REFLOW

"Phosphorus REcovery for FertiLisers frOm dairy processing Waste"

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Introduction

The fast-growing world human population may drive to ~10 billions of humans by 2050. Meet human needs for food is one of the greatest global challenges of the 21st millennium (UN World Population Prospects, 2019). It requires to maintain a high level of production in a restrictive context marked by a huge amount of issues for environment (soil, water, air qualities, water availability, climate global change,…), economy (prices energy, fertilizers, agroressources,…) and society (preservation of human and animal health, social expectations regarding agriculture production …). In this context, the European commission proposes a circular bio-economy action plan to promote new forms of development that encountered a more sustainable society and economy. Reconsidering the origin of plant nutrient, as waste or industrial by-products into bio-based fertilizers, contributes to the development of a sustainable circular bio-economy and agriculture.

Phosphorus (P) and Nitrogen (N) are essential macronutrients needed by all crops (Calderini et al., 1995). Fertilizers Europe estimates that, out of the 179 million ha of agricultural land available in the EU, 134 million ha (75 %) are fertilized with mineral fertilizers. The use of N and P fertilizers represent a major production cost for farmers, and field crop farms have the highest fertilizer costs (EU Agricultural Markets Briefs, 2019) and is 4% of the value of agricultural production between 2000-2009 (Lecuyer et al., 2013).

Beyond the financial cost of fertilizers, they also have environmental negative externalities. Indeed, added fertilizer may be lost through leaching (NO_3^-, PO_4^{2-}) or gases (N_2O, NO, NH_3), these losses may represent for example up to 50 to 60% of N added (Gutiérrez, 2012). Whereas N mineral fertilizers are mainly produced from atmospheric N, they consume high amounts of natural gas and energy (Ritter, 2008). Phosphorus mineral fertilizers used in agriculture are mainly derived from rock phosphate considered as a finite resource; rock phosphate has been added to the list of the critical raw material by EC in 2014 (European Commission, 2014). This context has strengthened the interest of nutrients present in organic waste. Although the use of manures is a current practice, the use of agro-industrial organic waste is less referenced. Agro-industrial organic wastes are mostly derived from agricultural processing industries such as dairy plant, which are the studied wastes in the Reflow project. According to raw materials and process, these wastes may be considered as a significant source of nutrients for crops. They may be transformed in different kind of biosolids that could constitute new fertilizers which we called Reflow fertilizers.

In Europe, the dairy industry represents the second largest agricultural sector after crops, accounting for more than 12% of total agricultural production. France, Germany, the Netherlands and the United Kingdom together produce between 58% and 92% of the EU's total production dairy products. The volume of cow's milk delivered to dairies is around 156 million tons in 2017 (Augère-Granier, 2018). This milk is processed into fresh products (drinking milk, yoghurts, cream, fermented milks, ...) and manufactured products like cheese, milk powder, butter, whey ... (Kolev Slavov, 2017) through different processes, such as pasteurization, coagulation, filtration, centrifugation, chilling.

The dairy industry is one of the main sources of industrial effluent generation in Europe (Demirel et al., 2005; Ahmad et al. 2019). For example, one liter of milk processed can produce up to 10 L of effluent depending on the end-product (Lateef et al, 2013). However, it should be noticed that the generated volume of dairy wastewaters and its characteristics is quite variable depending on the different types of industry, techniques, processes and equipment (Rico Gutiérrez et al., 1991). They contain different dilutions of milk or by-products, cleaning water (including sterilizing agents, acid

and alkaline detergent) (Carvalho et al., 2013). They have high nutrient concentration in organic and inorganic forms (Ahmad et al. 2019) that may present a large variability such as ranges observed for P (10-500 mg L^{-1}) and N (17-1120 mg L^{-1}) (Tawfik et al., 2008).

Regarding the diversity of (1) quality in raw material (milk, ...) in dairy plant, (2) milk transformation process, (3) dairy wastewater treatment strategies, and (4) sludge treatment, it appear clearly that a huge variety of products may be obtained and used as a source for the production of reflow fertilizers.

This document contains the Literature Reviews of the Early Stage Researcher Fellows active in Work Package 2 of the REFLOW European Training Network. This Work Package within the REFLOW Project has the objectives to evaluate (1) the interest of Reflow-fertilizers to provide available P to crop and enhance crop yield and (2) the impact of Reflow-fertilizers on soil biota and the different compartments of the Environnement

The Early Stage Researchers being trained in this workpackage are working on the consequences of reflow fertilizer application to soil on soil biological quality, their environmental effects and the recovery of P by plants. The literature reviews cover the following topics :

- Effect of the addition of different P fertilizers on soil biology and the consequence for plant growth and nutrition (ESR7)
- Environmental issues linked to the use of different fertilizers, specifically the questions of Nitrogen use efficiency, greenhouse gases mitigation (N_2O emission, C sequestration) and other environmental risks of N and P loss (ESR8)
- Importance of fertilizers application rate by considering fertilization modalities, P form in the fertilizer, behavior of P, its dynamics and transport in agriculture soils (ESR9)

The Literature reviews provide the Fellows with the essential background knowledge and current scientific state-of-the-art in order to clearly define research questions and hypotheses for testing. The Fellows will prepare technical reviews for publication from this review document.

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Impact of exogenous organic matter in fertilizers on Soil microbes (ESR7)

REFLOW project aims to recover several types of potential fertilizers from dairy wastewater. Dairy wastewaters contain milk biosolids, detergents, sanitizers, milk wastes, and cleaning waters. These wastes contain significant amounts of nutrients such as phosphorus (P), and thus, their utilization as fertilizers is being currently under study.

Animal or vegetal wastes are generally very heterogeneous based on the origin of the produces. Milk is known to be a rich product in elements with a great agronomical value such as calcium (Ca), P, sodium (Na), magnesium (Mg), Sulphur (S), chloride (Cl) and potassium (K) (Pashkova, 2009; Gulati et al., 2018). Furthermore, it contains trace elements in relatively high concentrations like iron (Fe), zinc (Zn), bromine (Br), rubidium (Rb) or strontium (Sr) (Pashkova, 2009; Gulati et al., 2018). Potentially, also organic pollutants may be found in the milk or its dairy production wastewaters like phthalates, surfactants, cleaning agents, polycyclic aromatic hydrocarbons (PAHs), dioxins, biocides or antibiotics (Huygens et al., 2018). Finally, also living organisms (pathogens) may be present in the recovered products from milk production such as bacteria, viruses, protozoa or worms (Huygens et al., 2018). Therefore, it should be considered the risk of precipitating undesired pollutants together with the intended products.

Perhaps at this stage and even if you are in the introduction part. It may be interesting to add a map of milk processing plant and position to have a better understanding of what are the waste water from dairy plant , their composition and the origin of the different components… it would be possible to increment this during your PhD.

The targeted materials recovered from such waste are the ones mentioned in the STRUBIAS (STRuvite + BIochar + AShes) report of 2018 (Huygens et al., 2018). Additionally, REFLOW will also evaluate the use of dairy wastewater sludge (DWWS) directly as a fertilizer. The STRUBIAS report differentiated the recovered products based on the recovery methodology. Thus, materials were divided into:

Dairy wastewater sludge (DWWS)

Precipitated phosphate salts and derivates

Thermal oxidation materials and derivates

Pyrolysis and gasification materials

Due to their richness in mineral elements, especially in P, these materials have an important potential as novel fertilizers. However, little is known about the effect they could have on soil microorganisms' communities, their functioning, and the consequences for the

processes they regulate, such as nutrient and carbon mineralization and feedbacks to plant uptake and productivity.

Understanding the effects REFLOW fertilizers may have on soil biology is crucial to achieve good yields and a good environmental welfare. Soil microorganisms are a key agent in nutrient cycling, contributing in chemical transformations of nutrients that allow them to be absorbed by plants (REFERENCES).Stevenson & Cole, 1999; Moreno-Lora et al., 2019). Moreover, soil microorganisms help in building soil organic matter stocks in soils which help in mitigating climate change and pollution of freshwater reservoirs (REFERENCES).Lehmann & Kleber, 2015; Kallenbach et al., 2017). REFLOW fertilizers should ideally contribute to maintain large and healthy soil microbial communities.

Many different techniques are available to assess soil biological health. Some methods measure the amount of soil microorganisms by extracting microbial C by fumigation with chloroform (Brookes et al., 1985). Other methods measure the activity of such organisms by assessing their enzymatic activities. Extracellular enzymes are proteins that hydrolyze specific types of molecules and allow nutrients to be utilized by soil microorganisms and plants (Burns et al., 2013). Enzymatic activities are commonly correlated with specific nutrient cycles in the soil (Burns et al., 2013). Functions of soil microorganisms can also be deciphered by using community level physiological profile (CLPP) tests (Rutgers et al., 2016). These analyses measure functional traits of soil microorganisms by analyzing how they degrade different C sources. Examples of this technique are MicroResp and BioLog (Rutgers et al., 2016; Moscatelli et al., 2018).

The phylogenetic composition of the microorganisms in the soil is also possible to be analyzed by doing DNA sequencing. Methods like metagenomics or the use of genetic markers such as 16S or 18S provide additional information about the composition of soil microorganisms (Bastida et al., 2019). Moreover, is it possible to measure phospholipid acids (PLFA) in which can be linked with the abundance of certain microbial or fungal taxa (Frostegård et al., 2011).

Finally, quantification of soil organisms provides useful information. Arbuscular mycorrhizal fungi (AMF) nodules or earthworm populations can be counted and are good indicators of soil health. Both AMF and earthworms are important players in the C, N and P soil cycles and are sensitive to agricultural practices (Bartz et al., 2013; Säle et al., 2015).

In this chapter, we aim to provide an insight into the potential effects of REFLOW fertilizers on microbial communities. Information about some materials to date is very limited, yet the available information may bring some hints on potential effects REFLOW fertilizers may cause on soil microbial communities.

1 Dairy wastewater sludge

Figure 1. Different types of dairy wastewater sludges (Ashekuzzaman et al., 2019).

Dairy wastewater sludge (DWWS) is the first residue obtained from dairy production. It is the basis for the recovery of the different materials that REFLOW project will produce. From this matrix ashes, hydrochar and phosphate salts will be recovered.

Milk processing activities produce large quantities of wastewater (up to 10 L of dairy wastewater per L of milk) (Wang & Serventi, 2019). From all the wastewater, only a small percentage of it is made by biosolids. The dry matter of these sludges is about the 10-25 % (Ashekuzzaman et al., 2019). This heterogeneous material is normally disposed as a fertilizer in Europe (Ashekuzzaman et al., 2019). There are different ways to treat this material, some of them include bio-chemical treatments, others use liming materials to precipitate the sludge (Wang & Serventi, 2019). To flocculate phosphorus in many cases aluminum or iron minerals are used, which are costly and may contain significant amounts of pollutants (Lopez-Mosquera et al., 2000; Ashekuzzaman et al., 2019). There are alternatives which employ biological methods to precipitate P, yet these type of filtering processes are less common compared to the chemical flocculants (Wang & Serventi, 2019).

DWWS is very rich in C, N, P, K and many other elements, yet their concentrations differ largely. N contents can vary from 17 mg: L to 1120 mg: L and P could range from 10 mg: L to 500 mg: L (Wang & Serventi, 2019). The techniques used in the processing of DWWS are known to influence their chemical composition (Ashekuzzaman et al., 2019). For instance, limed sludges have lower contents in N and higher contents in P compared to the biochemically treated ones (Ashekuzzaman et al., 2019). The high content in plant essential nutrients makes DWWS an interesting fertilizer.

The agronomic value of DWWS remains poorly studied to date (Ashekuzzaman et al., 2019). It is known that the application of untreated DWWS could be highly polluting, reducing crop yields and affecting soil physical structure (Lopez-Mosquera et al., 2000; De Jesus et al., 2015; Ahmad et al., 2019). Others found that DWWS has positive effects on plant growth, yet the number of studies is very limited (Lopez-Mosquera et al., 2000; Lopez-Mosquera et al., 2002; Omil et al., 2007; Challam & Chaturvedi, 2013). To date, no studies have assessed specifically how DWWS may affect soil microbial communities.

Most of the research conducted on organic effluents has been done on sewage sludge (SS). SS has been used as fertilizer and as a chelant in heavily contaminated soils (Agamuthu et

al., 2013). In general terms, SS, like DWWS contains large concentrations of beneficial nutrients for plant and microbial growth. SS application can modify physical, chemical and biological soil properties (Singh & Agrawar, 2008). Physical soil characteristics may be modified by an increase in soil aggregate stability, water holding capacity, porosity and humus content (Singh & Agrawar, 2008). Simillarly, bulk density and erosion are expected to be reduced after SS application (Singh & Agrawar, 2008). The effects on other characteristics, such as pH, are less clear with contrasting findings (Singh & Agrawar, 2008). Other chemical characteristics like N, P, soil organic carbon, cation exchange capacity, electrical conductivity and pollutants are known to increase with SS amendments (Singh & Agrawar, 2008). Finally, as mentioned, biological properties may be also affected by increases in pathogenic organisms, yeast populations and aerobic bacteria (Singh & Agrawar, 2008).

Specifically, SS, contains large concentrations of labile organic C, which contribute significantly to the growth of soil microorganisms (Demoling et al., 2007; Singh & Agrawal, 2008). Moreover, it contains large concentrations of labile N and P which are fundamental for both plant and microbial development (Demoling et al., 2007). The large amounts of labile C could also cause other nutrients such as N to be immobilized by the soil microbial community (Smith & Tibbett, 2004). Lastly, SS may contain large concentrations of heavy metals and other pollutants which may affect soil microbial communities (Singh & Agrawal, 2008; Mossa et al., 2017).

The effects of SS on soil microorganism's composition is dependent on the chemistry of the sludge and on their production method (Mattana et al., 2014; Lloret et al., 2016). Changes in the soil microbial have been addressed using different kind of techniques such as PLFA, 16S or 18S gene sequencing. PLFA assays have shown that the application of SS increases the content of bacterial and fungal biomass (Nicolás et al., 2014; Bastida et al., 2019). Moreover, it has been observed an increase in the bacterial gram+: gram− ratio after the application of SS (Nicolás et al., 2014; Bastida et al., 2019). 16S and 18S primers are commonly used in soil science to identify bacterial and fungal communities (Lindahl et al., 2013; Schöler et al., 2017). Analyses conducted on 16S and 18S sequencing revealed significant alterations on microbial communities after the application of SS (Lloret et al., 2016). Increases in microbial biomass have also been reported using the chloroform-fumigation method (Witter et al., 1993).

Soil enzymatic activities are known to be altered when soils are treated with SS. It has been reported that SS increases soil enzymatic activities (Siebielec et al., 2018). The large concentrations in labile C in SS may be behind the promotion of soil enzymatic activities (Allison & Vitousek, 2005). Yet, SS applications, with high contents in pollutants may also reduce the activity of soil microorganisms and thus their enzyme production (Sharma et al., 2017).

AMF root colonization is also known to be affected by SS, yet the results are contrasting. Some authors found that root colonization is diminished (Jacquot et al., 2000; Gryndler et al., 2008), whereas others have found positive effects (Zhu et al., 2016). Again, the large heterogeneity in the materials' composition may be the cause behind the scientific incongruences.

SS effect on earthworms has also been studied. Earthworms accumulate toxic elements from SS and tend to accumulate them in their casts where they are normally in a much available form for living organisms (Kızılkaya et al., 2004). Earthworms have been also used to remove pollutants from SS (Yang et al., 2014). The effects of SS on earthworms are known to be species-dependent, some species have been reported to increase after application whereas others have been found to decline (Vafa et al., 2016). It also has been noticed that high amounts of SS may cause mortality and malfunctioning in their immune system (Rorat et al., 2013).

The availability of pollutants in SS may also be modulated by the plant rhizosphere. Root exudates, which are composed by low molecular weight acids (LMWAs) among other substances, have the availability to enhance the solubility of many plant nutrients (Haoliang et al., 2007). The release of significant concentrations of LMWAs in soils fertilized with SS may cause soil pH to decrease and the availability of potential pollutants to increase (Koo et al., 2013; Vause et al., 2018). However, to date, it is unknown if the profile of root exudates is changed after the application of SS. Studies have shown that the emission of root exudates is affected by the availability of nutrients (Wang et al., 2013).

Another threat of SS disposal in soils is the inoculation of pathogens. SS, especially the ones coming from cattle production, may contain human and animal pathogens such as Escherichia coli, Listeria, Clostridium perfringens, Enterococcus or Salmonella (Brochier et al., 2012). These pathogens may have the availability to survive in plant tissues, soils and hydroponic systems when SS is applied (Brochier et al., 2012; Kyere et al., 2019). It has been reported that under realistic agricultural practices and following correctly adequate composting standards the risk of contamination is very small (Brochier et al., 2012). Moreover, native soil microbial communities are known to decrease the survival of potential pathogens (Xing et al., 2020). Nevertheless, specific strains of bacteria, such as Escherichia coli O104:H4, which caused over 50 deaths and near 4000 infected in Germany in 2011, may survive in soil for over a year after its inoculation (Knödler et al., 2016). Currently, there is no information on how viable pathogens will be in DWWS, this is one of the research questions that REFLOW aims to answer.

