in an increase of 2.7 in the GWP of PiS-SOL relatively to PiS. The use of both P additives with PoM reduced the impact in the CO_2eq, but the addition of SP had a higher impact, reducing to almost half the value of GWP obtained for raw PoM application to soil. On the contrary, CaS+PA emitted almost double the CH_4 than the raw CaS, which, consequently, generated a CO_2eq value 1.2 higher than the raw CaS.

3.3.5 Considerations for application on farm scale

It is important to, globally, analyse the impact of each MBF application after application to soil, in terms of N dynamics and GHG emissions, relative to the three raw manures (1:1 ratio) in order to minimize any pollution swapping or eventually induce any production brake.

Table 3.4 summarizes the results obtained here and translates them into a user-friendly language to allow a quick comparison of the MBFs which were studied. Raw manures (1:1 ratio) were first compared among themselves, using capital letters, and then the effect of their enrichment with N or P, in the MBFs with the 2:1 and 0.5:1 ratios, were compared with the manure of origin, using signals.

Relatively to the production of a MBF with PiS, the MBFs with mineral N addition, both urea and ammonium sulphate, increased the mineralization rates. Nonetheless, AS addition decreased the nitrification rate for the first week, indicating that, with this MBF, N will be continuously supplied during early growth stages, but at a lower rate. However, both MBFs increased GHG, contributing to an increase in GWP, making them less interesting, from an environmental and agronomic point of view. Similarly, PiS-SOL appeared as a good solution to optimize N availability in a short-time period, due to the increased nitrification rate, but again, it increased N_2O emissions with a negative impact on the GWP.

The addition of both urea and ammonium sulphate further increased N availability, compared to raw CaS, which will be a good solution for crops with higher N requirements. However, the use of urea increased the N_2O and CO_2 losses, increasing environmental concerns and reducing its agronomic value. To adopt this solution, it could be interesting to use an urease inhibitor. Concerning the use of a mineral P source, while superphosphate maintained the CaS behaviour, phosphoric acid slowed down N availability, which could be interesting to fertilize a crop with late N requirements, but the increase in CH_4 emissions, even if residual, increased the GWP, diminishing the interest on this MBF.

Among the 1:1 ratio MBFs, PoM proved to be the solution with the highest N and C losses after land application. However, with this manure, less quantity is required to supply the same amount of N, which can be positive and facilitates the manure storage, transport, and application. Nevertheless, it can be more heterogeneous, due to its higher solids content, making it difficult to mix and apply. The addition of a mineral N source, such as urea, to PoM, stimulated the N mineralization and nitrification, increasing the N availability and turning N quickly available to the crops. Contrary to the observed for the other MBFs, PoM+U diminished all the GHG emissions, thereby decreasing the GWP. This MBF presented promising results and should be further tested regarding its agronomic value. Relatively to the addition of P sources, both reduced the impact on the environment associated with the application of PoM. However, comparing superphosphate with phosphoric acid, the second decreased the N mineralization and nitrification rates, which was already low using raw PoM. This can result in a lower N exportation by the crop in earlier growth stages and may generate a deficit that can compromise the yield.

Table 3.4: Overview of the impact of each blend application to soil in terms of nitrogen dynamics and GHG emissions between the manure in 1:1 ratio and in the other two ratios the comparation with the correspondent raw manure. 1:1 ratio - H: Higher rates/emissions; M-Medium rates/emissions; L-Lower rates/emissions. 2:1 ratio and 0.5:1 - ↑: Higher availability/Increased in the emissions; ↓: Slower N availability/Less emissions; =: Equal result to the correspondent manure.

		Nitrogen dynamics		**Greenhouse gases emissions**			
		Mineralization rate	**Nitrification rate**	**N_2O**	**CO_2**	**CH_4**	**GWP**
1:1 Ratio	**PiS**	H	H	L	L	L	L
	CaS	M	M	L	L	L	M
	PoM	L	L	H	H	H	H
2:1 Ratio	**PiS+U**	↑	=	↑	=	↑	↑
	PiS+AS	↑	↓	↑	=	↑	↑
	CaS+U	↑	↑	↑	↑	=	↑
	CaS+AS	↑	↑	=	↓	=	↓
	PoM+U	↑	↑	↓	↓	↓	↓
	PoM+AS	↑	=	=	↓	↑	↑
0.5:1 Ratio	**PiS-SOL**	↓	↑	↑	=	↓	↑
	CaS+SP	=	=	=	=	=	=
	CaS+PA	↓	↓	=	=	↑	↑
	PoM+SP	↓	↑	↓	↓	↓	↓
	PoM+PA	↓	↓	↓	↓	↓	↓

PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

3.4 Conclusion

The on-farm production of MBFs with tailored N:P ratios, by combining manures and small amounts of conventional mineral fertilizers, has an interesting potential for farmers. Indeed, some of the MBFs tested led to higher N availability to the crop than the raw manures and/or avoided any increase of N and C gaseous emissions. The main findings were: i) PoM+U (2:1 N:P ratio), and the PoM+SP (0.5:1 N:P ratio), decreased the environmental impact by mitigating all the GHG emissions compared to PoM and thereby enhancing its agronomic value, ii) the P-enrichment of CaS with superphosphate, to obtain the 0.5:1 ratio, did not alter any of the parameters studied relative to raw CaS, turning this MBF, also, a potential solution to adopt when soils are poor in P or crops have a high P requirement, iii) some MBFs increased GHG emissions, namely N_2O emissions (e.g., PiS+U, PiS-SOL, CaS+PA), and iv) therefore, improvements to these MBFs should be considered, to avoid these emissions and the increase in the GWP.

Nevertheless, these results require validation and extrapolation to the field conditions. The agriculture ecosystem is contingent on weather conditions, e.g., rain, wind, and anoxic zones, which can alter drastically the nutrients dynamics, as well as the GHG emissions.

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Supplementary materials

Table S3.5: The net N mineralization (NNM) observed with each blend over the incubation time (mean values, n=4). Results for each ratio in each column followed by different letters differ significantly for P<0.05 (Tukey test).

							NNM					
		3	**8**	**14**	**21**	**28**	**35**	**42**	**49**	**63**	**77**	**91**
							mg N kg^{-1} soil					
1:1 Ratio	**PiS**	3.77^{a}	4.50^{bc}	4.28^{b}	8.37^{b}	17.38^{ab}	13.73^{b}	13.30^{b}	23.41^{a}	4.52^{c}	32.16^{a}	22.46^{b}
	CaS	3.18^{a}	11.97^{a}	10.86^{a}	21.82^{a}	24.29^{a}	20.83^{a}	39.05^{a}	27.41^{a}	27.32^{a}	30.75^{a}	37.22^{s}
	PoM	-4.53^{b}	6.15^{b}	11.83^{a}	2.08^{c}	15.59^{b}	11.88^{b}	11.90^{bc}	11.78^{b}	11.25^{b}	14.44^{b}	18.53^{c}
	SOIL	0.58^{a}	1.90^{c}	2.44^{c}	5.25^{b}	1.30^{c}	5.24^{c}	9.52^{c}	10.58^{b}	6.19^{e}	9.56^{c}	9.73^{d}
2:1 Ratio	**PiS+U**	6.72^{a}	7.69^{bc}	2.62^{b}	7.44^{bc}	6.07^{de}	17.55^{c}	29.00^{b}	16.77^{b}	0.53^{d}	19.57^{b}	19.00^{b}
	PiS+AS	-0.59^{d}	-0.73^{d}	11.04^{a}	5.83^{c}	6.86^{d}	5.03^{d}	6.15^{e}	8.56^{c}	3.54^{c}	23.91^{ab}	6.89^{d}
	CaS+U	3.01^{b}	10.34^{ab}	5.77^{b}	0.80^{d}	14.00^{c}	21.32^{bc}	31.33^{ab}	20.35^{ab}	16.78^{a}	24.40^{ab}	21.55^{a}
	CaS+AS	0.73^{cd}	7.80^{bc}	4.56^{b}	14.16^{a}	23.27^{a}	22.75^{b}	32.25^{a}	19.60^{ab}	14.78^{a}	26.47^{a}	27.05^{a}
	PoM+U	-5.73^{e}	15.06^{a}	14.14^{a}	10.94^{ab}	16.88^{b}	24.52^{ab}	19.86^{c}	20.44^{a}	15.79^{a}	27.19^{a}	23.67^{a}
	PoM+AS	2.16^{bc}	10.01^{ab}	2.88^{b}	5.43^{c}	3.49^{ef}	27.59^{a}	10.96^{d}	19.65^{ab}	11.85^{b}	10.15^{c}	16.31^{c}
	SOIL	0.58^{cd}	1.90^{cd}	2.44^{b}	5.25^{e}	1.30^{f}	5.24^{d}	9.52^{de}	10.58^{c}	6.19^{e}	9.56^{c}	9.73^{d}
0.5:1 Ratio	**PiS-SOL**	7.42^{a}	-8.50^{d}	-11.67^{e}	-5.18^{e}	-1.27^{d}	-0.78^{c}	-5.55^{c}	3.31^{d}	-7.06^{e}	12.73^{cd}	31.31^{b}
	CaS+SP	2.21^{cd}	22.96^{a}	33.35^{a}	26.13^{a}	27.96^{a}	30.78^{a}	36.38^{a}	26.05^{b}	32.55^{a}	37.36^{a}	30.58^{b}
	CaS+PA	5.34^{ab}	14.01^{b}	22.93^{b}	16.58^{b}	20.23^{b}	27.91^{a}	36.20^{a}	36.58^{a}	22.60^{b}	32.15^{b}	36.88^{a}
	PoM+SP	3.58^{abc}	1.46^{c}	11.76^{c}	7.84^{c}	4.21^{c}	8.32^{b}	10.04^{b}	12.24^{c}	13.04^{c}	15.78^{c}	16.13^{c}
	PoM+PA	-0.45^{d}	10.02^{b}	0.68^{d}	2.82^{d}	2.78^{cd}	7.30^{b}	11.70^{b}	13.40^{c}	2.89^{d}	12.75^{cd}	12.29^{cd}
	SOIL	0.58^{d}	1.90^{c}	2.44^{d}	5.25^{c}	1.30^{cd}	5.24^{b}	9.52^{b}	10.58^{c}	6.19^{d}	9.56^{d}	9.73^{d}

PiS: pig slurry; CaS: cattle slurry; PoM: Poultry manure; U: urea; AS: ammonium sulphate; PiS-SOL: pig slurry solid fraction; SP: superphosphate; PA: phosphoric acid; +: mix with.

Chapter IV Nutrient potential leachability in a sandy soil amended with manure-based fertilizers

Based on the published article:

Prado, J., Alvarenga, P., Ribeiro, H., Fangueiro, D. (2023). Nutrient Potential Leachability in a Sandy Soil Amended with Manure-Based Fertilisers. Agronomy 13, 990. https://doi.org/10.3390/agronomy13040990

Abstract

The application of manure-based fertilizers (MBFs) is considered an important practice to achieve agriculture sustainability. However, the potential losses of nutrients to the environment need to be thoroughly evaluated. This study aimed to assess nutrients' potential leachability from a sandy soil, fertilized with MBFs produced by mixing manure from one single animal species with N- or P-mineral fertilizers, to achieve target N:P ratios (1:1, 2:1 and 0.5:1). MBFs were prepared by combining pig slurry, cattle slurry or poultry manure with N- and P-mineral fertilizers, or slurry-derived materials, obtained by solid-liquid separation. A leaching experiment was set-up in soil columns treated with MBFs, for 59 days, with seven leaching events. Poultry manure application to soil led to higher potential N leaching, while pig slurry induced higher P leaching. All 2:1 MBFs decreased P leaching, relative to the original manure, with the higher reduction (52%) being observed for pig slurry with urea. The addition of urea to poultry manure also diminished its potential for N leaching. The behaviour of P-enriched materials, pig slurry solid fraction and both 0.5:1 MBFs obtained with phosphoric acid addition, showed a higher risk of P leaching, while the use of superphosphate as mineral-P source decreased the risk of P leaching. Concluding, it is possible to use specific MBFs, enriched with N and P from mineral sources, and have lower N and P leaching potential, reducing the risks associated with manure soil application, while increasing their interest as alternative fertilizers.

Keywords: Manure-based fertilizer; N:P ratio; nutrients leachability; macronutrients; micronutrients; nutrients availability.

4.1 Introduction

Since World War II, agricultural developments increased food production, with subsequent increases in the use of mineral fertilizers (Rayne and Aula, 2020). The higher food production resulted, nowadays, in non-sustainable agricultural practices and a reduction of soil's organic matter and nutrients content, which culminated in lower productivity (Rayne and Aula, 2020). The concept of circular economy has been promoted by the European Commission to achieve an eco-friendly agriculture, which would close the nutrients loop, by decreasing their losses, improving nutrients use efficiency, and diminishing the dependency on the importation of energy and raw materials for mineral fertilizers production (Ritzén and Sandström, 2017; Spiegal et al., 2020; Svanbäck et al., 2019). Onwards, an emphasis on the application of recycled organic materials to soil emerged, to close the nutrients cycles, and improve soil health and quality, while protecting the environment, climate, and ecosystems (Möller et al., 2018). Application of raw manure to soil is recognized as a practice to enhance soil's fertility and crop's yield, especially since it contributes to restore soil carbon reserve, improving carbon sequestration, and can also improve soil structure by increasing soil porosity (Hoover et al., 2019; Rayne and Aula, 2020).

To valorise manure as an essential resource, by maximizing the quantity of manure applied and nutrients recovery, it is necessary to alter some traditional practices and turn manure use more attractive to farmers. Nonetheless, manure utilization as fertilizer faces some constraints, like the fact that: i) nitrogen (N) and phosphorus (P) concentrations in manure are much lower than in mineral fertilizers, (Ozlu and Kumar, 2018) ii) only part of the nutrients are readily available for plants, the majority of the nutrients are present in the constitution complex organic molecules, which need to be mineralized, making it difficult to assess their availability (De Boer, 2017; Li et al., 2016), iii) the quantity and frequency of manure application to crops are lower than the usual practice with mineral fertilizers (Iqbal et al., 2019), and iv) the costs of transporting and applying manures, especially slurries (liquid manure), may turn the practice unsustainable (Silva et al., 2022). As stated, nutrients' availability to plants are difficult to predict and depend widely on the animal species and the water content of the material, i.e., if it is a solid manure or a slurry. For instance, according to the Portuguese legislation, predictably, the available N content ranges between 1.3 and 2.5 kg m^{-3} in a cattle slurry, between 14 and 21 kg t^{-1}

in a poultry manure, and between 3.0 and 4.2 kg m^{-3} in a pig slurry (MADRP, 2018)) Moreover, the availability of plants' nutrients, via manure application, also relies on the soil characteristics, namely, i) the soil colloidal complex, which can retain nutrients (Xu et al., 2016), ii) soil pH, which can influence the solubility of the nutrients (e.g. lower pH can increase P solubility) (Cerozi and Fitzsimmons, 2016), iii) soil composition, (e.g. soils with higher clay content will reduce nutrients losses, such as P (Girotto et al., 2013)) and iv) the microbiota, since soil microorganisms have extreme importance to the mineralization of manure and to the release of plant essential nutrients (Pahalvi et al., 2022).

However, manure application can also have negative impacts on soil and water (Iqbal et al., 2019), the most important of which is the non-point source pollution with nutrients to the receiving hydrological system. The recommendations for fertilizer application are usually based on crops' N requirements, hence, manure application to soil results, in most cases, in the overapplication of P. This is the result of an unbalanced N and P ratio relative to crops demands (Oenema et al., 2021). When P application exceeds the crop offtake, it can cause the buildup of large amounts of P in the soil profile (Chardon et al., 2007), a problem already faced by some countries in northwest Europe, which hinders the possibility of applying higher amounts of manures to soil (Vanden Nest et al., 2016), and ultimately can lead to potential P losses to the surface water bodies, causing eutrophication (Liu et al., 2019a).

To overcome some of these problems, the concept of bio-based fertilizer is becoming more acknowledged, which suggests an even nutrient flow, while recovering materials, such as manures, by improving its characteristics (e.g., nutrients concentration, nutrients availability, sanitization) (Chojnacka et al., 2020; Tur-Cardona et al., 2018), and maintaining the benefits of an organic fertilizer application. The production of a manure-based fertilizer (MBF) would combine the "more interesting" characteristics of the manures, i.e., organic matter content, and supply of both macro and micronutrients essential for crops' healthy productions, with some characteristics of mineral fertilizer, e.g., specific nutrients' ratio, higher nutrients concentrations and availability (Iqbal et al., 2019; Silva et al., 2022). The MBFs may, also, appear as a solution for the P overapplication to the soil, by transforming the manures, or altering their characteristics, to products that, for instance, can diminish the soil P saturation and restore soil P value to healthy levels, while taking advantage of their nutrients and organic matter content (Vanden Nest et al., 2016). Modifying the N:P ratio in the manures, by producing blends with an increase in the N:P ratio to values closer to the crop N needs, may avoid P surplus application. On the other hand, in some situations of soil P deficiency, it could be also interesting to evaluate the possibility of producing MBFs richer in P, for instance, producing blends with a 0.5:1 N:P ratio. The co-application of manure with mineral fertilizers, independently of the lower proportion of mineral fertilizers in the blend, may alter the leaching potential of the nutrients in the MBF compared to raw manures, or their mineralization rates, which may result in a decrease, or increase, risk of nitrate and P leaching from the soil and, also, induce an upsurge in the agronomic value of the MBFs (Huang et al., 2017; Li et al., 2016).

This study aimed to evaluate the potential nutrients leaching of several MBFs, produced by blending manures with small quantities of mineral fertilizers, to modify their N:P ratios to three specific ratios (1:1, 2:1, and 0.5:1). A first objective was to compare MBFs with the original raw manure, to identify if the addition of mineral fertilizer would induce an increase of the potential leaching, compared to the raw manure. A second objective was to compare the MBFs within the same N:P ratio, to identify which of the proposed MBFs would lead to the lower potential losses by leaching. The results will, also, be used to assess the agronomic efficiency of these MBFs, considering that both macro and micronutrients in the leachate are potentially available to the crop – plant available nutrients. A higher nutrient's leaching potential associated with a specific MBFs may indicate a higher risk of environmental problems and to a decrease in their agronomic value. More emphasis will be given to N, P and K, but other macro and micronutrients, and leachates' pH and electrical conductivity, will also be assessed.

4.2 Material and Methods

4.2.1 Manure sampling and characterization

The manures used in this experiment were all collected in farms representative of the Portuguese livestock system: i) cattle slurry (CaS), collected from a commercial dairy farm, at Palmela; ii) pig slurry (PiS), from a pig fattening farm, located at Montijo; and iii) poultry manure (PoM), sampled in a commercial farm specialized in the production of poultry meat, Herdade Daroeira, at Alvalade do Sado. Manures samples were stored at

4ºC, before characterization and use, and analysed in triplicate for their: dry matter content (DM), total organic carbon (TOC), total nitrogen (N_{Total}), ammonium nitrogen (NH_4^+-N), total phosphorus (P_{Total}, expressed as P_2O_5) and total K (K_{Total}, expressed as K_2O), using methodologies previously described by Prado et al. (2022). When the analytically determined NH_4^+ content of the manure (or MBF) was higher than the estimated $N_{\mathbf{av}}$, the measured NH_4^+ content was used as the value of $N_{\mathbf{av}}$. The composition of the three manures can be consulted in Table 4.1.

Table 4.1: Physicochemical characteristics of the raw manures (1:1 ratio) and of the manure-based fertilizers considered in the study (2:1 and 0.5:1 ratios), all expressed in fresh matter basis (mean value, n=3). The quantity of manure and mineral fertilizer applied in each column is also provided (with the percentage of the mineral in the blend), as well as the amount of nutrients vehiculated by those quantities per column.

		DM	**TOC**	**N_{Total}**	**NH_4^+-N**	**N_{av}**	**P_{Total}**	**K_{Total}**	**Blend composition**		**N_{Total}**	**NH_4^+-N**	**N_{av}**	**P_{Total}**	**K_{Total}**
		g kg^{-1}	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g P_2O_5 kg^{-1}**	**g K_2O kg^{-1}**	**g Manure**	**g Mineral**	**mg $column^{-1}$**	**mg $column^{-1}$**	**mg $column^{-1}$**	**mg $column^{-1}$**	**mg $column^{-1}$**
1:1 Ratio	**PiS**	129.7±2.16	55.0±2.16	8.4±0.07	5.6±0.02	5.6±0.04	5.7±0.05	3.41±0.03	13.87	-	116.67	78.24	70.0	34.40	39.26
	CaS	103.9±1.31	39.5±2.16	3.5±0.01	1.5±0.05	2.1±0.01	1.9±0.05	3.30±0.07	33.72	-	116.67	49.57	70.0	27.31	92.39
	PoM	749.4±1.11	352.1±2.16	20.3±0.39	3.5±0.12	10.2±0.19	11.8±0.42	18.49±0.10	6.90	-	140.00	21.66	70.0	35.45	105.86
2:1 Ratio	**PiS+U**	125.96	55.0	14.86	11.60	11.60	5.53	3.31	5.95	0.09 (1.3%)	86.47	70.00	70.0	14.75	16.83
	PiS+AS	125.76	55.0	14.6	11.34	11.34	5.52	3.30	5.98	0.19 (2.8%)	86.73	70.00	70.0	14.98	17.09
	CaS+U	103.59	39.5	5.13	3.15	3.75	1.85	3.29	18.6	0.07 (0.4%)	95.75	58.73	70.0	15.07	50.67
	CaS+AS	103.11	39.5	5.17	3.19	3.79	1.85	3.27	18.3	0.15 (0.8%)	95.32	58.91	70.0	14.82	50.13
	PoM+U	727.53	352.1	33.11	16.45	23.25	11.47	17.95	2.92	0.09 (2.9%)	99.67	49.51	70.0	15.02	44.86
	PoM+AS	702.53	352.1	32.16	16.07	22.64	11.07	17.33	2.90	0.19 (6.3%)	99.42	49.68	70.0	14.90	44.49
0.5:1 Ratio	**PiS-SOL**	232.50	96.7	10.93	5.47	5.47	10.91	4.40	12.81	-	140.00	82.62	70.0	60.84	46.75
	CaS+SP	103.45	39.5	3.44	1.46	2.07	3.94	3.28	33.72	0.17 (0.5%)	116.67	49.57	70.0	58.13	92.39
	CaS+PA	99.65	39.5	3.32	1.41	1.99	3.94	3.16	33.72	1.46 (4.2%)	116.67	49.57	70.0	60.38	92.39
	PoM+SP	743.67	352.1	19.9	3.08	9.95	19.81	18.13	6.90	0.14 (1.9%)	140.00	21.66	70.0	60.66	105.86
	PoM+PA	718.24	352.1	19.46	3.01	9.73	19.29	17.72	6.20	0.99 (13.8%)	140.00	21.66	70.0	54.34	105.86

PiS: pig slurry, CaS: cattle slurry; PoM: Poultry manure. PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid. DM: Dry matter; TOC: Total organic carbon; N_{Total}: Total nitrogen; NH_4^+-N: Ammonium nitrogen; N_{av}: Available nitrogen (calculated as a % of the total N); P_{Total}: Total phosphorus; K_{Total}: Total potassium.

4.2.2 Preparation of manure-based fertilizer blends

The ratios considered were obtained using the raw manures as a starting material for each potential MBF. The intended ratios, simply referred to as N:P ratios, were 0.5:1, 1:1 and 2:1, and were calculated considering the plant's available N content and the total P content. The plant available N content was assumed to be 60% of the total N, for manures with DM <20%, and 50% of the total N, for manures with DM> 20% according to the recommendations of the Portuguese legislation (MADRP, 2018). As for the total P content, and only to calculate the N:P ratio, P was expressed as P_2O_5, since it is the form traditionally used to express P in the formulations of commercial fertilizers, allowing a clearer perspective of which type of nutrients ratios could be achieved with manure-based fertilizers. The raw materials (PiS: pig slurry, CaS: cattle slurry; PoM: Poultry manure) were used as the MBFs with the 1:1 ratio, since, from their composition (Table 4.1), that corresponds to their approximate N:P ratios.

To obtain MBFs richer in N, i.e., with a 2:1 ratio, urea and ammonium sulphate were used as alternative materials, given rise to the blends: PiS+U (pig slurry with urea), PiS+AS (pig slurry with ammonium sulphate), CaS+U (cattle slurry with urea), CaS+AS (cattle slurry with ammonium sulphate), PoM+U (poultry manure with urea), and PoM+AS (poultry manure with ammonium sulphate).

To obtain MBFs richer in P, i.e., with a 0.5:1 ratio, three possibilities were evaluated, the use of the solid fraction of the manure, which, in the case of pig manure, was adequate to achieve the desired N:P ratio, and the addition of superphosphate or phosphoric acid, as alternative materials, given rise to the blends: PiS-SOL (solid fraction from pig manure), CaS+SP (cattle slurry with superphosphate), CaS+PA (cattle slurry with phosphoric acid), PoM+SP (poultry manure with superphosphate), and PoM+PA (poultry manure with phosphoric acid).

The composition of the MBFs prepared from the blending of the manures with the mineral supplementation is presented in Table 4.1 and was calculated based on the raw manure and mineral fertilizers composition.

4.2.3 Leaching experiment

The leaching experiment was performed with three repetitions per treatment, plus the control (soil without MBF). Each replicate was assembled with 1 kg of air-dried soil in PVC columns (30 cm long x 5.7 cm internal diameter). The soil used in this study was a sandy soil, classified as Haplic Arenosols (FAO, 2006), a sandy, very poor in both extractable P (9.92 mg P_2O_5 kg^{-1} soil), K (13.18 mg K_2O kg^{-1} soil), total organic carbon close to 4.3 g kg^{-1} soil, and an initial pH of 5.6. This soil was chosen to maximize the leaching potential and compare the MBFs within the worst conditions in terms of leaching. The soil in the columns was saturated with water from the bottom, by capillary rise, until it reached its full water holding capacity. The MBFs were applied to the soil top layer in the column, three days before the beginning of the leaching process, since manure can not be applied before rainfall events, considering a prediction of three-day meteorological forecast (Fangueiro et al., 2014). The amount of each material applied was calculated in order to supply the equivalent to 210 kg plant available N ha^{-1}. Considering the 0-20 cm top soil layer and a soil density of 1.5 t m^{-3}, the amount of N applied was equivalent to 70 mg N kg^{-1} soil. The amounts of each manure or MBF (with an indication of the percentage of mineral fertilizer), applied to the column are provided in Table 4.1, considering the referred, as well as the quantities of N_{Total}, NH_4^+-N, N_{av}, P_{Total} and K_{Total} vehiculated by each MBF.

Seven leaching events were planned, weekly during the first month, and every two weeks during the second month. Therefore, the leaching events corresponded to days 3, 10, 17, 24, 38, 51, and 59 after the MBFs application to soil. Columns were kept covered with perforated parafilm, to allow gaseous exchanges while minimizing water loss by evaporation. Before each leaching event, the column was weighed, and water was added to keep soil moisture at a constant rate. The leaching event was intended to simulate rainfall and used 200 mL of distillate water, corresponding to the soil's maximum water holding capacity. The water was added gradually to each column and the leachate was collected in vials. The collecting vials were weighed, to measure the total volume of leachate collected, to be converted to volume by assuming a density of 1 g mL^{-1}. The electrical conductivity (EC) and pH of the leachate were measured directly in the collecting vials (AL15, Aqualytic, Portugal) and, after that, the samples were stored at 4ºC before analysis of the nutrients' concentrations. The N concentration was measured directly in the leachate by segmented flow autoanalyzer SAN plus (San Plus System, Skalar, Nederland) with the modified Berthelot method to measure NH_4^+-N (Krorn, 1956) and rapid method for NO_3^--N (Singh, 1988). The macro (P, K, calcium (Ca), magnesium (Mg) and sulphur (S)) and micronutrient (iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn)) concentrations were also determined, directly in the leachate, by inductively coupled plasma optical emission spectrometry (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, USA).

4.2.4 Statistical treatment of the data

The statistical analyses were performed using one-way ANOVA for the 1:1 and 0.5:1 ratios, while for the 2:1 ratio, the factorial two-way ANOVA was used, to estimate the interaction between the type of manure and mineral N source. To determine the statistical significance of the means, a Tukey test was realized, with a $P<0.05$ and 95% degree of confidence. All statistical treatment of the data was performed with the software Statistix 7.

4.3 Results and discussion

4.3.1 Nitrogen potential leachability

In contrast with mineral fertilizers, organic materials have a significant amount of nutrients in organic forms, not readily available for plants (De Boer, 2017). The concentration of available nutrients relies on the mineralization dynamic of the organic fraction, which will be responsible for releasing nutrients during a short or longer period, depending on its characteristics. For instance, as stated in the introduction, N mineralization soil characteristics, i.e, soils richer in organic matter, stimulates microorganisms activity, hence contributing to a higher mineralization rate (Soler-Jofra et al., 2021). Soil porosity is also an important soil property that conditions N mineralization rate, because in soils with higher porosity, a higher oxygen content will promote a higher mineralization rate (Iqbal et al., 2019). On the other hand, higher N mineralization rates will increase the concentrations of N mineral forms more rapidly, increasing the risk of N losses by leaching, which will decrease the amount of available N to the plants and will decrease the manure fertilizing value (Padilla et al., 2018; Petersen, 2018). When fertilizing with MBFs prepared with manure and small amounts of mineral fertilizers, it is important to assess the concentration of nutrients already present in available forms, prone to plant uptake, and that can cause water pollution when they are leached, because the plants are not using them.