In conclusion, very little is known about the effects DWWS may have on soil microbial communities. It is assumed that it may affect soil properties in the same was as SS. However, the effects of SS on soils are very disparate as influenced by the differences in composition and production methodologies.

2   Precipitated phosphate salts and derivates (struvite-like salts)

Figure 2. Caption of struvite crystals (Das et al., 2017)

The precipitated salts are commonly referred to as struvite-like molecules. Specifically, most of the precipitated salts are considered calcium or magnesium phosphate salts (Huygens et al., 2018). However, aluminum and iron phosphates can also be formed, though their application as fertilizer is restricted in Europe since they can be toxic for plants and soil microorganisms (Huygens et al., 2018). Struvite, because of its low solubility, constitutes a promising and valuable fertilizer (Uludag-Demirer et al., 2005; Tao et al., 2016). Struvite is formed in wastewaters when phosphates are present together with magnesium or calcium and ammonia (simplified reaction: $Mg2+ + NH4+ + PO43- + 6H2O \rightarrow MgNH4PO4.6H2O$) (Uludag-Demirer et al., 2005). It is formed first as a powder and later can be bounded to form granules to facilitate agricultural application (Etter et al., 2011). Apart from struvite, other molecules called struvite analogs precipitate as well (Matthew & Schroeder, 1979). Analogs include for example: MgKPO4-6H2O, MgRbPO4-6H2O, MgTlPO4-6H2O, and MgCsPO4-6H2O (Matthew & Schroeder, 1979). However, the occurrence of these forms is less relevant because of their lower stability (Huang et al., 2011).

Many other elements (including beneficial and pollutants) can co-precipitate together with phosphate salts (Huygens et al., 2018). The presence of such impurities could reduce the potential use of struvite as a fertilizer (Tansel et al., 2018). In the STRUBIAS report, the risks of heavy metals and organic pollutants were considered low (Hutnik et al., 2013; Huygens et al., 2018; Tansel et al., 2018). The STRUBIAS report indicated that the content of metals and metalloids in precipitated phosphates was not considered as worrisome (Huygens et al., 2018). It should be noted that, conventional fertilizer is not free from heavy metals or other inorganic contaminations (Gimeno-Garcia et al., 1996).

Contaminations by organic pollutants can also be detected in struvite and other phosphate salts (Ronteltap et al., 2007; Huygens et al., 2018). However, the risks of contamination are

low when the carbon (C) content in the precipitated salts is below 3% (Huygens et al., 2018). Higher amounts of organic C are linked with a higher presence of impurities (Huygens et al., 2018).

Another aspect that should be considered is the particle size of struvite or other precipitated salts. It was noted that the particle size of struvite is not constant and depends on many factors during the crystallization process (Le Corre et al., 2005; Bauer et al., 2007). Generally, larger particle sizes are associated with slower dissolution rates (Degryse et al., 2017). Thus, a significant effect could be expected when the salts are applied with larger particle sizes. Faster dissolution rates may cause significant amounts of P and other nutrients to be lost due leaching and cause environmental damage.

There is little research conducted on the effect struvite has on soil microorganisms. Bastida et al., (2019) published one of the few papers that focuses directly on soil biota after struvite application. In their experiment, they compared the effects of struvite against sludge from a wastewater treatment plant (WWTP). They found that struvite affected significantly bacterial biomass and composition. Their phospholipid-derived fatty acids (PLFA) assay revealed an increase in the gram + : gram − ratio. Moreover, they conducted a metaproteomics analysis which showed increased populations of Verrucomicrobia, Cyanobacteria, and some orders within the Actinobacteria phyla. Both Verrucomicrobia and Cyanobacteria are known to be present in nutrient-poor soils (Bastida et al., 2016; Bergmann et al., 2011). Actinobacteria are considered important P solubilizing bacteria (Solans et al., 2019).

The paper of Bastida et al. (2019) is the only direct assessment on the effect's struvite cause on soil life but provides overall a partial understanding. It is not known whether microorganisms will react differently on soils with distinct properties and characteristics. Microbial communities, similarly to macroscopic organisms, are known to differ based on many soil factors across the globe (Martiny et al., 2005). Soil parameters such as pH are significantly correlated with soil microbial composition across the globe (Lauber et al., 2009). Therefore, it is unknown how native microbial populations of different soils will react to struvite amendments.

Moreover, the experiment of Bastida et al. (2019) did not include regular synthetic P fertilizer. Some studies have, as well, reported elevated microbial biomass under conventional fertilization (Huang et al., 2016; Liu et al., 2020). The use of fertilizers provides necessary nutrients for plant and microorganisms to enhance their productivity. Consequently, we do not know whether struvite applications increase microbial biomass at a similar or at a different rate compared to regular P fertilizers.

P-fertilizers, organic or inorganic, increase plant productivity. Higher plant yields could also be accompanied by higher root biomass (Hirte et al., 2018). The rhizosphere, which is the area of the soil in direct contact with plant roots, is known to be the richest part in the soil with regards to microbial activity and biomass (Ai et al., 2012). There are many complex mechanisms and relationships between roots and soil organisms. One of them is the exudation of different chemicals by plant roots, which have the ability to promote and/or decrease microbial growth (Preece & Peñuelas, 2020). Moreover, the application of

fertilizers is believed to affect the segregation of root exudates and change its chemical composition (Zhu et al., 2016). It is not known whether struvite or other precipitated salts will influence the production of such rhizodeposits and, thus, how the interaction between plants and microorganisms will be affected.

Furthermore, Bastida et al. (2019) used dry soil in their experiments which may have changed significantly the native soil bacterial and fungal populations (Gordon et al., 2008). Differences in microbial communities are crucial in the uptake of different macro and micronutrients by plants (de Santiago et al., 2019). Microbial communities are sensible to different management practices, latitudes and soil characteristics (Martiny et al., 2005; Lauber et al., 2009; de Santiago et al., 2019). In consequence, experiments which include undisturbed microbial communities from different soil types and latitudes may exhibit different patterns when struvite or other precipitated phosphate salts are applied.

Finally, as previously mentioned, struvite can be very different based on its origin. Therefore, different struvites with distinct concentrations in ions and granulometries are likely to show different effects on soil microorganisms. The C content in precipitated salts is commonly used as a proxy of the material quality (Huygens et al., 2018). In the STRUBIAS report it was mentioned the threshold of 3% in organic C content, so materials with more than 3 % would be discarded as directly applicable fertilizers (Huygens et al., 2018). Higher amounts of C are commonly associated with a higher degree of impurities in which most of the potential pollutants are found (Huygens et al., 2018). However, C is the main limiting factor for microbial growth (Demoling et al., 2007). In consequence, the effect of struvite or other precipitated salts with a very low C content is likely to have a small effect on microbial communities compared to other C-rich materials.

Other ions may precipitate as well with the targeted phosphate salts. It is difficult to predict which elements and at which proportion will also precipitate. It would be dependent on the parent material and the precipitation method used. Based on the chemical composition of dairy products, Ca, Mg, Fe and Na may be among the most relevant ions that could co-precipitate (Pashkova, 2009). With regards to Ca, it has been detected that Ca content in soil is positively correlated with many different PLFAs (Grayston et al., 2004). Calcium applied to the soil as an ameliorant of soil acidity in the form of gypsum ($CaSO_4·2H_2O$) was reported to increase arbuscular mycorrhizae fungi (AMF) in soils (Luo et al., 2018). Magnesium is an element that behaves similarly to Ca cations in the soil (Qadir et al., 2018). It is a crucial element for plant growth since it, among other functions, is a component of chlorophyll and mediates the activation of soil enzymes (Maguire & Cowan, 2002). However, when added in excess Mg can deteriorate significantly soil structure (Qadir et al., 2018). Sodium ions, when in excess, have the same soil structure deteriorating properties (Emran et al., 2020). Soil structure is linked to a better soil aggregation and, thus, enhanced microbial communities (Bach et al., 2010). Similarly, high concentrations of Fe are also known to reduce microbial biomass (Narendrula-Kotha & Nkongolo, 2017). Nevertheless, it is not known until what extent other ions will co-precipitate and how bioavailable they may be. In consequence, it is important first to conduct a chemical characterization of the REFLOW fertilizers and, second, to assess the mobility of such co-precipitates under a wide variety of soil types.

3   Thermal oxidation materials and derivates

Figure 3. Fly ash (www.indiamart.com)

General remarks:

 I often see that in some phrases, there is only one bibliographic reference. Either, there is only one, and therefore it must be specified that it is the only study, or if this is not the case, put at least 3 references.

This second group of materials included in the STRUBIAS report, they are defined as materials that were treated with thermal oxidation in an oxygen rich environment (Huygens et al., 2018). Materials produced with this technique are typically named ashes. Ashes are an interesting source of essential nutrients for plant growth such as P, potassium (K), Ca and silica (Si) (Huygens et al., 2018; Silva et al., 2019). Even though the application of ashes from biological wastes as fertilizer is considered promising, current European legislation still needs to be developed to consider ashes as viable fertilizers (Silva et al., 2019). There are still some knowledge gaps which need to be filled in order to create better regularizations, for example, the determination of the agronomic value and the assessment of its risks on human health and environmental welfare (Silva et al., 2019).   I suggest that at the end of the description of the different biofertilizers, make a characteristic / effect summary table

Ashes can be classified into bottom ash and fly ash: bottom ash is the one produced at the bottom of the furnace whereas fly ash represent the finer particles that remain at the top of the furnace and at its gas cleaning system (Silva et al., 2019). There are other ways of classifying ashes, one proposed way is based on its elemental composition which can be linked with its potential use (Figure 3) (Vassilev et al., 2013a; Vassilev et al., 2013b). Thus, ashes can be divided into:

K type: Rich in macronutrients such as P, K and S. Could be used as fertilizer.

C type: Rich in Ca and Mg. Could be used as liming material.

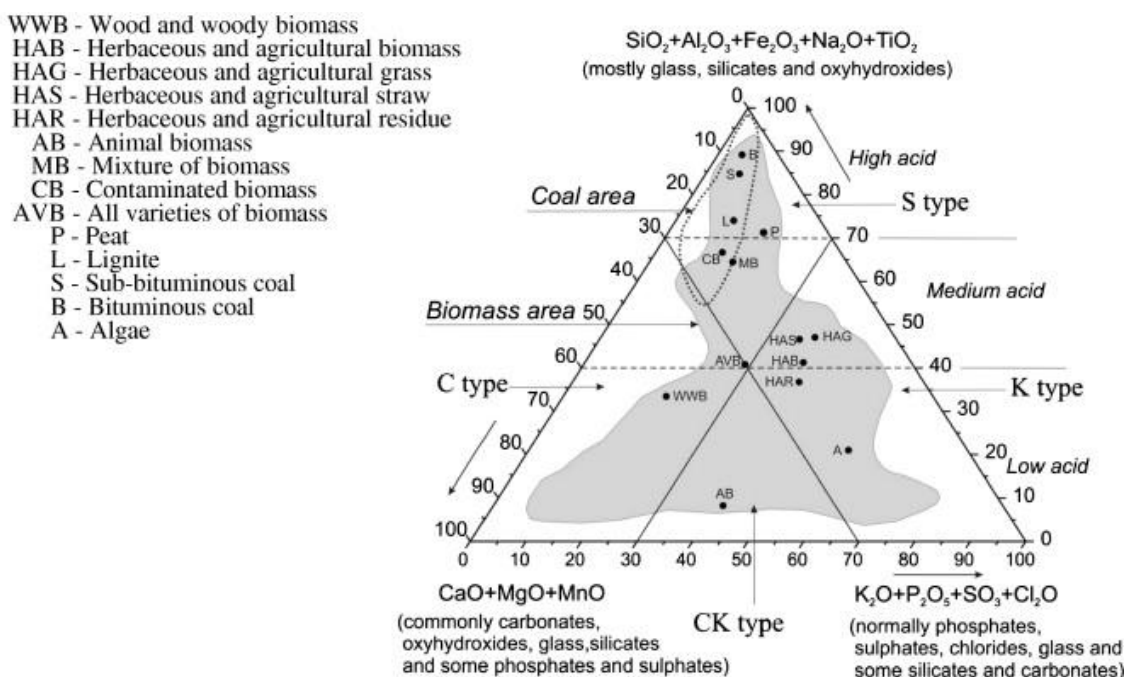S type: Rich in Si, Fe and Al. Unsuitable for either fertilizer or liming material.

Figure 4. Classification triangle of ashes (Vassilev et al., 2013b)

Commonly, the production of ashes is done at very high temperatures (>850 °C) allowing the suppression of all pathogens and other living organisms (Huygens et al., 2018). However, ashes are not free of other potential contaminants. For instance, the content of heavy metals, trace and minor elements are known to be concentrated in the ashes as the result of the combustion (Huygens et al., 2018). Nevertheless, the presence of these minor elements was not considered worrisome in the STRUVIAS report (Huygens et al., 2018). The situation could be different for organic pollutants such as polyaromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/F) or dioxin-like polychlorinated biphenyls (PCBs). These organic compounds are the result of incomplete combustions and they may be destroyed when higher temperatures are applied (>1000 °C) (Huygens et al., 2018). Yet, significant amounts of these compounds have been found in ashes, especially PAHs and PCDD/Fs (Huygens et al., 2018). PAHs and PCDD/Fs are known to impact negatively soil microbial communities (Murphy et al., 2016; Anyanwu & Semple, 2018).

Similarly to struvite, ashes composition differ greatly depending on the origin of the material. For example, the percentages of P can vary from the 0.1 % in the ashes of wood to 10.6 % in ashes from poultry manure (Huygens et al., 2018). Moreover, such variations can be detected even among the same type of materials, for example, P in ashes from poultry manure can range from 10.6 % to 4.6 % (Huygens et al., 2018). Consequently, it is expected that the effects derived from the application of ashes from dairy waste may be dependent on the chemical composition of the parent materials.

There are a few studies testing the effects of ash applications on soil microbial communities. However, there is no consensus in the results obtained. On one hand, some authors reported increased microbial biomass after ash applications (Saarsalmi et al., 2012; García-Sánchez et al., 2015a; Mortensen et al., 2019). On the other hand, others show a limited effect on microbial biomass (García-Sánchez et al., 2015b; Schönegger et al., 2018). Similar kind of incongruences arise when analyzing the effect of ashes on microbial communities' composition. For instance, some authors claimed significant differences in PLFA tests and changes in the AMF composition (Schutter & Fuhrmann, 2001; Peltoniemi et al., 2016; Cruz-Paredes et al., 2017), whereas others found no effect (García-Sánchez et al., 2015b; Noyce et al., 2016).

Specifically, applications of ashes on soil may increase the content of Actinobacterial communities and 18:2ω6 fungal specific phospholipid acid in peat soils (Peltoniemi et al., 2016). Previously, Schutter & Fuhrmann (2001) reported enrichments in 16:1ω5c, 17:0 cy and 16:1ω7c phospholipid acids and Athrobacter communities after the application of coal ash on a sandy-loam soil. Schutter & Fuhrmann (2001) also indicated that ash influenced positively gram-positive bacteria and fungal communities. Similarly, Cruz-Paredes et al., (2017) reported that the application of ash from wood and straw increased fungal communities in a loamy sand soil.

The changes in the soil biota profile due ash applications may be subject to the changes caused in soil pH. Ash has a strong effect in increasing soil pH (Hansen et al., 2017). Similarly, soil microorganisms are strongly affected by changes in the soil pH (Lauber et al., 2009). Thus, results that show alterations on soil microbial communities may be caused by the changes in the soil pH levels. Increasing soil pH by liming leads to an array of improvements in soil health, such as: improving P, Mg and Ca availability while at the same time reducing the availability of heavy metals and toxic elements such as Al (Fageria & Baligar, 2008). Yet, soils with very alkaline soils are also known to reduce the availability of macronutrients such as P (Penn & Camberato, 2019).

As mentioned, ashes have a great potential as liming agent, yet its effect as viable fertilizer is debatable. Contents in heavy metals, organic pollutants and limitations in the current legislation are some of the reasons behind their poor potential (Huygens et al., 2018). Yet, ashes are a good matrix to chemically recover P and produce phosphoric acid which can be then used as fertilizer. P-fertilizers recovered from ash seem to have less heavy metal concentrations than traditional phosphate rock derived fertilizers (Vogel et al., 2020). However, the low efficiency in P recovery, the relatively low content of P in the matrix and the large quantities of energy and chemicals needed make P-recovered fertilizers less environmentally friendly compared to traditional fertilizers (Pradel & Assani, 2019).

Currently, research did not address the different effects P-recycled fertilizer from ash may cause on soil microbial communities. It would be expected that fertilizers with a lower level in heavy metals compared to ordinary fertilizers would not affect negatively soil microbiome. Fertilizers recovered from ash effect on soil microorganisms against traditional fertilizer will be tested within the REFLOW project.