Both slurries used in this study presented similar dynamics concerning the potential leaching of NH_4^+ (Figure 4.1a), stabilizing the amount leached in the second week at ~4 mg NH_4^+-N kg^{-1} soil. On the contrary, PoM presented a peak of NH_4^+ leached on day 10, ~9 mg NH_4^+-N kg^{-1} soil. In both slurries and PoM, on day 38 a second peak was observed, which can be attributed to some organic N mineralization into NH_4^+, but also to previous low N leaching due to NH_4^+ immobilization as a result of microorganisms activity and, therefore, N accumulation. Such initial immobilization is usually observed after slurry or solid manure application to soil, as a result of the simultaneous application of C and N available for microorganisms (Zare and Ronaghi, 2019). Regarding the NO_3^- leached over the experiment (Figure 4.1b), only CaS presented a small amount of nitrate in the leachate (~2 mg NO_3^--N kg^{-1} soil) during the first 17 days, indicating that PoM and PiS presented a delay in nitrification, and consequently a lower risk of nitrate leaching was expected. Indeed, since these materials were very poor in nitrate, the nitrate leached was exclusively due to the nitrification process and accumulation of NO_3^--N in the soil (Fangueiro et al., 2014). Gómez-Garrido et al. (2018), also used pig slurry as a fertilizer and obtained similar results: a lower concentration of NO_3^- leached in the first weeks of the experiment. Nonetheless, especially with PoM application, the quantity of NO_3^- increased exponentially in the leachate until day 35. This was the material with the higher quantity of mineral N lost at the end of the experiment, ~54 mg N, representing ~67% of the Nav applied (Table 4.2), 2-times and 1.4-times higher than what was observed with CaS and PiS application, respectively. Consequently, in the absence of crop uptake or after sowing (reduced nutrient uptake), and with high precipitation, the potential risk of NO_3^- leaching may be high after the first month of PoM application. These results might also be used to appraise the N availability for plants, indicating that PoM application is more adequate for a crop with high N demands. For instance, it may be considered for a spring/summer crop, like maize, since it is a period when lower rainfall is expected, and crops have higher N requirements. Still, caution should be adopted when PoM is applied to the soil, because, even in the presence of a crop which absorbs part of the available N, it can contribute to an increase in the soil's N mineral forms, susceptible to be leached later (Zhang et al., 2019). On the other hand, CaS presented the lower quantity of total N lost at the end of the experiment, with only 30.82 mg N leached for 59 days, corresponding to the lower percentages of N_{av} and N_{Total} leached among raw manures applied. This can suggest that the MBF made with CaS will have a lower risk of potential NO_3^- leaching but might also induce a lower N availability to the crops. Still, the cumulative value of NO_3^- leached after the application of the three manures did not exceed the European legal limit for nitrate leaching in vulnerable areas, even applying the maximum quantity of 210 kg N indicated for these areas (EC, European Council, 1991).

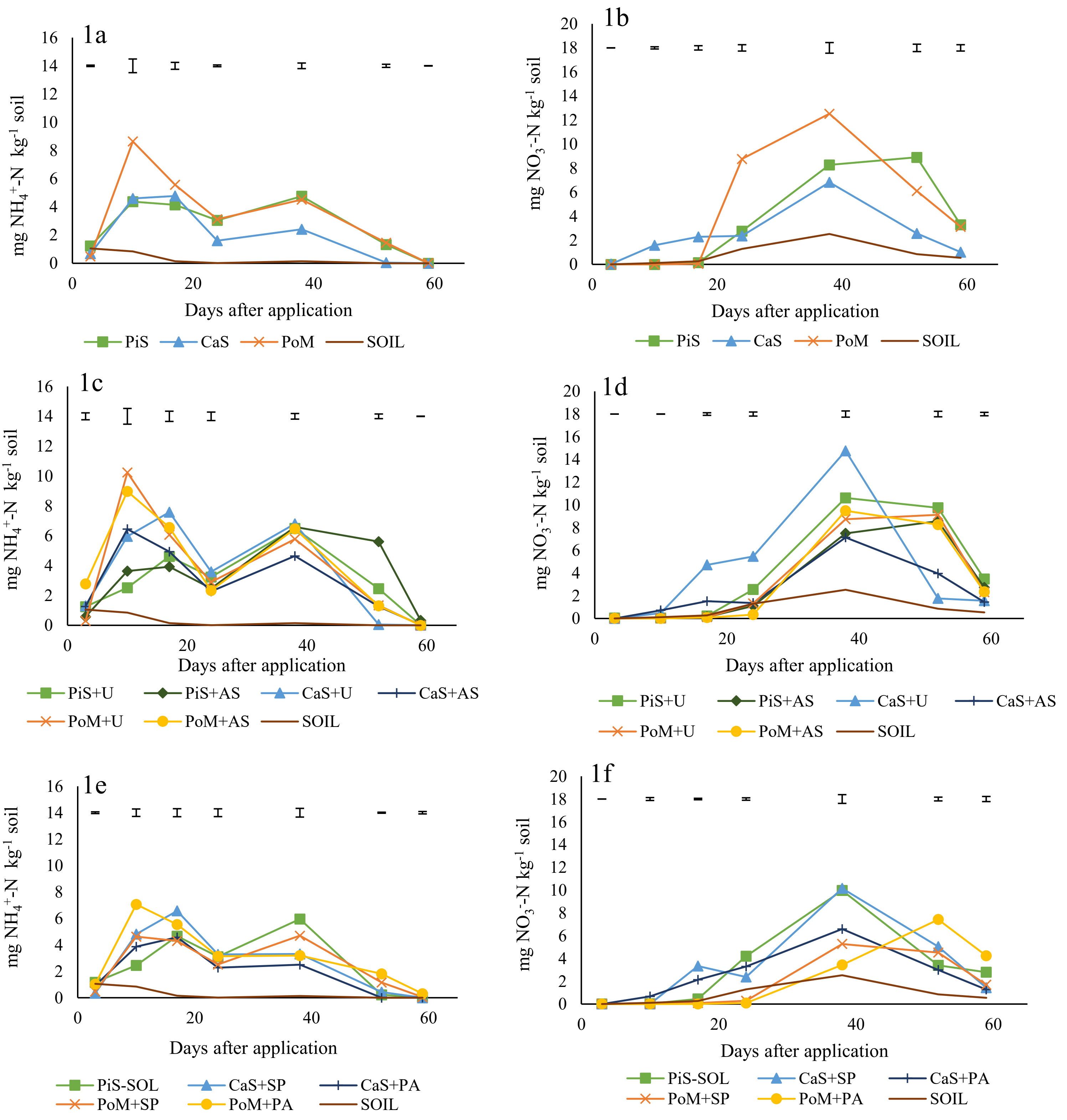

Figure 4.1: The NH_4^+ (left), NO_3^- (meddle) and mineral N (right) concentrations on the soil for each 1:1 ratio (1a to 1c), 2:1 (1d to 1f) and 0.5:1 (1g to 1i) during the incubation. Values presented are arithmetic means (n=4). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date. PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

Table 4.2: The cumulative values of the total mineral N (Min N), P, K, Ca, Mg and S leached over the experiment and the corresponding percentage of N, P and K conveyed by each blend. For each ratio in each column, the mean (n=3) followed by different letters differ significantly for $P<0.05$ (Tukey test), the capital letters are the differences between the diverse manure-based fertilizers compared to the manure of origin, while the small letters correspond to the differences within each ratio.

		Min N			**P**		K		**Ca**		**Mg**		**S**	
		mg N	**% Nav**	**% Nt**	**mg P**	**% applied**	**mg K**	**% applied**	**mg Ca**	**% applied**	**mg Mg**	**% applied**	**mg S**	**% applied**
1:1 Ratio	**PiS**	$^{BC}42.27^{b}$	$^{BC}49.21^{b}$	$^{C}29.53^{a}$	$^{B}1.66^{a}$	$^{B}2.45^{b}$	$^{A}26.05^{c}$	$^{A}60.79^{b}$	$^{A}32.93^{b}$	$^{B}25.11^{a}$	$^{A}6.74^{b}$	$^{B}12.85^{a}$	$^{B}7.05^{b}$	$^{B}43.78^{a}$
	CaS	$^{D}30.82^{c}$	$^{D}32.87^{c}$	$^{C}19.72^{b}$	$^{C}1.18^{b}$	$^{C}3.71^{a}$	$^{A}62.39^{b}$	$^{A}65.17^{b}$	$^{C}26.80^{c}$	$^{D}18.20^{a}$	$^{B}5.93^{b}$	$^{C}11.08^{a}$	$^{C}8.38^{b}$	$^{D}32.04^{a}$
	PoM	$^{A}54.49^{a}$	$^{A}66.67^{a}$	$^{B}33.34^{a}$	$^{BC}1.17^{b}$	$^{B}2.82^{b}$	$^{A}84.86^{a}$	$^{A}78.10^{a}$	$^{B}41.91^{a}$	$^{C}22.64^{a}$	$^{A}8.33^{a}$	$^{C}16.28^{a}$	$^{B}16.90^{a}$	$^{B}48.18^{a}$
	SOIL	7.82^{d}	n.a	n.a	0.17^{c}	n.a	2.18^{d}	n.a	17.29^{d}	n.a	3.46^{c}	n.a	2.98^{c}	n.a
2:1 Ratio	**PiS+U**	$^{A}47.23^{b}$	$^{A}56.30^{bc}$	$^{A}44.34^{ab}$	$^{C}0.77^{b}$	$^{A}4.16^{b}$	$^{B}12.67^{d}$	$^{A}58.70^{ab}$	$^{A}28.98^{c}$	$^{A}42.51^{bc}$	$^{A}5.88^{c}$	$^{A}21.42^{c}$	$^{B}5.07^{de}$	$^{A}50.91^{b}$
	PiS+AS	$^{AB}43.21^{c}$	$^{AB}50.57^{c}$	$^{B}39.83^{c}$	$^{BC}1.05^{a}$	$^{A}5.97^{a}$	$^{B}12.38^{d}$	$^{A}55.79^{abc}$	$^{A}29.89^{c}$	$^{A}43.63^{cd}$	$^{A}5.95^{bc}$	$^{A}21.79^{c}$	$^{A}19.36^{c}$	$^{D}1.09^{c}$
	CaS+U	$^{A}53.80^{a}$	$^{A}65.69^{a}$	$^{A}48.02^{a}$	$^{C}0.97^{ab}$	$^{B}5.32^{ab}$	$^{B}34.91$	$^{A}62.72^{bc}$	$^{C}29.12^{c}$	$^{B}40.89^{cd}$	$^{B}6.23^{bc}$	$^{B}22.46^{c}$	$^{C}6.94^{de}$	$^{C}42.37^{b}$
	CaS+AS	$^{C}36.99^{d}$	$^{C}41.68^{d}$	$^{B}25.01^{d}$	$^{C}1.00^{a}$	$^{B}5.63^{ab}$	$^{B}33.14^{bc}$	$^{A}60.25^{c}$	$^{B}36.07^{b}$	$^{A}65.69^{a}$	$^{A}7.44^{ab}$	$^{A}32.64^{ab}$	$^{A}27.46^{b}$	$^{E}0.55^{c}$
	PoM+U	$^{B}48.22^{b}$	$^{B}57.73^{b}$	$^{A}40.54^{c}$	$^{D}0.84^{ab}$	$^{A}4.51^{ab}$	$^{C}29.96^{c}$	$^{B}60.24^{c}$	$^{C}32.76^{bc}$	$^{B}32.59^{d}$	$^{B}6.53^{bc}$	$^{B}23.48^{bc}$	$^{B}11.77^{cd}$	$^{A}69.70^{a}$
	PoM+AS	$^{B}48.90^{b}$	$^{B}58.69^{b}$	$^{A}41.32^{bc}$	$^{CD}0.88^{ab}$	$^{A}5.82^{ab}$	$^{C}36.65^{a}$	$^{A}75.78^{a}$	$^{A}46.93^{a}$	$^{A}60.79^{ab}$	$^{A}8.36^{a}$	$^{A}36.54^{a}$	$^{A}43.96^{a}$	$^{D}5.42^{c}$
	SOIL	7.82^{e}	n.a	n.a	0.17^{c}	n.a	2.94	n.a	17.29^{d}	n.a	3.46^{d}	n.a	2.98^{e}	n.a
0.5:1 Ratio	**PiS-SOL**	$^{C}37.10^{b}$	$^{C}41.86^{b}$	$^{D}25.10^{a}$	$^{A}4.19^{b}$	$^{A}6.62^{b}$	$^{A}26.51^{c}$	$^{B}50.43^{c}$	$^{A}30.61^{b}$	$^{C}11.97^{b}$	$^{A}6.51^{ab}$	$^{C}6.95^{d}$	$^{B}8.71^{c}$	$^{B}31.75^{c}$
	CaS+SP	$^{B}41.11^{a}$	$^{B}47.56^{a}$	$^{B}23.78^{b}$	$^{B}1.77^{d}$	$^{D}2.76^{cd}$	$^{A}68.57^{b}$	$^{A}71.04^{ab}$	$^{A}40.71^{a}$	$^{C}34.25^{a}$	$^{A}8.40^{a}$	$^{B}25.80^{a}$	$^{B}15.39^{a}$	$^{B}59.06^{b}$
	CaS+PA	$^{D}31.10^{c}$	$^{D}33.26^{c}$	$^{C}16.63^{c}$	$^{A}5.92^{a}$	$^{A}9.53^{a}$	$^{A}63.73^{b}$	$^{A}65.80^{ab}$	$^{A}40.53^{a}$	$^{B}39.43^{a}$	$^{A}7.91^{ab}$	$^{C}19.15^{b}$	$^{B}14.89^{a}$	$^{A}67.69^{a}$
	PoM+SP	$^{D}29.70^{c}$	$^{D}31.26^{c}$	$^{D}15.63^{c}$	$^{B}1.25^{d}$	$^{C}1.79^{d}$	$^{B}70.98^{b}$	$^{B}64.28^{b}$	$^{D}29.03^{b}$	$^{D}9.92^{b}$	$^{B}6.20^{ab}$	$^{D}8.97^{c}$	$^{B}7.10^{c}$	$^{C}22.84^{d}$
	PoM+PA	$^{C}36.41^{b}$	$^{C}40.26^{b}$	$^{C}24.51^{ab}$	$^{A}2.92^{c}$	$^{B}3.90^{c}$	$^{A}81.55^{a}$	$^{A}74.27^{a}$	$^{C}33.59^{ab}$	$^{D}14.37^{b}$	$^{A}7.45^{ab}$	$^{CD}12.80^{c}$	$^{B}11.14^{b}$	$^{C}27.08^{c}$
	SOIL	7.82^{d}	n.a	n.a	0.17^{e}	n.a	2.94^{d}	n.a	17.29^{c}	n.a	3.46^{c}	n.a	2.98^{d}	n.a

PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid, n.a: not applicable.

The raw manures, with the 1:1 ratio, will be used as a reference to evaluate the results obtained for the NH_4^+ and NO_3^- potential leachability with the other two ratios used in the MBFs preparation: the 2:1 (Figure 4.1c and d) and the 0.5:1 ratios (Figure 4.1e and f). The amount of NH_4^+ leached with the 2:1 and 0.5:1 MBFs in the first leaching event was residual, similar to the raw manures, since most of the leachate was composed of water existing in the soil before MBFs application. In the second leaching event, the amount of NH_4^+ leached reflected the amount of mineral N applied via MBF. Also, as previously referred for the 1:1 manures, the peak of NH_4^+ leached on day 38, ~6 mg NH_4^+-N kg^{-1} soil, might be explained by organic N mineralization, since most of the applied NH_4^+ was lost in the previous leaching events (Figure 4.1c).

Regarding nitrate leaching, a common trend was observed for all treatments: residual NO_3^- leaching till day 27, followed by an increase, with a plateau on days 38-50, and finally, a decrease in the amount of nitrate leached, except for CaS+U and CaS+AS (Figure 4.1d). The combination of manure and mineral fertilizers seems to foment the NO_3^- leaching, emphasised by the results observed in CaS+U. Additionally, the different additives had repercussions on the slurries' potential NO_3^- leaching, but no disparity was observed relative to MBFs prepared with PoM. For instance, when ammonium sulphate was added to CaS, the N dynamic was similar to the raw CaS, leading to the lowest quantity of NO_3^- leached within the 2:1 ratio, yet higher than the 1:1 CaS. Therefore, it is possible to conclude that the addition of urea to slurries stimulated the nitrification process, especially after the first 17 days, increasing the NO_3^- leached. Indeed, the use of urea on its own, as previously described in a study by Scott et al. (2015), increased the risks of NO_3^- leaching compared to the application of bovine urine in two different soils (Scott et al., 2015). Also, a study demonstrated that by replacing 35% of the N applied via mineral fertilizer with chicken manure application, the N leachability was reduced when compared to the 100% of mineral fertilizer (Wang et al., 2019). Adding either U or AS to PoM, decreased the potential NO_3^- leaching associated with PoM in about ~13% of Nav and ~18% of N_{Total} leached, when compared to raw PoM (Table 4.2), suggesting that the nitrification of NO_3^- was delayed in this case, mitigating the risk of leaching. Hence, producing a 2:1 MBF with PoM and either U or AS has proven to be a good option for farmers to adopt, and they can choose which additive suits better their crop needs. A previous study showed that the use of pellets that combined composted cattle manure with urea, had a positive impact on the production of coriander, garden cress, and parsley plants (Souri et al., 2018).

P-enriched MBFs (0.5:1 ratio), with superphosphate or phosphoric acid addition, were also evaluated and, in these cases, the addition of a P source also diminished the NO_3^- leaching potential of PoM, indicating a possible nitrification inhibitor effect (Figure 4.1f). This could be attributed to the lower concentration of NH_4^+ observed during the experiments, which reduce the nitrification substrate (Figure 4.1e). Even though, it can be beneficial for the environment and increase its fertilizer value (i.e., lower losses, more nutrients will stay in the soil and, eventually, may be used by the crop), it may also suggest that a lower quantity of N will be available to the crop in the short term and that a higher quantity of mineral fertilizers will be necessary to suppress the crop needs. P-enriched MBFs with PoM decreased the NO_3^- present in leachates, which could: i) discard water pollution with NO_3^- and, thereby, have a material with a higher fertilizing value; and ii) adequate these blends for a less demanding N crop. When considering a MBF adequate for a soil with low P concentration. i.e., with a 0.5:1 ratio, PiS-SOL or CaS+PA can be considered good options, since they did not alter NO_3^- potential leaching, compared to the raw slurry (Table 4.2). Preserving the level of N leached, compared to the manure of origin, could indicate that, when these materials are applied, NO_3^- leaching problems are not expected, and that the N supply may be sufficient to satisfy crops' necessities (depending on the crop and soil utilized).

Relatively to P, in a short time, PiS-SOL, CaS+SP and PoM+PA will lead to an increase in P leachability. Therefore, the application of these MBFs should be performed consciousness, knowing they are only adequate for soils with lower P concentrations and with conditions that do not predispose leaching. Still, the long-term effects on the soil of the application of these MBFs are not yet known, even if, it is well known that, but manure can increase the nutrients levels in the soil (Svanbäck et al., 2019). Consequently, more studies are still needed to promote a safe use of these MBFs.

4.3.2 Phosphorus potential leachability

Phosphorus is an important macronutrient, which can become one of the major factors to limit crop growth (Gerke, 2015), and only a small part of the total P in the soil is available to the plant in, the form of free orthophosphate ions (Tiecher et al., 2020).

However the availability of this nutrient or its leachability depends on several factors, i) initial P concentration in soil, the reason why in this study a poor P soil was used, ii) manure or derived product characteristics or application rate, which was analyzed, iii) tillage practices, iv) irrigations regimes, which was kept constant between treatments, v) soil pH, that influence the orthophosphate ions availability and vi) the clay content that can increase P adsorption (Cerozi and Fitzsimmons, 2016; Ju and Zhang, 2017; Liu et al., 2019b). The amount of P applied via animal manure is, generally, higher than the amount exported by the plant, which may have two consequences: i) P leaching, if the weather and soil conditions enhance this phenomenon, or ii) P can be adsorbed onto the surface of reactive particles in the soil such as iron or aluminium oxides (Wang et al., 2020). The evaluation of the three raw manures, in terms of P leaching potential, can be observed in Figure 4.2a. Except for a peak at day 24 for PiS, the P dynamic was similar between the three organic materials. Nonetheless, the cumulative amount of P lost by leaching over the 59 days of the experiment presented some differences. Bi et al. (2020), explained that the combined application of organic and inorganic fertilizers formed a complex of stable phosphate, which mobilized the bacteria community and increased the phosphatase activity, improving P solubility. Indeed, PiS presented the higher quantity of P leached, 1.66 mg P kg^{-1} soil, while CaS presented the higher losses of total P applied (3.71%) (Table 4.2), in agreement with the fact that CaS supplied the lower amount of P when applied to the column (Table 4.1). It might still be due to the fact that the P forms present in CaS are eventually more soluble and, therefore, more susceptible to be leached. Nevertheless, this is still a very low percentage of P leached, and the conditions of the experiment do not account for the P absorption by the crop, which would also counteract the P leaching. Therefore, CaS has proven to be the manure with the higher P availability, but caution must be adopted when considering the soil where CaS is going to be applied, because the higher P availability can lead to higher P leaching. The soil used in this study was a sandy soil, with low clay content, to maximize the leaching conditions. Nevertheless, independently of the manure applied to the soil, the amount of P leached was low, in accordance with the results from a study by Tiecher et al. (2020), which also reported negligible P losses, in a field experiment, after successive application of PiS. Still, McDowell et al. (2021), determined that the application of superphosphate, with or without cattle manure, resulted in lower P sorption and the leaching of P was substantial. Hence, when applying MBFs, the fertilization plan should consider this risk.

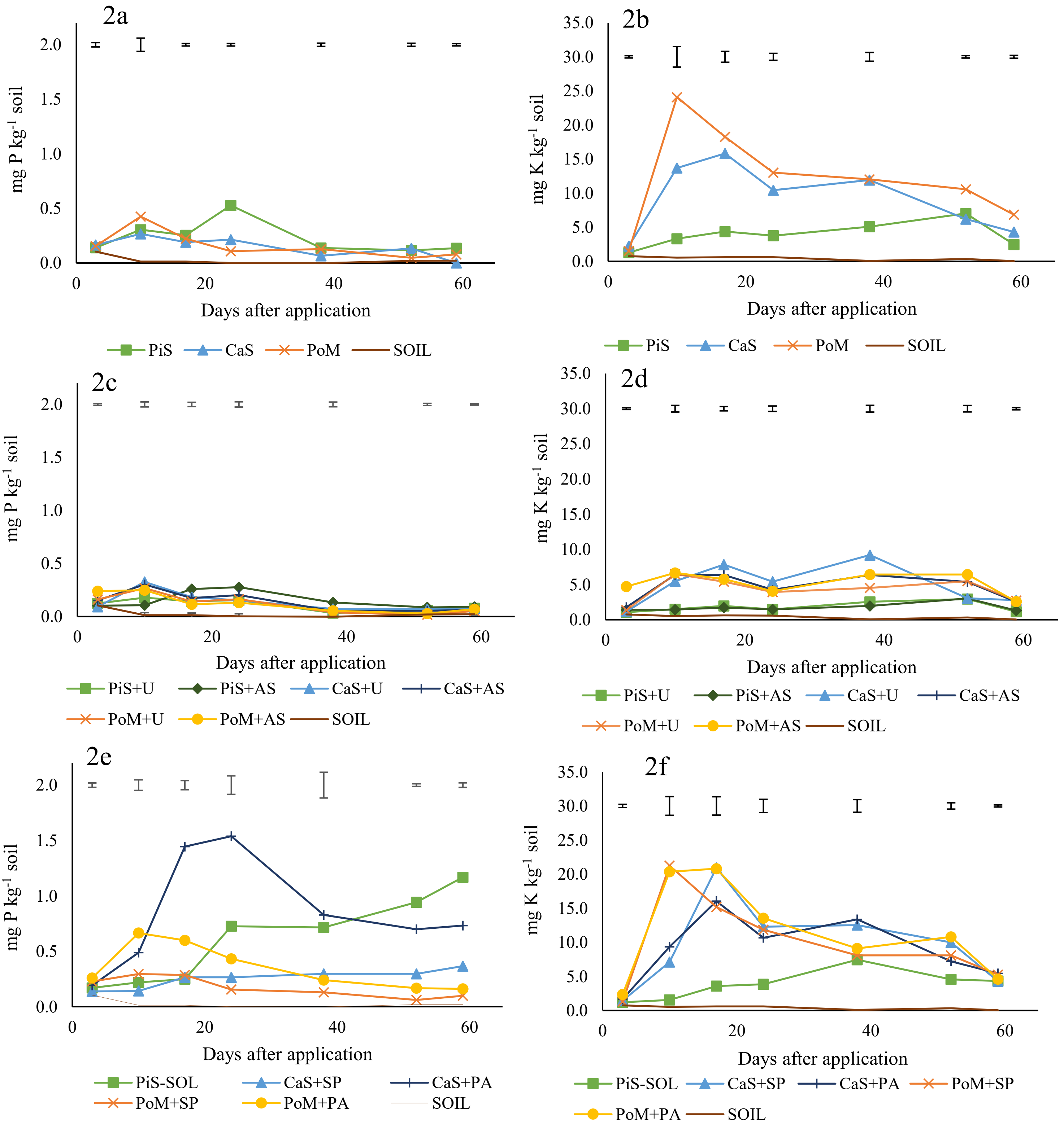

Figure 4.2: The P (left) and the K (right) concentrations on the leached for each 1:1 ratio (2a and 2b), 2:1 (2c and 2d) and 0.5:1 (2e and 2f) during the leaching events. The vertical bars represent the standard errors for the means when performing the Tukey test (n=3). PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with

phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid.

Comparing the P leached by the N-enriched MBFs (i.e., 2:1 ratio), over the 59 days of leaching events, no differences could be observed between the two sources of N, urea and ammonium sulphate, with no differences observed in terms of cumulative P leached (Figure 4.2c and Table 4.2). Nonetheless, ammonium sulphate added to PiS slightly increased the quantity of P leached, compared to the addition of urea, with 1.05 and 0.77 mg P kg^{-1} soil of cumulative P leached by PiS+AS and PiS+U, respectively, but PiS+U decreased that value relative to the raw PiS, which was 1.66 mg P kg^{-1} soil.

The addition of ammonium sulphate, an acidic mineral fertilizer, to PiS might have increased the P solubility, as commonly observed in acidified slurry (Regueiro et al., 2016), which can explain why a higher amount of P was lost in PiS+AS relative to PiS+U (Figure 4.2e), and with considerably higher cumulative P losses (Table 4.2). But it should be highlighted that, independently of the manure used or the N source, the quantity of P leached from the MBF application was considerably lower than after the application of the corresponding raw material (even if the difference was statistically significant only for PiS+U). This could be attributed to the quantity of fertilizer required to supply the same amount of N to the soil being lower using a 2:1 ratio fertilizer. Hence, using a manure supplied with a N source diminished the risk of P leachability, adequate to be used in P saturated soils, avoiding environmental problems and guaranteeing healthy soils (Vanden Nest et al., 2016). The PiS+U, PiS+AS, and CaS+AS MBFs may avoid the overapplication of P to the soil, and reduce the risk of leaching, which is a major concern in northern Europe (Vanden Nest et al., 2016).

Kang et al. (2011), after their study with poultry manure and pig slurry, demonstrated that, in the long-term, P losses via leaching were the consequence of organic P mineralization. Hence, having MBFs P-enriched with a part of P via mineral fertilizer might have reduced P losses, in some part, since the P mineralization will take longer to occur. The P potential leachability dynamics were quite different for the different solutions evaluated (Figure 4.2e), and with considerably higher cumulative P losses (Table 4.2). Organic P compounds, once dissolved, have a lower affinity to sorption onto the soil particles, which occurs when these materials are applied to the soil (McDowell et al., 2021) and, on the other hand, the addition of a mineral P source may have stimulated P leaching. For the first 10 days, the addition of phosphoric acid to PoM and CaS enhanced the potential of P leaching, more pronounced in the case of CaS+PA, reaching a plateau from days 17 to 24, at ~1.50 mg P kg^{-1} soil (Figure 4.2e). The addition of a liquid P source, phosphoric acid, to liquid manure, CaS, may have strengthened the P leachability when compared to its addition to PoM, also because of the acidic properties of phosphoric acid, which will increase P solubility in slurry (Regueiro et al., 2016). Independently of the manure used, the addition of phosphoric acid increased the cumulative P leached, four times for PoM+PA, and almost six times for CaS+PA, relative to the original manures (Table 4.2). Nonetheless, the enhancement of PoM with superphosphate maintained the risks of P leaching compared to PoM (1:1 N:P ratio). This is an advantage compared to the use of raw manure, which resulted in the over-application of P and in extreme cases culminated in P environmental problems. From day 17 onward, the use of PiS-SOL started to induce P losses with an exponential growth from day 38 onward. Therefore, the application of PiS-SOL to the soil also increases, considerably, the quantity of P potentially leached, relative to the raw slurry (PiS in 1:1 ratio), with, approximately, a 4-fold increase, enhancing the environmental risks. It is known that the application of manure can improve P availability by enhancing the biological cycle (Gichangi et al., 2009). Therefore these MBFs (0.5:1 ratio) were planned for soils that are very poor in P, and this increase of plant available P, can be beneficial, agronomically speaking, since there would be a crop to assimilate P. Based on the total amount of P lost, P will be more prone to leaching, particularly in sandy soils like the one used in this study, when using PoM+PA, PiS-SOL and CaS+PA, in ascending order. Still, to note, the cumulative value leached, even if higher than the value observed in 1:1 raw manure, was still low.