4   Pyrolysis and gasification materials

Figure 5. Hydrochar (Yao et al., 2017)

The last group of materials covered in the STRUBIAS report are the pyrolysis and gasification materials. The outputs are often referred as (bio)chars. (Bio)chars are created by exposing organic residues to high temperatures for long periods of time in anoxic conditions. In the REFLOW project, because of the high moisture content of the dairy waste products the pyrolysis is conducted generally in very moist matrixes. The terminology of the specific procedure is wet pyrolysis and the generated char is referred as hydrochar. Hydrochar production is characterized by operating temperatures of 180-260 ºC with a residence time ranging from 5 minutes to 12 hours (Huygens et al., 2018)

Likewise as all the products covered by the STRUBIAS and REFLOW projects, the composition of chars and specifically hydrochar is highly dependent on the composition of their parent materials. Materials with high content in contaminants are discarded for char production (Huygens et al., 2018). Pyrolysis and gasification materials in the same way as thermal oxidation ones are commonly free of pathogens but could contain significant amounts of heavy metals and organic pollutants such as PAHs, PCDD/Fs or PCBs (Huygens et al., 2018). As mentioned, PAHs and PCDD/Fs are known to impact negatively soil microbial communities (Murphy et al., 2016; Anyanwu & Semple, 2018). Yet, alike thermal oxidation materials, the removal of pollutants from pyrolysis and gasification materials is not achievable (Huygens et al., 2018). Moreover, recovery of fertilizers from pyrolysis and gasification materials is more challenging than thermal oxidation ones (Huygens et al., 2018). Hence, dairy waste wasters with high content in contaminants should be discarded to produce hydrochar.

Pyrolysis and gasification materials, unlike ashes, contain large amounts of C. The ratio between organic hydrogen (H) and organic C is a good indicator of C stability. C stability is crucial in chars, ratios above 0.7 (H:Corg > 0.7) indicate higher presence of volatile organic compounds (VOCs) and other organic pollutants in the chars. VOCs are a large group of labile organic molecules such as benzene, toluene, pyrazines, pyridines, pyrroles or furans (Huygens et al., 2018). These are toxic compounds are known to harm soil microorganisms and plants (Huygens et al., 2018; Ahmed et al., 2019; Li et al., 2019). Therefore, the

STRUBIAS report highlighted the importance of standardized methods which produce materials with a H:Corg < 0.7 (Huygens et al., 2017).

Pyrolysis and gasification materials with stable (recalcitrant) C are applied to soils with the objective of increasing C stocks. This aspect has been deeply studied by the scientific community in the recent years as a technique to sequester C and mitigate the exacerbating levels of atmospheric CO_2. A search in ScienceDirect including the terms biochar and carbon sequestration retrieves almost 400 papers. Biochar, ideally, is formed by stable C molecules which are able to avoid microbial degradation (Majumder et al., 2019). Hence, adding biochar would result in increasing the C levels of soil. Nevertheless, application of pyrolysis and gasification materials could lead to a priming effect (Kuzyakov, 2010). Pyrolysis and gasification materials contain as well until a certain extent some concentrations of labile C which may cause microbial communities to shift their nutritional demands towards the native soil organic matter (SOM) (Cui et al., 2017). This priming effect would have a negative effect on the C sequestration purposes.

Similar issues may arise when pyrolysis and gasification materials are applied into the soil as fertilizers. Biochars may contain significant amounts of valuable nutrients for agriculture such as P, K, Ca, Mg, S and small amounts of N (Huygens et al., 2018). Except for N, which is very unavailable in biochar, the rest of nutrients are considerably available (Huygens et al., 2018). The concentration of these nutrients is also very variable depending on the parent material, according to the STRUBIAS report the concentration of these nutrients may range from the 15 % to the 75 % of the biochar content (Huygens et al., 2018). Because of potentially significant amounts of labile C in pyrolysis and gasification materials microbial communities may be enhanced in the short term and cause the immobilization of plant nutrients such as N (Fang et al., 2018). This immobilization effect, yet temporary, may prevent farmers to shift the application of ordinary fertilizers towards pyrolysis and gasification materials alone (Huygens et al., 2018).

Biochars and hydrochars are used, like ashes, to increase soil pH and thus are used as liming agents. Pyrolysis and gasification materials have been considered by some authors as superior compared to standard lime when correction acidic soils (Wu et al., 2020). Application of biochar proved to be effective in restoring heavy metals contaminated soils by raising soil pH (Houben et al., 2013). Other researchers, on the other hand, pointed out that biochar applications have a lower potential in increasing soil pH compared to lime (Raboin et al., 2016; Huygens et al., 2018). Therefore, the application of pyrolysis and gasification materials may not be of economic interest compared to other liming materials for farmers (Huygens et al., 2018).

Amendments with pyrolysis and gasification materials aside of raising soil pH have a positive effect on soil structure. Biochar are porous materials that increase soil porosity and water holding capacity in a wide variety of soils (Glaser et al., 2002; Heikkinen et al., 2019; Campos et al., 2020). Biochar's pores provide specific habitats for the development of soil microbial communities (Gul et al., 2015; Huygens et al., 2018). The effect of pyrolysis and gasification materials may have on soil structure or microbial communities is also dependent on the type of biochar applied (Gul et al., 2015; Campos et al., 2020).

In summary, pyrolysis and gasification materials have a good potential as soil amenders. They increase effectively soil pH, provide essential nutrients for plants and can improve significantly soil structure. However, applications of chars are in certain cases not economically attractive compared to traditional amendments. Moreover, the presence of labile amounts of C could cause native SOM to be reduced and/or nutrient immobilization. The STRUBIAS report provided guidelines to label chars based on the availability of C and the content of nutrients (Huygens et al., 2018).

With regards to the direct effects pyrolysis and gasification materials have on microbial biomass, some researchers have indicated increases in microbial biomass (Zhang et al., 2014; Mackie et al., 2015; Liao et al., 2016). Others, on the other hand, have reported negative effects on microbial biomass (Dempster et al., 2012; Ameloot et al., 2014; Andrés et al., 2019). The differences in labile C in the biochars used may be one of the reasons behind the lack of consensus among the research community. Consequently, materials with a higher content in labile C may promote microbial communities and accelerate SOM decomposition, whereas materials with very stable C molecules would contribute to build up SOM stocks (Andrés et al., 2019). For the specific case of hydrochar, the targeted type of pyrolysis and gasification material in the REFLOW project, the studies on microbial biomass are much more limited. Yet, researchers have concluded that hydrochars have a stronger compared to biochar in stimulating microbial biomass (Gajić et al., 2012; Andert & Mumme, 2015; Schimmelpfennig et al., 2017).

Total enzymatic activity after the application of hydrochar has been reported both to decrease (Andert & Mumme, 2015) and to increase (Ren et al., 2017; Taskin et al., 2019). Similarly, enzyme activities have been reported to increase after biochar applications (Khadem & Raiesi, 2017), to decrease (Ameloot et al., 2014) or to cause no significant effect (Elzobair et al., 2016). Again, the differences in the biochar composition (e.g. amount of labile C) may be behind the differences found (Khadem & Raiesi, 2017).

The effects hydrochar have on the composition of groups in soils has also been studied. It has been reported that hydrochar increases the abundance of Bacteroidetes and Proteobacteria, while decreasing Acidobacteria and Firmicutes groups (Andert & Mumme, 2015). The effects of hydrochar on AMF colonization are not clear, it has been shown both colonization inhibition (George et al., 2012) and promotion (Rillig et al., 2010).

The studies on the effects that biochar has on microbial communities are more studied than the ones on hydrochar. For example, PLFAs have been studied after the application of biochar. In the same way as ashes, biochar also promotes fungal communities PLFAs (Steinbeiss et al., 2009; Xu et al., 2018; Dangi et al., 2020). Biochar applications also have been reported to increase the number of gram-negative bacteria and actinomycetes (Prayogo et al., 2014) and nitrogen-fixing and denitrifying bacteria (Ducey et al., 2013). However, other studies showed no effect on soil microbial composition (Andrés et al., 2019). Many factors may influence the effects biochar has on microbial communities' composition, such as soil pH, the type of biochar or the soil type (Muhammad et al., 2014; Andrés et al., 2019). Finally, the effects of biochar on macrofauna have also been assayed; it has been reported that the addition of biochar may cause earthworm population to decrease (Sanchez-Hernandez et al., 2019; Zhang et al., 2019).

Hence, there are still many knowledge gaps on the potential effects hydrochar may cause on soil microbial communities. Since the effects of pyrolysis and gasification materials are dependent on their chemical composition, it is still unknown the potential effects dairy wastewater hydrochars may cause on soil biological indicators.

5   Conclusion

There are very few papers covering the effects of DWWS or its derivates on soil biological indicators. Nevertheless, it is expected that, on average, the effects REFLOW fertilizers may cause on soil microorganisms will be comparable to similar recovered fertilizers from other organic sources.

The general effects on microbial communities and contents of potential pollutants are summarized in Figure 6. It would be expected that the materials with the higher amounts in labile C would cause the strongest effects in soil microbial communities, yet these effects would be accompanied by higher presence of impurities and pollutants. Therefore, materials with higher contents in C are expected to contain larger amounts of heavy metals, organic pollutants and pathogens. Finally, also the materials with the higher contents of labile C are expected to have the strongest effect on soil pH.

Struvite or other precipitated salts are expected to have lower amounts of heavy metals compared to chemical fertilizers or organic C rich products. Since the precipitation of salts can be achieved selectively, co-precipitation of pollutants may be restricted. Moreover, parent materials with lower contents in contaminants can be selected for precipitation of phosphate salts.
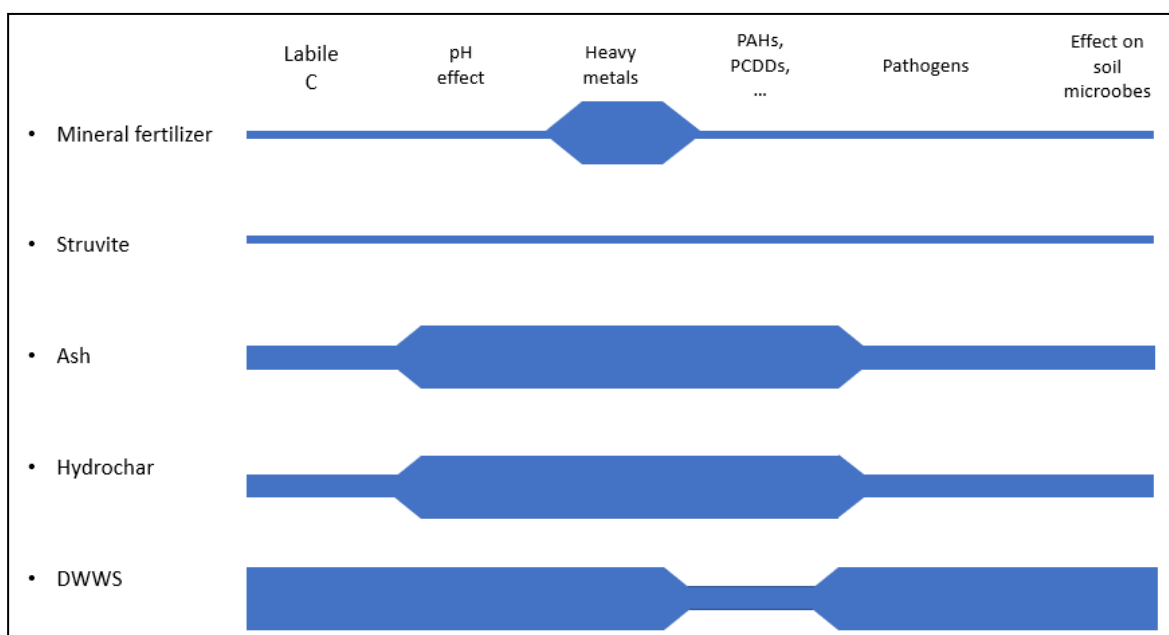

Figure 6. Summary figure. Broader strains represent a higher abundance or stronger expected effect.

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Nitrogen transformation and GHG emissions from soils amended with organic waste and derived fertilizer products (ESR8)

Biofertilizers such as biochar and hydrochar derived from organic waste can provide plant nutrients to crops like other organic fertilizers, and at the same time have the potential to mitigate greenhouse gas (GHG) emissions. Before using novel organic fertilizer produced from dairy waste, a strategy of biofertilizer application must be developed for farmers and decision-makers. This article introduces a suite of indicators for evaluating the nitrogen use efficiency (NUE) of biofertilizers and other organic fertilizers, and the mineral fertilizer equivalent (MFE) values giving the amount of mineral fertilizer nitrogen that the biofertilizer can substitute. The carbon sequestration for the application of various organic fertilizers in soil has been reviewed and negative side effects in form of GHG production and emissions due to application of different N and organic fertilizers are presented and discussed. The potential risks of organic fertilizer application were briefly stated. The aim of the review is to provide information to researchers, decision-makers and farmers about sustainable management of organic fertilizer applications in soil, so that they can develop and design the best application strategies for biofertilizers.

Nitrogen (N) is an essential macronutrient required by plants, crops, and animals, and N are worldwide one of the most crucial crop-yield limiting factors (EU Nitrogen Expert Panel, 2015; LeBauer & Treseder, 2008) . Thus, N-fertilizers have been applied to fields by farmers throughout the world - they have been available and affordable for farmers in developed countries since the 1950s and is now used by farmers in most countries (Smil, 2004). The application of N fertilizers has greatly increased the productions of food, feed, fiber and biofuel, thereby reducing the pressure on food resources of a growing global population (Smil, 2004; Xu et al., 2012). However, huge amounts of N input and mismanagement of N can have negative and harmful impacts on our environment, health, and economy (Galloway et al., 2004; Galloway et al., 2008; Sutton et al., 2011b). Therefore, it is important to properly manage N, especially in agriculture, which is the worldwide largest N user. It is important to develop agricultural N management that simultaneously fulfil agronomic goals (agricultural income, high crop and animal productivity) and environmental goals (minimum N losses) (EU Nitrogen Expert Panel, 2015) . However, the N management is not simple as agricultural N is easily lost into the environment during the N cycling. Since the 1990s the agri-environmental policy of the European Union (EU) has restructured the European N use and focused on reducing the N losses (expressed as N balance, which is a measure of N input minus N output) from agricultural land to the environment (Figure 1-1), and the N losses decreased from 2000 to 2010. However, there were no more decreases between 2010 and 2015, and N losses to the environment were 52 kg N ha^{-1} yr^{-1} of utilized agricultural area, which is considered to be unacceptable high (Eurostat, 2018b).
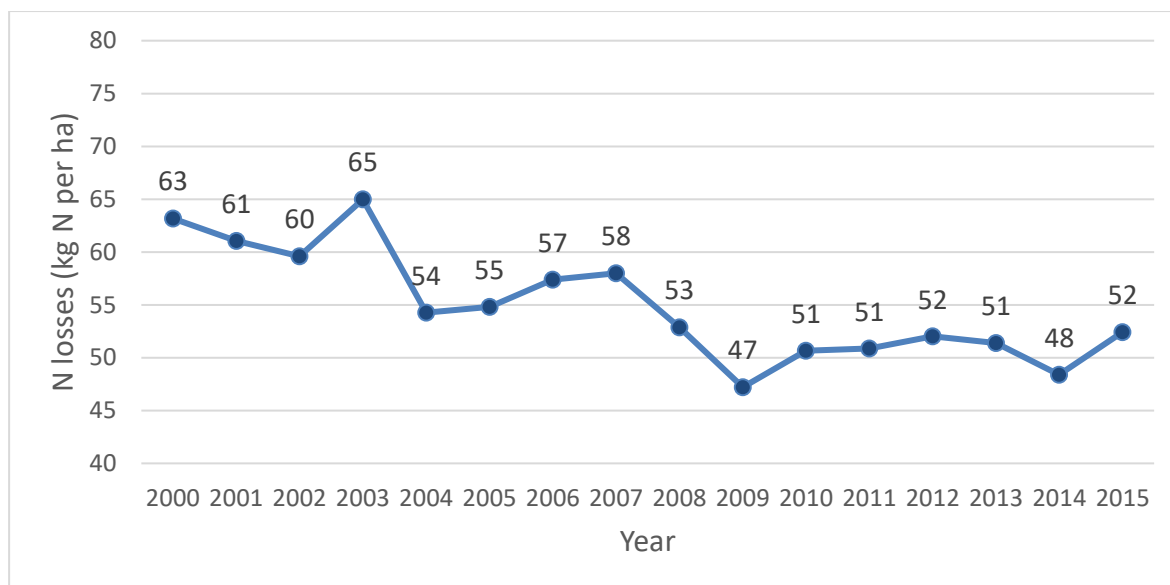
Figure 6-1 Nitrogen accounted for in the gross nitrogen balance (i.e. N losses to the environment) for EU agriculture (Eurostat, 2018b).