4.3.3 Potassium potential leachability

The origin of K to produce mineral fertilizers is not renewable since it comes from potash ore, with its biggest reserve in Canada, and the EU is dependent on its importation (Manning, 2015). Therefore, it is very important to consider alternative materials, rich in K, to change fertilization practices, and use manure, or these MBFs, as an alternative K-fertilizer.

PoM was the manure that led to the higher K losses by leaching in the first ten days, reaching a peak at ~25 mg K kg^{-1} soil (Figure 4.2b), which also indicates a higher K availability to the crops from the application of that manure. This was explained by some authors that referred that K in poultry manure is more labile than in mineral fertilizers (Ashworth et al., 2020). After analysing K dynamics over the 59 days of the experiment (Table 4.1), it appears that PoM leached 80% of the total K applied, while the slurries had lower potential leaching, ~66% of the total K applied. Potassium is a macronutrient required in large quantities to optimize growth and productivity since it is essential for physiological mechanisms and metabolism processes in plants (Torabian et al., 2021). A large amount of manure's K is in a mineral form, and K is exported in high quantities by the plants. Therefore, when applied to a crop, it is expected that K concentration in leachate would be expected to be residual (Girotto et al., 2013). Comparing the two slurries, from an agronomical perspective, CaS will supply a higher amount of K than the other slurry, which can be essential for the fertilization of some crops in soils with low plant available K concentrations.

The dynamic of K was relative similar among the different 2:1 MBFs (Figure 4.2d), and the percentage of K leached, relatively to the applied, was also statistically the same, but with a clear reduction in the quantity of K leached relative to the raw manures (Table 4.2). The explanation was the same already provided for P: the reduction in the quantity of the 2:1 blend, which is necessary to apply when using the 2:1 ratio to supply the same amount of mineral N (Table 4.1). These differences are important to be considered when assessing the use of these MBFs in different soils, with different extractable K concentrations. Since K present in the soil is mostly in unavailable forms for plants, crystalline structures, the addition of an organic material, which can "release" soluble K more easily and in higher amounts (e.g., the raw slurries, CaS and PoM), would make a difference in K-poor soils (Torabian et al., 2021). Macholdt et al. (2019) observed, in a long-term field experiment, a positive effect of combining mineral fertilizers and manures, with an increase in the total and plant available nutrients concentration in the soil.

Since day one, the amount of K leached increased exponentially when a P source was added to either PoM or CaS (Figure 4.2f), decreasing onwards. Alfaro et al. (2004), observed that after slurry application, K was lost immediately, as observed in the raw manure and after 0.5:1 N:P ratio MBFs application. However, adding superphosphate to PoM slightly reduces the amount of K leached, compared to PoM, while the combination of CaS and superphosphate resulted in a small increase of the K leached, compared to CaS (Table 4.2). Independently of the MBFs produced, the behavior of the mixture (manure + mineral fertilizer), did not present a trendy behavior, making difficult to state what can be expected. Even if K is not, as problematic as N and P, in terms of environmental impacts, this nutrient is a mobile ion and significant losses by leaching might occur, namely in sandy soils (Alfaro et al., 2004).

4.3.4 Macronutrients potential leachability

The shortage of macronutrients has repercussions on crops' yield and quality (de Bang et al., 2021). Due to the rich composition of manure, with one single application crops receive all macronutrients. This effect can be observed through the concentration of micronutrients in the leachates (Table 4.2, more complete information is in the supplementary material file, Figure S4.4).

From the three raw manures (1:1 ratio), PoM presented the higher amount of Ca leached, ~42 mg Ca column^{-1} , while CaS presented the lower cumulated quantity of Ca leached, ~27 mg Ca column^{-1} (Table 4.2). This can be important when considering that Ca, in the form of calcium carbonate, is important to occlude soil organic carbon and subsequently ameliorates soil aggregation (Rowley et al., 2018). Yet, it was reported that, after intensive fertilization with cattle slurry, the percentage of Ca leached was between 47-51% of the total Ca applied (Tripolskaja et al., 2016). That study was conducted after several years of manure application, which increased the nutrients leaching. Hence, the long-term effect of MBFs application in different types of soil should be assessed, to better understand what the consequences of their use in agriculture fields might be. The use of a N source, producing the 2:1 ratio MBFs, increased the percentage of Ca released from the mixture and the addition of a P source to manure doubled the concentration of Ca in the leachate in CaS+SP and CaS+PA, but the opposite was observed in PoM+SP and PoM+PA.

Magnesium is an important nutrient for the formation of chlorophyll and the structure of the chloroplast (Wang et al., 2018). To allow crops to absorb Mg, it is essential to convert the mineral and organic magnesium into water-soluble, which is the form represented in Table 4.2. Since the unique exogenous source of Mg was the manure, no major differences were observed by applying different MBFs to the soil. It can be stated that the enrichment of N or P in the blends did not affect the potential leaching of Mg or, from a different perspective, the potential availability of Mg to the plant. Caution should be taken when considering this because in the long term close to 50% of the total Mg can be lost via leaching (Tripolskaja et al., 2016).

The application of PoM to the soil resulted in a double amount of the S leached, compared to the slurries application (Table 4.2). In the case of PoM, the higher S availability can induce an improvement in nutrients assimilation by the plant, especially N fixation, and S is part of the organic metabolites and is a cellular component (de Bang et al., 2021). In the 2:1 ratio, the major differences were obtained when ammonium sulphate was used, due to higher enrichment in S, in the form of sulphate, relative to the use of urea. This improvement resulted in an increase in the amount of S leached 2.8-, 2.3-, and 2.6-times higher in PiS+AS, CaS+AS and PoM+AS, respectively, relative to the original raw manure. This extra S addition to soil might be relevant, since in the last decades, in Northern Europe, S content in the soil has diminished (Pötzsch et al., 2019). Even though the amount of S leached was higher when ammonium sulphate was added to the manures, the percentage of S leached, compared to the S amount applied to soil, was close to 1% in the slurries and close to 5% in PoM. This indicates that, only a small part of S was leached, and that S was slowly released. The addition of a P mineral source to the manures, to obtain the 0.5:1 ratio, doubled the quantity of S in the leachate in the CaS-blends, while in PoM-blends the S leached was reduced to half, more noticeable with superphosphate (Table 4.2). This demonstrates that the amendment of a liquid or solid manure with a P source had different consequences: in slurries it stimulated the S leaching while for solid manure it delayed its release. In soils with S deficiency PiS+AS, CaS+AS and PoM+AS can be a good solution to consider, if the crop has higher S demand, otherwise S will be leached.

The deficiency of micronutrients may not have such a notorious effect on crop yield, as the shortage in macronutrients (de Bang et al., 2021), but they are important when other nutrients are missing, and ameliorate plants' homeostasis (Prather et al., 2020). The leaching of Fe, Cu, Zn and Mn can be consulted in supplementary material (Figure S4.5 and Figure S4.6). This information was also pertinent since manure can vehiculate heavy metals such as Zn and Cu, which are regulated by law (Liu et al., 2019b). Liu et al., (2019b), referred that the application of manure, by providing heavy metals, can alter the biogeochemical cycle of these elements, and, if manure is applied at a higher rate, can create a potential risk of heavy metal leaching and, ultimately, contaminate soils. Still, the values observed, as cumulative leaching, were residual, even after one application of MBFs and in conditions that promote a maximum leaching. Therefore, it is possible to state that the application of MBFs did not promote a risk of heavy metal contamination. Nonetheless, this was a first study, conducted with these MBFs, and no long-term effects were evaluated. For instance, in a study where cattle manure was applied for 5 consecutive years, the authors concluded that it was vital to adopt mitigation measures, due to the concentration increase in the soil (Qian et al., 2020). Also, Fe can alter the P solubility, and reduce P leachability by precipitation of complex of Fe and P (Sohrt et al., 2017).

4.3.5 Effects on leachate electric conductivity and pH

Electric conductivity (EC) and pH can affect plants' growth, since both properties affect nutrients' availability and uptake (Cameselle et al., 2019). In this study, the major differences were observed in the EC of the leachates (Figure 4.3), and not in their pH values (Figure S4.7). The EC is an indicator of soil soluble salts concentrations, which, when in excessive concentrations, may hinder the soil's health, affecting the plant's ability to absorb water and nutrients from the soil (Osyczka and Rola, 2019; Seaton et al., 2021). The application of fertilizers results, necessarily, in an increase in soil salinity (secondary salinization), with the concomitant increase in the EC of the leachate produced from that soil, which could also serve as a nutrient availability indicator (Wei et al., 2018).

The EC of the leachates gradually decreased along the 59 days of leaching, being higher on the first two leaching events, days 3 and 10 (Figure 4.3). The MBFs which presented a higher EC were PoM, CaS, PoM+SP and CaS+PA in agreement with the results shown in the previous section (Figure 4.1 and Figure 4.2). Nevertheless, it is important not to neglect the contribution of the micronutrients to this increase in the EC of the leachate (e.g., Fe, Cu, Zn and Mn; Figure S4.5 and Figure S4.6), some added by the fertilizers' application to the soil.

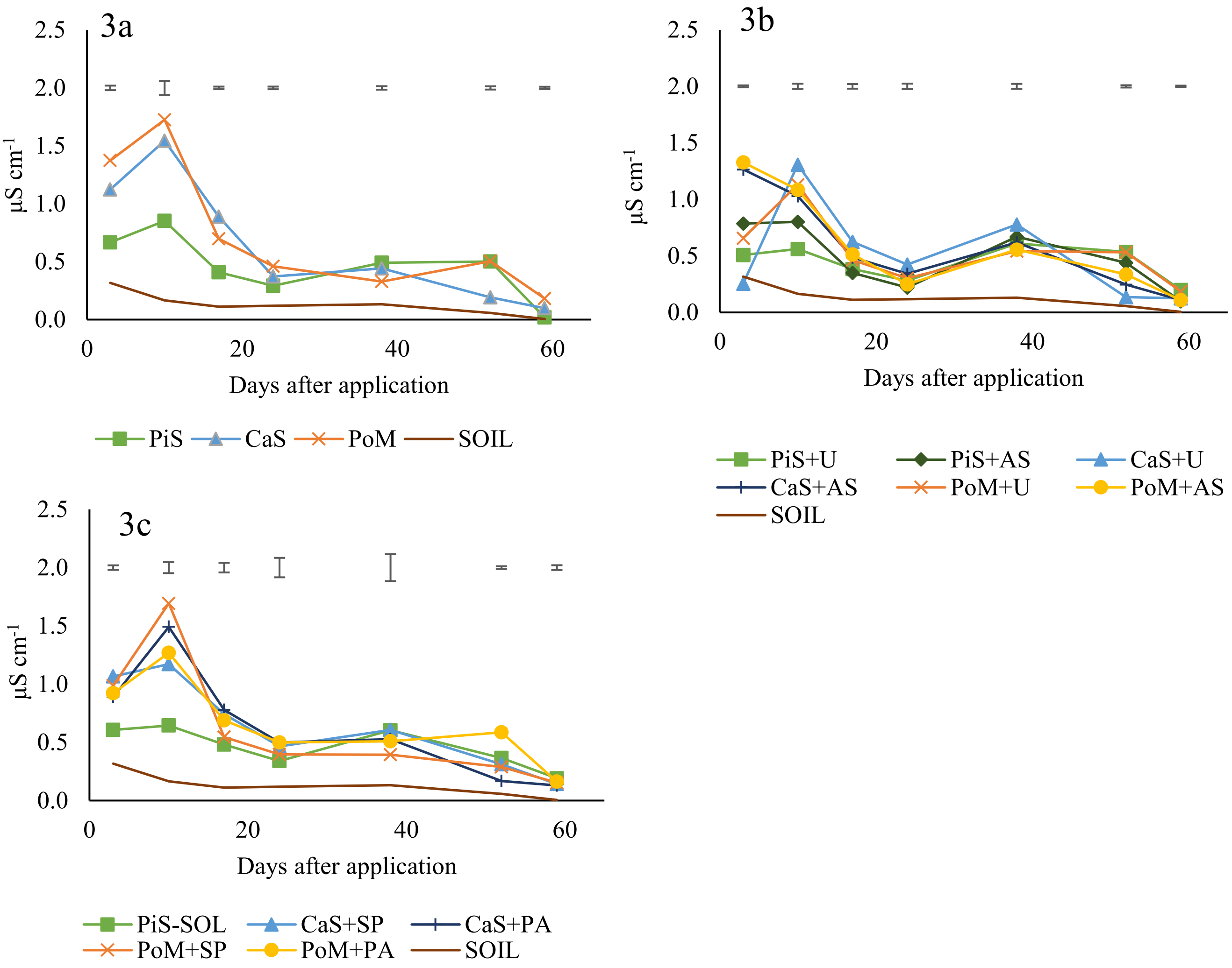

Figure 4.3: The electric conductivity in the leachate over the experiment for the 1:1 ratio (3a), 2:1 ratio (3b), and 0.5:1 ratio (3c). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date. PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid.

4.4 Conclusion

The soil application of the proposed MBFs, with distinct N:P ratios, had repercussions on the potential nutrient leaching, demonstrating that the use of these MBFs might have specific impacts on water quality. The potential of nutrients leaching decreased with time indicating that the first 24 days after application are the more problematic in terms of potential leaching.

Within the 1:1 ratio, PoM led to the highest N, K, Ca and S potential leaching, indicating that this manure has, on one side, the higher concentration of nutrients available for plants, but, on the other side, represents a higher risk of water pollution in soil prone to leaching.

The application of the urea or ammonium sulphate to PoM mitigated the potential of NO_3^- leaching compared to raw PoM, which may predispose these MBFs for winter crops. On the other hand, urea addition to slurries, especially to CaS, increased exponentially the risks of NO_3^- leaching. An important feature, common to all the N-enriched MBFs was the decrease in the P leaching potential, turning them into an important option to lower the risk of their use in P-saturated soils. One other important feature, not addressed in this study, was the benefits of organic matter application, which are also dependent on soil characteristics.

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Sustainable Alternatives to Minimize Gaseous Emissions. doi:10.3390/agriculture12071021

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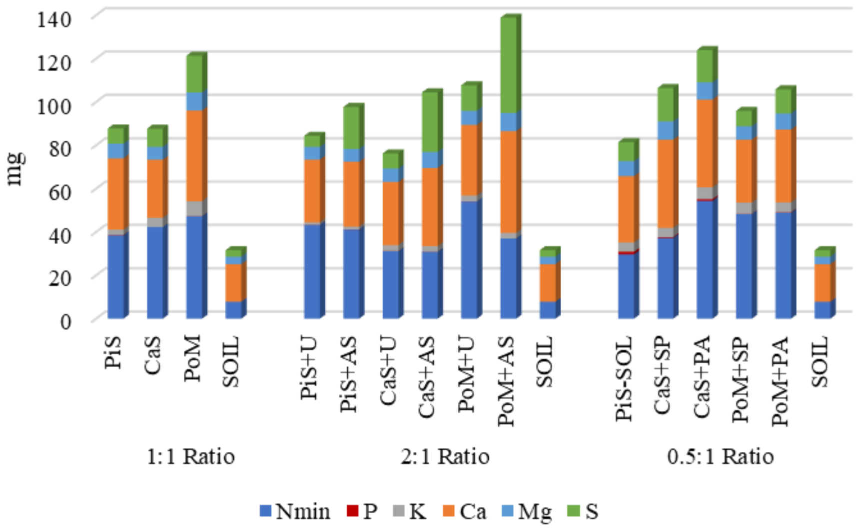
Supplementary material



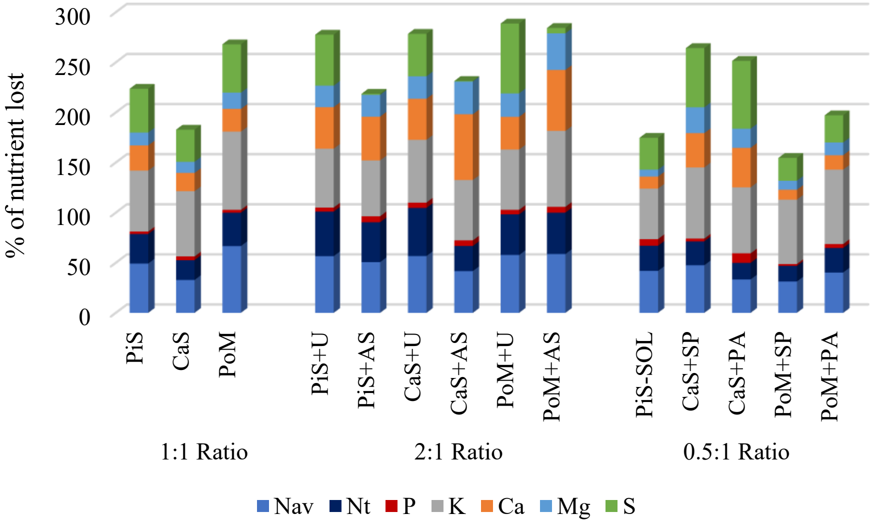


Figure S4.4: The cumulative nutrients leached (top) and the percentage of nutrient lost relative to the total applied (bottom) for each 1:1, 2:1 and 0.5:1 ratio during the leaching events. PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid.

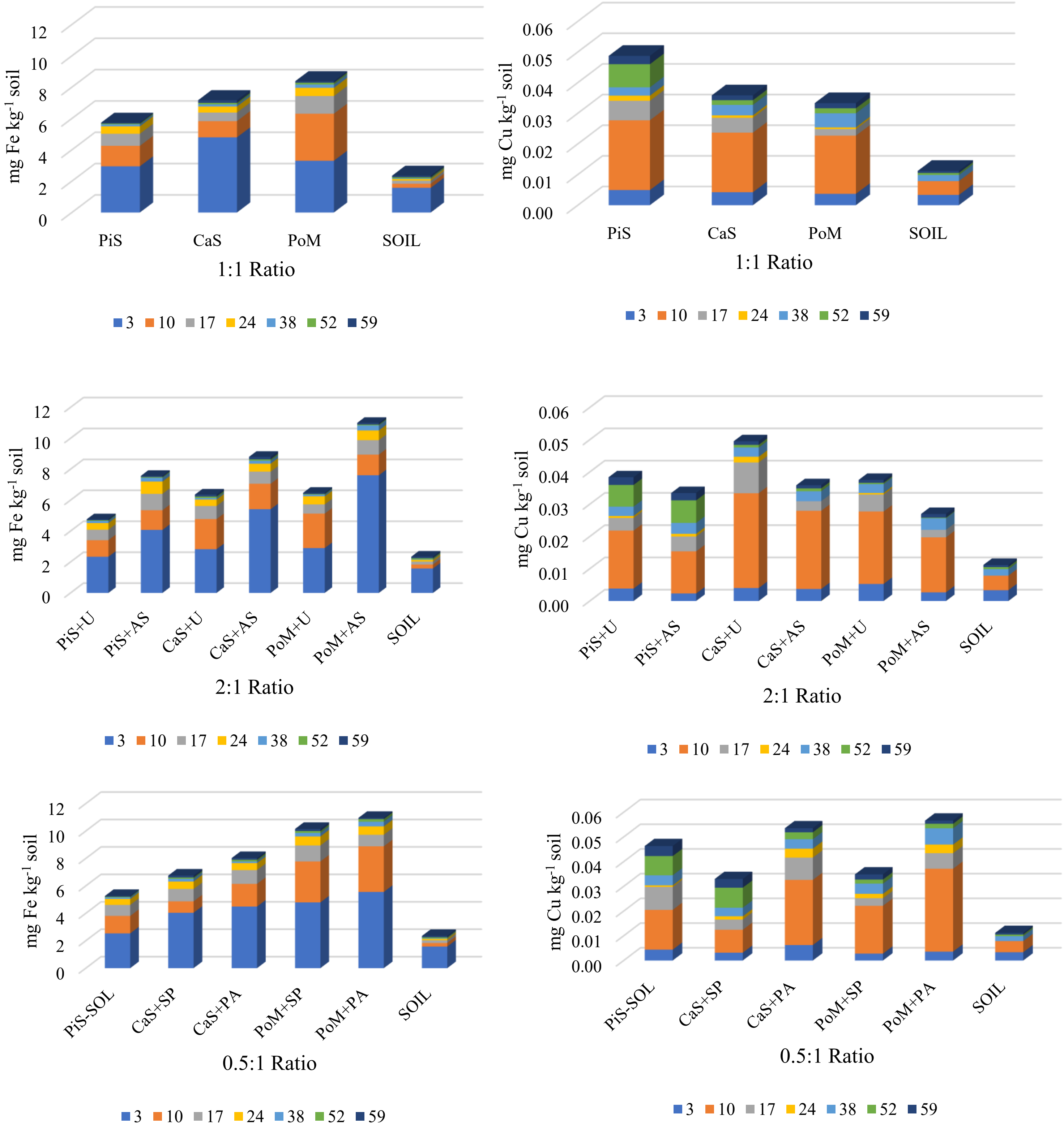


Figure S4.5: The iron (left) and the copper (right) concentrations on the leachate for each 1:1, 2:1 and 0.5:1 ratio during the leaching events. PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid.

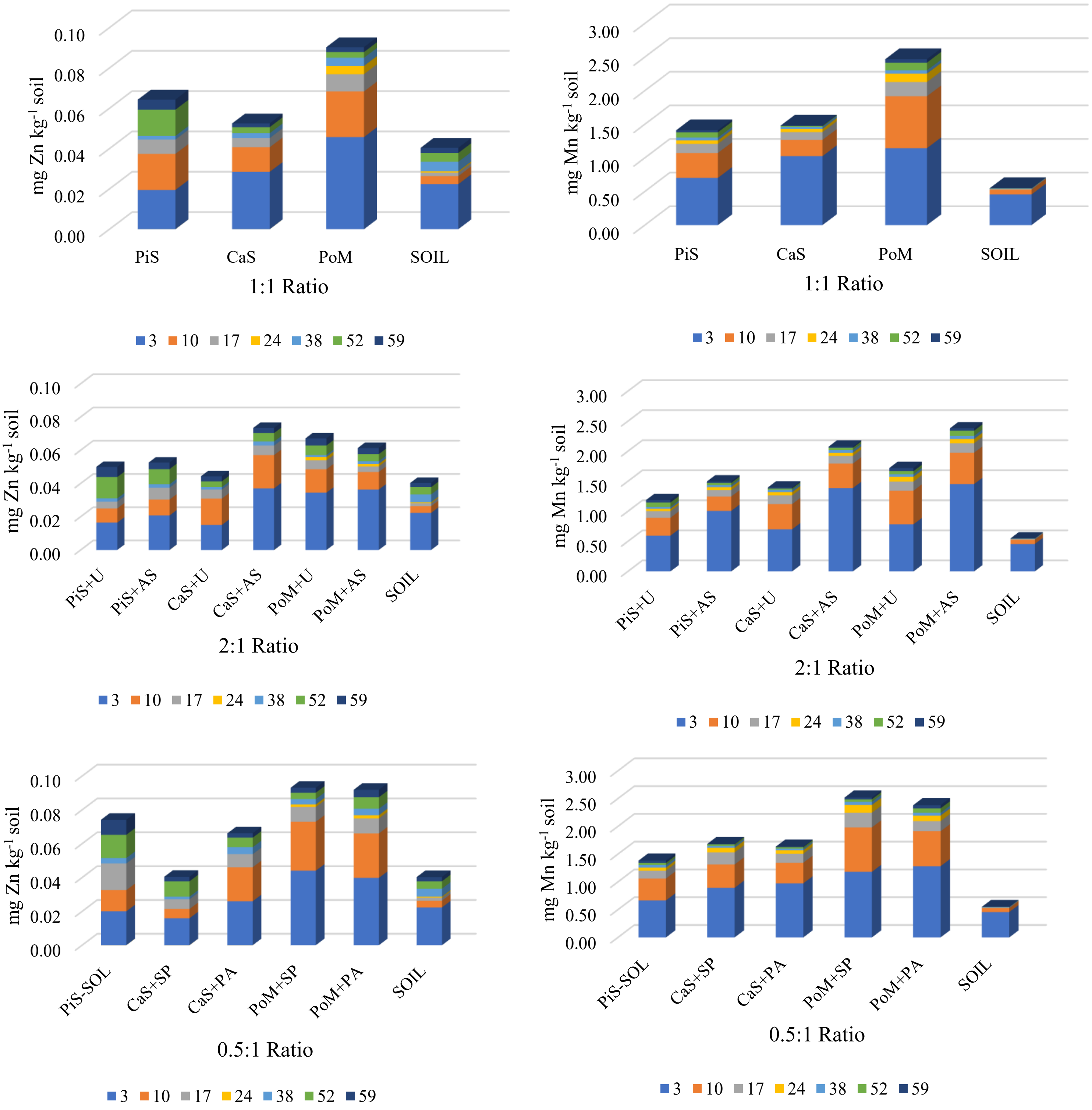


Figure S4.6: The zinc (left) and the manganese (right) concentrations on the leachate for each 1:1, 2:1 and 0.5:1 ratio during the leaching events. PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid.

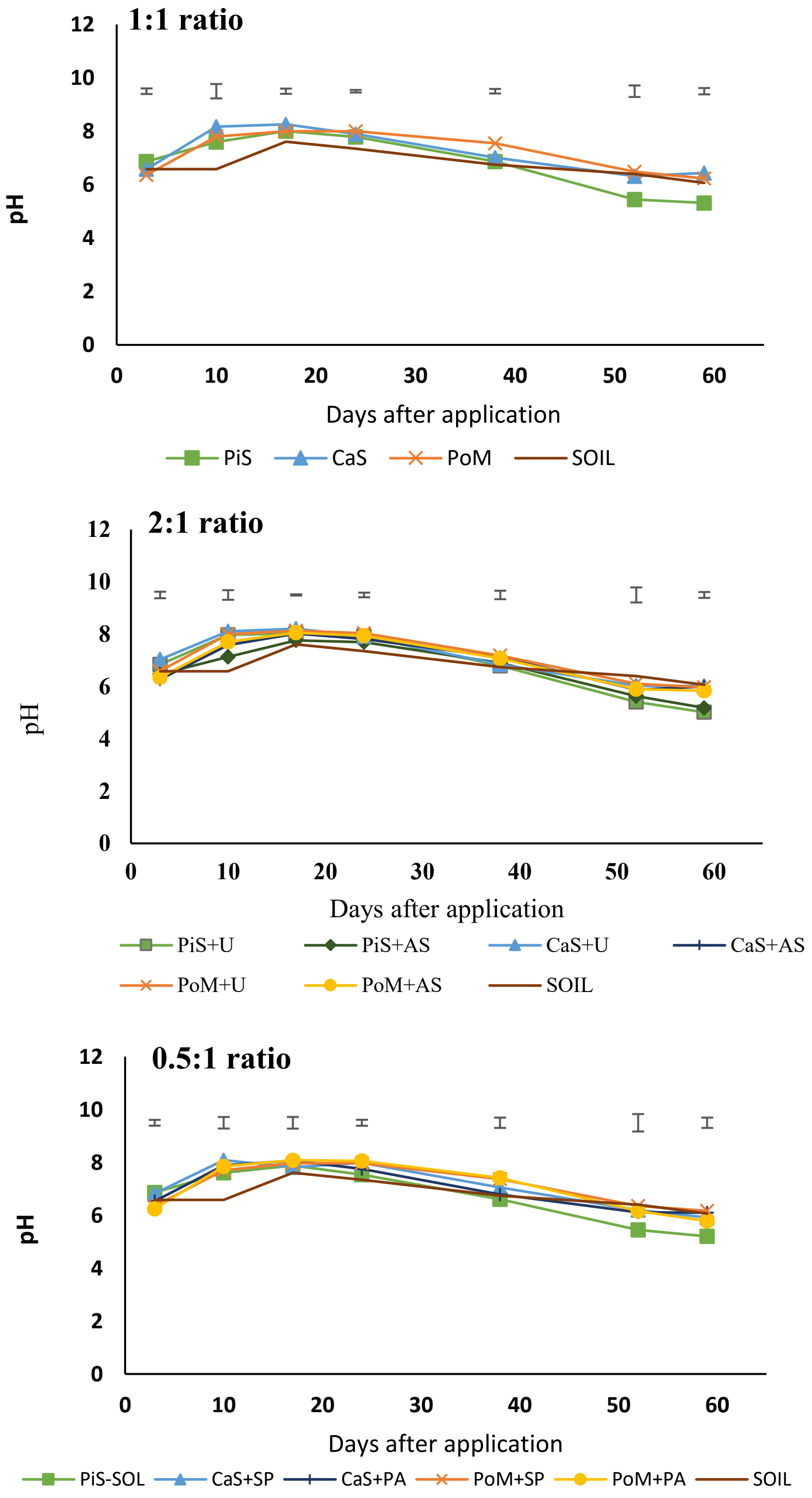


Figure S4.7: The pH dynamics in the leachate for each 1:1, 2:1 and 0.5:1 ratio during the leaching events. The vertical bars represent the standard errors for the means when performing the Tukey test (n=3). PiS+U: pig slurry with urea, PiS+AS: pig slurry with ammonium sulphate, CaS+U cattle slurry with urea, CaS+AS: cattle slurry with ammonium sulphate, PoM+U: poultry manure with urea, PoM+AS: poultry manure with ammonium sulphate, PiS-SOL: solid fraction from pig manure; CaS+SP: cattle slurry with superphosphate, CaS+PA: cattle slurry with phosphoric acid, PoM+SP: poultry manure with superphosphate and PoM+PA: poultry manure with phosphoric acid.