Nitrogen in the form of dinitrogen (N_2) gas is the most abundant element in the atmosphere (78%). Most living organisms cannot take up and use N_2, except for some archaea and bacteria with the ability to convert N_2 to bioavailable N via N fixation. The N-fixation is a process achieved by free-living bacteria and archaea (i.e. diazotroph) or a result of symbiotic relationships between N-fixing microorganisms and eukaryotes, such as unicellular haptophyte algae, termites, bivalves, and legumes (Kuypers et al., 2018). In agricultural and food systems, the major N losses are from ammonia (NH_3) volatilization, nitrate (NO_3^-) leaching, and emissions of N_2, nitrous oxide (N_2O) and nitrogen oxides (NO_x) through nitrification-denitrification reactions (EU Nitrogen Expert Panel, 2015) . Galloway et al. (2004) defined these N forms except N_2 as the reactive N (Nr) – the N components which are photochemically reactive, radiatively active, and biologically active (Galloway et al., 2004). The processes of Nr potentially endanger our health and environment are: atmospheric NH_3 and NO_x contributing to formation of particle matters (PM2.5) that is a risk to human health, tropospheric ozone that damage plants and crops, deposition of NH_3 and NO_x decreasing species diversity in natural ecosystems (eutrophication and soil acidification), NO_3^- leaching to surface and groundwater and causing eutrophication, N_2O emission leading to global warming and stratospheric ozone destruction (Galloway et al., 2008; Qiao et al., 2012; Sutton et al., 2011a).

Economic issues must be considered when developing strategies for N management. In 2017 European farmers used 11.6 million tons of N fertilizers and 1.3 million tons of P fertilizers, and this was an increase of 8 % (10.7 million tons) for N and a reduction of 9 % (1.5 million tons) for P since 2007 (Eurostat, 2019a). According to FADN (Farm Accounting Data Network), fertilizer costs account for around 10 % of the intermediate consumption (i.e. costs of agricultural raw and auxiliary materials, repairs and maintenance, etc.) in 2016 (EC, 2019). Across the EU, the fertilizer cost was 7500 EUR/farm in 2016, and as an average of all farms, it has increased by 4 % annually between 2006 and 2016. For dairy farms, the cost of fertilizer use per hectare increased by 1.7% annually during this period (EC, 2019).

Inefficient use of the mineral fertilizer and an associated reduction in N use efficiency will lead to substantial financial losses, and it is estimated that a 1% reduction in N use efficiency will increase the global cost for plant production with around $1.1 billion (Kant et al., 2010).

For the purpose of reducing negative environmental effects and increased costs of agricultural practices, there is a need for decision-makers and farmers to develop ideal strategies for recycling N and P by designing novel organic fertilizers that replace mineral fertilizers, and develop production systems with high fertilizer use efficiency, low N leaching and GHG emission.

The European Union (EU) is the largest milk producer in the world with an annual production of 172.2 million tons of raw milk corresponding to 20% of the global milk production in 2018 (Eurostat, 2019b). This makes the European dairy industry a key sector in the agri-food industry and is one of the most important industries in the EU (Bórawski et al., 2020). The European dairy processing industry is one of the large sources of industrial wastewater in Europe (Carvalho et al., 2013), and with the increase in the production of dairy products, the volume of dairy effluent is expected to increase. An average-size dairy factory in the EU produces approximately 180,000 m^3 of wastewater per year (Kothari et al., 2017), containing 14 - 830 mg L^{-1} of total nitrogen (TN), 9 - 280 mg L^{-1} total phosphorus (TP), 40 - 8,240 mg L^{-1} biological oxygen demand (BOD), 430 - 18,045 mg L^{-1} chemical oxygen demand (COD), and 24 - 4500 mg L^{-1} suspended solids (SS) (Ashekuzzaman et al., 2019). Transformation of dairy processing wastewater into organic fertilizers is an excellent pathway for not only reducing the high polluting potential but also recycling nutrients (such as N and P), which contributes to the EU goal for mitigation of negative effects in our environment and economy. The intention of this review is to collect and present the information and knowledge about N recycling in organic waste/biofertilizers (defined as the organic fertilizers recycled/produced from organic waste containing biomass) and the use of N-biofertilizer applications. The objective is to provide key indicators for management of N biofertilizers that can be used by decision-makers and farmers, and present the effects of biofertilizer applications on soil quality and risk of pollution swopping when recycling the waste with a focus on carbon sequestrations and mitigation of GHG emissions.

1   Nitrogen use efficiency for biofertilizer application

1.1   The nitrogen use efficiency (NUE) indicator

Globally most (around half) of the anthropogenic produced N is used by farmers in the form of N fertilizers (Schlesinger, 2009). Thus, optimizing N management to achieve high yields of crops with high N fertilizer efficiency is a key constituent of food security, environmental sustainability, and economic profit maximization. In consequence N efficiency indicators should be developed to track and evaluate the magnitude of N use efficiency. The EU nitrogen expert panel (2015) introduced the nitrogen use efficiency (NUE) that can be applied to evaluate the relative transfer of N inputs into agricultural products and assess

the risks of environmental N losses in agriculture at the farm, regional or national level. The concept is based on the mass balance principle (i.e. a ratio of N outputs over N inputs) and it can be estimated through a series of measures, such as the amount of crop growth per unit of N applied (e.g., mineral N fertilizers and manure); N in meat or milk production as a fraction of N added in food to the animals producing the milk; or the amount of N exported per unit of N imported on a farm. In order to estimate the NUE the data needed are:

a) total inputs of N into the system and total outputs of N stored in harvested or in animal products,

b) the system nature (such as crop system, livestock feeding system, food processing and distribution system, and farm) and its boundaries,

c) the time periods of the studies, and

d) likely changes in N stocks in the system.

The NUE indicator is displayed through a two-dimensional input-output diagram (Figure 2-1), which allows the NUE, N output and N surplus as well as possible reference/target values to be presented in a coherent manner.
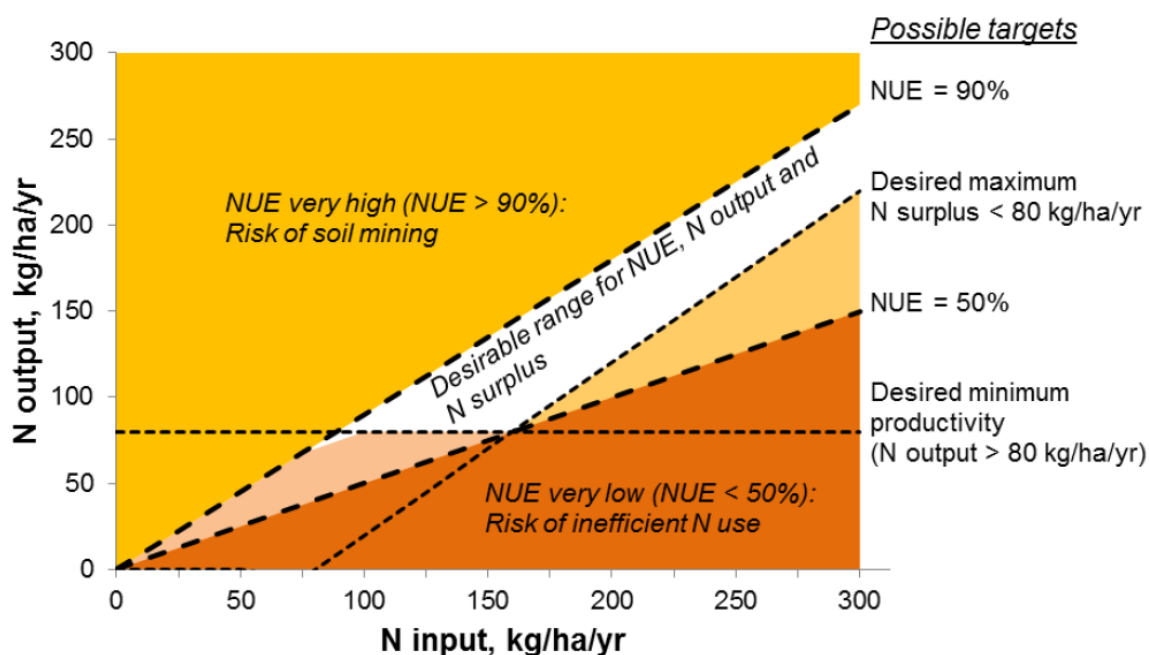
Figure 1-1 Conceptual framework of the Nitrogen Use Efficiency (NUE) indicator (EU Nitrogen Expert Panel, 2015) .

In Figure 2-1, there are three areas separated by the two dotted lines "NUE = 90%" and "NUE = 50%", i.e. an area (top) with very high NUE (> 90%), and an index between with desirable range for NUE, N output and N surplus, and an area (bottom) with very low NUE (< 50%). The principle of these three areas is that very high and very low NUE are both not desirable. "NUE very low" reveals low resource utilization efficiency and high N losses;

"NUE very high" reveals resource exhaustion - soil N depletion (i.e. soil nutrient mining). In developing regions such as rural Africa, the mining of N as well as other nutrients from soils are a common phenomenon, resulting in soil degradation, erosion and poverty. On the other hand, in some developed countries, mining nutrients from high-fertile soils can be considered an ideal way, as it leads to the intensive use of resources and may reduce potential nutrient losses (EU Nitrogen Expert Panel, 2015) . The pre-set reference values are suggested by the EU nitrogen expert panel (2015): the reason why the NUE of 90% set as the upper limit is that some N losses in practice are inevitable. Moreover, the lower limit that NUE of 50% is set since many cropping systems have demonstrated that value is achievable. However, the reference lines are tentative as they depend on agricultural and food systems as well as environmental conditions. There is a horizontal dotted line setting in the figure, illustrating a target N output value of 80 kg N per ha per year, which is the desired minimum productivity from its cropping system. That line is also variable and depends on the system nature (e.g. crop and soil type, climate, etc.) and in some cases (e.g. for livestock systems) depends on animal type, species and animal feed nature. The dotted line across the "NUE = 50%" and the "desired minimum productivity" is set to indicate a maximum level of N surplus, which serves as a proxy for potential total N losses via volatilization of NH_3, leaching and denitrification of NO_3^-, possible changes in system N stocks, and uncertainties in estimating N inputs and N outputs in the mass balance of N. The target value for maximum N surplus also depends on the regional and system conditions, such as the balance of N loss pathways and thresholds of N species concentrations in surface and ground waters, the vulnerability of habitats, and the contribution and impacts of NH_3 volatilization and N_2O emissions into the air.

1.2   The NUE indicator used at different scales

The proposed NUE indicator is suitable for determining realistic targets and monitoring the progress of systems at national, regional and field scales, especially in relation to food and nutrition security, sustainable consumption and production, marine ecosystems and terrestrial ecosystems. The reference value of NUE, N output and N surplus will be estimated based on the type of agricultural systems, climate-soil-environmental conditions, and the type of N inputs. It is an excellent indicator for academic researchers, extension service officers, farmers, industrial engineers and policy decision-makers. To use the NUE indicator, the N input and N output values of the systems will be mapped into the framework (Figure 2-1) and the results can illustrate the productivity and environmental impacts of the corresponding systems. Examples for various systems at different scales illustrating the NUE indicator and its related interpretations with the application of the two-dimensional input-output diagram (Figure 2-1) are discussed.

1.2.1   The NUE indicator used at the field scale

The example for the concept of the NUE indicator used at the field scale is the Broadbalk experiment at Rothamsted in the UK, which is the oldest continuous agronomic experiment in the world where they have grown winter wheat annually since 1843 (EU Nitrogen Expert Panel, 2015) . Besides, the fertilizer treatments and a control plot have been maintained every year, to which there was not been added fertilizer, manure, compost or other residues. In that experiment, a single winter wheat variety (Hereward) was planted

annually from 1996 until 2012, and the mean results were plotted in the framework of the NUE indicator (Figure 2-2). Due to the increased incidence of soil-born fungal diseases in crop rotations, it is notable to recognize that the wheat yields as well as N yields are lower for the continuous wheat (green circle dots) compared to the first year wheat (purple square dots). When the application rates were low (0 to 50 kg ha^{-1} yr^{-1}), the NUE was slightly higher than 90%, implying that there might be other N sources (such as N from soil and atmosphere) being utilized besides N fertilizers. The application rates ranging between 100 and 200 kg ha^{-1} yr^{-1} made the NUE falling in the "desirable range" but when the application rates increased over 200 kg ha^{-1} yr^{-1}, the NUE values tended to decrease and N surplus could be higher than the expected target, resulting in N losses. This example properly demonstrates the usefulness of the NUE indicator, and the results show that for the "high inputs – high outputs" winter wheat cultivation, the tentative reference values of NUE, N output and N surplus can be achieved simultaneously (i.e. the dots fallen in the desirable range). What is more, this two-dimensional input-output diagram also suggested the importance of crop rotations for simultaneously achieving the expectations of high NUE, high N outputs, and low N surplus.
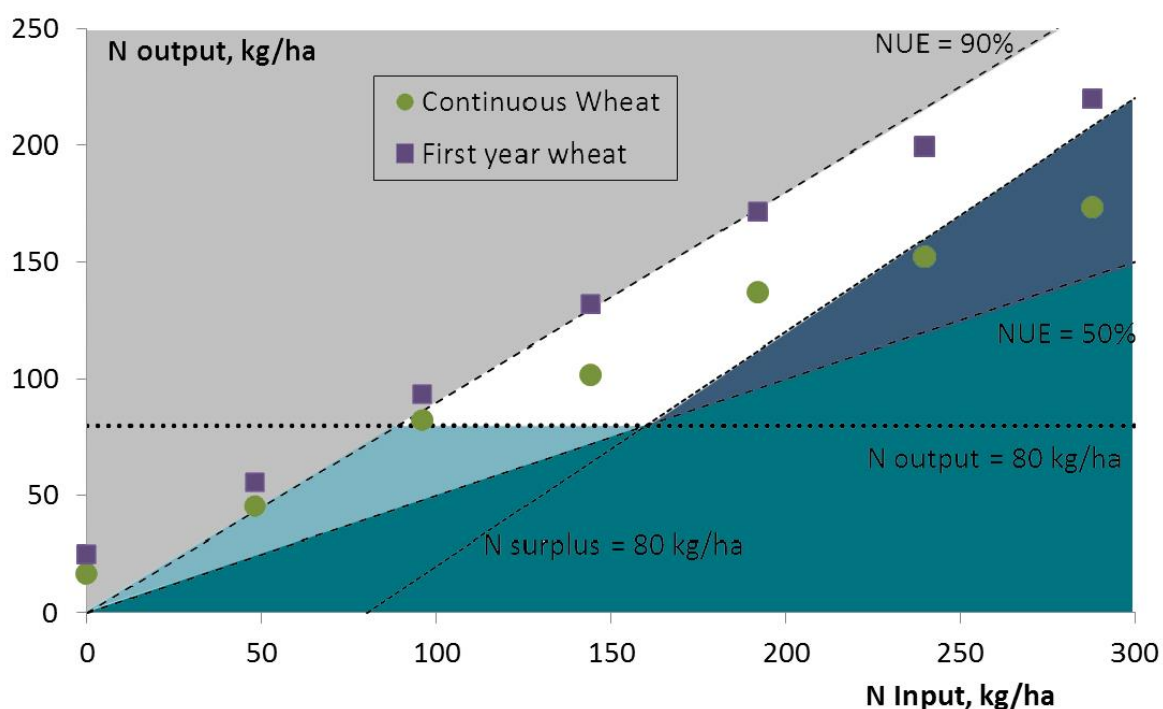
Figure 1-2 Relationship between annual N input via N fertilizer and annual N output via harvested grains and straw for continuous wheat and first-year wheat in a rotation of oats-maize-wheat-wheat-wheat from 1843 onwards (EU Nitrogen Expert Panel, 2015) .