Chapter V Manure-based fertilizers production: nitrogen and phosphorus dynamic

Abstract

The manure-based fertilizers (MBF) emerge as a solution to overcome the surplus of manure and to implement more sustainable practices in agriculture. This study aimed to assess the nitrogen (N) and phosphorous (P) dynamics in a sandy soil amended with MBF with specific N:P ratios, commonly used in agriculture, 1:1, 2:1 and 0.5:1. A central of manure processing, where manures would be combined between them, was considered here. An holistic approach (including three experiments) was used to assess the potential impacts of MBFs application on air, water and soil quality. An aerobic incubation was performed to estimate the N mineralization and nitrification rates as well as the emissions of greenhouse gases, namely nitrous oxide emissions, and a leaching experiment, to assess potential leaching of several elements, namely nitrate and phosphorus. Two MBFs and one mineral fertilizer (MF), used as reference, were evaluated for each ratio. The treatments considered were **1:1 ratio** - poultry manure (PoM) + cattle slurry (CaS), PoM + pig slurry (PiS) and MF 10:10:10; **2:1 ratio** - liquid fraction of PiS (PiS-LIQ) + PiS, liquid fraction of CaS (CaS-LIQ) + CaS and MF 13:06:18; **0.5:1 ratio** - cattle manure (CaM) + PiS, CaM + PoM and MF 07:14:14. The application of PiS+PiS-LIQ, PoM+PiS and PoM+CaS resulted in similar N mineralization rate to MF, and after 20 and 40 days of incubations, respectively, the concentration of N mineral in the soil was either equal or superior to the corresponding MF. However, the application of MBF had a higher impact on the GWP compared to MF, which may suggest the necessity of adopting mitigation techniques. The potential of N leaching was always superior with the MF, and, in the case of P leaching, a direct relationship was observed between the amount of P leached and the MBF P content. It is possible to create MBF with the three ratios, however treatment such as slurry acidification must be considered to reduce the GWP.

Keysword

Nutrient cycle; N mineralization; P potential leaching; Greenhouse gases

5.1 Introduction

The manure-based fertilizer (MBF) emerges as a new concept in the European Union (Tur-Cardona et al., 2018). The application of organic materials will promote soil restructuration and improve soil's health while retrieving nutrients and closing the nutrient cycle (Chojnacka et al., 2020a; Sigurnjak et al., 2019). Further, adding a fertilizer rich in organic matter (OM) has extreme importance in the Mediterranean area, where the soil degradation and low OM contents are recurrent (Mata et al., 2019; Silva et al., 2022a). Furthermore, the increase in soil's OM may mitigate some of the impacts of agriculture on the environment (Pardo et al., 2017). The creation of MBFs will face three main issues that are problematic either for the agriculture or for the livestock sector i) the necessity of importing nutrients in the form of mineral fertilizers (MF), which are originally from non-renewable sources (Malomo et al., 2018), ii) diminish the heterogeneity of manures, increasing farmers acceptance of using animal manure as a replacement of mineral fertilizers (Tur-Cardona et al., 2018) and iii) settle the imbalance N:P ratio of manures relatively to crops necessity, especially in the north of Europe, where years of constant application of manure culminated in the overapplication of phosphorus (P) (Sigurnjak et al., 2017c). MBF appear as a solution to increase manure export out of the farm and overcome the surplus of manure, providing a solution for a more sustainable agriculture, and ameliorating soil's health. Formulating MBF with specific nutrient ratios for basal fertilization, similar to the commonly used mineral fertilizer, will increase manure acceptance by farmers. It is to refer that the adoption of low-technology treatments may be sufficient to modify the nutrients ratios without increasing exponentially the cost, as observed by Prado et al. (2022). Solid-liquid separation of manure demonstrated to be appropriate to obtain two materials with distinct nutrient ratios i) liquid fraction rich in nitrogen (N) and potassium (K) with a higher N:P ratio, relevant for soil with excessive P concentration and ii) solid fraction rich in P with a lower N:P ratio (Dennehy et al., 2017).

Even if much is known about the manures application to soil, e.g., benefits and awareness of their application, the knowledge regarding manure-based fertilizers is insufficient to sustain their adoption for basal fertilization in crops such as cereals or forages. The blending of manure to produce MBF may alter the nutrients dynamics after soil application, namely N and P, and therefore diminish or enhance the nutrient availability for plants and/or losses to the environment. A detailed assessment of the N and P cycle is necessary to completely understand the impact that MBF may have after soil application on nutrient availability for the crops or the associated environmental impacts.

One of the major constraints of using organic materials is that part of the nutrients is in the organic form, implying the necessity of nutrient mineralization to be assimilated by crops (Dalias and Christou, 2020) while the nutrients present in the MF are already in assimilable forms. On one side, the use of MFs may allow a quicker release of nutrients, but, on the other side, the application of manures, and likewise manure-based fertilizers, due to the necessity of nutrients mineralization, may ensure that nutrients will be released slowly for longer periods, increasing the soil nutrients reserve (Fangueiro et al., 2016). According to Portuguese legislation, it can be considered that 60% and 50% of the total N is in available forms in slurries and in solid manures, respectively (MADRP, 2018). This information is essential to prepare the MBF, but it is imperative to assess the dynamic of N mineralization, which may suffer alteration due to manure blending. This will increase the acceptance of farmers by offering reliable information relative to the product and the top-dressing fertilization that will be necessary (Zavattaro et al., 2017).

The tailor-made MBFs can contribute to the mitigation of the environmental impact of the livestock sector (Prado et al., 2022) but their application to the soil should not foment greenhouse gases (GHG) emissions, namely nitrous oxide (N_2O), methane (CH_4) or carbon dioxide (CO_2), (Fangueiro et al., 2021; Prado et al., 2020). Fertilization is, firstly, based on N requirements, and consequently, due to the unbalance N:P ratio of manure, overapplication of P to the soil is common (Liu et al., 2019). P is an element that can be adsorbed by colloids in the form of iron and aluminium oxides, but it can also be leached when soils are saturated and become a source of water contamination (Vanden Nest et al., 2016; X. Wang et al., 2020). The formulation of a MBF that conceptualizes a nutrient ratio with a lower P concentration is important to avoid both, the overapplication of P and the risk of potential P leaching.

The scenario considered here, a central manure processing plant, implies the following premises i) the manures will be mixed between them or with the subproducts of manure treatment to achieve some desired N:P ratios (1:1; 2:1; 0,5:1) ii) only low technology treatments will be conducted, to avoid increasing the costs and iii) the manures considered are accessible all year, which mean that only manures with higher production were considered. The aim of the study was to determine the effects of MBFs soil application to i) assess the N availability through the evaluation of mineralization and nitrification rates, ii) study potential alterations of the N or C air losses by gases emissions, iii) appraise the potential of nitrate and P leaching and iv) the integration of all the information will permit to ascertain the sustainability of replacing MF, in basal fertilization, with MBFs.

5.2 Material and methods

5.2.1 Manure sampling and characterization

Four manures were utilized: i) cattle slurry (CaS), cattle manure (CaM), pig slurry (PiS) and poultry manure (PoM). The fours materials were characterized, according to the methodology described in Prado et al. (2022), in terms of dry matter content (DM), pH, total organic carbon (TOC), total nitrogen (N_{Total}), ammonium nitrogen (NH_4^+-N), total phosphorus (P_{Total}, expressed as P_2O_5) and total K (K_{Total}, expressed as K_2O). P and K were expressed as P_2O_5 and K_2O , respectively, to approximate the formulation of MBF with MFs. The CaS and PiS were treated by solid-liquid separation as described in Fangueiro et al. (2015a), to obtain the liquid fraction (LIQ) used to produce MBF. The main characteristics of the four manures and the respective LIQ of CaS and PiS are shown in Table 5.1.

Table 5.1: Composition of the raw manures and the sub-product of solid-liquid separation, all expressed in fresh matter basis (mean value ± standard deviation, n=3).

	DM **g kg^{-1}**	**TOC** **g kg $^{-1}$**	**N_{Total}** **g N kg^{-1}**	**NH_4^+-N** **g N kg^{-1}**	**N_{av}** **g N kg^{-1}**	**P_{Total}** **g P_2O_5 kg^{-1}**	**N:P**
PiS	49.8±0.30	19.8±0.31	4.6±0.04	3.4±0.01	3.4±0.02	2.3±0.01	1.2
PiS-LIQ	16.6±1.81	7.3±0.43	3.8±0.10	3.3±0.07	3.3±0.09	0.2±0.01	11.3
CaS	61.9±0.62	13.0±0.87	2.6±0.06	1.4±0.01	1.6±0.04	1.0±0.01	1.6
CaS-LIQ	35.9±0.90	13.0±0.87	1.8±0.07	1.2±0.01	1.2±0.07	0.4±0.00	2.7
CaM	800.4±0.95	412.6±1.50	14.3±0.13	0.9±0.02	7.2±0.18	7.4±0.05	0.9
PoM	772.8±1.10	582.0±1.30	19.2±1.27	3.9±0.08	9.6±0.35	15.3±0.30	0.6

PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure. DM: Dry matter, TOC: Total organic carbon, N_{Total}: Total nitrogen, NH_4^+-N: Ammonium nitrogen, N_{av}: Available nitrogen (calculated as a % of the total N), P_{Total}: Total phosphorus (expressed as P_2O_5).

5.2.2 Manure-based fertilized preparation

The manure-based fertilizers were designed according to the three N:P ratios, usually find in MF used by farmers: 1:1, 2:1 and 0.5:1. The amount of available N (N_{av}) was considered to calculate the ratios, and the N_{av} was estimated as 60% or 50% of the total N, for manures with DM<20% and manure with DM>20%, respectively (MADRP, 2018).

As previously referred to, the MBFs were designed considering the scenario of a central manure processing plant receiving different kinds of manure and producing some derived products as solid and liquid fractions.

For the 1:1 ratio, the following MBFs were considered i) poultry manure with cattle slurry (PoM+CaS) and ii) poultry manure with pig slurry (PoM+PiS). For the 2:1 ratio the mixtures were i) pig slurry with liquid fraction of pig slurry (PiS+PiS-LIQ) and ii) cattle slurry with liquid fraction of cattle slurry (CaS+CaS-LIQ). For the 0.5:1 ratio, i) cattle manure with pig slurry (CaM+PiS) and ii) cattle manure with poultry manure (CaM+PoM). The manure-based fertilizers composition was calculated using the nutrients' concentration of the raw materials. The DM, TOC, N_{Total}, NH_4^+-N, N_{av} and P_{Total} can be seen in Table 5.2.

Table 5.2: The manure-based fertilizers composition, calculated value based on the raw manures and sub-products nutrients concentrations. All nutrients were expressed in fresh matter. The quantity of total and available N and total P applied.

		DM	**TOC**	**N_{Total}**	**NH_4^+-N**	**N_{av}**	**P_{Total}**	**N:P**	**N_{Total} applied**	**N_{av} applied**	**P_{Total} applied**
		g kg^{-1}	**g C kg^{-1}**	**g N kg^{-1}**	**g N kg^{-1}**	**g N kg^{-1}**	**g P_2O_5 kg^{-1}**		**mg N kg^{-1} soil**	**mg N kg^{-1} soil**	**mg P_2O_5 kg^{-1} soil**
1:1 Ratio	**PoM+PiS**	410.5	258.2	11.9	3.7	6.2	5.0	1.20	134.8/67.4*	70.0/35.0*	56.7/28.4*
	PoM+CaS	498.4	515.3	12.8	2.9	6.5	5.1	1.30	137.8/68.9*	70.0/35.0*	55.3/27.7*
	10:10:10	-	-	100.0	75.0	100.0	100.0	1.00	70.0/35.0*	70.0/35.0*	70.0/35.0*
2:1 Ratio	**PiS+PiS-LIQ**	35.6	13.0	4.2	2.5	2.5	1.1	2.20	116.7/58.4*	70.0/35.0*	31.8/15.9*
	CaS+CaS-LIQ	30.8	13.0	2.1	1.3	1.3	0.6	2.30	16.7/35.4*	70.0/35.0*	30.5/15.3*
	13:06:18	-	-	130.0	105.0	130.0	60.0	2.17	70.0/35.0*	70.0/35.0*	60.0/30.0*
0.5:1 Ratio	**CaM+PiS**	790.4	346.8	8.7	0.9	4.4	7.5	0.58	139.8/69.9*	70.0/35.0*	120.9/60.5*
	CaM+PoM	799.6	354.7	9.0	1.0	4.5	7.6	0.59	140.0/70.0*	70.0/35.0*	118.1/59.1*
	07:14:14	-	-	70.0	50.0	70.0	140.0	0.50	70.0/35.0*	70.0/35.0*	140.0/70.0*

PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure. DM: Dry matter, TOC: Total organic carbon, N_{Total}: Total nitrogen, NH_4^+-N: Ammonium nitrogen, N_{av}: Available nitrogen (calculated as a % of the total N), P_{Total}: Total phosphorus (expressed as P_2O_5).

*: The quantity of nutrients applied for the greenhouse gases emissions experiment.

5.2.3 Experiments

Three experiments were conducted simultaneously to assess the overall impact of MBF application to soil on:

1- N mineralization and nitrification through an anaerobic incubation with destructive soil sampling.
2- GHG emissions through a second aerobic incubation with no soil perturbation.
3- The potential of N and P leaching, through a soil column experiment.

The soil used in the three experiments was a sandy soil, classified as Haplic Arenosol, with a pH of 5 and considered very poor in P and K according to Veloso et al. (2022). For both aerobic incubations, the soil at 30% of water holding capacity (WHC) was pre-incubated at 20ºC for 7 days . During the incubation period, the soil was kept at 70% of soil water holding capacity and maintained constant by regular weighting and moisture correction.

In the three experiments, the amount of N applied was equivalent to 210 kg N_{av} ha^{-1}.

The application of manure-based fertilizer for each ratio was compared with the equivalent mineral fertilizer, usually utilized by farmers. For every N:P ratio two MBFs and a MF was used as a reference as follow:

i) 1:1 ratio: PoM+CaS, PoM+PiS and MF 10:10:10 (10% N of which 2.5% NO_3^- and 7.5% NH_4^+, 10% P_2O_5, and 10% K_2O).
ii) 2:1 ratio: PiS+PiS-LIQ, CaS+CaS-LIQ and MF 13:06:18 (13% of N of which 2.5% NO_3^- and 10.5% NH_4^+, 6% of P_2O_5 and 18% of K_2O).
iii) 0.5:1 ratio: CaM+PiS, CaM+PoM and MF 7:14:14 (7% of N of which 2% NO_3^- and 5% NH_4^+, 14% of P_2O_5 and 14% of K_2O).

5.2.3.1 Incubation experiment

An aerobic incubation was performed to assess the N dynamics after the incorporation of the blends into the soil, emphasizing the N mineralization/immobilization and nitrification rates. The application of each material was replicated four times and soil without treatment was used as the control.

The methodology used here is fully described in Prado et al. (2022a). Briefly, for each replicate, 1000 g of air-dried soil was mixed with the MBFs, or MF and the mixtures were incubated for 90 days at 20ºC. 26 g of amended soil were sampled on days 1, 4, 8, 11, 15,22, 29, 36, 43, 50, 64, 78 and 92, to assess the concentration of mineral N (NH_4^+ and NO_3^-) according to the methodology followed by Fangueiro et al. (2016).

The net N mineralization (NNM) and the apparent net N mineralization (ANNM) were calculated as follows, where t is a specific day and t_1 the first sample day:

NNM (mg N kg^{-1}) = min N (t) – min (N) (t_1)

ANNM (% organic N applied) = $\frac{(\min N(t)-\min N(t1))treatment-(\min N(t)-minN\ (t1))soil)}{Organic\ N\ applied}\ x\ 100$

Where min N (t) represents the mineral N (NH_4^+ and NO_3^-) at each sampling date, and min N (t1) represents the mineral N (NH_4^+ and NO_3^-) on day 1.

The ANNM was calculated considering the organic N applied with the manure-based fertilizers since the aim was to determine how much of the organic N from the manure-based fertilizer application would be mineralized. The ANNM values presented here corresponded to the end of the first, second and third months of the incubation experiment. Also, since the fertilization rate is based on N_{av}, the ANNM was also calculated according to N_{av} applied with the manure-based fertilizers and mineral fertilizers.

ANNM (% available N applied) = $\frac{(\min N(t)-\min N(t1))treatment-(\min N(t)-minN\ (t1))soil)}{available\ N\ applied}\ x\ 100$

The net nitrification (Nit), was calculated for specific time intervals [ti; tf], using the following formula (Fangueiro et al., 2016).

$$\text{Nit (mg } NO_3^-\text{-N } kg^{-1}) = NO_3^-\ (t_f) - NO_3^-\ (t_i)$$

5.2.3.2 Greenhouse gases emissions experiment

The GHG emission experiment was assembled with three repetitions per treatment. Each jar glass used in this incubation was filled with 0.5 kg of dried soil amended with MBF or MF. The jars remained open between measurements and were stored in an incubator for 90 days at 20ºC. GHG emissions rates (CH_4, CO_2 and N_2O) were measured on days 1, 3, 8, 10, 13, 15, 20, 23, 27, 34, 41, 48, 62, 76 and 90, after application.

GHG emissions rates were measured as described by Fangueiro et al. (2015). Briefly, at each sampling date, the jars were hermetically closed, and headspace air sampling was performed immediately (T_0), 30 minutes (T_{30}), and 60 minutes (T_{60}) after closure. The emissions rates were calculated by linear regression, through the T_0, T_{30} and T_{60}, and adjusted for the 20ºC. The gases concentrations in the air samples were measured by gas chromatography (GC) using a GC-2010 Plus (Shimadzu, Japan), equipped with an electron capture 63Ni to detect N_2O, a thermal conductivity detector for CO_2, and a flame ionization detector for CH_4.

Cumulative gas emission was calculated for each of the sampled gases by summing the daily emissions. In that calculation, when no daily data was available, concentrations were estimated assuming that the daily gas flux changed linearly between the nearest sampling dates. Also, the global warming potential (GWP) was determined for the N_2O and CH_4, and expressed as CO_2 equivalents CO_2eq, using the GWP convertor factor for a 100-year time horizon, which, for CH_4, is 28-times higher than for CO_2, while for N_2O, is 265-times higher than for CO_2 (IPCC, 2016).

5.2.3.3 Leaching experiment

The leaching experiment was performed with three repetitions per treatment plus the control (soil without MBF or MF). For each replicate, a PVC column was filled with one kg of air-dried soil, that was saturated prior MBFs and MFs application. Leaching events were performed on days 4, 9, 16, 23, 30, 37 and 44 after MBFs and MF application.

For each leaching event, the amount of distilled water added to each column was 200 mL, corresponding to the soil maximum water holding capacity. The water was added gradually to each column and the leachate was collected in vials. On each collecting date, the volume of leachate was recorded for each column. A leachate sample from each column was stored at 4ºC to analyse the nutrient concentrations. The NH_4^+-N and NO_3^--N concentration were measured directly in the leachate and quantified by segmented flow autoanalyzer SAN plus (San Plus System, Skalar, Nederland) with a modified Berthelot method. The P, potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S)) and micronutrient (iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn)) concentrations were also determined, directly in the leachate, by inductively coupled plasma optical emission spectrometry (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, USA). The concentration of K, Ca, Mg, S, Fe, Cu, Zn and Mn are presented in the supplementary material (Figure S5.4).

5.2.4 Statistical Analyses

The statistical analysis was performed using one-way ANOVA. To determine the statistical significance of the means values, with the use of Statistix 7 a Tukey test was realized with a $P<0.05$ and 95% degree of confidence.

5.3 Results and discussion

5.3.1 N Mineralization

For the 1:1 ratio, the evolution of the concentration of NH_4^+-N and NO_3^--N in the soil amended with the MBF presented clear differences relative to the MFs (Figure 5.1a and b). In soil amended with the 1:1 MBF, mineral N was present exclusively in the NH_4^+ form until day 22, while after day 30 NO_3^- was the only form of mineral N. The higher increase in the NO_3^- concentration demonstrated in soil with PoM+PiS and PoM+CaS relative to 10:10:10, can be explained by the higher nitrification rates observed in these treatments between the days 22 to 29, ~25 mg NO_3^--N kg^{-1} soil (Table 5.3). The present results were similar to those observed in other studies, where a quick decrease in the NH_4^+-N concentration was attributed to nitrification (Sigurnjak et al., 2017b). The NH_4^+ is the subtract to obtain NO_3^-, if NH_4^+ was only consumed by the nitrified bacteria, at some point the NO_3^- concentration in the soil would decrease. However, the NO_3^- concentration kept rising, which suggests that the mineralization and nitrification processes occurred simultaneously in these treatments. Both manure and MBFs are a source of soluble NH_4^+ and organic N that are transformed by the soil biotic community through the mineralization-immobilization turnover (Whalen et al., 2019). Hence, MBF stimulates the NH_4^+ nitrification and the mineralization of the organic N since with their application there was a vehiculation of organic matter that stimulated the soil microorganism activity, while with the application of MF, by not adding organic matter to the soil they do not stimulate the microorganism activity, and thereby mineralization.

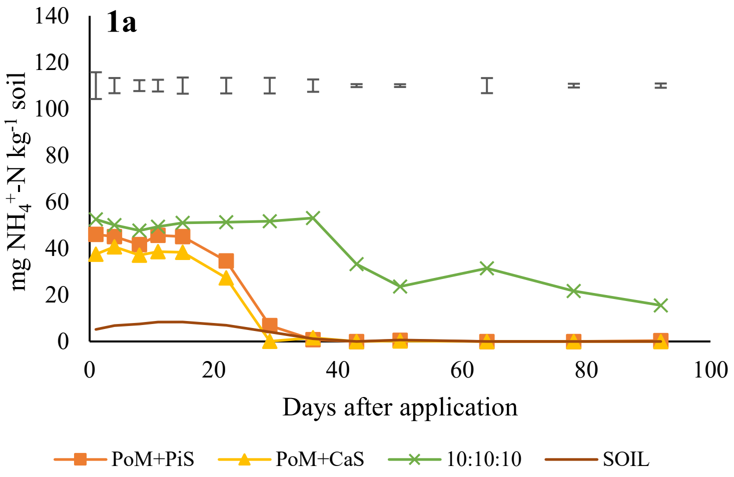
The 10:10:10 treatment led exclusively to mineral N immobilization while mineral N immobilization was observed till day 15 in PoM+PiS and day 29 in PoM+CaS. Later, organic N mineralization was observed in these two MBFs and close to 10% of the total organic N applied was mineralized in these two treatments. In a study where both pig slurry and calcium ammonium nitrate were considered, the net N release from the organic material was much higher than from the MF, and relative to the initial N applied, the authors demonstrated long periods of N immobilization with MF (Luo et al., 2021). This initial phase of N immobilization followed by N mineralization has been reported in several other studies (Dalias and Christou, 2020; Fangueiro et al., 2015d). The concentration of NH_4^+ and NO_3^-, in soil immediately after application of MF was higher than MBF options on day one. This will increase the risk of NO_3^- leaching from day one, a feature also observed in the leaching experiment and discussed in the leaching chapter.

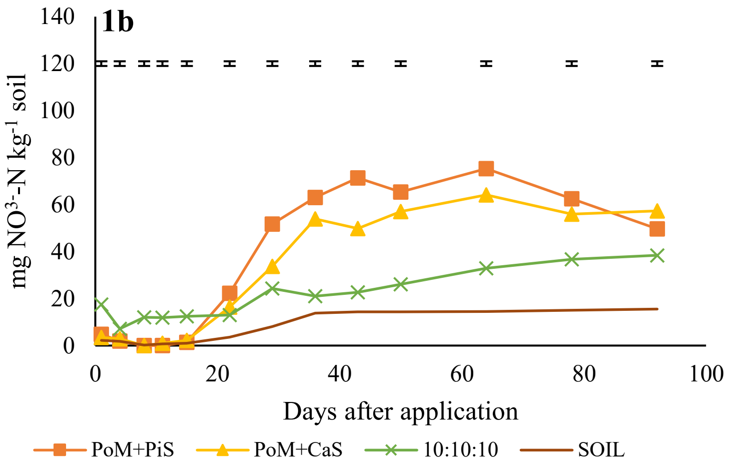
Table 5.3: Nitrification rates observed with each blend during the significant time intervals of the incubation (mean values, n=4). Results for each ratio in each column followed by different letters differ significantly for P<0.05 (Tukey test).

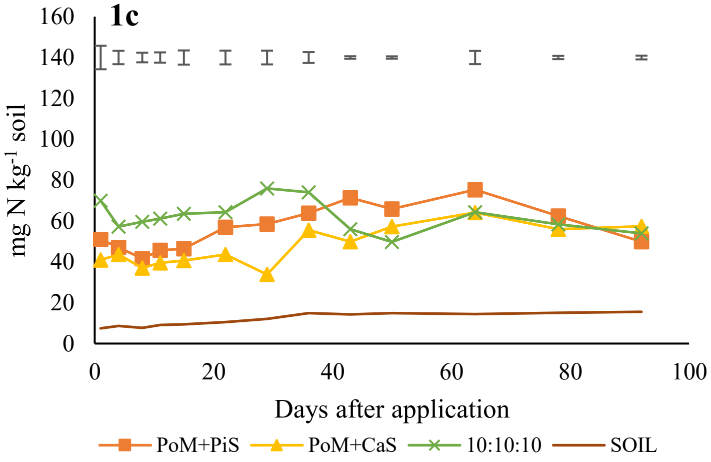
	22 to 29	**29 to 36**	**36 to 43**	**43 to 50**	**50 to 64**	**64 to 78**	**78 to 92**
	mg NO_3^--N kg^{-1} soil						
PoM+PiS	29.39^{a}	11.27^{a}	11.42^{a}	-6.03^{a}	4.79^{a}	38.57^{a}	-59.11^{b}
PoM+CaS	21.79^{ab}	10.29^{a}	-4.10^{b}	2.61^{ab}	2.69^{a}	-8.12^{b}	7.24^{a}
10:10:10	11.29^{bc}	-3.28^{b}	0.67^{ab}	4.53^{ab}	6.78^{a}	3.81^{b}	1.74^{a}
SOIL	4.45^{c}	5.66^{ab}	-0.59^{ab}	-2.34^{ab}	2.77^{a}	-9.48^{b}	10.57^{a}
PiS+PiS-LIQ	15.24^{a}	18.66^{a}	3.77^{ab}	7.86^{a}	28.48^{a}	-11.98^{b}	-13.32^{b}
CaS+CaS-LIQ	-2.29^{b}	-2.53^{b}	6.93^{ab}	-8.40^{b}	7.36^{b}	20.07^{a}	-14.68^{b}
13:06:18	3.29^{b}	3.54^{b}	12.35^{a}	-3.93^{b}	8.40^{b}	28.72^{a}	-6.12^{b}
SOIL	4.45^{b}	5.66^{b}	-0.59^{b}	-2.34^{b}	2.77^{b}	-9.48^{b}	10.57^{a}
CaM+PiS	8.95^{a}	3.48^{a}	22.87^{a}	-10.54^{a}	11.43^{a}	20.22^{a}	-12.15^{c}
CaM+PoM	5.85^{b}	0.97^{a}	7.33^{b}	-0.48^{a}	8.72^{ab}	13.74^{ab}	7.51^{a}
07:14:14	5.33^{b}	1.78^{a}	0.30^{b}	1.81^{a}	6.43^{ab}	5.88^{b}	-5.13^{a}
SOIL	4.45^{b}	5.66^{a}	-0.59^{b}	-2.34^{a}	2.77^{b}	-9.48^{c}	10.57^{a}

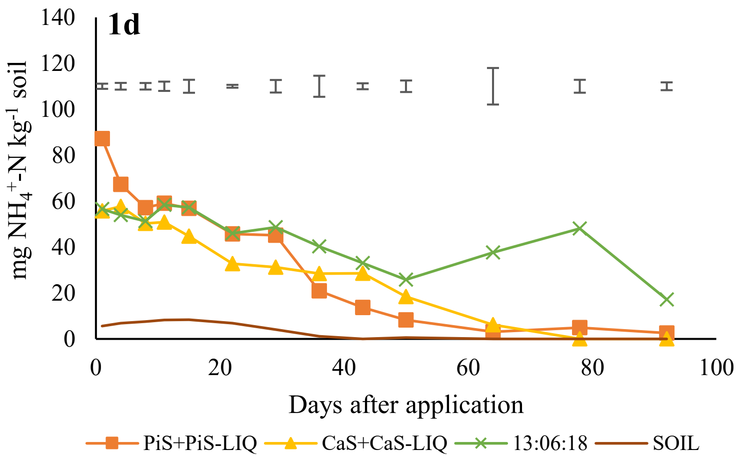
PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

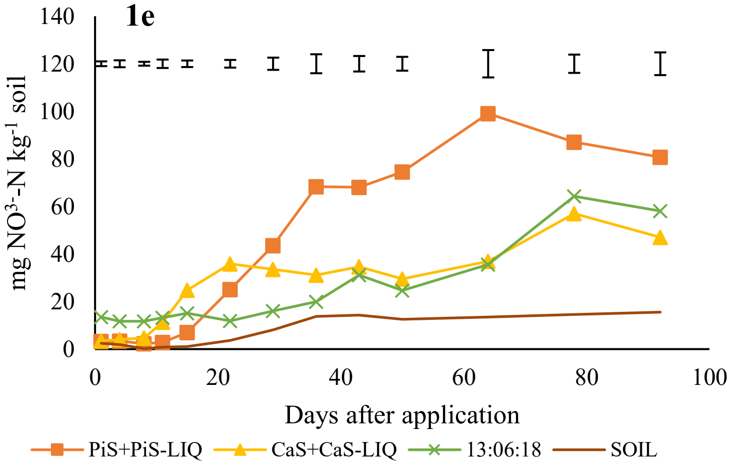
These features, N mineralization and NH_4^+ nitrification, were considered in this study as a form to predict and survey the plant N availability, an important information to deliver when presenting possible fertilizers to farmers, which contain a higher quantity of organic N susceptible to be mineralized (Ribeiro et al., 2010b). The present results demonstrates that the combination of the two manures, PoM and PiS, resulted in higher nitrification rates relative to isolated application of PoM or PiS. Zare and Ronaghi, (2019), stated that the use of PoM presented a low nitrification rate relative to the use of PiS, which they attributed to the higher C/N ratio of PoM, due to the higher content of total organic carbon (Table 5.1). The C/N ratio has a direct influence on N mineralization since soil microbes feed on organic N and C at fixed stoichiometry when incorporating organic materials into the soil microbes tend to balance the C/N ratio (Bonanomi et al., 2019). The present results may indicate a slow initial N release when applying PoM+PiS to the soil, while with PoM+CaS, the N started to be available after the second month, so it may be necessary to complement the N supply with a MF adequate for topdressing fertilization. Both potential MBFs presented a higher N nitrification rate than the 10:10:10, indicating that the application of these organic fertilizers should be sufficient to supress crops' necessities. Indeed, the mineral N mineralization rates was similar to the denoted with 10:10:10 (Figure 5.1c). Luo et al. (2021), acknowledged that MBFs can substitute MFs and contribute to a more sustainable agriculture, by supporting a circular economy. However, and as referred by the same authors, few studies dealing with the application of bio-based fertilizer are available.