1.2.2 The NUE indicator used at the national scale

The concept of NUE indicator may not only be used at the field scale but also at larger scales such as the national scale. The relationships between agricultural N inputs and N outputs for the 28 member states of the EU (EU-28) from 2004 to 2011 is illustrated in the two-dimensional input-output diagram of the NUE indicator (Figure 2-3). The eight-year means N inputs and outputs ranged between 60 - 370 kg ha^{-1} yr^{-1} and 40 – 190 kg ha^{-1} yr^{-1}

respectively, with the NUE ranging between 48 – 112%. The upper value (112%) of the NUE (for Romania) suggested that N outputs were larger than N inputs and implied the soil mining occurred. This diagram notably indicated substantial differences between countries for the NUE, and the NUE values of most countries were fallen between the reference values (50 – 90%). It also revealed that when N output was less than 80 kg ha^{-1} yr^{-1}, the N surplus was always less than the desired maximum value (80 kg ha^{-1} yr^{-1}). Moreover, the results were considerably different between the new and old member states, and for some new member states, soil N mining was possible to occur.
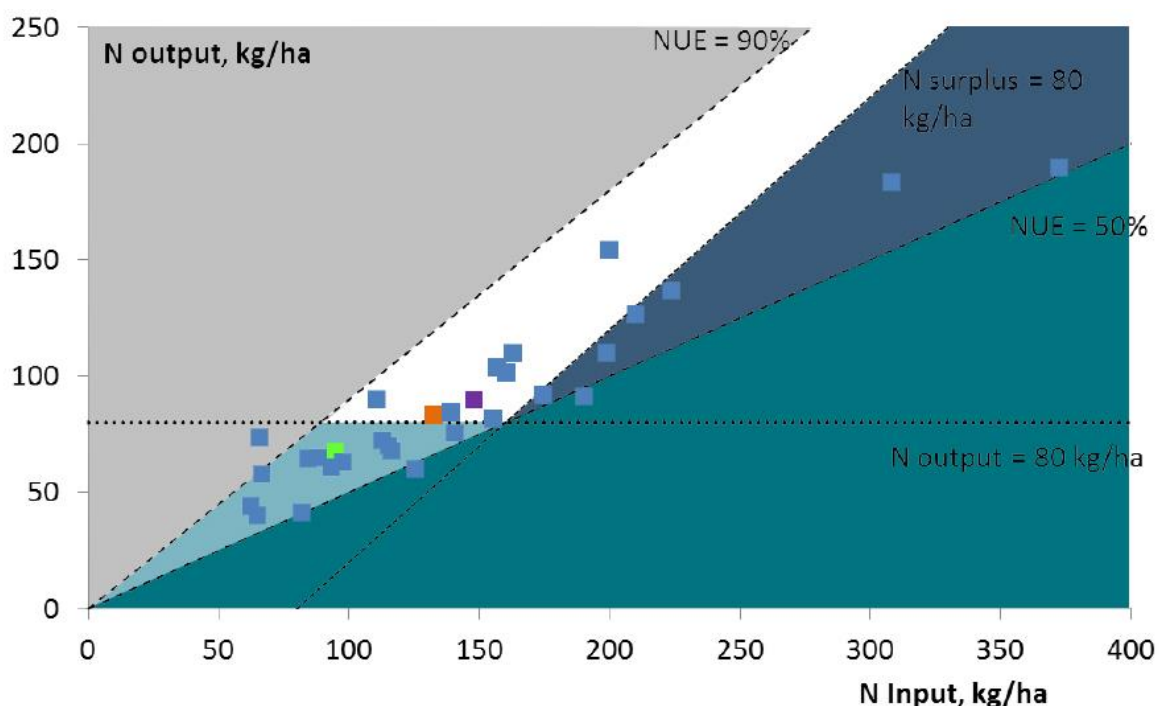
Figure 1-3 Relationships between N output and N input of agriculture in the Member States of the EU (each blue dot represents a member state). Green dot – the mean for the 12 new member states (EU-12); orange dot – the mean for the EU-28; purple dot – the mean for the 15 so-called old member states (EU-15) (Eurostat, 2018a).

1.2.3 The NUE indicator for mixed systems

In addition to each specific crop type or various crop production systems, the concept of NUE indicator can be also applicable to the systems of mixed crop-livestock production, where livestock consume some or all of the crops grown in the farm, and livestock products are N outputs of the farm (Figure 2-4). The total N inputs and outputs for mixed crop-livestock production systems ranged between 80 – 450 kg ha^{-1} yr^{-1} and 50 – 200 kg ha^{-1} yr^{-1}, respectively. The N surplus ranged between 50 – 300 kg ha^{-1} yr^{-1}, and the NUE values ranged between 20 – 50%, which tended to rise with time. Obviously, the reference values for NUE, N output and N surplus were not achieved simultaneously. The main reasons included the relatively low conversion efficiency of agricultural protein N in milk and meat, and the relatively high N losses due to NH$_3$ volatilization, N leaching and denitrification. Because of increases of productivity, decreases of N losses through NH$_3$ volatilization and N leaching, and decreases of N inputs through N fertilizers and animal feed, the NUE increased from 28% (a mean during 1998 – 2001) to 38% (a mean during 2010 – 2013)

(Oenema, 2013). On livestock production farms, as the NUE is a balance of N inputs (e.g. N feeds to animals, N in manure to fodder crops, etc.) and outputs (e.g. N in animal products and by-products, N manure to crop production), it can be conveniently used to monitor changes in the farming system, for changes in both crop production and animal production. Based on this example, it is clear that the NUE concept is applicable for mixed crop-livestock production systems but may need different reference values.
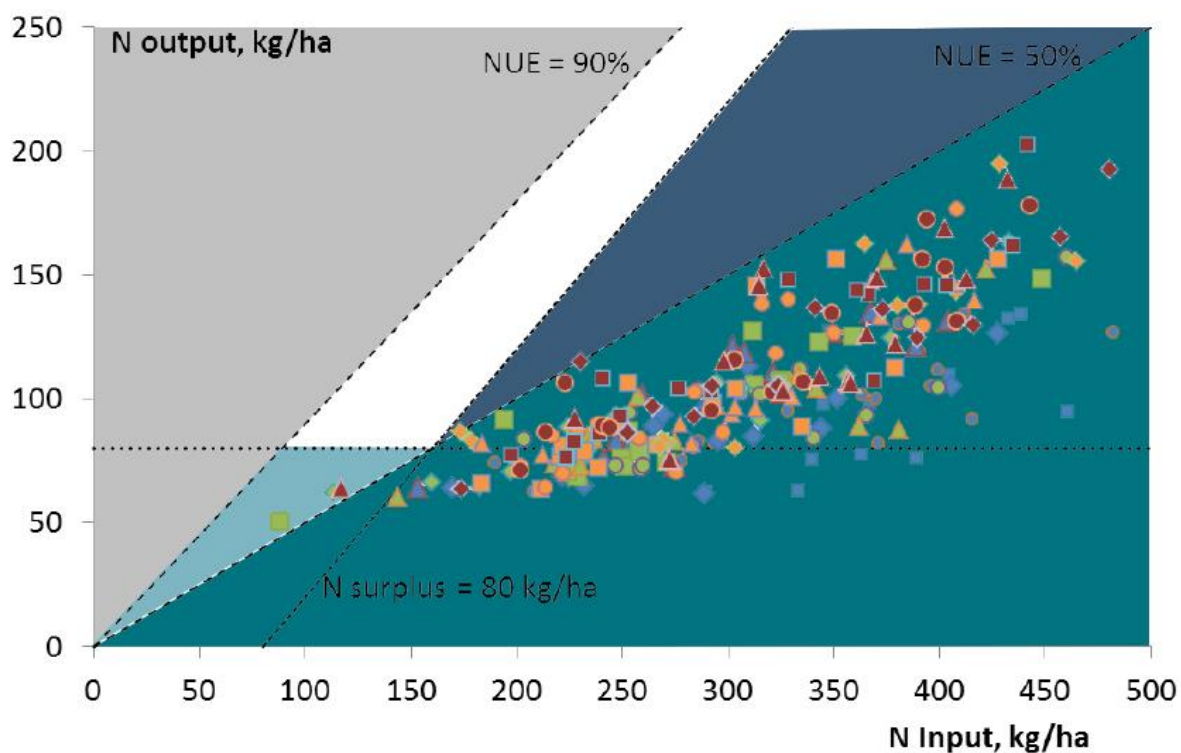
Figure 1-4 Relationship between N inputs through N fertilizers, animal feed, clover N fixation, and atmospheric N deposition and N outputs through sales of milk and cattle on 16 customized dairy farms in the Netherlands from 1998 to 2013. Different symbols indicate different years; blue symbols - 1998-2001; green symbols - 2001-2005; orange symbols - 2006-2009; and red-wine symbols 2010-2013. The data was from (Oenema, 2013).

1.3   Other indicators illustrating N fertilizer use efficiency

Based on the concept of the NUE indicator, there is a range of indicators or factors being derived from the literature (Cormier et al., 2016; Ladha et al., 2005; Sommer et al., 2013) and broadly used for calculation of N fertilizer use efficiency. The most typical indicators include the agronomic efficiency (AE) of N, the fertilizer N uptake efficiency (NUpE), and the N mineral fertilizer equivalent (N-MFE) value.

1.3.1   Agronomic efficiency (AE)

The N agronomic efficiency (AE) can be defined as the amount of extra grain harvested per kilogram of N applied to crops, which reflects the direct production impact with fertilization and also guides for the economical use of fertilizers (Ladha et al., 2005; Sommer et al.,

2013). So the AE is calculated as the ratio of yield increase (ΔY) to N supply (ΔN), with the unit of kg grain produced kg^{-1} N fertilizer applied:

$$AE = \frac{(Y_T - Y_0)}{(N_T - N_0)} = \frac{\Delta Y}{\Delta N}$$ *(Equation 1-1)*

where Y_T is grain yield (kg ha^{-1}) in treatment with N fertilizer applied (N_T, kg ha^{-1}), Y_0 is grain yield (kg ha^{-1}) in control with no N fertilizer applied or only a supply of N from the environment (N_0, kg ha^{-1}).

1.3.2   Fertilizer N uptake efficiency (NUpE)

The fertilizer N uptake efficiency (NUpE) can be defined as the amount of extra N assimilated by the crop from per kilogram of N fertilizer applied to the crop, i.e. the ratio of additional N absorbed to N fertilizer supply (Ladha et al., 2005; Sommer et al., 2013). It can be calculated with the differences in N uptake (ΔU) divided by the differences in N fertilizer supply (ΔN), expressed as a ratio or a percentage:

$$NUpE = \frac{(U_T - U_0)}{(N_T - N_0)} = \frac{\Delta U}{\Delta N} * 100\%$$ *(Equation 1-2)*

where U_T is the total N uptake (kg ha^{-1}) in treatment with N fertilizer applied (N_T, kg ha^{-1}), U_0 is the total N uptake (kg ha^{-1}) in control with no N fertilizer applied or only a supply of N from the environment (N_0, kg ha^{-1}).

The two commonly used efficiency indicators (AE and NUpE) are regularly calculated by the N balance, also known as the N difference or the apparent N efficiency of applied N fertilizer method (Ladha et al., 2005). Meanwhile, the NUpE can be also measured via the ^{15}N isotopic analysis ($NUpE_{15N}$). This technique can measure the N recovery rate in subsequent crops, but since it is costly and complicated to implement, the N balance method is more broadly adopted (Ladha et al., 2005).

In agricultural practices, generally only the total amount of N in aboveground biomass or the N uptake in the crops at harvest is measured. The NUpE calculation (Equation 2-2) is sometimes defined as the apparent N recovery (ANR), since it is based on the assumption that the additional N uptake (ΔU) comes only from the additional fertilizer applied, which implies that the N uptake from the soil (or environment) does not change with increasing fertilizer addition. This is not always the case, as increased crop growth may promote the growth of roots and absorption of additional mineral N from the soil; or vice versa, increased N supply will reduce root development and reduce crop uptake of native soil N (Sommer et al., 2013).

1.3.3   N mineral fertilizer equivalent (MFE) value

The N mineral fertilizer equivalent (MFE) value is a vital indicator for organic N waste/biofertilizer application as it describes how efficient organic fertilizers, biofertilizers or animal manures are in providing N available to crops compared to mineral N fertilizers. An indicator N fertilizer replacement value (NFRV) is also widely used in the literature,

which is a synonym for the N-MFE value (Sommer et al., 2013). The N-MFE specifies that, under a certain series of conditions (i.e., climate, time of fertilizer application, type of crops, etc.), how much organic N fertilizer can be used to replace a given amount of mineral N fertilizer that can achieve the same effect on crop productivity/N uptake. The mineral N fertilizer used as reference fertilizer is always a popular and highly efficient one, such as ammonium nitrate based fertilizers (Sommer et al., 2013).

There is a range of methods to measure and calculate the N-MFE based on different methods of the NUE calculation as discussed above. One method is based on the crop yield response to organic and mineral fertilizers, respectively. It uses the AE of organic N fertilizer divided by the AE of mineral N fertilizer and thus the MFE value of N can be defined as the ratio between both indicator values:

$$MFE_{AE} = \frac{AE_{org-N}}{AE_{min-N}} \times 100\% \qquad \textit{(Equation 1-3)}$$

where AE_{org-N} is the agronomic efficiency for organic N fertilizer application and the AE_{min-N} is the agronomic efficiency for mineral N fertilizer application. The MFE_{AE} can also be determined as the ratio between two slope coefficients, i.e. the ratio of AE of organic fertilizer and mineral fertilizer (Figure 2-5a). However, this method is not faultless, since the AE must be determined at the same level of yield or N input and when it is close to the maximum yield, the N-MFE value may have a huge error. Furthermore, the N-MFE sometimes will be over 100% due to the effects of non-N fertilizers (Sommer et al., 2013).

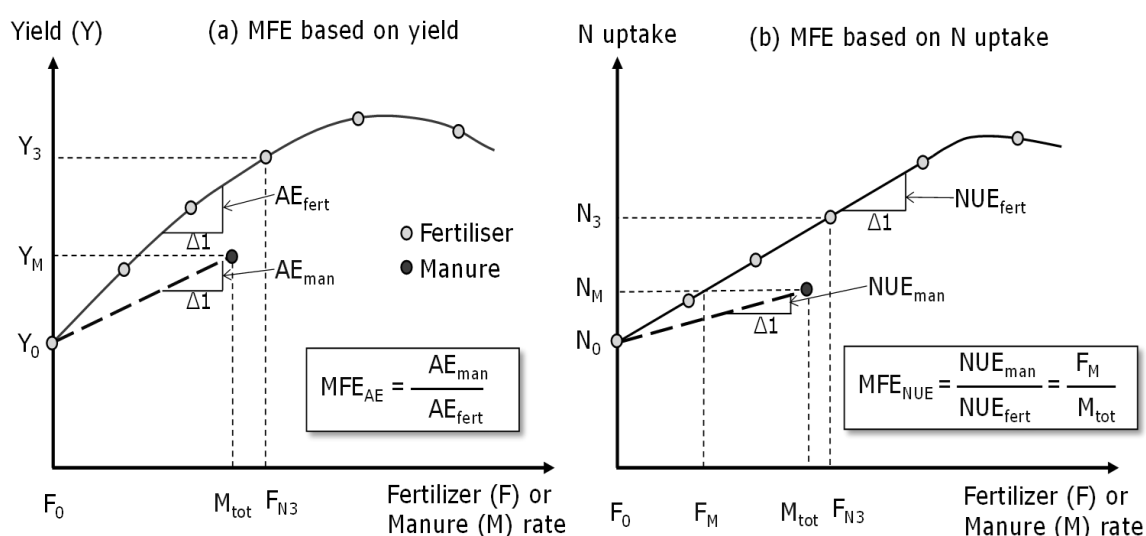**Figure 1-5 Illustration of the concepts for MFE value determination, based on (a) crop yield response or (b) crop N uptake (Sommer et al., 2013).**

To avoid those problems, another alternative method for calculating the N-MFE is derived. It is based on the crop N uptake from organic and mineral N fertilizers, respectively. The N-MFE thus equals to the NUpE of organic N fertilizer divided by the NUpE of mineral N fertilizer:

$$MFE_{NUpE} = \frac{NUpE_{org-N}}{NUpE_{min-N}} \times 100\% \qquad \textit{(Equation 1-4)}$$

where $NUpE_{org-N}$ is the fertilizer N uptake efficiency for organic N fertilizer application and the $NUpE_{min-N}$ is the fertilizer N uptake efficiency for mineral N fertilizer application. Meanwhile, the MFE can be also calculated via the N input level of mineral fertilizer divided by the level of organic N fertilizer added which can yield the same crop N uptake as the former one does:

$$MFE_N = \frac{F_m}{M_{tot}} \times 100\% \qquad \textit{(Equation 1-5)}$$

where F_m is the mineral fertilizer N input level and the M_{tot} is the organic fertilizer N input level that leads to a common amount of crop N uptake as the mineral N fertilizer does.

The advantage of the concept of MFE_{NUpE} is that in a relatively large range of N fertilizer application rates, the N uptake is generally linearly related to fertilizer N input (Figure 2-5b) compared to that of MFE_{AE}, and therefore, not like the MFE_{AE}, the MFE_{NUpE} will not be affected by N input and yield levels. However, this method also has several disadvantages. It requires representative sampling of the entire plant (including roots) for chemical analyses. However, since that is extremely costly, it generally only samples the aboveground biomass or even less - just the harvested grain for chemical N analyses. That will lead to inaccurate results if the sampled parts cannot represent the entire crops (Sommer et al., 2013).

It is notable that because of the differences in protein content of crops that absorb N from organic and mineral sources, the MFE values based on the N uptake (i.e. MFE_{NUpE}) are usually slightly lower than the MFE values based on the yield response (i.e. MFE_{AE}). What is more, the MFE values are tentative and depend on the experimental conditions such as soil type, crop type, climate, application method and rate, etc (Sommer et al., 2013). Thus, it is vital to determine the specific MFE values for each type of organic fertilizers.

1.4   The N-MFE values for organic waste and animal manure

To meet the demands of sustainable agriculture, the huge amount of mineral N fertilizer application should be limited, and more types of organic wastes and animal manures can be used as replacements of mineral fertilizers. Therefore, it is extremely essential to have an overview of the NUE and N-MFE values for various organic fertilizers before application.

There is a range of factors having effects on the N-MFE values for different organic fertilizers (Sommer et al., 2013; Webb et al., 2013), including

a) the proportion of ammonium N ($NH4+$-N) in the total N content of each organic fertilizer (the most important and decisive factor for the magnitude determination of the MFE values);
b) the organic fraction and the C/N ratio;
c) the time of application;
d) the crop types and species;

e) the application method; and

f) the method to calculate the MFE values.

In addition, it is notable that when calculating and reporting the N-MFE values, the first-year N-MFE values and the cumulative MFE values after long-term fertilizer application can be different, since many organic fertilizers are "slow-release" fertilizer and its organic N content will be available for the crop due to N-mineralization in the years after application.

In the literature on may find N-MFE values of different organic fertilizers (Table 2-1). The organic fertilizers reviewed below include animal manure/waste (cattle deep litter and slurry, human urine, meat and bone meal, and pig slurry), sewage sludge, and compost (composted fish sludge, neutral and acid compost, and source-segregated and composted organic household waste). The N-MFE values of these bio-fertilizers range from 7 to 101% indicating how important it is to know this factor when using them for crop production. The cited data are from both short-term pot/field studies and long-term field studies and for bio-fertilizers, and in short-term studies the bio-available N is the growth-limiting factor, which was confirmed by Brod et al. (2012) and Gomez-Munoz et al. (2017). They also found that the MFE values of meat and bone meal (MBM) and composted fish sludge (CFS) decreased from 76 to 65% and 67 to 53% with the increase of fertilizer application rates. Those results confirmed previous studies stating that increasing amounts of organic fertilizers led to a decrease of fertilizer effectiveness, for manure, composted biowaste, and liquid biogas residues (Brod et al., 2012). They indicated that the two industrial composts (neutral and acid Dynea composts) with lower MFE values (7 - 30%) was more like soil amendments instead of N fertilizers, as the N mineralization rate was pretty slow due to reduced availability of carbon in composts for growth of soil microorganisms (Brod et al., 2012). In addtion, after acidification, the NUE and MFE value of Dynea composts (added acids) were higher than those of neutral compost (Brod et al., 2012). Similarly, acidification of pig and cattle slurry which reduces NH_3 emission may also increase the MFE values after surface-spreading of pig slurry from 74 to 101% and of cattle slurry from 39 to 63%. In long-term studies adding sewage sludge to silage maize the MFE values were 55% at low application rate and 64% at high application rate (Černý et al. (2012). This result compared with the finding by Brod et al. (2012) implied that in short-term fertilizer application, doubling the application rate might not have a higher NUE and MFE values, but in the long-term application, an increase of the rate would work for improving the NUE and MFE values. The reason can be that higher application rate in short-term studies leads to emission of easily available N (NH_3 emission, denitrification) and that reduces MFE values, while in long-term studies the initial losses of these components are not affecting the total N available so much, due to N-mineralization of added organic N will contribute much available N during later years. Gomez-Munoz et al. (2017) found in the long-term experiment that continuous application of agricultural and urban wastes improved soil quality, and not like the short-term N availability, long-term N availability was more related to the accumulation of N and C in soil. They reported the values of NUE and MFE in the final year (2013) were generally increased compared to those in the first year (2003), except for composted household waste and cattle deep litter (Table 2-1).

Based on those findings reviewed above, we can conclude that for biofertilizer application,

a) low C/N ratio wastes can be better for improvement of soil N availability, crop N uptake, the NUE and the MFE values;

b) long-term application of biofertilizer should be a better solution to achieve higher MFE values;

c) acidification in advance can be considered into the strategy of biofertilizer application; and

d) application rate is supposed to be appropriate and reasonable for both crop growth and MFE maximization.

Table 1-1 Comparisons of N-MFE values of various organic fertilizers for agricultural productions in different regions in the EU

Region	Study type	Crop type	Fertilizer type	pH	Total N (g kg^{-1} DM)	Inorganic N (mg kg^{-1} DM)	C/N ratio	Application rate	NUE
Copenhagen, Denmark	Long-term field study	Spring oats (*Avena Sativa* L.)	Human urine	7.1	1.86	8.7	9.1	171 kg N ha^{-1} yr^{-1}	44% (2003), 73% (2013)
Copenhagen, Denmark	Long-term field study	Spring oats (*Avena Sativa* L.)	Sewage sludge	7.0	2.05	12.8	9.3	314 kg N ha^{-1} yr^{-1}	11% (2003), 51% (2013)
Copenhagen, Denmark	Long-term field study	Spring oats (*Avena Sativa* L.)	Source-segregated and composted organic household waste	7.3	2.57	12.0	9.4	303 kg N ha^{-1} yr^{-1}	27% (2003), 11% (2013)
Copenhagen, Denmark	Long-term field study	Spring oats (*Avena Sativa* L.)	Cattle deep litter	7.4	2.21	12.6	10	232 kg N ha^{-1} yr^{-1}	22% (2003), 16% (2013)
Copenhagen, Denmark	Long-term field study	Spring oats (*Avena Sativa* L.)	Cattle slurry	7.3	1.83	8.7	9.1	119 kg N ha^{-1} yr^{-1}	32% (2003), 55% (2013)
Ås, Norway	Short-term pot study	Italian ryegrass (*Lolium multiflorum*)	Meat and bone meal	6.5	90	312.8	5	150 & 300 kg N ha^{-1}	68% (150), 57% (300)

Ås, Norway	Short-term pot study	Italian ryegrass (*Lolium multiflorum*)	Composted fish sludge	5.7	69	2586.9	7	150 & 300 kg N ha^{-1}	59% (150), 48% (300)
Ås, Norway	Short-term pot study	Italian ryegrass (*Lolium multiflorum*)	Neutral Dynea compost (Industrial compost)	7.3	73	928.7	5	150 & 300 kg N ha^{-1}	11% (150), 5% (300)
Ås, Norway	Short-term pot study	Italian ryegrass (*Lolium multiflorum*)	Acid Dynea compost (Industrial compost)	3.5	78	2437.0	5	150 & 300 kg N ha^{-1}	25% (150), 17% (300)
Prague, Czech Republic	Long-term field study	Silage maize (*Zea mays* L.)	Sewage sludge	-	36.6	-	-	120 & 240 kg N ha^{-1} yr^{-1}	29% (120), 20% (240)
Foulum, Denmark	Short-term field study	Winter wheat (*Triticum aestivum* L.)	Pig slurry	7.45 & 6.17	4.37 & 4.43	3660 & 3640	-	100 kg NH$_4^+$-N ha^{-1}	-
Foulum, Denmark	Short-term field study	Winter wheat (*Triticum aestivum* L.)	Cattle slurry	7.03 & 6.01	2.65 & 2.58	1500 & 1520	-	100 kg NH$_4^+$-N ha^{-1}	-

[1] (Gomez-Munoz et al., 2017); [2] (Brod et al., 2012); [3] (Černý et al., 2012); [4] (Sorensen & Eriksen, 2009)

2 Carbon sequestration in application of organic waste and biofertilizers

The soil organic matter (SOM) in farmland is one of the most important components of soil. Its physical and chemical properties, and decomposition and fixation processes can directly or indirectly determine the soil quality (such as the stability of soil structure, soil fertility, etc.), which is the basis for high and stable crop yields (Melillo et al., 1995). In addition, soil organic carbon (SOC) is the main source and sink of carbon dioxide (CO_2) in the atmosphere, and soil carbon pools are crucial carbon pools and sinks in ecosystems, whose carbon reserves are over 2.70 trillion tons. Changes in the SOC and soil carbon pools thus play a key role in the exchanges of global greenhouse gases between soil and atmosphere (Franzluebbers, 2005; Powlson et al., 2011).

The soil carbon pool consists of organic and inorganic carbon pools. In humid and semi-humid areas organic carbon pools are major components and composed of animal and plant residues, microbial debris and soil humus. In arid and semi-arid areas inorganic carbon pools are larger fractions and mostly in the form of carbonates (Post et al., 1990). It is estimated that about 1500 Pg of organic carbon and 1200 Pg of inorganic carbon are stored in the global soil within the range of 0-1 m (Kirschbaum, 2000). Due to the large soil carbon pools, even small changes will have a great impact on the concentration of greenhouse gases in the atmosphere and terrestrial ecosystems (Smith, 2008). For example, a 10% reduction in the SOC pools is equivalent to the amount of CO_2 emitted during 30 years of human activities (Kirschbaum, 2000); a 5 to 15% increase of the SOC content in the 0 to 2 m layers of soil will reduce the atmospheric CO_2 concentration by 16 to 30% (Baldock, 2007). Moreover, global warming and soil degradation is one of the major reasons for crop yield reduction (Fungo et al., 2017; Luo et al., 2016; Sheng et al., 2016).

Therefore, adding carbon when applying biofertilizers to soil is beneficial for our agricultural production and environmental health. The addition of SOC will have notably effects on the improvement of soil fertility because it improves the physical structure and water holding capacity of the soil, but it enhances the soil nutrient supply capacity, thereby promoting high and stable crop yields. In addition, the increase of SOC is important for the reduction in atmospheric CO_2 concentrations and mitigation of climate change.

2.1 Carbon addition

In agricultural production, it is necessary to apply N fertilizers and organic fertilizers (such as organic waste and animal manure), in order to increase the effective N content in soil, which contributes to high and stable crop yields (Pan et al., 2009). Many studies have shown that increasing the applied amount of N fertilizers can increase the content of SOC in the top layers of soil (via increased uptake of carbon by crops - capture) (Halvorson et al., 1999). Alvarez (2005) developed a regression model to evaluate the effects of N fertilizers applied on SOC sequestration using a data set containing characteristics of 137 soil from different climates, soils categories and management. This study showed that application of N fertilizers could increase the SOC storage by approximately 12.0 t C ha^{-1}

when crop residues were returned to the fields. This result is confirmed by a range of studies showing that application of organic fertilizer increases the SOC content in the world by about 33%, which translates to the SOC has been increased by 699.6 in upland soils and 401.4 kg C ha^{-1} yr^{-1} in paddy soils (Rudrappa et al., 2006; Xia et al., 2017; Zhang et al., 2010; Zhang et al., 2012b).

Long-term field fertilization experiments in China showed that the application of organic fertilizers combined with chemical fertilizers increased the SOC content of dry farmland, increasing at a rate of about 0.1 to 0.4 g kg^{-1} yr^{-1} (Zhang et al., 2010). In similar crop rotation system of the long-term experiments in Ohio, USA, the rate of SOC sequestration is significantly higher in soil added organic fertilizers (392 kg C ha^{-1} yr^{-1}) than soils added mineral fertilizers (355 kg C ha^{-1} yr^{-1}) (Jarecki et al., 2005). Furthermore, the long-term experiment in Oregon (started in 1931) showed that in a wheat (*Triticum aestivum* L.)-fallow rotation system, the annual yield and the C and N content of the soil surface layer (0-30 cm) are higher in fields that in all years are added organic fertilizer than in those under other fertilization treatment. The content of SOC decreased gradually in the soils that were not added organic fertilizer (Parton & Rasmussen, 1994). A study revealed that the SOC was an important factor in crop production, and mentioned the importance of increasing SOC by farm compost amendment (carbon addition) (D'Hose et al., 2014). Another study further evaluated the main factors affecting carbon sequestration through long-term trials in the semi-arid area of the northwestern United States. It pointed out that compared to N fertilizers, the application of organic fertilizers could reduce the loss of SOC and have a significant impact on SOC content, because organic fertilizers could provide a large amount of exogenous C input (i.e. carbon addition), which could increase the total C input by 30% to 80% (Rasmussen et al., 1998).

2.2 Carbon removal

Carbon removal and storage or sequestration of CO_2 is another vital process involved in carbon sequestration besides carbon addition, in order to slow or reverse atmospheric CO_2 pollution and to mitigate or reverse global warming (Minx et al., 2018; Sedjo & Sohngen, 2012). A range of research reported that some kinds of organic waste (biofertilizer, e.g. biochar) had the potential to contribute to carbon removal by increasing SOC (Kambo & Dutta, 2015; Libra et al., 2011; Nguyen & Lee, 2016; Plaza et al., 2016; Xu et al., 2016b).

Biochar is a highly aromatic carbonaceous materials produced from pyrolysis of organic wastes (biomass) under 250-700 °C anoxic or hypoxic conditions. The organic waste used in the process includes agricultural wastes, forestry wastes, aquatic organisms, human and animal manure, industrial and municipal wastes (Colantoni et al., 2016; Tripathi et al., 2016). It is estimated that with the biochar process 7.6 t ha^{-1} yr^{-1} of CO_2 could be recycled to soil and by 2100, 9.5×10^{10} t of carbon potentially may be stored in the soil globally (Kuppusamy et al., 2016), which could offset 12% of the anthropogenic C emissions (Fungo et al., 2017). Microporous materials (e.g. biomass chars), and especially N-doped carbon materials, have a potential for adsorbing large amounts of CO_2 at temperatures between 150 and 500°C (Lee et al., 2008). Biochar can be used as a low-cost and highly selective solid adsorbent in air filters to adsorb CO_2 in the gas phase and thus contributes to reduce CO_2 emitted to the atmosphere(Day et al., 2005). The efficiency of biochar is due to its high stability even at

high temperatures where it is used to adsorb CO_2 (Xu et al., 2016b). In the soil the large specific surface area and composition of of biochar (such as $FeOH$, $CaCO_3$, etc.) have a significant physical and chemical adsorption effect on CO_2 (Nguyen & Lee, 2016; Xu et al., 2016b). Meanwhile, as biochar is made of carbonaceous materials and in the soil the average retention time of carbon in biochar is between 617 and 2829 years, it is important to use biochar for carbon sequestration (Wu et al., 2016).

Hydrochar is a carbonaceous material produced from the conversion of organic waste or renewable resources into a solid peat-like material via hydrothermal carbonization (HTC) (Libra et al., 2011). The HTC, also known as wet pyrolysis, is a thermochemical process that converts organic raw materials into solid products with C-rich content. It is conducted at a temperature range between 180 and 260 °C, at which temperature the biomass is immersed in water and heated in a closed system under pressure between 2 and 6 MPa for 5 to 240 minutes (Kambo & Dutta, 2015). Hydrochar has been shown to contribute to carbon storage and sequestration when applied to field/soils (Owsianiak et al., 2018; Reza et al., 2014). Hydrochar has a small surface area in relation to volume and is not ideal for CO_2 adsorption in contrast to biochar, therefore, hydrochar activation is necessary – the lignocellulosic biomass is activated with hydrochar activation (i.e. activating hydrochar with a mildly oxidizing gas such as CO_2 or steam at high temperatures in an inert atmosphere), leading to the formation of highly porous activated carbons, which are essential for carbon removal and storage (Fagnani et al., 2019). However, due to the sufficient degradable C substrate in hydrochar, some studies pointed out that the application of hydrochar stimulated microbial activities and emissions of carbon dioxide, nitrous oxide, and methane to the atmosphere (Kammann et al., 2012; Malghani et al., 2013; Schimmelpfennig et al., 2014). Therefore, it is important to consider optimizing the hydrochar application in soil when estimating the carbon sequestration potential of different hydrocarbons, thereby improving the stability of the soil, reducing GHG emissions, and having a positive impact on agricultural production.

3   Greenhouse gas emissions in application of organic waste and biofertilizers

Methane (CH_4) and nitrous oxide (N_2O) are important atmospheric GHGs, contribute 15% and 5% of the total radiative forcing of long-lived GHGs, respectively (Rodhe, 1990). From 1951 to 2010, GHG caused global surface warming by 0.5 to 1.3 °C (Stocker et al., 2013). The global atmospheric CO_2, CH_4 and N_2O concentrations have increased since industrialization and is expected to increase in the coming years and the emission of GHG gases will lead to a further increase in global temperature in the future. Compared with that the average temperature in the period between 1986 and 2005, the global average temperature is expected to rise by 0.3-4.8 °C in the period from 2081 to 2100 (Stocker et al., 2013). The increase of GHGs in the atmosphere comes from the anthropogenic emissions caused by the use of fossil fuels and changes in land use. During 2007-2016, GHG emissions from agriculture, forestry and land use contribute about 23% of total net anthropogenic emissions, and CO_2, CH_4 and N_2O from agricultural areas account for 13%, 44% and 81% of anthropogenic emissions, respectively (IPCC, 2019). Reduction of GHG emissions from agricultural soils with new effective mitigation strategies (e.g. applying

organic wastes as N fertilizers) within the framework of the Paris Agreement (UNFCCC, 2015) is therefore vital for achieving the goals for reducing global warming.

3.1   Processes of CO2, CH4 and N2O production

The process of CO_2 production in soil is commonly referred to as soil respiration, and its emission intensity mainly depends on a) the content and mineralization rate of organic matter in soil; b) the number and activity of soil microbial communities; c) and the respiration of soil animals and plants (Mosier, 1998; Smith et al., 2008). Among them, soil organic matter is the main carbon (C) source for soil respiration. Organic carbon not only provides energy for microbial activities, but as mentioned in the previous chapter it has a profound impact on soil physical, chemical and biological properties. The soil temperature is one of the main driving factors of soil respiration, so the soil CO_2 emission flux shows obvious seasonal changes, with the highest flux in summer and the lowest in winter (Mosier, 1998; Smith et al., 2008).