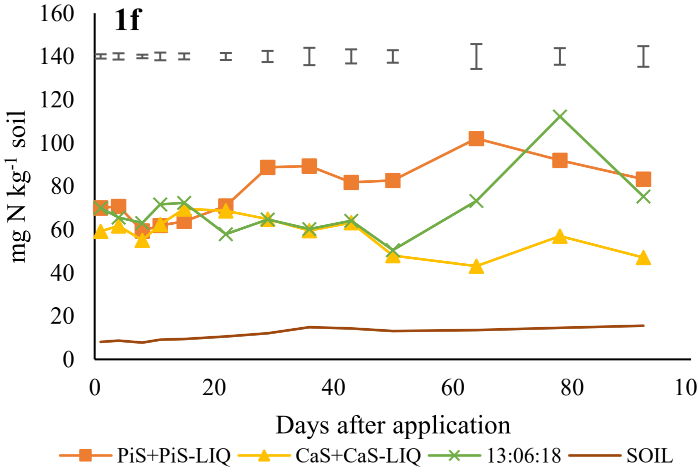

1a
mg NH$_4^+$-N kg^{-1} soil
0
20
40
60
80
100
120
140
0
20
40
60
80
100
Days after application
PoM+PiS
PoM+CaS
10:10:10
SOIL


1b
mg NO^{3-}-N kg^{-1} soil
0
20
40
60
80
100
120
140
0
20
40
60
80
100
Days after application
PoM+PiS
PoM+CaS
10:10:10
SOIL


1c
mg N kg^{-1} soil
0
20
40
60
80
100
120
140
160
0
20
40
60
80
100
Days after application
PoM+PiS
PoM+CaS
10:10:10
SOIL


1d
mg NH$_4^+$-N kg^{-1} soil
0
20
40
60
80
100
120
140
0
20
40
60
80
100
Days after application
PiS+PiS-LIQ
CaS+CaS-LIQ
13:06:18
SOIL


1e
mg NO^{3-}-N kg^{-1} soil
0
20
40
60
80
100
120
140
0
20
40
60
80
100
Days after application
PiS+PiS-LIQ
CaS+CaS-LIQ
13:06:18
SOIL


1f
mg N kg^{-1} soil
0
20
40
60
80
100
120
140
160
0
20
40
60
80
100
Days after application
PiS+PiS-LIQ
CaS+CaS-LIQ
13:06:18
SOIL

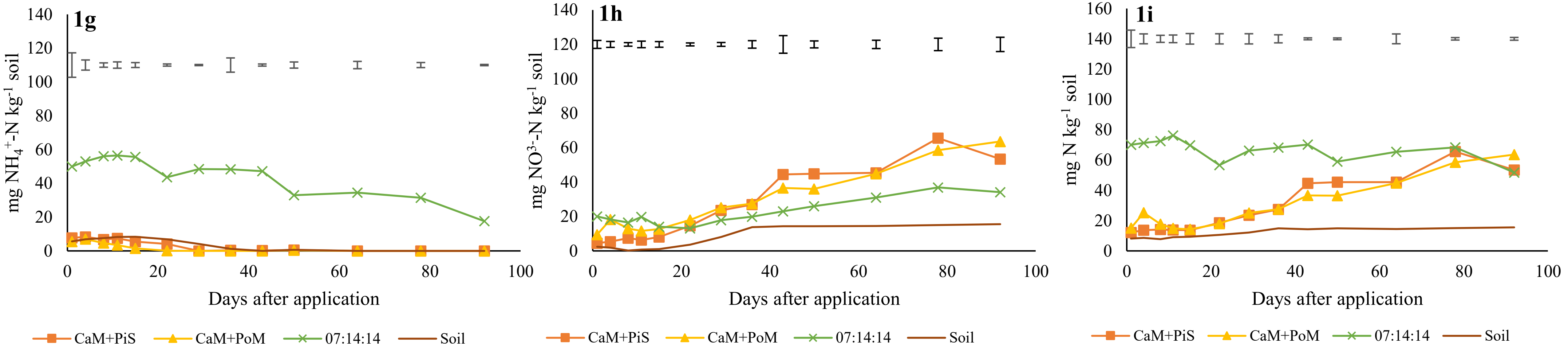


Figure 5.1: The NH_4^+ (left), NO_3^- (meddle) and mineral N (right) concentrations on the soil for each 1:1 ratio (1a to 1c), 2:1 (1d to 1f) and 0.5:1 (1g to 1i) during the incubation. Values presented are arithmetic means (n=4). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date. PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

The NH_4^+-N concentration in the soil amended with the 2:1 ratio reached values close to zero only after 60 days. While with the application of the other ratios the levels of NH_4^+ reached faster concentrations close to zero, after 20 days (Figure 5.1a,d and g). This can indicate either i) N mineralization rate was higher in the 2:1 and NH_4^+ was accumulated in the soil or ii) that the nitrification rate was slower compared to the other ratios. For the PiS+PiS-LIQ, the N mineralization rate was lower than the observed in the other ratio and the nitrification rate was higher, while for the CaS+CaS-LIQ the nitrification rate was slower until day 50, increasing the accumulation of NH_4^+. The MF maintained the NH_4^+-N concentration in the soil with a range of 60 and 30 mg NH_4^+-N kg^{-1} soil during the last 50 days (Figure 5.1d). The NO_3^--N concentration was two or three-time higher with PiS+PiS-LIQ compared to CaS+CaS-LIQ and 13:06:18, respectively, between days 36 and 64 (Figure 5.1e). This may indicate that PiS+PiS-LIQ mineralizes N quicker, since the product of N mineralization is the subtract to N nitrification. Also, CaS+CaS-LIQ after the end of the first month presented a similar nitrification rate as 13:06:18, indicating a tendency for a positive N nitrification similar to the mineral fertilizers. Nonetheless, the higher NNM was observed when 13:06:18 was applied. This can be explained, mainly, by the maintenance of higher NH_4^+ concentration in the soil after the application of CaS+CaS-LIQ and the fact that after four days the N was available in the soil (Table 5.4 and Figure 5.1d). This aspect had repercussions on the ANNM, which presented lower periods of N immobilization after the application of 13:06:18 (Table 5.5). The N immobilization has been observed with manure but is usually temporary (Halassy et al., 2021). Nevertheless, it can lead to crop N deficiencies and impair yield production (Dalias and Christou, 2020).

Table 5.4: The net N mineralization (NNM) and the apparent N mineralization (ANNM) observed with each manure-based fertilizer, over the incubation time (mean values, n=4). Results for each ratio in each column followed by different letters differ significantly for $P<0.05$ (Tukey test).

	NNM												ANNM					
	4	**8**	**11**	**15**	**22**	**29**	**36**	**43**	**50**	**64**	**78**	**92**	**% N_{av} applied**			**% $N_{organic}$ applied**		
	mg N kg^{-1} soil												**29**	**64**	**92**	**29**	**64**	**92**
PoM+PiS	-7.87^{b}	-11.43^{c}	-9.23^{b}	-8.59^{a}	6.36^{a}	4.16^{a}	8.91^{a}	14.27^{a}	10.99^{a}	22.14^{a}	47.91^{a}	-4.93^{bc}	< 0	19.33^{b}	< 0	< 0	8.61^{a}	9.88^{a}
PoM+CaS	3.82^{a}	-3.94^{b}	0.18^{a}	-0.03^{a}	-0.47^{a}	-5.06^{b}	10.13^{a}	11.60^{a}	14.46^{a}	26.03^{a}	11.36^{b}	16.45^{a}	< 0	26.55^{a}	15.01	< 0	3.80^{b}	< 0
10:10:10	-12.78^{c}	-10.33^{c}	-16.04^{c}	0.22^{a}	-6.73^{b}	5.93^{a}	4.08^{a}	-14.08^{c}	-20.28^{c}	-5.71^{c}	-11.61^{c}	-16.04^{c}	1.79^{a}	< 0	< 0	n.a.	n.a.	n.a.
Soil	1.18^{a}	0.01^{a}	0.70^{a}	2.63^{a}	3.57^{a}	5.35^{a}	7.46^{a}	5.93^{b}	5.63^{b}	7.85^{b}	7.55^{b}	9.66^{ab}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
PiS+PiS-LIQ	-19.76^{d}	-31.01^{d}	-28.53^{b}	-26.58^{b}	-19.59^{d}	-1.68^{b}	-4.82^{ab}	-8.68^{b}	-7.68^{b}	15.60^{a}	-3.03^{b}	-7.19^{b}	< 0	15.14	< 0	< 0	< 0	< 0
CaS+CaS-LIQ	2.53^{a}	-4.15^{b}	3.05^{a}	8.07^{b}	7.49^{a}	5.93^{a}	0.33^{ab}	8.09^{a}	-11.24^{bc}	-16.11^{c}	-3.84^{b}	-12.09^{b}	2.29	< 0	< 0	6.07	< 0	< 0
13:06:18	-3.86^{c}	-8.37^{c}	5.37^{a}	2.30^{a}	-12.07^{c}	-7.73^{c}	-9.74^{b}	-5.90^{b}	-19.52^{c}	3.21^{ab}	42.28^{a}	5.37^{a}	< 0	< 0	< 0	n.a.	n.a.	n.a.
Soil	1.18^{b}	0.01^{a}	0.70^{a}	2.63^{a}	3.57^{b}	5.35^{a}	7.46^{a}	5.93^{a}	5.63^{a}	7.85^{ab}	7.55^{b}	9.66^{a}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
CaM+PiS	0.37^{c}	0.89^{a}	-0.16^{b}	1.47^{ab}	6.45^{a}	12.78^{a}	15.17^{a}	30.85^{a}	33.15^{a}	33.15^{a}	49.36^{a}	36.39^{b}	10.45^{a}	40.27^{a}	48.27^{a}	6.62^{a}	14.53^{a}	27.80^{b}
CaM+PoM	13.51^{a}	1.63^{a}	-1.17^{b}	-0.74^{ca}	3.03^{ab}	8.12^{b}	14.52^{a}	17.97^{b}	21.45^{a}	29.81^{a}	43.55^{a}	45.53^{a}	8.76^{b}	35.50^{b}	58.81^{b}	4.90^{b}	13.18^{a}	32.92^{a}
07:14:14	1.26^{b}	0.72^{a}	5.93^{a}	-1.74^{bc}	-13.30^{b}	-3.54^{d}	-4.16^{c}	0.22^{c}	-12.44^{c}	-4.77^{c}	-1.48^{b}	-17.28^{d}	< 0	< 0	< 0	n.a.	n.a.	n.a.
Soil	1.18^{b}	0.01^{a}	0.70^{b}	2.63^{a}	3.57^{ab}	5.35^{c}	7.46^{b}	5.93^{c}	5.63^{d}	7.85^{b}	7.55^{b}	9.66^{c}	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.

PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

Table 5.5: Total cumulative emissions of nitrous oxide, carbon dioxide and methane observed after application of each blend to soil, and respective amount of CO_2 equivalents (CO_2eq), quantifying the global warming potential (GWP) of each blend (mean values, n=3 or 4). Results for each ratio in each column followed by different letters differ significantly for P<0.05 (Tukey test).

	Greenhouse Gases Emissions									**Potential N and P Leaching**				
	N_2O Emissions		**CO_2 Emissions**		**CH_4 emissions**		**GWP**			**mineral N leached**			**P leached**	
	mg N kg^{-1} soil	**% N_{Total} applied**	**mg C kg^{-1} soil**	**% C applied**	**mg C kg^{-1} soil**	**% C applied**	**mg CO_2eq kg^{-1} soil**	**% N_2O**	**% CH_4**	**mg N column^{-1}**	**%N_{av} applied**	**% N_{Total} applied**	**mg P column^{-1}**	**% P applied**
PoM+PiS	0.51^{a}	0.75^{a}	145.94^{b}	9.70^{b}	0.53^{a}	0.02^{a}	148.59^{a}	90.09^{a}	9.91^{c}	61.91^{b}	74.56^{b}	38.72^{b}	3.52^{b}	13.05^{b}
PoM+CaS	0.32^{b}	0.47^{b}	161.95^{a}	9.70^{a}	0.33^{b}	0.02^{a}	95.14^{b}	90.38^{a}	9.62^{c}	69.22^{ab}	85.00^{b}	43.17^{b}	2.75^{b}	10.16^{b}
10:10:10	0.12^{c}	0.33^{c}	24.74^{c}	n.a.	0.20^{c}	n.a.	36.59^{c}	84.56^{ab}	15.44^{b}	77.39^{a}	96.67^{a}	96.67^{a}	7.46^{a}	23.50^{a}
SOIL	0.07^{d}	n.a.	11.66^{d}	n.a.	0.21^{c}	n.a.	25.46^{d}	77.18^{b}	22.82^{a}	9.99^{c}	n.a.	n.a.	0.30^{c}	n.a.
PiS+PiS-LIQ	0.22^{b}	0.38^{b}	21.88^{b}	12.16^{a}	0.09^{c}	0.05^{a}	60.66^{b}	95.96^{a}	4.04^{d}	73.31^{a}	90.86^{a}	54.52^{b}	1.96^{b}	11.98^{b}
CaS+CaS-LIQ	0.38^{a}	0.71^{a}	60.03^{a}	16.67^{a}	0.35^{a}	0.01^{b}	119.89^{a}	91.80^{a}	8.20^{c}	58.61^{b}	69.86^{b}	41.92^{c}	1.30^{c}	7.51^{b}
13:06:18	0.10^{c}	0.29^{b}	19.78^{b}	n.a.	0.32^{a}	n.a.	35.57^{c}	75.06^{b}	24.94^{a}	76.61^{a}	95.57^{a}	95.57^{a}	3.09^{a}	19.80^{a}
SOIL	0.07^{d}	n.a.	11.66^{c}	n.a.	0.21^{b}	n.a.	25.46^{d}	77.18^{b}	22.82^{b}	9.99^{c}	n.a.	n.a.	0.30^{d}	n.a.
CaM+PiS	0.22^{b}	0.32^{a}	150.54^{b}	5.47^{a}	0.27^{b}	0.01^{a}	66.24^{b}	88.67^{a}	11.33^{c}	45.59^{c}	50.87^{c}	25.47^{b}	8.20^{b}	15.01^{b}
CaM+PoM	0.31^{a}	0.45^{a}	159.38^{a}	5.73^{a}	0.35^{a}	0.01^{a}	93.04^{a}	89.47^{a}	10.53^{c}	60.68^{b}	72.42^{b}	36.21^{b}	9.32^{b}	17.55^{a}
07:14:14	0.04^{c}	0.10^{b}	15.06^{c}	n.a.	0.22^{c}	n.a.	15.61^{d}	61.36^{c}	38.64^{a}	77.49^{a}	96.43^{a}	96.43^{a}	28.64^{a}	46.50^{a}
SOIL	0.07^{c}	n.a.	11.66^{c}	n.a.	0.21^{c}	n.a.	25.46^{c}	77.18^{b}	22.82^{b}	9.99^{d}	n.a.	n.a.	0.30^{c}	n.a.

PiS: pig slurry, PiS-LIQ: pig slurry’s liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry’s liquid fraction, CaM: cattle manure, PoM: Poultry manure. n.a.: not applicable.

The NH_4^+ concentration in the soil was always higher in 07:14:14 treatment than with the application of either CaM+PiS or CaM+PoM, but the NO_3^- in the soil was superior with the MBF than with the MF, only after day 29. Even though the MBFs led to lower initial NH_4^+ concentrations, ~ 5 mg NH_4^+-N kg^{-1} soil, then with the MF, the nitrification rate was superior with MBFs to that observed with the application of 07:14:14 (Table 5.3). The increase observed for the NO_3^- concentration in the soil was sufficient to obtain positive values in the NNM in CaM+PiS and CaM+PoM. This also had repercussions in the ANNM, which was only positive with the MBF, still with no discriminatory pattern between them, (Table 5.5). However, the N availability with these MBFs was inferior to the referred for the other MBFs in the other two ratios. The lower mineral N concentrations in soil with the MBFs (Figure 5.1i) in this ratio may be attributed to the presence of one or two solid manure in the blend, that are rich in straws and therefore increase the C/N ratio. Adding a material with a high C/N ratio, like manure, alters the C/N ratio of soil and since microbes consume both N and C, the higher C concentration imbalances the biota ratio, increasing the their mineral N uptake, hence making N unavailable to the crops, by temporarily N immobilization (Bhogal et al., 2016; Luo et al., 2021).

5.3.2 Greenhouse gases emissions

5.3.2.1 Nitrous oxide emissions

The sustainability of agriculture also depends on maintaining a clean and safe environment, which needs to act in response to the new energy and climate targets decreed by the EU, which aims to reduce 40% of GHG emissions compared to 1990 (Fangueiro et al., 2021). The use of manures, more specifically MBFs as a partial substitute for mineral fertilizers should promote the achievement of those targets (Chai et al., 2019). The application of organic materials, like manures, to the soil is known for emitting significant amounts of GHG (Fangueiro et al., 2018). Planning MBFs enriched in N may intensify the N_2O emissions and caution must be taken when suggesting such materials as fertilizers. The N_2O emissions occur in anaerobic conditions derived from denitrification but can also occur in aerobic incubations, due to the presence of anaerobic pocket in the soil (Anderson et al., 2021). This experiment was conducted with the soil at

70% WHC which allows both nitrification and denitrification processes, nonetheless the emissions were low.

The exponential increase in the N_2O emissions observed with the PoM+PiS and PoM+CaS (Figure 5.2a), corresponded to periods of higher nitrification rates (Table 5.3). Due to the absence of soil-crop interaction, NO_3^- is accumulated in the soil being susceptible to denitrification. The N_2O emissions observed with these two MBFs were seven and eight-times higher than those observed with the 10:10:10 and the control, respectively (Figure 5.2a). Consequently, the cumulative values of N_2O emitted were higher from the soil with PoM+PiS, 0.51 mg N kg^{-1} soil, followed by PoM+CaS, 0.32 mg N kg^{-1} soil, against 0.12 mg N kg^{-1} soil with the MF 10:10:10.

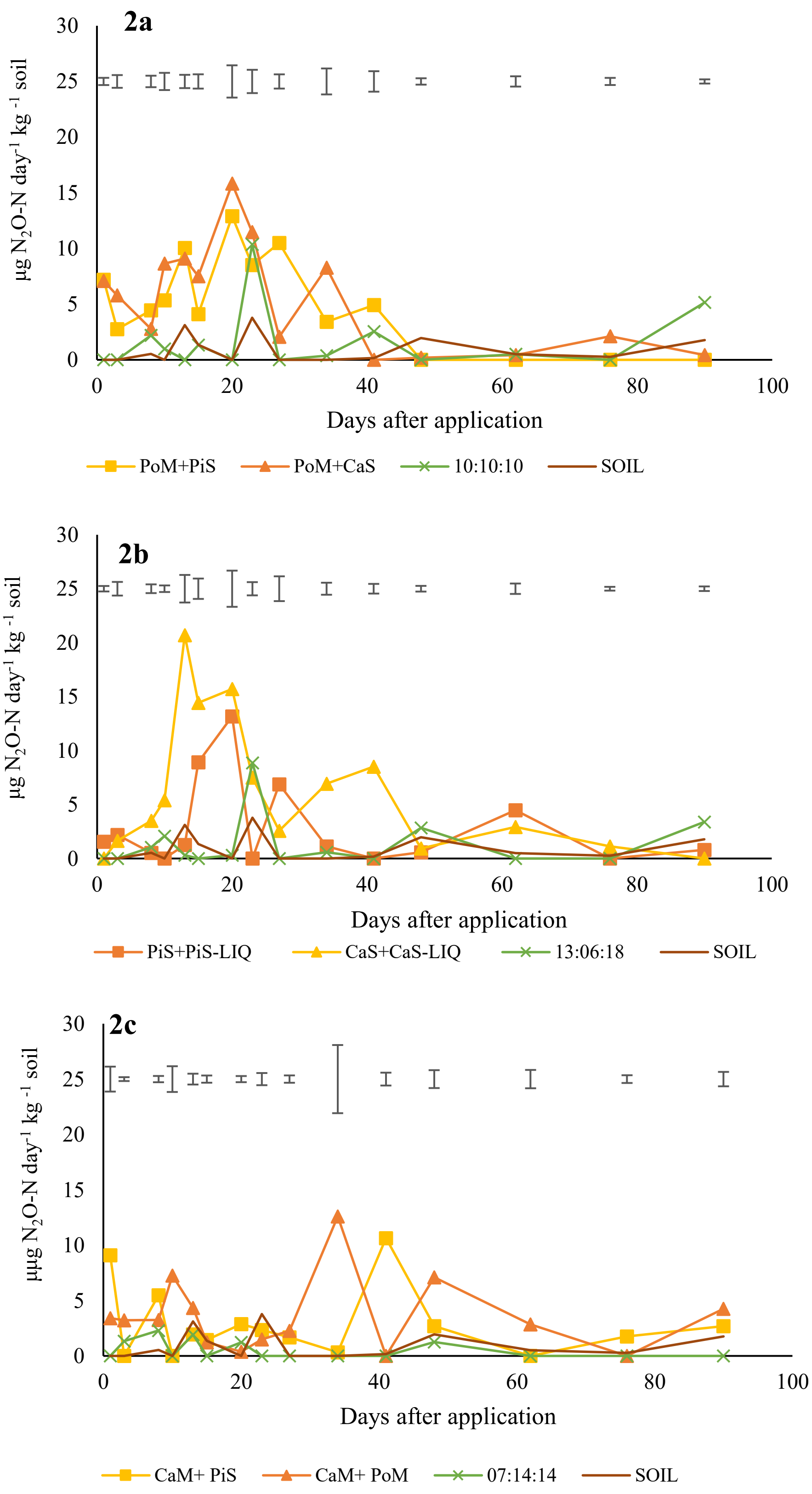


Figure 5.2: Nitrous oxide daily emissions rates observed after the application of each blend to the soil of each sampling date, during the 90 days of experiment for each 1:1 ratio (2a), 2:1 (2b) and 0.5:1 (2c). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date (n=3). PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

Similar to the observed with the application of MBFs with 1:1 N:P ratio, the cumulative increase in the N_2O emissions of CaS+CaS-LIQ was the result of a higher nitrification rate (Table 5.3) and in the absence of crop-soil interaction (Figure 5.2b). Still, the emissions of CaS+CaS-LIQ were eighth-time and the PiS+PiS-LIQ was three-time superior to the emissions observed with the application of 13:06:18. The use of PiS+PiS-LIQ emitted half the N_2O, 0.22 mg N kg^{-1} soil compared to CaS+CaS-LIQ, 0.38 mg N kg^{-1} soil, and released almost the double compared to the 13:06:18, 0.10 mg N kg^{-1} soil. The production of MBFs richer in N did not foment the N_2O emissions since the emissions observed were inferior to the ones observed in the previous ratio, 1:1 N:P. This may be attributed to the higher quantity of N_{Total} present in the 1:1 MBFs relative to the 2:1 MBFs (Table 5.2). The application of 1:1 N:P MBFs, led to the vehiculation of higher amounts of organic N, which is used in N mineralization and is also the substrate for N nitrification. As seen the ANNM with the 1:1 MBFs was higher than the with the 2:1 (Table 5.4). Hence, N was more prone to nitrification with the application of the 1:1 MBFs, which consequently increased denitrification.

The N_2O emissions observed with the application of CaM+PiS and CaM+PoM to the soil were higher than in the MF 7:14:14 (Figure 5.2c). The slow crescent increases in the quantity of N_2O emitted were coherent with the slow nitrification rate observed, (Table 5.3). The 07:14:14 was the MF with the lower amount of N_2O emitted, with values comparable to the control (Table 5.5). The daily and cumulative emissions of N_2O in the CaM+PiS and CaM+PoM were superior to the registered values of 07:14:14. However, when reporting the amount of N_2O emitted relative to the N_{Total} applied, CaM+PiS led to the lowest value (< 0.32%), among all MBFs analysed, that have values between 0.38 and 0.75%.

As reported in other studies, the use of manures relatively to MF increased the N_2O emissions, consequently several solutions can be utilized to mitigate the emissions (Wu et al., 2019). Considering that this study was based on a hypothetical scenario with a central solution, the addition of a nitrifying inhibitor can be considered when producing the MBF, which is a well-known mitigation solution (Wu et al., 2019). Slurry acidification is also a solution propose to mitigate N_2O emissions (Fangueiro et al., 2018). The nutrients recovery and the recycling of subproducts are essential to green agriculture and more sustainable practices (Mažeika et al., 2021; Souri et al., 2018). An alternative to the use of strong acids, commonly used in slurry acidification, could be to incorporate bioacidification into the process, also important to diminish the cost associated with manure-based fertilizers production (Prado et al., 2020).

5.3.2.2 Carbon dioxide and methane emissions

The CO_2 emissions are mainly due to soil respiration (Pardo et al., 2017), which is stimulated by adding a C source to the soil due to the increased organic matter degradation, increasing microbial respiration (Galic et al., 2020; Shakoor et al., 2021). Thereby the treatments with MFs, independently of the ratio analysed, presented the lowest CO_2 emissions, under the 25 mg C kg^{-1} soil. The application of the 2:1 ratio MBFs, which vehiculated the lower amount of C to the soil, culminated in lower daily CO_2 emissions compared to the other two ratios (supplementary material, Figure S5.5). Consequently, the cumulative value of CO_2 emitted were low with PiS+PiS-LIQ, ~22 mg C kg^{-1} soil, and reached ~60 mg C kg^{-1} soil with the application of CaS+CaS-LIQ. With the application of the other MBFs, the cumulated amount of CO_2 emitted was around the 150 mg C kg^{-1} soil, which corresponds to a maximum of ~10% of the total C applied emitted with PoM+PiS or PoM+CaS (Table 5.5). These represents significant CO_2 emissions but more than 80% of the C applied with MBFs remained in the soil and might contribute to the C sequestration, imperative to diminish the impact of agricultural activities and manure application (Galic et al., 2020). The C sequestration in the soil, mitigates the pollution effect of CO_2 emissions, which is favoured when the soil organic carbon increases (Triberti et al., 2016). In previous studies, a 34 years long-term experiment and another of 9 years, with the combined effect of manure and MF application to the soil, increased the soil organic carbon to values superior to the single application of MFs, which resulted in higher C sequestration, supporting with the present results (Qaswar et al., 2020; Triberti et al., 2016). The application of 1:1 and 2:1 MFs stimulated the emissions of soil's C and did not restore the soil C reserve, contrary to the MBFs. Hence, the long-term application of MBFs in basal fertilization will contribute as a mitigation solution to balance the CO_2 emissions of the agriculture sector.