Most agricultural sources of CH_4 come from enteric fermentation by ruminants, and rice fields and natural wetlands are also important sources of CH_4. Organic matter in animal manures and bedding materials may be transformed under anaerobic conditions where microorganisms produce CH_4 (Chadwick et al., 2011; Smith et al., 2008). The production of CH_4 from those sources is affected by environmental factors such as temperature, biomass composition, animal manure management and number of microorganisms adapted to the environment (Chadwick et al., 2011; Sommer et al., 2013). There is limited CH_4 production occurring in the application of biofertilizers (e.g. biochar and hydrochar) as the production process is strictly anaerobic (Kiyasudeen et al., 2016).

The biological processes of N_2O production in agriculture include nitrification, denitrification (Chadwick et al., 2011) and nitrifier denitrification (ND) (Colliver & Stephenson, 2000), nitrification–coupled denitrification (NCD) (Kremen et al., 2005), fungal denitrification (FD) (Shoun et al., 1992) and dissimilatory nitrate reduction to ammonium (DNRA) (Kraft et al., 2014). It is generally believed that denitrification and nitrification are the dominant pathways to produce N_2O in soil during the application of fertilizers (Chadwick et al., 2011). The denitrification is a process in which denitrifying microorganisms reduce NO_3^- and NO_2^- in the soil to gaseous NO_x and N_2 under anaerobic conditions, where N_2O is an intermediate product of incomplete denitrification (Chadwick et al., 2011; Khalil et al., 2004). When applying organic material not containing NO_3^- the organic N and TAN in the added material has to be transformed to NO_3^- by nitrifiers in the oxidation process of NH_4^+ in the soil to NO_2^-, and NO_3^-. The diffuses may cause NO_3^- into the anaerobic zone where anaerobic microorganism produce N_2O or N_2 (Chadwick et al., 2011; Khalil et al., 2004).

3.2   Greenhouse gas emissions in fertilizer application

Organic fertilizers and biofertilizers are bound to have an impact on GHG (CO_2, CH_4 and N_2O) emissions, because the production of these gases are affected the way biofertililizer affects soil physical properties, SOC and soil microbial biomass, optimize the structural composition of soil microbial communities, and improve soil enzyme activities.

Soil CO_2 emission flux is affected by soil physical, chemical and biological processes, and is related to soil C and N content and cation exchange capacity (CEC) (La Scala Jr et al., 2000). The application of N fertilizers and organic fertilizers (e.g. animal manure, biochar, crop residues and municipal compost) have in some studies been shown to increase CO_2 emissions (Bol et al., 2003; Chadwick et al., 1998; Iqbal et al., 2009), and in other studies the use of organic fertilizers and biofertilizers (e.g. vegetable fiber, digestate and biochar) have been shown to reduce/capture CO_2 (Meijide et al., 2010; Xu et al., 2016b; Zhang et al., 2016), or may not have an effect on CO_2 emission/capture (Karhu et al., 2011; Zhang et al., 2012a). The explanation for increasing CO_2 emission include that a) the high content of labile dissolved organic C compounds in the applied fertilizers (including liquid manures and biochar) (Bol et al., 2003; Chadwick et al., 1998; Jones et al., 2011), b) applied fertilizers promoted the decomposition of SOC (Singh & Cowie, 2014), and c) applied organic fertilizers improved the microbial and enzyme activities to enhance soil respiration (Iovieno et al., 2009). In contrast, some types of organic fertilizers such as biochar had no impact on CO_2 emission or decreased CO_2 emission and improved carbon sequestration via the property of physical and chemical adsorption of CO_2 (Karhu et al., 2011; Xu et al., 2016b). As the effects of fertilizers on CO_2 emission are various depending on many factors such as sources of fertilizers, treatments on fertilizers, and soil properties, more studies under different conditions are needed to clarify its principles and mechanisms.

The effects of N fertilizers on CH_4 emissions are complicated, and may increase, decrease the emission, or have no effect. The process and production or absorption are related to soil properties, crop varieties, fertilizer types, and application time, method and amount of fertilizers (Zou et al., 2005). Application of N fertilizers promoted plant growth, and at the same time inhibited soil CH_4 oxidation, thereby enhancing CH_4 emissions (Xu et al., 2016a). However, the presence of NH_4^+ in N fertilizers could also stimulate the growth and activities of methane oxidizing bacteria, promote CH_4 oxidation, and thereby reduce CH_4 emissions (Bodelier et al., 2000; Christensen et al., 1997). The production and emission of CH_4 require adequate methanogenic substrates and a suitable growth environment for methanogenic bacteria, and organic fertilizers can increase carbon in soil and improve the availability of C and N sources for use by methanogenic bacteria (Blair et al., 1995; Yang et al., 2010). Most methanogenic bacteria are suitable to grow up from 35-37℃ to 55-60℃, and the application of organic fertilizers can improve the thermal characteristics of the soil to absorb more radiant energy, which increases the soil temperature, thereby producing more CH_4 (Parashar et al., 1993). However, CH_4 emission mainly occurred in paddy fields where is an anaerobic environment, with less emission in drylands, and the emission in drylands are largely unaffected by organic fertilizers (Malghani et al., 2013; Zhang et al., 2012a). Similar to adsorption of CO_2, biochar as an organic fertilizer shows the ability to adsorb CH_4 and mitigate its emission (Karhu et al., 2011). In general, the application of most organic fertilizers (crop straw, animal manure, compost, biogas residue, etc.) can increase CH_4 emissions from paddy fields but has little effect on drylands, and different organic fertilizers have various effects on CH_4 emission.

It is reported in the literature that N fertilizers can increase N_2O emissions from farmland soils (Richardson et al., 2009), and as the amount of N fertilizers increases, a growing number of articles reported that soil N_2O emission increases exponentially, not linearly, since N_2O emission factors are not constant but increase monotonically with N additions

(Kim et al., 2013; Shcherbak et al., 2014). The application of N fertilizers can significantly increase the content of NH_4^+ and NO_3^- in soil, and then enhance the extent of nitrification and denitrification, thereby promoting the production and emission of soil N_2O. A meta-analysis of field trials indicated that the application of urea produced higher N_2O emissions than ammonium sulfate (Siqueira Neto et al., 2016). The application of high-use-efficiency N fertilizers such as coated N fertilizer can significantly reduce soil N_2O emissions as when using the coated fertilizer, the inorganic N is slowly released for nitrification and denitrification, where the N2O is a by-product (Hyatt et al., 2010).

It has been proved that the N_2O emission factor of controlled-release urea is significantly lower than that of urea, ammonium sulfate and calcium ammonium nitrate (Liu et al., 2017), and the application of controlled-release N fertilizer can reduce N_2O emissions by 38.3% as a whole (Xia et al., 2017). A meta-analysis showed that organic fertilizers increase N_2O emission by an average of up to 32.7% compared with mineral N fertilizers only (Zhou et al., 2017). Regarding the application of organic fertilizers, the research showed that the formation and emission of N_2O in soil were affected by both C and N content in reaction substrates. When organic fertilizers are applied based on C demand, N_2O emission is mainly restricted by the level of external N supply, while when the organic fertilizers are applied based on the N demand, N_2O emission is mainly restricted by the level of external C supply (Chadwick et al., 2000). The application of animal manure in soil with low C/N ratio could lead to an increase in N_2O emissions (Rochette et al., 2004). However, under the high soil C/N ratio, the gaseous N loss is mainly N_2 (Bhandral et al., 2007). These differences are due to the growing or decreasing concentrations of soil NH_4^+ and NO_3^- stimulate or reduce the extent of nitrification and denitrification and thus affect the N_2O emission (Ma et al., 2013). As plants take up NH_4^+ and NO_3^- for their growth, optimizing the fertilizer type, application time, rate, and method based on the demands of crops, and adjusting the ratio of NPK and the C/N ratio can improve the NUE and effectively reduce N_2O emission (Di et al., 2009; Yao et al., 2010).

Application of biochar in soil would significantly reduce N_2O emission (Kammann et al., 2012; Malghani et al., 2013; Zhang et al., 2012a). It is due to that biochar can change soil physical (gas diffusion, agglomerates, water retention, etc.), chemical (pH, soil redox potential Eh, availability of organic and mineral nitrogen, dissolved organic carbon, etc.) and biological properties (microbial population structure, microbial biomass and activity, enzyme activity related to the N cycle, etc.) to mitigate N_2O emission (Van Zwieten et al., 2015). It is indicated that biochar can increase the abundance of nosZ gene (nitrous oxide reductase gene) in soil microorganisms, thereby promoting the reduction of N_2O (Harter et al., 2014). In order to effectively mitigate N_2O emission, it is required to optimize fertilization time and method according to the characteristics and demands of crops, adjust the application rate of NPK, choose long-acting slow-release N fertilizer, and improve the NUE of N fertilizer. For organic fertilizer application, the application strategy is relatively complex and mainly depends on the physical, chemical and biological properties, C/N ratio and application methods of different organic fertilizers

4 Potential risks in application of organic waste and biofertilizers

The conversion of organic waste into fertilizers is an important pathway of the comprehensive utilization of agricultural waste. Sewage such as dairy processing waste are rich in organic matter and plant nutrients, and can be an important source of commercial organic fertilizers. However, bio-fertilizer produced from dairy waste may contribute to nutrient losses and environmental pollution. Inappropriate application of organic fertilizers will cause non-point pollution of farmland and destroy our ecosystems.

4.1 Potential risks of nutrient (N and P) losses

When using organic fertilizers and biofertilizers on farmland, the main environmental issue is that the surface water and groundwater may be contaminated with excessive amounts of N and P. Because of the high content of N in animal manure, slurry, and anaerobic digestates, the N leaching has attracted extensive attention from researchers (Chantigny et al., 2008; Möller et al., 2008). These high N levels are attributed to the relatively low animal N use efficiency (EU Nitrogen Expert Panel, 2015). The research also reported that manure application without any pretreatment would result in severe nitrate leaching (Giola et al., 2012). Due to contamination by nitrate leaching, the edible parts of some vegetables may accumulate high concentrations of nitrate, especially if excessive nitrogen fertilizer is applied. Eating these crops can damage the health of human beings (Li et al., 2017). Meanwhile, the huge amount of P in organic fertilizers could lead to eutrophication of surface waters (Bloem et al., 2017).

4.2 Potential risks of soil contaminants

The application of dairy waste or biofertilizers from dairy waste on the farmland has potential risks, because it may lead to the incorporation of phytotoxic compounds (ammonia, volatile organic acids, phenolic compounds and salts), pathogens and heavy metals (Ni, Pb, Cr, Cd) into the soil (Nkoa, 2014). The concentration of trace elements in the organic nutrient sources is usually higher than that in agricultural soil, resulting in the accumulation of trace elements in the soil after repeated application of fertilizers or sewage sludge (Bloem et al., 2017). The trace elements including arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), nickel (Ni), lead (Pb), zinc (Zn) are potentially toxic and must be under the regulations of the EU (Bloem et al., 2017). The literature reported that thermo-chemical processes could be the best solution and significantly reduce detrimental components in the organic waste and derived fertilizers to mitigate all health and environmental risks (Bloem et al., 2017).

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Modelling soil P dynamics and P cycling in soils amended with organic waste (ESR9)

Phosphorus is a vital element for all living organisms, which is widely used in agriculture to meet increasing crops demands. Bioavailable P concentration in agricultural soils is managed by fertilization with larger fractions of commercial fertilizer used in agriculture being based on rock phosphate, which is a non-renewable resource with limited mining opportunities in the EU region.

To mitigate the negative economic and environmental impact of rock phosphate usage in agriculture the circular economy approach can be used with returning P to agricultural soils in form of recycled waste. A number of papers discussing potential of sewage sludge and animal manures application to soil as a nutrient source was published, with very little data available on P efficiency of dairy processing waste recycling and reusing on grazed systems. To date there is no clear recommendation on dairy processing waste application time, rates, and methods which would maximise plants uptake and avoid P losses to environment. To create such guidelines for farmers soil-waste interaction needs to be studied to evaluate not only P content in recycled waste, but also bioavailable P and P fate and transport in agricultural soils depending on soil type. In Western Europe with large areas devoted to pasture-based systems, returning dairy processing waste to soil would allow to transit to closed loop system returning most of consumed P back to fields potentially reducing by this cost on ferritization and reducing amount of waste being discharged to water sources or landfills. However, careful assessment of the waste efficiency as an alternative P source for crops is required with consideration of both soil properties and external factors influencing P transport in agricultural soils such as soil temperature and rainfall.

In this chapter, the potential of a circular economy approach to managing soil fertility from P perspective is discussed. Soil P dynamics and transport model is shown, with the links between soil pools and processes governing soil P transformation. Analytical tools available for P dynamics and availability study is discussed to develop protocol of soil and dairy processing waste analysis and investigation of P fate and transport in pasture soils amended with DPW.

1   Circular economy in agriculture

With the world's population growing and industry developing a negative impact on environment and climate new approaches are needed to mitigate pressures on environment. Approaches are required to meet population demand in goods while reducing usage of non-renewable resources and decreasing pollution. As a solution to the rising concerns on technologies sustainability a circular economy model can be applied changing a course from "take – produce – use – dispose" to "make – use – recycle" (Figure 1). Transition to a closed loop system would allow mitigation of a lack of resources and negative impact of agricultural sector on the environment by reducing the amount of waste.
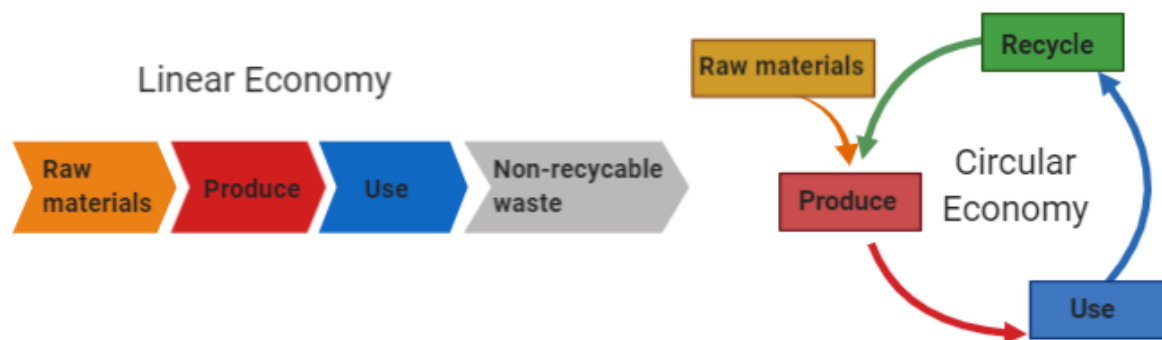
Figure 1 – Linear and circular economy approaches.

In the EU the introduction of the EU Circular Economy Package (2016) aimed to ensure sustainable use of materials and resources embracing development of resource efficient and environmentally sustainable technologies.

For agriculture, the introduction of the new course of development means increased interest in returning recycled nutrients to soil as fertilisers resolving the need for waste management and soil fertilization, which currently are primarily managed by application of mineral fertilizers.

One of the crucial soil nutrients which was included to the list of EU critical materials is phosphorus (EU Commission, 2014) based on its high supply risk and importance of the resource. Agricultural sector in the EU is highly dependent on imported P sources with less hen 20% of P being mined in the EU (EU Commission, 2017). To mitigate risks related to increasing P demand and limited mining opportunities use of the alternative P sources such as recycled animal manure, human waste, biochar can be employed in agriculture. Besides the potential of recycled waste to reduce consumption of rock phosphate, recycling of P-containing waste can help mitigate negative environmental impact of food industry and wastewater treatment plants (Amann et al., 2018). Therefore, transition of the P lifecycle to the circular economy by increasing sustainable P management and recycling is one of the prioritised sectors highlighted in the EU 2020 Strategy (Kanter and Brownlie, 2019).

The largest amount of all mined P is used in agriculture as fertilizer (Belboom et al., 2015) supplying soil with nutrients and on different levels being a raw material for crops production and lifestock. In such systems P is being removed from soils with crops and only part of it can be returned to soil with animal manure, while the other part is passed to food industry with crops, meat, and dairy products(Noya et al., 2017). In Western Europe a larger portion of agricultural areas are used for lifestock feeding with 60% of the UK and up to 73% of Irish agricultural areas being pasture based grasslands (Peeters, 2009). In grazed pastures and grasslands P is a vital soil nutrient and addition application of P fertilizer can benefit biomass production in such systems (Frossard et al., 2000).