This experiment was conducted in aerobic conditions, thereby, the CH_4 emissions were residual in all the ratios. Even when adding material rich in C, the emissions of CH_4 by the MBFs were always equal to or lower than 0.05% of the total C applied (Table 5.5). PoM is a material rich in both N and C (Table 5.1), which could enhance the emissions of CH_4 if anaerobic conditions are proportionate (Anderson et al., 2021). On the other hand, the use of solid manure as a base of a MBF will improve soil aeration and reduce its compaction, fomenting aerobic conditions and lowering the potential for CH_4 emissions (Domingo-Olivé et al., 2016; Risberg et al., 2017).

5.3.2.3 Global warming potential

The higher increases in N_2O emissions after the application of the possible MBF resulted in higher GWP independently of the ratio analysed (Table 5.5). Indeed, the emissions of N_2O were responsible for ~90% of CO_2eq observed in all the MBFs. If the use of manures improved the soil aeration, CH_4 is less emitted as well as N_2O (Silva et al., 2022b).

Comparing the several options analysed, PoM+PiS (1:1) and CaS+CaS-LIQ (2:1) presented the higher GWP with 149 and 120 mg CO_2eq kg^{-1} soil, indicating that mitigations techniques much be considered to turn these MBFs into viable solutions. Counter wise, PiS+PiS-LIQ (2:1) and CaM+PiS (0.5:1) demonstrate that their N and P enrichment did not impair their GWP, demonstrating their interest as the solutions with a lower pollution impact. When comparing the values obtained in this studied, with others, with the application of both cattle slurry and cattle slurry liquid fractions in a sandy soil, the combination of the two material reached GWP lower than the reported by the authors (Fangueiro et al., 2015d). Still, awareness should be taken into account when using these materials, since compared to the MFs the potential for pollution was greater with all the solutions presented. The authors Regueiro et al. (2016), demonstrated the efficient use of slurry acidification to mitigate the GHG emissions on both pig slurry and on the liquid and solid fractions, which reduced the impact on GWP.

5.3.3 Leaching experiment

5.3.3.1 Potential N leaching

One of the major constraints of using manures is the potential risk of NO_3^- leaching after soil application, which regulates the amount of manure that can be applied to the soil (Fangueiro et al., 2021). For the first two leaching events, independently of the ratio analysed, the MFs presented higher concentrations of NO_3^--N in the leachate (Figure 5.3a, c and e). One of the major disadvantages of the MFs was the amount of NO_3^--N leached for the first 16 days, while the use of MBFs, independently of the ratio considered, led to residual NO_3^- leaching. As a result, the total amount of NO_3^--N leached with the MFs was between 90 and 97 mg N column^{-1} , above the total N applied (Table 5.5), indicating that MFs are more prone to NO_3^- leaching than MBFs. This effect had more emphasis in the 0.5:1 ratio, where the 07:14:14 treatment lost 80 % more mineral N than the 0.5:1 MBFs tested. Therefore, it can be concluded that the application of CaM+PoM and CaM+PiS (0.5:1 MBFs) have a diminished potential of N leaching but it can also be interpreted as a lower N availability. Hence, these two MBFs should be adequate for crops with lower N necessities, or their application could be combined with legumes, to increase N fixation due to rhizobium nodules (Lindström and Mousavi, 2020). On the other hand, these MBFS should be preferred to MFs for winter crop fertilization.

Contrary to the MFs, the concentration of NO_3^--N in the leachate from the MBFs treatments, was low, for the first 16 days, especially with the 2:1 MBF and PoM+PiS. Onwards, MBFs started to leach NO_3^-, due to the nitrification rates observed before (Table 5.3), this is in agreement with the application of pig slurry to the soil, which also presented lower NO_3^- leached during the first weeks (Gómez-Garrido et al., 2018). While the 0.5:1 and 2:1 MBFs had a slow NO_3^--N release, the use of PoM+PiS presented an earlier peak on day 16, ~17 mg NO_3^--N column^{-1}, and PoM+CaS peaked at day 23, ~14 mg NO_3^--N column^{-1} (Figure 5.3a). This had repercussions on the total mineral N leached, which after 44 days, was equal to the total amount of N leached from the MBFs in the 2:1. As expected, considering the total amount of mineral N leached, 1:1 and 2:1 MBFs were more prone to N losses than the 0.5:1 MBFs. From the MBFs considered, PoM+CaS (1:1 ratio) and PiS+PiS-LIQ (2:1 ratio) were the only solutions that presented a cumulative N leaching similar to the respective MFs. This predisposes their applications to situations where either the crop is high N demanding and their application is more adequate for spring crops. Nonetheless, it is to stress that the majority of the MBFs tested did not increase the potential risk of NO_3^- leaching relative to MF. This is in agreement with recent studies that reported that the use of manures can mitigate the leaching of NO_3^- compared to MFs (De Boer, 2017). Changes have been conducted to equal the NO_3^- regulation referring to the application of animal manure sub-products to the application of the MF (Huygens et al., 2020). This indicates that i) MBFs may not have a greater contribute to groundwater contamination and ii) N should have a higher availability to the crops.

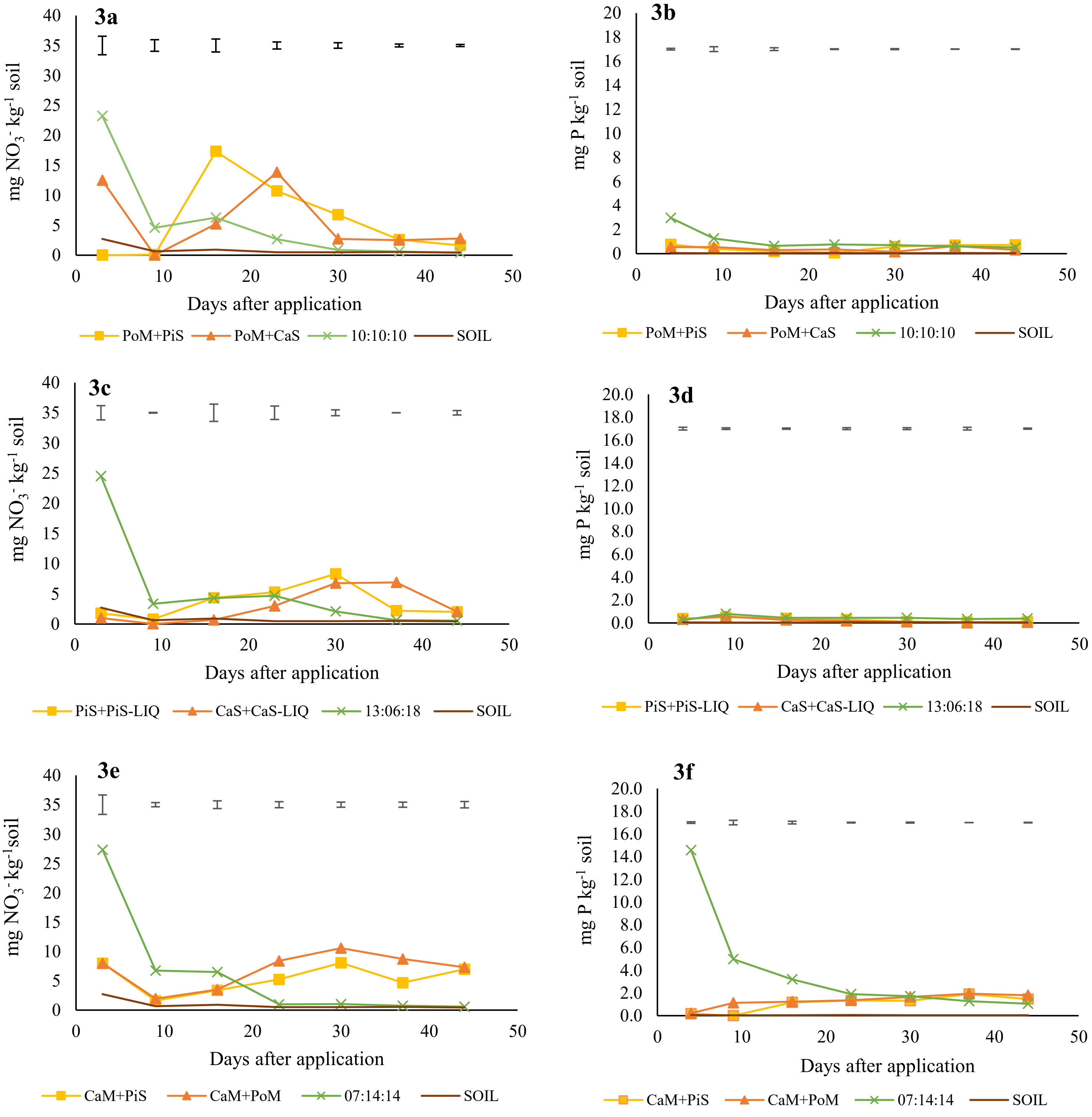


Figure 5.3: Nitrate (left) and phosphorus (right) potential leaching observed after the application of each blend to the soil during the 49 days of experiment in each 1:1 ratio (3a,3b), 2:1 (3c,3d) and 0.5:1 (3e, 3f). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date (n=3). PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

Portugal's legal limits to consider water as NO_3^- polluted is 50 mg l^{-1}, which was not fulfilled with the MBFs or MFs contribution to NO_3^- water concentration (APA, 2020). It is still to refer that the majority of MBFs lost between 71 and 91% of the N_{Total} in the 1:1 and 2:1 MBFs and 46-61% of the N_{Total} in the 0.5:1 MBFs, while the percentage of N_{Total} leached in MFs was above 100%. This indicates that MF stimulated N leaching. This indicates that soil's N was also stimulated to be lost through leaching. Indeed the application of MF to the soil is associated with NO_3^- leaching (Padilla et al., 2018). The lower percentage of N_{Total} lost in MBFs treatment is one of the benefits of applying these materials, in which a large part of N is in organic form, needing few weeks for mineralization and nitrification to occur, as observed above in the first section. This is an important feature of conceptualizing MBF, since the NO_3^- leached was low for the first two weeks, contrary to the observed with MFs. This initial period corresponds to the time when crops are starting to grow, and N assimilation is lower. Onwards, with the crop's growth, the N requirement increases, and so does the N supply by MBF, assuming that the amounts of N leached in our experiment should be used by plants in real conditions.

5.3.3.2 Potential P leaching

The potential P leaching was not significant when observing the 1:1 and the 2:1 ratios with <15% of the total P applied lost by leaching, (Figure 5.3b and d and Table 5.5). The total P leached, was almost double with both the MFs used, 10:10:10 and 13:06:18, compared to the blends in the respective ratios. One of the reasons that led farmers to discard manures as a fertilizer was the excessive P in soil due to the fertilization recommendations based on N content and the unbalanced N:P ratio in the manures (Svanbäck et al., 2019). Our results have proven that the application of MBFs from the ratios like 1:1 and 2:1 do not increase the potential of P leaching considering the equivalent mineral fertilizer and thereby the potential of P overapplication. The lower amount of P in the leachate was observed in the 2:1 ratio, indicating that the P in these MBFs will not be released that easily. Therefore, the use of PiS+PiS-LIQ or CaS+CaS-LIQ did not present significant risks of P leaching.

On the other hand, the use of the 0.5:1 MBFs appears as a solution to solve P deficiencies in soils poor in P. Nevertheless, the blend richer in P, should not foment P leaching and consequent risk of P pollution. Regarding the P leaching over the 44 days after fertilizers application, the MF 07:14:14 led to higher P losses than any of the MBFs tested (Figure 5.3f). Moreover, the P leached with 07:14:14 was ~3-times higher than the amount of P leached in the MBF in this ratio, with a loss of 47% of the P applied. However, the amount of P leached was higher with CaM+PiS and CaM+PoM, ~9 mg P column^{-1}, than with any of the other MBFs analysed (Table 5.5). McDowell et al. (2021), observed an increase of P leaching with CaM compared to the use of superphosphate, that they associate with the lower affinity of organic P to soil sorption compared to orthophosphate, a mineral form assimilated by the crops. The differences observed in our study can be the consequence of manures blending that decreased the P potential leaching of CaM, or the fact that P is a very immobile element that can be absorbed on iron or aluminium oxides in the soil (Wang et al., 2020). In short, the production of MBFs with enrichment in P did not foment the potential P relative to MFs application.

5.4 Conclusion

As referred, these results presented here aimed to overcome two problems, i) the surplus of manures and ii) the need to replace mineral fertilizers in agricultural and simultaneously increase soils health.

Some of the MBFs proposed in this study as potential MBF, demonstrated a promising opportunity to be more explored. In the 1:1 ratio, PoM+PiS even if presenting higher nitrification rates, did not increase the N leaching compared to the 10:10:10. Indeed, this MBF had the lower potential of N leaching in the ratio, indicating that N will be available without compromising the surrounding ecosystem. However, solutions should be adopted to mitigate the GWP value, specially the N_2O emissions. Also, PoM+CaS led to nitrification rates similar to the homologous MBF in the 1:1 ratio, and the potential of N leaching was equal to the MF. The application of PiS+PiS-LIQ, a N-enriched MBF, improved the NNM and nitrification rates to values that may equal this MBF to the MF 13:06:18, and the potential of N leached was similar to the MF 13:06:18. Also, among the two blends enriched in N, this was the one with the lower GWP value but was, still, higher than the MF option. Additionally, independently of the MF analysed, the N leaching potential was always superior with MF compared to the majority of the MBF. The MBFs analysed more prone to P leaching were the ones from 0.5:1 N:P ratio, due to their P-enrichment, the results demonstrated that the 07:14:14 had a higher P potential leaching than either CaM+PoM or CaM+PiS. Still, these possible MBFs presented a higher GWP, and N was mineralized at slower rate, hence some mitigation solutions should be considered. Obtaining such information, like N mineralization and nitrification rates or the potential of nutrients leaching, is crucial to increase farmers acceptance to use the MBFs.

The results indicate that it is possible to design MBFS for most of the N:P ratio usually used in mineral fertilizers. Nevertheless, some of these MBFs might led to an increase of some N or C losses, through GHG emissions. Hence in future studies the MBFs need to be designed to improve their features and minimize the risk of nutrients losses, resourcing to technologies that achieve this.

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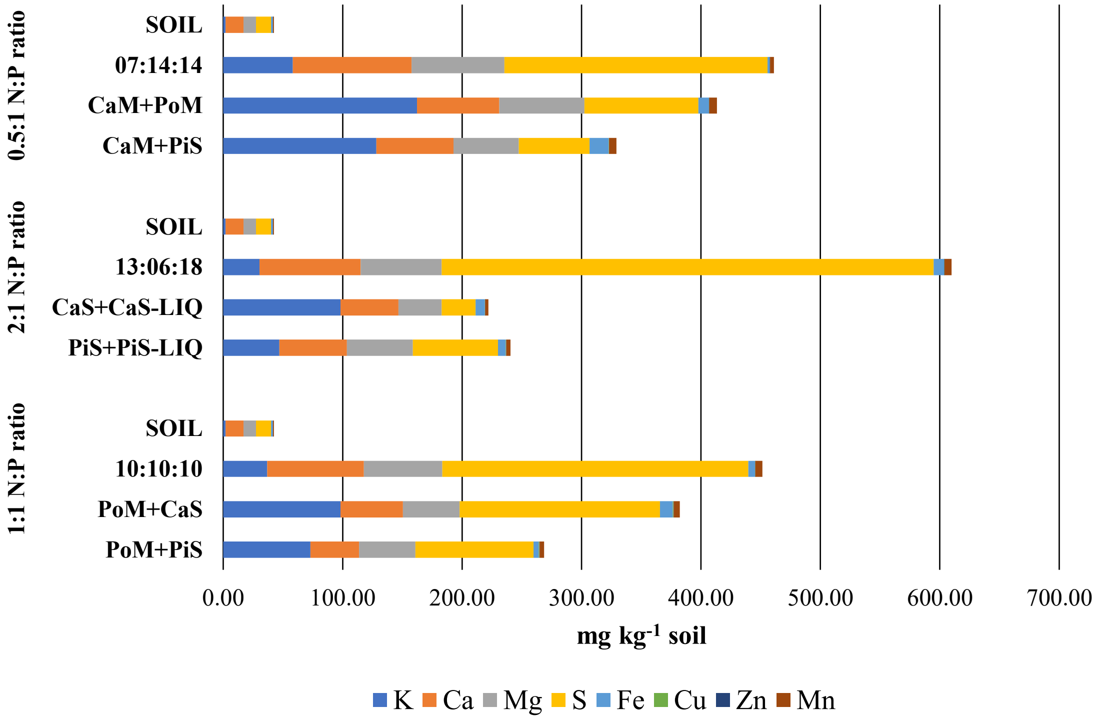

Figure S5.4: The cumulative concentration of potassium (K), calcium (Ca), magnesium (Mg), sulphur (S), iron (Fe), copper (Cu), zinc (Zn) and manganese (Mn) leached after the application of each blend to the soil during the 49 days of experiment in each N:P ratio, 1:1, 2:1 and 0.5:1. PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

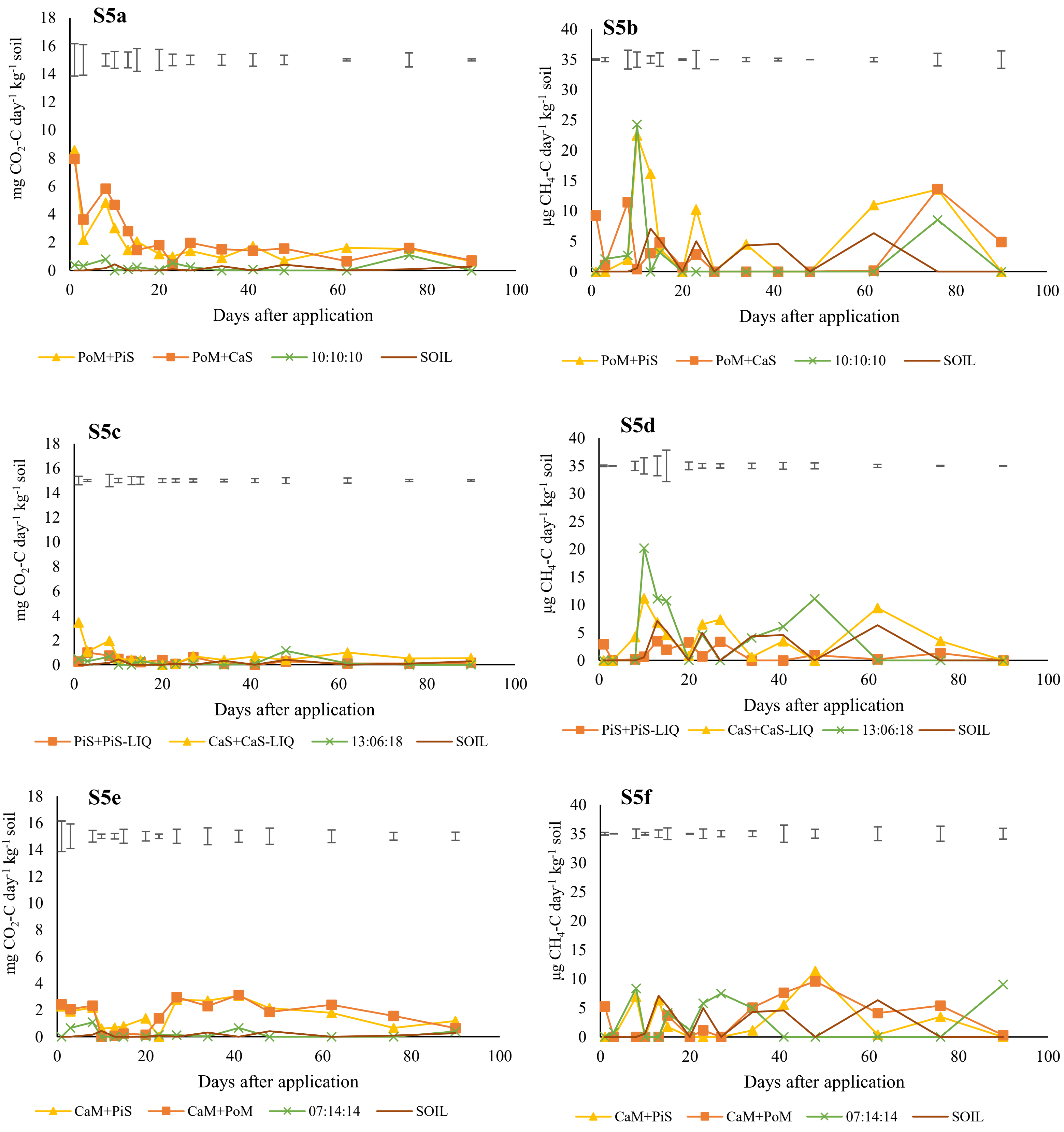

Figure S5.5: Carbon dioxide (left) and methane (right) emissions observed after the application of each blend to the soil during the 90 days of experiment in each 1:1 ratio (S1.a,S1.b), 2:1 (S1.c,S1.d) and 0.5:1 (S1.e, S1.f). Bars represent the standard error values used for comparison of the treatments in the Tukey test at each sampling date (n=3). PiS: pig slurry, PiS-LIQ: pig slurry's liquid fraction, CaS: cattle slurry, CaS-LIQ: cattle slurry's liquid fraction, CaM: cattle manure, PoM: Poultry manure.

Chapter VI Assessment of the agronomic value of manure-based fertilizers

Based on the published paper to Agronomy

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Abstract

Producing manure-based fertilizers (MBFs) with specific nutrients ratios is a solution to overpass the imbalance of nitrogen and phosphorus in manures, and to recycle manure's nutrients, promoting sustainable agricultural practices. Several MBFs with different tailored N:P ratios (0.5:1, 1:1 and 2:1) were considered. This study aimed to determine their agronomic value, in a pot experiment with oat, comparing their performance with conventional mineral fertilizers (MFs) in Haplic arenosol soils with different nutrients requirements. For the 0.5:1 ratio, the MBFs prepared were: cattle manure with pig slurry (CaM+PiS), cattle manure with poultry manure (CaM+PoM) and poultry manure with superphosphate (PoM+SP); for the 1:1 ratio: poultry manure with cattle slurry (PoM+CaS) and poultry manure with pig slurry (PoM+PiS) and for the 2:1 ratio: cattle slurry with the liquid fraction of cattle slurry (CaS+CaS-LIQ), pig slurry with the liquid fraction of pig slurry (PiS+PiS-LIQ) and poultry manure with urea (PoM+U). Oat fertilized with PoM+SP (0.5:1) and PoM+PiS (1:1) led to yields similar to those obtained with the use of MFs (6.3 and 7.2 mg DM, respectively). The referred MBFs, PoM+SP and PoM+PiS, as well as PiS+PiS-LIQ (2:1), were agronomically equivalent to the MFs. After their application the N uptakes were equivalent to those obtained with the MFs, ~16 g N kg^{-1} DM, 13.9 g N kg^{-1} DM and ~24 g N kg^{-1} DM respectively. Replacing MFs with MBFs in the basal fertilization of oat, demonstrated to be a solution to turn agriculture more sustainable, by recycling nutrients efficiently.

Keywords

Manure-based fertilizer; Tailor N:P ratio; N uptake; P uptake; Sustainable agriculture

6.1 Introduction

The growing demand for food worldwide led to the urge of improving crop production and, concomitantly, agriculture became more dependent on nutrients application in the form of mineral fertilizers (MFs) (Iqbal et al., 2019). Simultaneously, soil became more deficient in organic matter and nutrients.

Over the last decades, what once was a cohesive ecosystem, is now becoming two separate sectors, where agriculture and livestock grew separately, culminating in an open nutrient cycle, with imbalanced flows of nutrients between these two activities, which led to serious environmental and economic issues (Hills et al., 2021; Luo et al., 2021). One of the consequences is the surplus of manure production in some areas, which remains marginally applied to crops due to this separation between livestock production and agriculture (Spiegal et al., 2020). The application of manure to the soil is known to have several benefits, such as an increase in the soil's nutrient reserve, enhancing microbial activity, and improving the soils' chemical and physical properties (Hayashi et al., 2022). The high agronomic value of manure should encourage farmers to apply it to soil (Rayne and Aula, 2020), but its lower nutrients concentration and variability in terms of composition, relative to MFs, as well as, the lack of knowledge regarding the plant nutrients' availability in manures, culminates in a low acceptance by farmers on the use these materials (Fangueiro et al., 2018). Moreover, most manure fertilization recommendations are based on the crops' nitrogen (N) requirements, which caused, in many situations, an overapplication of phosphorus (P) to the soil, especially in Flanders and in the Netherlands, with serious environmental consequences concerning surface and groundwater contamination (Sigurnjak et al., 2019).

The concept of a manure-based fertilizer (MBF) emerges as a solution to solve several problems associated with manure application, namely, diminishing the differences between manure and mineral fertilizer, increasing the acceptance of manures by farmers, and enhancing the manure agronomic value, by increasing the availability of specific nutrients. Nonetheless, the use of MBFs will also reduce the necessity of applying MFs, which in some cases led to soil overexploitation (Keskinen et al., 2020; Pahalvi et al., 2022). The use of MBFs with a tailored N:P ratio, will address the main issues pointed out by farmers as limitations to the use of manures as fertilizers, since MBFs can contribute to: i) obtain a material with lower variability in terms of composition, ii) design

N:P ratios, close to values usually found in MFs and adapted to different soils' fertility classes, iii) increase the nutrients concentrations, and iv) supply both nutrients and organic matter to the soil, improving soil's health. Also, livestock farmers have been struggling to dispose manure, since the production overpasses the demand (Valentinuzzi et al., 2020). Hence, the production of MBF will solve the problems of manure surplus.

The importance of having a distinct N:P ratio in a fertilizer is to adjust nutrient concentrations, not only to crops necessities, but also to the soil fertility class (Oenema et al., 2021). Additionally, the application of MBFs would straighten the link between livestock and agriculture sector, since by recycling manure's nutrients to produce MBFs with similar concentration as the MFs, it will repropose nutrients from an available resource, reduce nutrient losses and will contribute to a circular economy by closing the nutrients cycle (Lesschen et al., 2020). For instance, up to 80% of the N from manure can be lost (Chojnacka et al., 2020a). Contrary to MFs, which need to be produced from non-renewable sources, like phosphate rocks (Powers et al., 2019), MBFs are produced from an organic material, which is available all year round, offering a sustainable management option for its management. The partial replacement of MFs with MBFs can be one first step to guarantee food security, since nutrients are recovered to the food chain, which implies they are dependent on using new land products (Mažeika et al., 2021). Concomitantly, the production of MBFs might allow the transformation of manure into a manure-derived material that accomplishes the RENURE criteria (N mineral / N total > 90%, or total organic carbon (TOC) / N total ≤ 3). If these criteria are achieved, the soil application of the MBFs in nitrate-vulnerable areas would not be so restrictive (170 kg N ha^{-1} $year^{-1}$), allowing N-application ratios similar to those when using MFs (Huygens et al., 2020). This will facilitate the farmers' acceptability of MBFs, since the application of MBFs or MFs needs to comply with the same legal requisites.

The aim of this study was to determine the agronomic efficiency of a set of MBFs with specific N:P ratios in their ability to replace MFs, for basal fertilization. These MBFs were obtained considering two hypothetical scenarios: i) "on-farm", where manures produced by a single animal species can be mixed with small amounts of mineral fertilizer to tailor their N:P ratios, and ii) a "central-solution" for manure processing, where manures, and derived products obtained by treatment (solid-liquid separation), can be mixed to optimize their N:P ratios. The two scenarios considered two perspectives, one where the farmers would produce the MBFs on their own, and the second, more similar to MFs, where the production of MBFs would be at a centralized plant, but still, using available manure resources. The second scenario appears as a solution to the surplus of all the manures, since the continuous production of these organic materials can become an environmental problem, causing their accumulation (Keskinen et al., 2020). In previous studies, Prado et al. (2022) indicated that N availability in soils fertilized with MBFs depends on the type of manure considered, as well as on the mixture produced. However, the referred results were obtained in a laboratorial incubation of soil with MBFs, with no plants. As the properties of the soil and the plant behavior affect the nutrients availability, it is important to evaluate the performance of MBFs in soils with different properties, also considering the soil-plant interactions. The application of these MBFs in the basal fertilization for oat (*Avena strigose* cv saia) was evaluated, assessing the nutrients' availability to the plant, by ascertaining the nutrients uptake, apparent recovery, and concentrations in the soil. Oat is an important cereal for human and livestock feeding (Wang et al., 2020b), and its fertilization with manure, or manure-derived fertilizers, would be very important as a strategy to close the nutrients cycle and increase sustainability in agriculture and livestock production.

The novelty of this study is that it offers a solution to the use of manures which have an imbalanced N:P ratio compared to the crops' nutrient demand and converets them to a material with a known N:P ratio, a feature currently attributed only to MFs. Recently investigations focusing on MBFs have gained more interest as in some cases MBFs were obtained with new advanced technological solutions (Chojnacka et al., 2020). The solutions tested in this study intended to increase the manure potential as MBF, while trying to make the most of its intrinsic characteristics.

6.2 Materials and Methods

6.2.1 Manure

The manures utilized in the present study were pig slurry (PiS), cattle slurry (CaS), cattle manure (CaM) and poultry manure (PoM), collected at the same farms, as the ones used in previous studies conducted by Prado et al. (2022). These manures were selected according to the Portuguese reality, as a result of their surplus in the livestock sector, which indicates their availability all year. The CaS and CaM were collected at an intensive dairy, in Benavente. PiS was collected at a fattening pig farm in Montijo. The CaS was richer in straw bedding, which increased its dry matter content. PoM was collected at a poultry meat farm located at Alvalade-Sado. PoM had a high content of rice hull bedding, which may have diluted some of the nutrients and increased its richness in dry matter content. The slurries were treated by solid-liquid separation, to use the resulting liquid fraction (LIQ), as described by Prado et al. (2022). All animal manures and subproducts were analyzed for their pH, dry matter (DM), total organic carbon, total N (N_{Total}) and ammonium N (NH_4^+), total P (P_{Total}, expressed as P_2O_5), total K (K_{Total}, expressed as K_2O) concentrations, following the methodologies described in Rodrigues et al. (2021). The manures composition can be consulted in Table 6.1.

Table 6.1: Physicochemical characteristics of the raw manures considered in the study (mean value ± standard deviation, n=3). The composition of each manure-based fertilizer was calculated to provide the desired N:P ratios, and the nutrients' concentrations and ratios were calculated considering the values of the raw manure and derived fractions. All concentrations were expressed on a fresh matter basis.

		DM	**TOC**	**N_{Total}**	**NH_4^+-N**	**N_{av}**	**P_{Total}**	**N:P**	**K_{Total}**
		g kg^{-1}	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g kg^{-1}**	**g P_2O_5 kg^{-1}**		**g K_2O kg^{-1}**
Raw Manures	**PiS**	32.4 ± 0.61	11.7 ± 0.35	3.32 ± 0.05	2.47 ± 0.01	1.99 ± 0.05	0.99 ± 0.02	2.0	2.14 ± 0.08
	PiS-LIQ	16.4 ± 0.39	5.2 ± 0.21	2.60 ± 0.05	2.26 ± 0.03	1.56 ± 0.04	0.09 ± 0.01	17.5	1.86 ± 0.01
	CaS	160.9 ± 3.29	48.8 ± 1.86	3.87 ± 0.05	1.70 ± 0.01	2.32 ± 0.03	0.75 ± 0.02	3.1	7.16 ± 0.22
	CaS-LIQ	48.2 ± 1.92	20.8 ± 2.50	3.96 ± 0.01	1.80 ± 0.02	2.38 ± 0.03	0.32 ± 0.01	7.4	0.87 ± 0.10
	CaM	197.4 ± 0.09	88.8 ± 0.10	5.85 ± 0.16	2.75 ± 0.04	2.93 ± 0.05	4.14 ± 0.12	0.7	11.03 ± 1.12
	PoM	742.5 ± 3.29	356.7 ± 5.95	23.34 ± 0.50	3.67 ± 0.13	11.67 ± 0.02	9.96 ± 0.46	1.2	16.50 ± 0.93
0.5:1 Ratio	**CaM+PiS**	187.8	83.8	5.8	2.7	2.9	4.1	0.7	10.9
	CaM+PoM	204.4	91.9	6.3	2.8	3.1	4.3	0.7	11.2
	PoM+SP	724.4	348.0	22.8	3.6	11.4	22.8	0.5	16.1
	07:14:14	-	-	70.0	50.0	70.0	140.0	0.5	140.0
1:1 Ratio	**PoM+PiS**	419.7	199.9	14.2	3.1	7.3	5.9	1.2	10.0
	PoM+CaS	517.3	238.3	15.9	2.7	8.1	6.5	1.2	11.3
	10:10:10	-	-	100.0	70.0	100.0	100.0	1.0	100.0
2:1 Ratio	**PiS-LIQ+PiS**	29.8	11.3	3.2	2.4	1.9	0.8	2.3	2.1
	CaS-LIQ+CaS	144.1	45.5	3.9	1.1	2.3	0.9	2.5	2.7
	PoM+U	728.0	349.7	31.9	12.6	20.5	9.8	2.1	16.2
	13:06:18	-	-	130.0	105.0	130.0	60.0	2.2	180.0

PiS: pig slurry; PiS-LIQ: liquid fraction from pig slurry; CaS: cattle slurry; CaS-LIQ: liquid fraction from cattle slurry; CaM: cattle manure; PoM: poultry manure; PoM+U: poultry manure with urea; PoM+SP: poultry manure with superphosphate; +: mixed with; DM: dry matter; TOC: total organic carbon; N_{Total}: total nitrogen; NH_4^+-N: ammonium nitrogen; N_{av}: available nitrogen (calculated as according to MADRP, 2018); P_{Total}: total phosphorus, expressed as P_2O_5; N:P: shorten for N_{av}:P_2O_5; K_{Total}: total potassium, expressed as K_2O.

6.2.2 Manure-based fertilizer preparation

The target ratios for the manure-based fertilizer used were 0.5:1, 1:1 and 2:1, considering the available N (N_{av}) and content of P was expressed in P_2O_5, for the ratios in order to follow the same criteria used for mineral fertilizers. To facilitate the reading the N_{av}:P_2O_5 ratio will be referred to as N:P ratio. N_{av} was calculated according to the

Portuguese legislation, where the amount of N_{av} is equal to 60% of the total N content of the manure (N_{Total}), for animal manures with DM content < 20%, and 50% of the N_{Total}, for animal manures with DM > 20% (MADRP, 2018).

The MBFs used were designed according to two scenarios previously referred to: i) on-farm, with only one type of manure, enriched with a small amount of mineral fertilizer to obtain the desired N:P ratios and ii) central-solution, where the processing of different kinds of manures will be done, and, in the case of slurries, the solid-liquid separation will be considered. In the case of the 0.5:1 ratio, the blends utilized were i) cattle manure with pig slurry (CaM+PiS), ii) cattle manure with poultry manure (CaM+PoM) and iii) poultry manure with superphosphate (PoM+SP). For the 1:1 ratio, the following mixtures were considered i) poultry manure with cattle slurry (PoM+CaS) and poultry manure with pig slurry (PoM+PiS). In the 2:1 ratio, it was considered i) cattle slurry with the liquid fraction of cattle slurry (CaS+CaS-LIQ), ii) pig slurry with the liquid fraction of pig slurry (PiS+PiS-LIQ) and iii) poultry manure with urea (PoM+U). The main characteristics of the blends are presented in Table 6.1.

6.2.3 Pot experiment

The crop utilized in the present study was oat (*Avena strigose* cv saia). To remove bacterial pathogens and some viruses, a preventive treatment with bleach was performed on the seeds (Rodríguez-García et al., 2020).

Three sandy soils, classified as Haplic Arenosol, with very distinct extractable P content, which corresponded to three different fertility classes, were used: poor (soil 1), fertilized with 0.5:1 MBFs and MF; medium (soil 2), fertilized with 1:1 MBFs and MF; and rich (soil 3), fertilized with 2:1 MBFs and MF. The three soils were fully characterized (Table 6.2). The fertilization was planned according to the crop needs for basal fertilization and the soil type (Table 6.2). The basal fertilization was based firstly on the plant requirement regarding N, and it was checked if P was not being over-applied. The Portuguese recommendation for oat fertilization is to add 1/3 to 1/2 of the N needs via basal fertilization (Veloso et al., 2022). In this study, the decision was to add 1/2 of the N needs, to maximize the N input. Apart from the MBFs, three commercial MFs were applied to the soil, selected in accordance with the soil, N:P ratios and crop's needs: 07:14:14 (0.5:1 N:P ratio) for soil 1, 10:10:10 (1:1 N:P ratio) for soil 2 and 13:06:18 (2:1

N:P ratio) for soil 3. The MF 10:10:10 had 10% of N (2.5% NO_3^- and 7.5% NH_4^+), 10% of P and 10% of K, the 13:06:18 presented 13% of N (2.5% NO_3^- and 10.5% NH_4^+), 6% of P and 18% of K (13:06:18) and the 07:14:14 presented 7% of N (2% NO_3^- and 5% NH_4^+), 14% of P and 14% of K (07:14:14). Each soil type, without mineral or organic fertilizer, was considered as control. The quantity of N, P and K applied per pot with each MBFs or MFs was calculated (Table 6.3).

Table 6.2: Soil initial characteristics and the respective basal fertilization recommendation.

		Soil 1	**Soil 2**	**Soil 3**
Soil characteristics	**NO_3^--N (mg kg^{-1} soil)**	1.9	2.8	10.6
	Extractable P_2O_5 (mg kg^{-1} soil)*	22.5	62.1	418.1
	Extractable K_2O (mg kg^{-1} soil)*	14.7	76.0	627.9
Basal fertilization recommendation	**Nitrogen available applied (mg pot^{-1})**	117.0	117.0	117.0
	Phosphorus applied (mg P_2O_5 pot^{-1})	200.0	100.0	50.0
	Potassium applied (mg K_2O pot^{-1})	133.0	83.0	50.0

*Extracted with Egner-Rhiem method.

Table 6.3: Fertilizer application rate in each treatment (g pot^{-1}) and the correspondent nutrients applied (g pot^{-1}).

Soil		**Quantity of fertilizer**	**Amount of nutrient applied (mg pot^{-1})**			
N:P ratio	**Fertilizer**	**(g pot^{-1})**	**N_{av}**	**N_{Total}**	**P_2O_5**	**K_2O**
	Control	no fertilizer	-	-	-	-
Soil 1	**CaM+PiS**	40.1	117.0	232.8	163.6	435.6
0.5:1 ratio	**CaM+PoM**	37.2	117.0	233.3	159.1	414.9
	PoM+SP	10.3	117.0	233.3	233.9	165.0
	07:14:14	1.7	117.0	117.0	233.0	234.0
	Control	no fertilizer	-	-	-	-
Soil 2	**PoM+PiS**	16.0	117.0	228.5	94.2	163.2
1:1 ratio	**PoM+CaS**	14.5	117.0	229.0	94.4	160.0
	10:10:10	1.17	117.0	117.0	117.0	177.0
	Control	no fertilizer	-	-	-	-
Soil 3	**PiS+PiS-LIQ**	60.8	117.0	194.4	51.0	127.0
2:1 ratio	**CaS+CaS-LIQ**	50.1	117.0	194.4	47.1	136.1
	PoM+U	5.7	117.0	181.9	55.7	92.3
	13:06:18	0.9	117.0	117.0	53.8	162.0

PiS: pig slurry; PiS-LIQ: liquid fraction from pig slurry; CaS: cattle slurry; CaS-LIQ: liquid fraction from cattle slurry; CaM: cattle manure; PoM: poultry manure; PoM+U: poultry manure with urea; PoM+SP: poultry manure with superphosphate; +: mixed with.

The experiment was conducted between January and March 2022. The pots used in this study were circular, 21 cm heigh, and had a surface area of 115.5 cm^2 with a volume of 5 L. Four repetitions per treatment were considered, with a total of 56 pots each filled with 5 kg of soil. Pots were randomly distributed on a growing bench and moved every week to ensure that light exposure was the same for all pots throughout the whole experiment. The soil moisture was kept at 70% of their maximum water holding capacity during the whole experiment. MBFs and MFs in each treatment were incorporated into the soil four days before sowing. This short period between application and sowing is a common practice in winter crops in Portugal and aims to minimize nutrient losses due to heavy rainfall in winter, typical of the Mediterranean climate. Fifty seeds were sown per pot, which were reduced to a total of 25 plants per pot after germination.

6.2.4 Plant analyses, Yield, and Nutrient Uptake Calculations

After two months of growth, the aboveground part of the plants was cut, and the fresh weight, the dry weight (DM) (after three days in an oven at 60 ºC), and the nutrient content were evaluated. Total N content was measured by the Dumas method using close to 80 mg of dry plant material (FAO, 2021) in a NDA 702 DUMAS Nitrogen Analyzer (VELP Scientific, Usmate Velate, Italy). The other elements were analyzed by inductively coupled plasma optical emission spectrometry (iCAP 7000 Series ICP Spectrometer, Thermo Fisher Scientific, Waltham, MA USA) after a wet digestion of close to 0.2 g of dry plant material with aqua regia (nitric acid:hydrochloric acid ratio 3:1 v/v) at reflux conditions, maintained for 2 h in a block digestion system (Digipress MS, SCP Science, Canada).The N, P and K uptake was calculated in accordance with Federolf et al. (2017):

$$\text{Uptake} = \text{Dry Matter (kg)} * \text{Nutrient content (g kg}^{-1}\text{ DM)}$$

The apparent N recovery was calculated and expressed as a percentage of the N_{av} and N_{Total} applied to the soil by the fertilizer, following Shah et al. (2016).

$$\text{Apparent N recovery (\%)} = (\text{N Uptake}_{\text{treatment}} - \text{N Uptake}_{\text{control}} * 100)/ \text{ N applied}$$

The yield increase, relative to the control ($Yield_{increase}$), obtained for each soil was calculated following Cai et al. (2019):

$$\text{Yield}_{\text{increase}}\text{ (g)} = \text{Yield}_{\text{treatment}}\text{ (g)} - \text{Yield}_{\text{control}}\text{ (g)}$$

6.2.5 Soil Analyses

At the end of the experiment, a sample of each pot soil was collected. The pH and electric conductivity (EC) were measured in a water suspension (1:2 w/v, soil:water ratio), after 1hour of agitation (pH meter model: Orion 3 Star and EC meter model: Orion star A212, Thermo Fisher Scientific, USA). The NO_3^--N was determined after one-hour extraction with KCl 2M (1:5 w/v, soil:solution ratio). The supernatant analysis provided information on the NO_3^--N concentration in the soil, after reading in the segmented flow autoanalyzer (San Plus System, Skalar, Nederland) with a modified Berthelot method (Krorn, 1956). The extractable P and K concentrations were assessed by the Egner-Rhiem method (Egnér et al., 1960).

6.2.6 Statistics

The statistical analysis was performed using one-way ANOVA for each N:P ratio and soil type. Whenever significant differences were found, a Tukey test was performed at $p<0.05$ using the Statistix 7 software, to further illustrate differences among the means.

6.3 Results

6.3.1 Plant yields

The yields obtained in soil 3 were always higher than those obtained in soil 1 or soil 2 (Figure 6.1). This was even verified for the Control, because soil 3 had a high level of fertility, as a result of its nutrients content (Table 6.2). Hence, to accurately compare the different treatments, the yield increase was calculated. Comparing those results, it is possible to observe that the fertilizer application had a higher impact on yields in: soil 1 > soil 2 > soil 3, i.e., the fertilization was more important in the “poorer” soil.

Assessing the treatments in each soil type, the major differences in total yield were observed between fertilized pots and the control without any fertilizer (Figure 6.1). In soil 1 (fertilized with the 0.5:1 N:P ratio MBFs and MF), the application of PoM+SP produced similar yields as the mineral fertilizer 07:14:14, ~6.6 g DM. The lower yield, compared to PoM+SP and 07:14:14, was observed with the application of CaM+PiS and CaM+PoM, which presented a $\text{Yield}_{\text{increase}}$ of ~3.1 g.

On soil 2 (fertilized with 1:1 N:P ratio MBFs and MF), the application of PoM+PiS led to a total yield without significant differences from the one obtained with the MF 10:10:10 application. The PoM+CaS treatment led to a lower yield, compared to the MF, even if not statistically different from PoM+PiS. This had consequences on the relative yield increase observed, which was significantly lower for PoM+CaS than for the mineral fertilizer 10:10:10, 2.88 and 5.53, respectively.

On soil 3, the impact of the application of the fertilizers, both organic and mineral, on yield, was lower than in soil 1 and soil 2, when compared with the results obtained for the control, because soil 3 was richer in nutrients. Nonetheless, PiS+PiS-LIQ led to higher relative yield increases, ~3 g, which was lower in the case of CaS+CaS-LIQ or PoM+U application, with ~1 g yield increase.

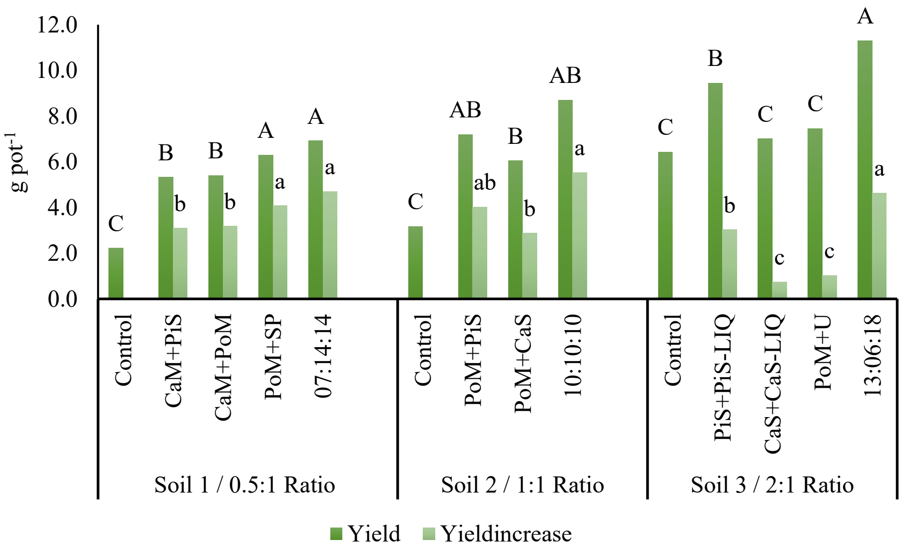

Figure 6.1: The average total yield production (DM) and yield increase ($\text{Yield}_{\text{increase}}$) observed. Results for each ratio in each column followed by different letters differ significantly for $P<0.05$ (Tukey test). PiS: pig slurry; PiS-LIQ: liquid fraction from pig slurry; CaS: cattle slurry; CaS-LIQ: liquid fraction from cattle slurry; CaM: cattle manure; PoM: poultry manure; PoM+U: poultry manure with urea; PoM+SP: poultry manure with superphosphate; +: mixed with.

6.3.2 Nutrients uptake

6.3.2.1 Nitrogen

Plants grown on Soil 1 (0.5:1 ratio) fertilized with the MBFs presented N concentration significantly similar to that found in the MF 07:14:14 (~15 g N kg^{-1} DM; Table 6.4). The combination of PoM with SP resulted in a N uptake equal to the one obtained after the application of the 07:14:14 MF. On the contrary, the absence of a mineral source in the MBFs, resulted in significantly lower N uptake (Table 6.4). Additionally, the apparent N recovery in PoM+SP was identical to the 07:14:14 MF, i.e. close to 70% of the Nav (Figure 6.2). Nonetheless, oat plants fertilized with MBFs with the 0.5:1 ratio presented the lower N$_{Total}$ apparent recovery (between 20-36%) compared to the 07:14:14 MF.

Table 6.4: Nitrogen and phosphorus, concentrations in the plant material as well as nutrient uptake calculated based on plant yield and nutrients concentrations in the plant. Results for each ratio in each column followed by different letters differ significantly for $p<0.05$ (Tukey test).

Soil N:P ratio	Treatments	N g kg^{-1} DM	N uptake mg N pot^{-1}	P g kg^{-1} DM	P uptake mg P pot^{-1}	K g kg^{-1} DM	K uptake mg K pot^{-1}
Soil 1 0.5:1 ratio	Control	12.56^{c}	27.95^{d}	2.46^{b}	5.41^{d}	11.32^{d}	24.74^{c}
	CaM+PiS	16.65^{a}	88.74^{b}	2.48^{b}	13.12^{c}	23.94^{b}	127.20^{ab}
	CaM+PoM	14.09^{bc}	76.08^{c}	2.36^{b}	13.43^{c}	25.40^{a}	132.61^{a}
	PoM+SP	17.04^{a}	112.31^{a}	4.39^{a}	29.32^{b}	18.66^{c}	117.90^{b}
	MF 07:14:14	15.63^{ab}	106.66^{a}	4.98^{a}	34.51^{a}	17.77^{c}	123.00^{ab}
Soil 2 1:1 Ratio	Control	12.36^{a}	33.63^{c}	3.70^{a}	10.62^{c}	12.84^{b}	40.64^{c}
	PoM+PiS	14.41^{a}	96.29^{ab}	3.88^{a}	28.67^{a}	18.65^{a}	133.86^{a}
	PoM+CaS	15.04^{a}	91.03^{b}	4.31^{a}	24.66^{b}	19.39^{a}	116.42^{ab}
	MF 10:10:10	13.38^{a}	116.58^{a}	3.61^{a}	28.91^{a}	12.87^{b}	111.53^{b}
Soil 3 2:1 Ratio	Control	15.26^{d}	93.59^{d}	8.64^{b}	53.30^{c}	25.56^{c}	152.18^{d}
	PiS+PiS-LIQ	24.37^{a}	229.83^{a}	8.24^{b}	81.66^{a}	34.03^{b}	327.65^{a}
	CaS+CaS-LIQ	21.73^{ab}	145.60^{c}	9.90^{a}	68.35^{b}	38.24^{a}	262.57^{c}
	PoM+U	18.92^{bc}	145.18^{c}	9.13^{ab}	66.20^{b}	31.01^{b}	240.91^{c}
	MF 13:06:18	16.67^{cd}	185.74^{b}	7.07^{c}	79.85^{a}	26.25^{c}	306.47^{b}

PiS: pig slurry; PiS-LIQ: liquid fraction from pig slurry; CaS: cattle slurry; CaS-LIQ: liquid fraction from cattle slurry; CaM: cattle manure; PoM: poultry manure; PoM+U: poultry manure with urea; PoM+SP: poultry manure with superphosphate; +: mixed with; n.a.: not applicable.

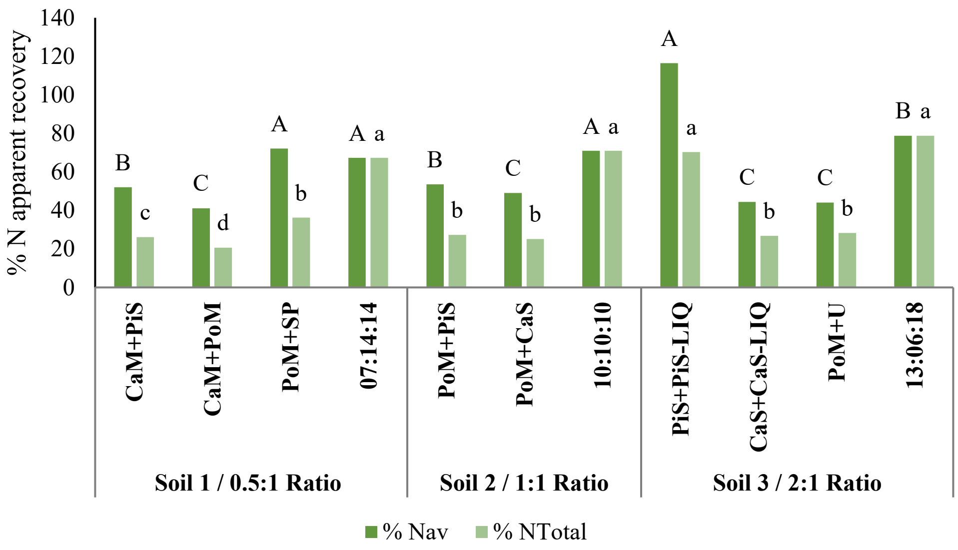

Figure 6.2: The apparent nitrogen recovery for oat fertilized with the different MBFs and MFs, expressed as a percentage of available N (Nav) and total N (NTotal) applied. Results for each ratio in each column followed by different letters differ significantly for $P<0.05$ (Tukey test). PiS: pig slurry; PiS-LIQ: liquid fraction from pig slurry; CaS: cattle slurry; CaS-LIQ: liquid fraction from cattle slurry; CaM: cattle manure; PoM: poultry manure; PoM+U: poultry manure with urea; PoM+SP: poultry manure with superphosphate; +: mixed with.

On soil 2 (1:1 N:P ratio), N concentration in the plants from all treatments (including the control) was similar. However, significant differences were observed regarding the N uptake (Table 6.4). As occurred with the yields, the N uptake was not significantly different between PoM+PiS and PoM+CaS, but only PoM+PiS led to a N uptake similar to the higher uptake observed for MF 10:10:10. Despite the available N applied in all the treatments being equal (Table 6.3), the plants fertilized with the 10:10:10 MF had an apparent recovery close to 70% of the N_{av} applied, while in the plants fertilized with PoM+PiS or PoM+CaS the values were only of, approximately, 50%. The apparent N recovery, as a percentage of the N_{Total}, was even lower following MBFs application to Soil 2 (i.e., only 30% was utilized), while oat plants fertilized with the MF 10:10:10 used 70% of the total N applied.

Considering soil 3 (2:1 N:P ratio), plants fertilized with PiS+PiS-LIQ and CaS+CaS-LIQ, presented higher N concentrations (~23 g N kg^{-1} DM) than the other two fertilized treatments. N uptake was considerably higher when PiS+PiS-LIQ was applied, ~230 mg N pot^{-1} relative to all other fertilizers. Nonetheless, the N uptake by plants fertilized with 13:06:18 was superior to the one observed with the two MBFs from the 2:1 ratio

(CaS+CaS-LIQ and PoM+U). Apparent N recovery from plants fertilized with PiS+PiS-LIQ corresponded to ~116% of the N_{av} applied and to ~70% of the N_{Total} applied, while those from mineral fertilizer 13:06:18 were able to achieve an apparent N recovery relative to N_{Total} of ~79%. Comparing the MBFs in the 2:1 ratio, the apparent N recovery, compared with N_{av} and N_{Total}, was 2.6 times higher with the application of PiS+PiS-LIQ (Figure 6.2).

6.3.2.2 Phosphorus

On soil 1, plants fertilized with CaM+PoM and CaM+PiS, which did not receive a P mineral source, had the lower P concentration, with values similar to the control plants, ~2.4 g P kg^{-1} DM. The addition of a mineral P source to PoM (PoM+SP), was efficient to stimulate a higher P concentration in the plants (Table 6.4), achieving a concentration similar to the plants fertilized with the MF 07:14:14. In fact, P uptake by plants fertilized with PoM+SP, ~29 mg P pot^{-1}, was more than the double compared to CaM+PoM and CaM+PiS, ~13 mg P pot^{-1}.

On soil 2, a similar P content in the plants was observed in all treatments, including the control (Table 6.4).

On soil 3 (2:1 ratio fertilizers), the plants from treatments CaS+CaS-LIQ and PoM+U had the higher P concentration, (~9.5 g P kg^{-1} DM) against ~8 g P kg^{-1} DM in the plants in control and in PiS+PiS-LIQ, and only 7 g P kg^{-1} DM in the MF 13:06:18 (Table 6.4). Yet, considering the P uptake, plants fertilized with MF 13:06:18 and PiS+PiS-LIQ were able to export a higher amount of the applied P (~80 mg P pot^{-1}) than in CaS+CaS-LIQ and PoM+U (~67 mg P pot^{-1}).

6.3.2.3 Potassium

The application of fertilizers with the 0.5:1 N:P ratio (soil 1) resulted in a higher K concentration in plants when CaM+PoM was applied, 25 mg K kg^{-1} DM (Table 6.4). As demonstrated in Table 3, the quantity of K applied was different between the treatments in this ratio. Still, in terms of K uptake, no statistical differences between the MBFs and the MF 07:14:14 were noted, not even for the PoM+SP, where the quantity of K applied to the soil was lower.

The K concentration in the plants in the 1:1 N:P ratio (soil 2) was higher when the MBFs were applied to the soil, ~19 g K kg^{-1} DM, against the concentration observed in the plants fertilized with the 10:10:10 pots and the control, ~13 g K kg^{-1} DM (Table 6.4). The K uptake was around the ~120 mg K pot^{-1} with the application of PoM+PiS or PoM+CaS. Regardless, there was a similar uptake in plants where PoM+CaS and MF 10:10:10 were applied.

The application of 2:1 N:P MBFs (soil 3), made it possible for plants to have a higher K concentration, ~33 g K kg^{-1} DM, relative to the 13:06:18 pots and the control (Table 6.4). An enhancement in the K uptake was observed with the application of PiS+PiS-LIQ, ~328 mg K pot^{-1}, while the lowest value for K uptake, when MBFs were used, was observed after PoM+U or CaS+CaS-LIQ application to the soil, ~250 mg K pot^{-1}.

6.3.3 Soil properties at the end of the experiment

Comparing the soil properties at the end of the experiment, no specific trends or clear benefits were observed (Table 6.5), which is evident by the fact that the soil C content did not increase because of the replacement of mineral by organic fertilizers. This indicates that a onetime application of MFs or MBFs to soil may have the same outcome and is not sufficient to observe the expected benefit of the use of organic fertilizers, which is only possible with a continued practice.

Nevertheless, it is to highlight that in soil 1, which had the lower EC, no significant increase in this value was observed after MBFs application to soil, and the contrary was true after MF application, meaning that the risk of soil secondary salinization when organic fertilizers, as MBFs, are used, is prevented.

Also, a positive effect was observed in soil pH after MBFs application to soil 1 and 2, relative to the control and to the MFs application, counterbalancing its acidity. This is important since MFs 07:14:14 and 10:10:10 induced a slight reduction in the soil pH, which was already acid in soil 1 and 2.

In soil 1, the soil P content at the end of the experiment was significantly higher in MF treatment than in CaM+PiS, CaM+PoM and the control, which indicates a higher risk of P leaching in the soils fertilized with MF. The opposite situation was observed regarding K, although the risk of non-point source pollution with K is lower than with P.

Table 6.5: Soil chemical properties at the end of the experiment (mean values, n=4). Results for each ratio in each column followed by different letters differ significantly for $P<0.05$ (Tukey test).

Soil N:P ratio	Treatments	pH	EC mS	NO_3^--N g kg^{-1} soil	Ext. P_2O_5 g kg^{-1} soil	Ext. K_2O g kg^{-1} soil	C %
	Soil 1	5.90^a	124.60^b	7.28^a	26.48^b	14.37^c	0.38^a
Soil 1	**CaM+PiS**	6.04^a	117.85^b	7.43^a	23.02^b	35.52^a	0.39^a
0.5:1 ratio	**CaM+PoM**	6.01^a	136.23^{ab}	7.28^a	24.97^b	30.93^{ab}	0.32^a
	PoM+SP	5.93^a	132.73^{ab}	7.26^a	28.05^{ab}	18.03^{bc}	0.36^a
	07:14:14	5.54^b	165.53^a	7.26^a	34.63^a	19.00^{bc}	0.34^a
	Soil 2	5.95^{ab}	130.90^a	7.43^a	66.92^a	24.77^a	0.43^a
Soil 2	**PoM+PiS**	6.22^a	136.67^a	7.30^a	63.09^a	31.14^a	0.39^a
1:1 ratio	**PoM+CaS**	6.10^{ab}	137.43^a	7.34^a	75.80^a	25.28^a	0.40^a
	10:10:10	5.86^b	137.13^a	7.38^a	75.55^a	23.60^a	0.38^a
	Soil 3	6.62^a	138.80^a	7.40^a	334.21^a	48.41^a	0.74^a
Soil 3	**PiS+PiS-LIQ**	6.74^a	126.27^a	7.24^a	405.54^a	39.97^a	0.73^a
2:1 ratio	**CaS+CaS-LIQ**	6.66^a	155.05^a	7.55^a	364.59^a	50.53^a	0.73^a
	PoM+U	6.63^a	138.34^a	7.24^a	401.73^a	47.40^a	0.75^a
	13:06:18	6.51^a	133.25^a	7.33^a	385.69^a	42.53^a	0.69^a

PiS: pig slurry; PiS-LIQ: liquid fraction from pig slurry; CaS: cattle slurry; CaS-LIQ: liquid fraction from cattle slurry; CaM: cattle manure; PoM: poultry manure; PoM+U: poultry manure with urea; PoM+SP: poultry manure with superphosphate; +: mixed with.

6.4 Discussion

6.4.1 Plant yield

The willingness of farmers to use MBFs depends on several factors, but the most relevant is the need to guarantee the same plant yields as when MFs are applied. This condition can only be met if the nutrients in the MBFs are delivered to the plants at the right time and in the right dose.

The soil application of PoM+SP (0.5:1 N:P ratio, soil 1) and PoM+PiS (1:1 N:P ratio, soil 2) led to plant yields similar to those obtained with the MFs 07:14:14 and 10:10:10, respectively. This suggests that it is possible to apply MBFs for basal fertilization of oat and obtain similar plants' yields as the correspondent MFs. However, only the plants fertilized with PoM+SP obtained a relative yield increase similar to that observed in the respective MF (07:14:14). It might be expected that N-enrichment of PoM with urea, would lead to similar yields as PoM+SP and the mineral option (13:06:18) (Iqbal et al., 2020). Still, the urea in this experiment did not stimulate oat production to a yield equal to that observed for 13:06:18. This can be attributed to the solid nature of PoM and the fast enzymatic hydrolysis of urea. Urea hydrolysis releases two ammonia molecules, which might result in a N loss due to the fact that PoM has an alkaline pH, stimulating ammonia volatilization. Therefore, urea may not be the most adequate N source to combine with PoM.

The highest yields were obtained in soil 3 (ratio N:P 2:1), when PiS+PiS-LIQ and 13:06:18 were applied, but, in this experiment, the control (soil 3) also led to higher total yields from all controls. Consequently, the values of relative yield increase obtained in soil 3 were the lowest among all MBFs tested. PiS+PiS-LIQ may have obtained the highest yield from the 2:1 N:P MBFs tested, still with lower values than the correspondent MF (13:06:18), This effect could be attributed to the fact that, even applying a higher amount of N_{Total}, a fraction of N in the MBFs is in organic molecules, which need to be mineralized to be available to the crop (Reuland et al., 2022).

This may have created a nutrient deficiency and, therefore, reduced crop yield. Hence, the application of MBFs in rich sandy soils may not be the best option, and several alternatives should be considered: i) perform the top-dressing fertilization at an earlier stage, ii) use the 2:1 MBFs in cultures less nutrient demanding, or iii) intercropping with legumes, to assimilate part of the N through rhizobium, improving crop healthy growth (Lindström and Mousavi, 2020).

6.4.2 N uptake and apparent recovery

Another important feature that MBFs should comply with, to be considered by farmers, is the N plant uptake, evidencing that the N provided was sufficient for the plants' needs. The N oat's uptake in treatments with MBFs was similar, or even higher, relative to the use of an equivalent mineral fertilizer. Hence, the results suggest that it is possible to replace MFs with MBFs. In the 0.5:1 N:P ratio (soil 1), the yields obtained with PoM+SP and MF 0.7:14:14 resulted in equivalent N concentrations in the aerial part of the plants fertilized with those materials. Indeed, a positive correlation between the production and the N uptake is usually observed (Prather et al., 2020). The combination of PoM with SP stimulated the N mineralization and, subsequently, the N_{av} apparent recovery, which achieved values similar to the mineral fertilizer (Figure 6.1). This was, also, supported by the similar N uptakes between PoM+SP and MF 07:14:14 (Table 6.4). The apparent stimulation of N mineralization by PoM+SP could be attributed to the increase in the microorganisms' activities. Indeed, microorganisms need both N and P, and adding a

MBF rich in nutrients to a poor P soil, may have stimulated their activity and increased the N mineralization, as reported by Heuck et al. (2015). Furthermore, the application of PoM+PiS (1:1 N:P ratio) to soil 2 led to a N uptake similar to the MF 10:10:10, and, consequently, the plants presented an equal N concentration. The apparent N recovery from plants fertilized with PoM+PiS was much lower than when ap-plying MF 10:10:10, especially when expressed in terms of N_{Total}. This indicates that organically bound N from MBFs requires longer periods to become plant available and that approximately 70% of NTotal in MBFs remained in the soil for the next crops. Nevertheless, the use of MBFs did not limit N use by plants, as N uptake values did not appear to be a limitation. Furthermore, since N will be mineralized and nitrified at a lower rate, the risk of nitrate leaching following MBFs application should be lower (Fangueiro et al., 2008).

Overall, PoM+PiS appears as one of the best options for the MBFs with the 1:1 N:P ratio and may support the replacement of MFs for N basal fertilization. For the 2:1 N:P ratio (soil 3), the application of PiS+PiS-LIQ induced a higher N uptake within this ratio, meaning that the N_{av} applied was more promptly assimilated by the plants (Figure 6.1). The percentage of N apparent recovery, considering N_{Total}, was ~78%, which indicates that 22% was in an organic form in the soil, available for a slow mineralization, while a major part of the N was released rapidly. In previous works, the incorporation of the pig slurry liquid fraction into soil induced an enhancement in the N use efficiency, compared to the incorporation of pig slurry (Fangueiro et al., 2017), which is in agreement with the present results. Therefore, enriching PiS in N with its liquid fraction appears as an optimal solution to overcome the imbalance N:P ratio of manure in specific situations where it is required more N. However, when combining two liquid materials, the quantity of material needed to supply the same amount of nutrients as a solid material is much higher.

One of the features of MBFs is that they may improve soil health and close the nutrients' cycles (Luo et al., 2021). The lower percentage of the N_{Total} applied recovered by the plants when the MBFs were applied, indicates a slower N mineralization. Yet, compared to the control, independently of the MBF applied, the N uptake was always higher in plants amended with them. Hence, the nutrients were better used by the plants fertilized with the MBFs compared to the MFs, which could indicate fewer N losses, and therefore a more efficient N cycle.

No differences were observed in the concentration of NO_3^--N when the soil was analyzed after the 60 days of the experiment. This aspect could be the result of N losses not considered in this study, for example, nitrous oxide emissions, which were not measured (De Rosa et al., 2018) (Table 6.5). The application of MBFs to soil has the benefit of vehiculating nutrients, not only in mineral forms but also in organic forms. This implies that part of the nutrients will become available only after mineralization (Ribeiro et al., 2010b). The duration of the experiment and the fact that the soil used had a sandy texture, may have hampered the observation of results, i.e., mineralization may have been delayed and can affect negatively yields and the crops' quality (de Bang et al., 2021). For instance, in another study, where the application of PiS was combined with MF, the yield increased with the combination of the two materials was only observed after two years of application, allowing a higher N uptake and N use efficiency, compared to the single use of mineral fertilizers (Yang et al., 2020). As referred earlier, the organic fraction contains a large part of nutrients that need to be mineralized. The fact that the plants fertilized with CaS+CaS-LIQ or PoM+U only presented an apparent N recovery of ~20% of the N_{total} applied, may indicate that these manure-based fertilizers will release N more slowly. These results indicate that to implement the application of CaS+CaS-LIQ or PoM+U, early top-dressing fertilization may be needed, to avoid nutrient deficiencies and maintain yields similar to those obtained with MF application.

6.4.3. Phosphorus recovery

One of the major disadvantages of manure application as fertilizer is the nutrient imbalance, relative to crops' requirements, which, in most cases, led to the overapplication of P (Sigurnjak et al., 2019). Designing MBFs with different P contents is important to overcome this problem, but it is crucial to bear in mind that P is a macronutrient, essential for crop metabolism and healthy growth (Wang et al., 2020a). To consider MBFs as substitutes for P mineral fertilizers in basal fertilization, it is imperative that: i) oat P concentration and P uptake are, at least, equal to the value obtained with the MFs; and ii) that P concentration in the soil should not enhance P leaching.

PoM+SP was the 0.5:1 N:P MBFs that led to P concentration in oat plants similar to the values observed with the MF 07:14:14 application. The amount of P vehiculated with PoM+SP was equal to the conveyed at 07:14:14 (Table 6.3). However, P uptake by oat plants after PoM+SP application was lower than that after applying MF. Therefore, P availability was lower with PoM+SP than with MF, but this did not affect P assimilation by oat. This indicates that the addition of P from a mineral source to the manure was not expected to cause P deficiencies for the plants.

The present results were coherent with Iqbal et al. (2019) stating that the application of MBFs tended to release nutrients more slowly. At the end of the experiment, the soil extractable P in the PoM+SP treatment was statistically similar to MF 7:14:14 (Table 6.5). This could indicate that if a second crop was sown on that soil, there would be P remaining to meet this P demand. In addition, the soils' extractable P concentration in the PoM+SP treatment was similar to the other two MBFs applications and to the control. The lower P availability can be attributed to the material present in solid manures, which is rich in fibers and lignin, and may hinder the mineralization of the organic compounds (Bhogal et al., 2016). Manure or MBFs contain P bound in organic forms, which require mineralization as mentioned before. Mineralization can be a prolonged process, which consequently can lead to P deficiencies after applying MBFs, especially in soils poor in P, like soil 1. Nevertheless, the combination of PoM with SP appeared to be sufficient to overcome this constraint. The application of MBFs like CaM+PiS or CaM+PoM, without the addition of a P mineral source, may have resulted in P deficiency for oat, even if these were the MBFs with higher P application (Table 6.3). Also, P availability depends on the presence of certain ions in the soil, such as iron (Fe) or aluminum (Al), that can bind P into Fe or Al oxides (Tiecher et al., 2020). Azevedo et al. (2018), stated that soil with lower clay content has a diminished P adsorption capacity, increasing its availability. Since a sandy soil was used in this experiment, the formation of ox-ides should not play a relevant role , and the P was not adsorbed as much, making it more available to the plant. MBFs like CaM+PiS and CaM+PoM were tailored for situations where there would be a necessity of applying high P contents or in soils with a P deficiency. Based on the results of our pot experiment it can be concluded that the application of CaM+PiS or CaM+PoM might be reasonable for winter crops, when the potential of P leaching especially on sandy soils is higher due to higher precipitation, diminishing the risk of P leaching (Tiecher et al., 2020). It is important to keep in mind that if P was not immediately available for oat, it may be available for the next crop in the rotation. Nevertheless, the application of such MBFs needs to be also assessed in soils with different properties.

The best options regarding P fertilization using MBFs (e.g. the one that released P more quickly) were PoM+PiS for the 1:1 N:P ratio (soil 2), and PiS+PiS-LIQ for the 2:1 N:P ratio (soil 3). Indeed, both treatments allowed P concentrations in the plant similar to those obtained with MF, indicating equivalent agronomic value in terms of P, which is important to achieve quality yields and healthy crops to meet human nutritional demands (Wang et al., 2020a). With the application of these MBFs, P is released at a rate that allowed oat plants to assimilate this nutrient as required: i) the different P uptakes observed indicates that some MBFs are more suitable for plants with high P requirements, while others should be more adapted to plants with low P requirements, and ii) the release of these nutrients is highly dependent on the materials blend. Tailoring MBFs, like those suggested in the 2:1 ratio for soils with higher P concentrations, (e.g., PiS+PiS-LIQ), is important to avoid P overapplication (Vance et al., 2021).

Oat fertilized with the 2:1 MBFs (Soil 3) presented no P deficiency, which can be attributed to: i) The high extractable P content in the soil; ii) The extractable P forms found in PiS more easily used by plants (Azevedo et al., 2018); and (iii) to the richness of LIQ in available P (Prado et al., 2022). Indeed, the single use of pig slurry liquid fraction has proven to result in higher P uptake in oat forrage (de Bang et al., 2021). Therefore, when considering the application of PoM+PiS or PiS+PiS-LIQ, two aspects should be considered: i) The fact that P becomes available more quickly, which means it will be more susceptible to leaching; ii) These MBFs should be considered for crop species with a higher P need.

MBFs with 1:1 and 2:1 ratios may have helped to retrieve P from manure to plants, meaning a more efficient P cycle and the adoption of sustainable agriculture practices with MBFs application.

In a previous study, where granulated poultry manure was used to fertilize wheat, spring rapeseed and potato, the soil P content for treatments with the organic fertilizer application was the same as that of the soil with MFs at the end of the production cycle, after the harvest of the last crop (Mažeika et al., 2021). Indeed, other authors pointed out that an increase in soil P content was more frequent after the second year of manure application (Antoniadis et al., 2015).

6.4.4 Potassium recovery

Potassium is also an essential macronutrient important to maintain cells' osmotic regulation, produces ions vital for metabolic reactions and facilitates protein production (de Bang et al., 2021). The application of CaM+PoM or CaM+PiS to soil 1 stimulated K dissolution, relative to the others analyzed in the 0.5:1 ratio (Table 6.5). This aspect was observed with the concentration of extractable K in the soil from pots with CaM+PoM or CaM+PiS, since it was almost 2-times higher than in pots with MF 07:14:14. Still, the amount of K applied was two times higher with MBFs. This may be due to CaM, a rich K manure, mix with other manure, such as PoM, also rich in K (Table 6.1). The CaM+PoM and the 1:1 MBFs formulated from PoM presented higher K concentration and K uptakes, either equal to or higher than the correspondent MF. The formulation of MBFs with manure rich in K stimulated K availability to values comparable to MFs, demonstrating the potential of MBFs to replace mineral K-fertilization. For instance, the application of PoM+CaS or PoM+PiS resulted in higher K concentrations in the oat plants, due to the higher K uptake compared to MF or the control. In summary, the CaM+PoM and the 1:1 MBFs have demonstrated a potential to substitute K mineral fertilization in basal fertilization, providing a substrate for metabolic and physiologic functions to occur properly (Torabian et al., 2021).

Since this fertilization was planned to focus on N requirements, the quantity of MBFs was sufficient to suppress the firstly N oat’s necessities. Hence, producing MBFs enriched in N, led to an amount of K applied in the 2:1 ratio lower compared to the other ratios. Nonetheless, after solid-liquid separation treatment, the K migrates to the liquid fraction (Regueiro et al., 2016a). The 2:1 MBFs not only used manures, like CaS, richer in K, but also utilized their corresponding LIQ, rich in K (Table 6.1). The oat fertilized with CaS+CaS-LIQ presented a higher K concentration and the oat amended with PiS+PiS-LIQ presented a higher K uptake within the ratio, predisposing these MBFs to crops with higher K requirements. The fact that soil 3 was considerably rich in K, may have disguised some of the results. Still, according to some authors, even in soils rich in K, the crop K uptake after manures application was enhanced compared to MF (Antoniadis et al., 2015). Nonetheless, this MBF is considered for soil richer in P and did not consider the K vehiculated.

No differences were observed in terms of extractable K in the soil in the N:P 1:1 and 2:1 ratios (Table 6.5). On one hand, this indicates that K fertilization was not affected by the application of MBFs, and, on the other hand, that can be attributed to the fact that K is mostly in unavailable forms, as a result of the combination with feldspars and mica in crystalline structures (Torabian et al., 2021). Consistent with the present results, in a previous study, after a year of PoM application to soil, no differences were observed in the soil (Mažeika et al., 2021), and it may be necessary another growth cycle until differences can be stated (Antoniadis et al., 2015). Yet, relative to soil 1, the application of 1:1 MBF improved the final concentration of extractable K in the soil, at the end of the experiment (Table 6.5). This could be attributed to the fact that the MBFs in that N:P ratio presented a higher content of organic C, sufficient to stimulate the soil microfauna activity, namely the bacteria responsible for K solubilization (Meena et al., 2016).

6.4.5 Soil properties at the end of the experiment

Farmers in the European Union, and especially in the Mediterranean area, are demonstrating more interest in applying organic material, more awareness relative to soil degradation and the importance of restoring soil organic matter content. Hence, offering solutions that combine the benefit of manure, e.g. organic matter, macro and micronutrients, with the characteristic of mineral fertilizers, e.g. specific N:P ratios adequate for crops, is the key to increase the adoption of sustainable agriculture practices.

The use of organic fertilizers in this study, after a single application, did not result in differences in terms of C content in the soil, or on the nutrients reserve, since the organic fraction has a higher impact in the long term and only after repeated applications (De Rosa et al., 2018; Qaswar et al., 2020). The soil application of manures, and transposing the knowledge to MBFs, might improve soil aggregation, soil health and, subsequently, the quality of crops (Rayne and Aula, 2020), but the benefits are more noticeable in the medium/ long term. For instance, the application of cattle manure for 12 years increased C content in the soil, and improved soil aggregation, while pig slurry application did not significantly increase C concentration in the soil (Domingo-Olivé et al., 2016). The timeline of this study was not sufficient to observe the expected impact on soil properties

Portugal is a county where the majority of the soils have an acidic nature (Gomes et al., 2019). The application of manure started to regain importance as an organic amendment to raise soil pH (Köninger et al., 2021). Based on the results obtained in this study, the MBFs have not modified the soil's pH, but the contrary was true with the MFs 07:14:14 and 10:10:10, where the pH was lowered relative to the control. These results are in agreement with those obtained by other authors (Iqbal et al., 2019). This can be a concern for soil health, since the soil microorganisms' metabolism function under specific conditions, and this pH decrease can impair their activity (Seaton et al., 2021; Yang et al., 2019). One other aspect of manure application, and also of MBFs, is the accumulation of soluble salts in soil (Prado et al., 2022), easily assessed by an increase in the soil EC (Wei et al., 2018). Based on the present results, the application of either MBFs did not significantly increase soil's EC, and, consequently, the risk of soil secondary salinization associated with organic fertilizers application was mitigated (Sigurnjak et al., 2017c).

The application of manure is known to ameliorate soil structure and improve soil water holding capacity. Therefore, in the long-term, the application of MBFs could lead to an improvement of soil water holding capacity, which is fundamental to overcome the more frequent drought periods in the Mediterranean region (Heuck et al., 2015).

6.4.6 Practical implications of MBFs application

Manure is produced all year, but it is used as organic fertilizer in specific periods, e.g. before crops' sowing or eventually all year round in permanent grassland. Hence, the use of MBFs as those designed for this study, mainly for the on-farm solution, may require farmers to i) increase the storage time of manure or ii) store MBFs. It is known that the storage of manure has effects on the manure composition and therefore impacts their agronomic value (Ali et al., 2019). The storage time of manure might therefore be useful for the design of some specific MBFs with more stabilized compositions. Similarly, the storage of MBFs after production will have an effect on nutrients (namely N and P) availability for plants, so this point should be considered by farmers. Regarding the central-solution, it may not require an increase of manure storage at farm scale, but will need the transportation of large amounts of manures to one central place, with environmental and economic impacts, especially when transporting slurries, due to their water content (Silva et al., 2022b).

Independently of the scenario considered in this study, it will be necessary to rent or acquire specific equipment to produce and/or apply MBFs to the soil.

The benefits of applying MBFs can also benefit farmers economically. Within the on-farm scenario, the cost would be the acquisition of mineral fertilizer to be used in the blend, but in a considerably lower amount than that required when using only MFs. Therefore, the costs of fertilization are expected to decrease. On the other hand, for the centralized solution, an economic analysis is essential to understand the price difference between the production of these MBFs and the MF. Yet, farmers might be willing to pay more for this type of fertilizer, but they also expected it to be a product of excellency (Hills et al., 2021).

Some other important features of manure that need to be considered before the implementation of MBFs are the odours associated with manure handling, as well as the potential risks of viable weeds seeds. In future works, these aspects should be considered and answered, since nothing was conducted in this study to control these aspects.

6.5 Conclusion

Tailoring MBFs with different N:P ratios demonstrated the relevance of combining livestock and crop production sectors to better respond to the crops' nutrient needs, while contributing to a sustainable agriculture. The results determined the potential of applying MBFs as partial substitutes for MFs in basal fertilization, which established their agronomic efficiency independently of the scenario analyzed. The application of PoM+SP (0.5:1 ratio), PoM+PiS (1:1 ratio) and PiS+PiS-LIQ (2:1 ratio), led to the production of oat plants with similar characteristics to the plants fertilized with the MFs. The lower DM yield obtained with the MBFs was the result of i) their slow nutrient release, which can be interesting for winter crops to avoid nutrient losses, and ii) the fact that high nutrient availability in soil 3 impaired the results in the 2:1 ratio. This indicates that these MBFs need to be further refined. Overall, the application of MBFs is a viable solution to recycle nutrients while promoting sustainable agricultural practices by partially substituting MFs.

Nevertheless, more studies should be conducted to properly assess the application of these manure-based fertilizers and ascertain the viability of their application.

6.6 References

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Chapter VII Conclusions and Final Considerations

7.1 Conclusions

Manure is a valuable resource, but its characteristics such as low nutrient concentration, imbalance N:P ratio relative to the crop's necessities, difficult its implementation at farm-scale. This thesis promotes the transformation of manures in MBFs, by trying to close the nutrients cycle and repurposing a material rich in all the nutrients essential for crops' healthy grow. The solution developed in this study was the design of MBFs for basal fertilization with specific N:P ratios, 1:1, 2:1 and 0.5:1, commonly utilized by farmers with mineral fertilizers. The proposed MBFs can be prepared considering two hypothetical scenarios: on-farm, mixing a single manure type with mineral fertilizers or manure-derived materials obtained after treatment (separation or acidification) and a central-solution, where different types of manure and derived materials could be combined.

Prior to the MBF design, a first stage of manure scooping was important to determine the manures with the higher surplus in Portugal, which would be available all year to farmers for soil application. The results demonstrated significant differences between the manures studied. CaS, PiSF and PoM presented a N:P ratio close to 1, reaching one of the desired ratios. Also, a possibility to achieve the intended 0.5:1 ratio would be to combine CaM with other raw manures. Other solutions were required to obtain the 2:1 ratio, since by mixing raw manures, it was not possible to reach the desired ratio. Thereby low technology treatments, solid-liquid separation, acidification, and the combination of both, were performed to understand if the alterations on N:P ratio would be sufficient to achieve the desired ratios or would promote the MBF formulation. The slurries liquid fractions obtained demonstrated, as expected, higher N:P ratios, but too high to respond to the 2:1 ratio and the amount of material necessary to supply N content was too high, discarding their application on its own. However, the use of LIQs combined with other manures or derived products demonstrated a potential to produce the desired 2:1 MBFs. One other positive outcome of this low-technology treatment application was that the solid fraction of PSF already presented the intended 0.5:1 ratio, ideal for soil with lower P content.

The on-farm scenario obtained interesting results, proving that it is possible to obtain MBF by mixing manures with mineral fertilizers. For instance, PoM+SP (0.5:1 N:P ratio), demonstrated that enriching poultry manure with superphosphate, not only mitigated the environmental impact associated with poultry manure application but had an agronomic performance equal to the correspondent fertilizer. The results demonstrated that it is possible to obtain MBF for the three ratios, but not for all manures. Some of the solutions proposed, exponentiated the risks of N losses through N_2O emissions, like the PiS+U or C losses by CH_4 emissions, CaS+PA. Hence, for a farmer to substitute MF with MBF obtained with all manures, it may be necessary a few adjustments, such as manure treatment to avoid nutrients losses. This should also improve nutrient use efficiency, since nutrients losses would be diminutive. Further studies are required to analyse the effect of manure treatment on nutrients losses and compared the economic effects of adopting the use of MBFs, with and without treatments.

The central-solution demonstrated that it is possible to combine different manures to obtain the 1:1 and 0.5 N:P ratios. To achieve the 2:1 N:P ratio, it was necessary to use the slurries LIQs. Based on the results, mixing the manure between them, presented several potential MBFs, like PiS+PiS-LIQ, PoM+PiS or CaM+PoM. The MBFs, especially the ones in the 2:1 ratio, reduced the potential of P leaching compared to MFs. This feature was extremely important since it is one of the features that discard manure use, due to P overapplication. However, using material rich in organic matter did foment the N and C losses through GHG emissions. Hence, similar to the on-farm scenario, combining MBF with treatments like acidification may reduce the potential N and C emissions. Nonetheless, after an initial period of 15 days of applying these MBFs, the concentration of NO_3^- in the soil was always higher when applying MBFs than with MF application. This point is highly relevant since it shows that, despite most of the N being in the form of organic N molecules, N availability for plants was not compromised, but was improved compared to MF.

Still, some questions are, unanswered, such as the costs of MBF. Manure, at this time, is a free material, but if solutions like this regain importance, it may be associated with costs. Also, transporting manure could have cost, but so does the transport of mineral fertilizers. The application of MBFs is also an advantage since adds organic matter to the soil and contribute to the C sequestration, improve water holding capacity, ameliorates microbiota community and healthier soil. Also, there is a potential to reuse the nutrients, with more emphasis on N and P, present in an organic and available material such as manure. On the contrary to MF, which needed to be produced the nutrients. Therefore, applying MBFs can contribute to close the nutrients cycles and circular economy. Nonetheless, the MBFs tested in this thesis, especially the ones in central-solution, are new. Therefore, the present results suggest that applying MBFs as a substitute for MF in basal fertilization is a possibility, but further studies are required.

7.2 Final Considerations

The hypothesis analysed in the present study were tested predominantly on a laboratory scale. Even if a small pot trial was conducted, field validation is essential to guarantee the veracity of the present results, since the uncontrol weather conditions of fieldwork alter the nutrients' dynamics and the results may differ from the observed. Also, the interaction of MBF with different soil types could survey important data to properly inform farmers of their features to plan a correct fertilization.

It would also be interesting to determine the GHG emissions in the presence of the crop, to understand if the N_2O emissions would still have such an impact on the GWP or if the value of cumulative emissions could be reduced. Also, to reduce the impact of N_2O emissions it could be important to incorporate acidification, which does not alter the N:P ratio but mitigates the GHG emissions. However, acidification should be performed with industrial sub-product to perform bio-acidification and contribute to the circular economy and product sustainability.

The first step of MBF production is the manure composition analyse. There are tables within the Portuguese law that regulates the manure composition, however, to better suit crops, manure and soil, it is essential to analyse manure. Creating a method or equipment that analysed manure's composition in a short period, would facilitate the correct application of those manures into the soil. This is something that comes from the necessity of always having to analyse the manure composition before applications, which is a long process. Perhaps this is not something within the thesis theme, but it is a necessity. Also, even though it is not possible to address all problems at once, heavy metal concentrations or pathogenic organisms that can contaminate crops can be concerning after soil application, and therefore considering these characteristics is also imperative to implement more sustainable practices in the agriculture sector.