In grazed systems P is removed from systems with livestock as after P uptake by plants it is then transferred to livestock and later is transported (removed) from the system as animal or animal products. In a linear economy P exported from such systems wold not be returned to the system and further P application would be required to maintain optimal P concentration in soil. However with the circular approach part of the exported P can be

recycled and returned to the system as fertilizer. In grazed system closing the loop would mean returning food production waste such as DPW to soil in form of pre-treated (biochar, granulated biosolids) or raw waste (wastewater treatment sludge). Sustainability of such approach requires closer investigation of organic P utilization in pasture-based systems to evaluate bioavailability and possible losses of P returned to the system.

2　Closing the loop: understanding agricultural potential of recycled P

The P cycle in soil passes through inorganic and organic cycles which are connected to each other. The inorganic cycle is slower than organic and take millions of years and consists of such processes as erosion of P-containing rock and soil, P transportation, sedimentation, tectonic activity, soil -containing rock transformation and transport to soil etc (Filippelli, 2002). In organic cycle P transformation and transport is rapid and typically takes up one-year time (Bennett and Carpenter, 2002). However, these cycles are natural, and they do not account artificial soil fertilization, as well as extraction of soil P in agricultural which is not returned as waste applied to land. This leads to P losses to water or losses to wastewater treatment sludge when the sludge is not being applied to agricultural lands. Currently only 50% of P removed from pasture-based systems is being returned to fields as an organic waste, while the rest of P removed from system is discharged within wastewater or lost with an incinerator ash (Science Communication Unit, 2013). This leads to permanent removal of P applied to soil which then is being replenished by applying mineral fertilizers. In pasture-based phosphorus is continuously transferred between soil, plants, and animals therefore returning plant and animal waste to soil has potential to create closed loop system. Returning decomposed plants debris and animal products can provide significant P supply to soil taking up to 50% of total soil P (Shutter et al., 2012). In grazed pastures returning of manure to soil as fertilizer can allow to return up to 85% of P taken up by plants, and can contribute as much as 280kg P ha- 1 annually in cattle grazed systems (Haynes and Williams, 1993).

Currently predominant wastes which are returned to soil as fertilizers are animal manures. Depending on the waste origin and solids content, total P content in biosolids varies greatly: 23.6 – 27.8 g kg-1 in poultry manure, 16.22 – 29.7 g kg-1, 2.94 – 4.02 g kg-1 in beef manure, and up to 26.21 g kg-1 in dairy manure (Fuentes et al., 2006). An alternative P rich effluent which can allow to return to field as a pat of circular economy approach is dairy processing waste (DPW). It is known that at least 2-3% of incoming milk is lost to wastewater treatment facilities due to losses in cleaning operations and intermediate operational steps, milk leaks, and spills. In addition to the milk losses at operational steps listed above, production of cultured dairy products contributes to P-rich effluents production (Vourch et al., 2008). P content in DPW with 15.3% total solids content can reach 5.6 g kg-1 (Glanbia Ingredients Ireland, 2012), in untreated wastewater treatments sludge from dairy processing plant. To resolve logistic complication of the DPW transport and spreading on agricultural fields, such techniques as chemical and biological precipitation or incineration can be employed for DPW recycling allowing to produce product with higher P content comparing to raw DPW (Ryan et al., 2016).

Currently most of the recycled P in Western Europe and in Ireland in particular (Ryan et al., 2016) is being used as fertiliser or soil enhancer, however, despite the EU Circular economy action plan which embracing returning of waste to agricultural soils as P fertilizer, the approach of direct application of biosolids require further evaluation to address concerns regarding risk associated with application of biological waste to soil, and bioavailability of nutrients applied to soil (Tarayre et al., 2016). Despite high concentration of P in DPW and its potential in agriculture it is remines unclear what fraction total P in waste can be utilized by crop when applied to agricultural soils.

3   Soil P cycling and transport in agricultural soils.

In cropped agricultural systems P cycle involves chemical transformation of soil P between the following pools: soluble P pool (organic and inorganic) which is considered to be immediately available for plants (1), labile or weakly adsorbed P (2), insoluble P chemically bound with Ca ions in calcareous and alkaline soils or occluded be Fe and Al oxides in acidic soils (3), strongly adsorbed by hydrous oxides of Fe and Al (4), and insoluble organic P within soil organic matter (not available for plants) (5) (Stevenson&Cole, 1999). A simplified diagram reflecting P soil cycling and interactions between the pools is presented on Figure 2.
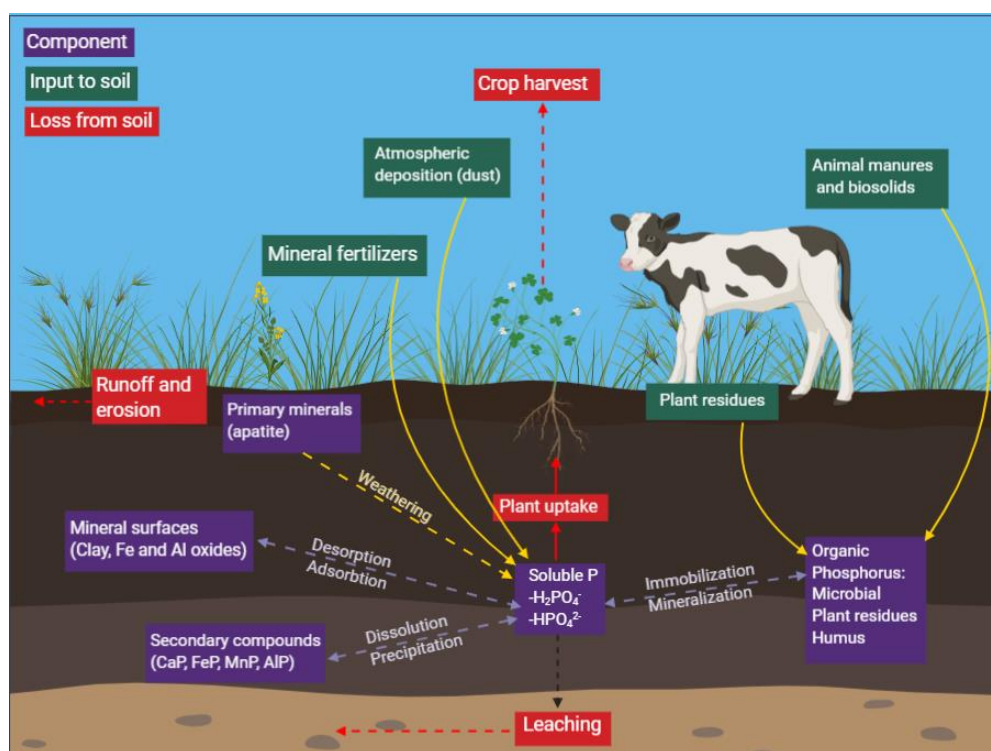
Figure 2 – Soil phosphorus cycling.

From an agronomical perspective it is important to ensure that phosphorus which enters the system is available for plants. Only the soluble P pool (Figure 2) is considered to be readily available for plans, and 3 other pools which are not immediately available for plants are: adsorbed P (minerals and secondary compounds), primary minerals, and organic P. As it is shown on the diagram, P uptake by plans occurs from the soluble P pool size of which is regulated by number of processes. And briefly soil P cycle consists of the following

processes: weathering and precipitation, mineralization and immobilization, adsorption and desorption, and P losses through leaching and runoff.

Weathering and precipitation are pathways for P containing in P-rich minerals which through weathering can enter soluble P pool. Opposite process is P Precipitation which describes processes of soluble P chemical reaction with positive charged ions which leads to soil mineral formation and limits P availability. This process can be impacted by a number of factors, including geochemical composition and soil pH. For instance, in acid soils P precipitation occurs in presence of iron, aluminium, and manganese, and soluble P in such soil can be limited, while in alkaline soils precipitation is primarily occurs through reactions involving Ca2+ compounds (Prasad et al., 2014).

Mineralization and immobilization of P are processes which describe pathways between soluble and organic P pools. Mineralization is a process of transformation organic H2PO4- or HPO42- to soluble orthophosphates supplying by this available P pool. Mineralization of organic P maintains slow release of soluble P, which is crucial during growing season as it allows to achieve continuous supply of crops with P. Mineralization is mainly occurs during growth season when soil temperature ranges between 18 and 40 0C and is mainly driven by release of enzymes produces by soil microflora. The factors which can limit mineralized P availability are precipitation, when released P reacts with soil positively charged ions and form inorganic complexes, or immobilization. Immobilization is a biotic process of soluble P consumption by soil microorganisms. Besides the soil temperature the processes are also depend on soil moisture, pH, and energy supply (Prasad et al., 2014).

The last group of processes which may impact soil P availability for crops are adsorption and desorption. Desorption is release of P which is bound with soil minerals to soil solution which increases soluble P pool. Adsorption, or fixation, is a chemical processes of binding soluble P compounds to soil particles. Unlike precipitation, this process is reversible, and P does not involve permanent change in chemical and structural changes in P-containing compounds. These processes are also depending on soil geochemical composition and pH, and the optimum pH when most of soluble P will remain in soluble pool is in range between 6-7. Decreasing of pH can lead to P adsorption by Fe and Al oxides. Fixation of P by mineral presented in soil is limited, and it must be considered when applying P fertilizer to soils to ensure sustainable use of the P source: precise application of P fertilizer such as band application can maximise plant uptake of supplied P, and avoid P losses through run off and leaching, which increases with soil P saturation level. The interactions between the pools and processes which may impact the P dynamics in soil are shown on Figure 3.
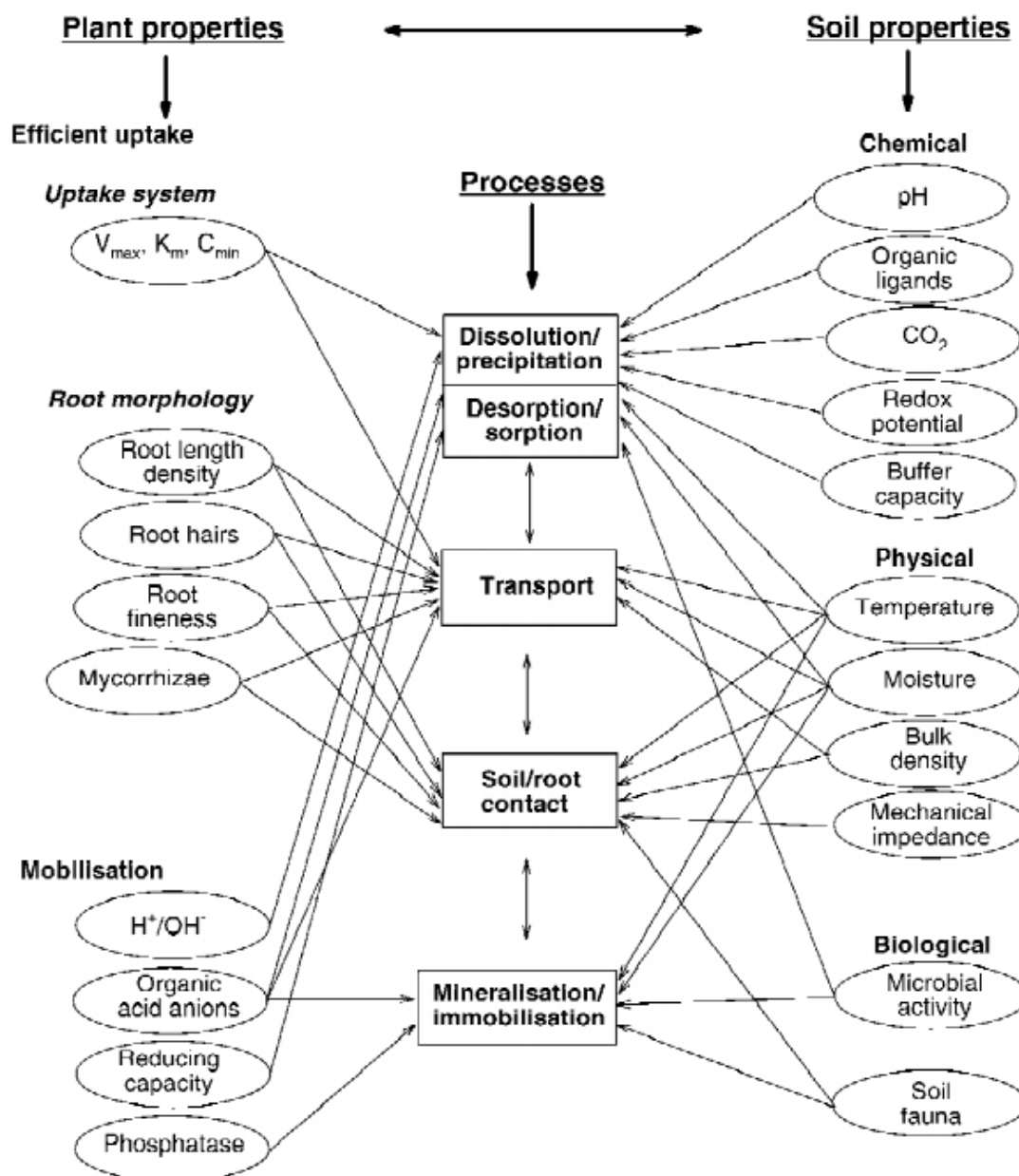
Figure 3 – Processes, plant and soil properties affecting native soil P and fertilizer P dynamic and transport in agricultural soils, adapted from (Horst et al., 2001).

To evaluate the sustainability of DPW application to pastured soils as additional P supply it is important to consider whether productivity of the system can be sustained by organic P. The answer to this concerns lies in understanding bioavailability of organic P and factors which regulate it. P turnover in soils implies inorganic orthophosphate assimilation by plants and further release of biological materials, their maturing, and decomposition (Condron et al., 2005). Typically, organic P pool consists on the following P forms: phosphomonoesters (i), phosphodiesters (ii), and organic polyphosphates (iii). Since these forms needs to be transformed to soluble P through mineralization, availability of P delivered from DPW will be impacted by microbiological activity as well. Besides this, soluble P released from organic fertilizer can enter not available P pool through precipitation or adsorptions (Figure 2) therefore effectiveness and P uptake by crops will also be impacted by application soil geochemical composition, pH, and texture. It is also

known, that unlike other nutrients, immediately after application P will be fixed, and P diffusion in soil is slow with average transport speed about 0.13 mm/day (Syers et al., 2008). At this diffusion rate application of P must be applied with consideration of volume of soil explored by crops rhizosphere to enhance roots contact with available soil P.

Application of DPW to pasture based soil except of returning nutrients to field and providing crops with P source may potentially have other benefits. Due to the form of the P in the DPW, it may potentially act like slow release P fertilizer continuously supplying crops with P during grass growth period. The mechanism of P transport from organic fertilizer may also be a useful tool in mitigating P losses thorough leaching in coarse soils, soils with high water content, and regions with high rainfalls. This may also improve crops response to P fertilizer in soils with low P content prone to P fixation (Malhi et al., 2002).

However, despite the fact DPW is a P-rich effluent, which provides an attractive opportunity to implement circular economy and return P to pasture soils, further studies of its potential are required to ensure effective and sustainable recycling of the waste products. While treated and untreated sewage sludge and composted food waste application to soil as a fertilizer have been previously reported and is widely studies across the world, there is very little information on DPW recycling. To evaluate the full agronomical potential of the DPW returning to pasture system the following aspects need to be studied:

- P forms in DPW, and their transformation after application to soil

- P dynamics in soils amended with DPW

- P mineralization rate in soils amended with DPW

- Bioavailability of P delivered from DPW in soils with different P status

- In pasture soils: grass curve and soil P dynamics during grass growth year

This information will to model chemical interactions between native soil P and P delivered from DPW, evaluate agronomical potential in soils with different fertilization history. Importantly, this information will allow us to develop recommendation regarding optimal application strategies to maximise crops response to recycled DPW application to soil and minimize P losses.

In order to collect data required for developing the model of DPW P dynamics and transport in soil, incubation, pot, and field experiments can be employed with involvement of a number of tests which can be used for available P pool and organic P mineralization rate determination, as well as soil P speciation allowing to develop models of interactions between native soil P and DPW for various systems and create robust recommendations for DPW application to maintain soil quality in pasture-based systems.

4   P speciation and evaluation of available P pool size in agricultural soils

To study transformation of native soil P and P delivered from fertilizers several techniques were developed. The simplest fractionation method lays in separation soil P into three fractions: soil solution P, and solid phase soil P which comprises of organic and inorganic fraction. The method offers simple solution with application of spectrophotometry for determination of soluble and inorganic P pools (Murphy&Riley, 1962), and organic P is calculated as difference between total P and inorganic P. while the method offers simple and rapid basic speciation of P in soils, it has been reported to overestimate organic P fraction in presence of inorganic polyphosphates underestimating inorganic P pool (Dick&Tabatabai,1977). More precise method of soil speciation which involves separation of total soil P into seven fractions was developed by Hedley (Hedley et al., 1982) and is widely used to investigate soil P nature and transformation in soils. The Hedley's extraction protocol allows to separate soil P into the following groups: labile P (available for plants), moderately labile P (not readily available for plants, but can be a source of P for the soluble P pool), and stable P (not available for plants); and can provide information of chemical nature of P compounds within each of the groups (fig. 4)
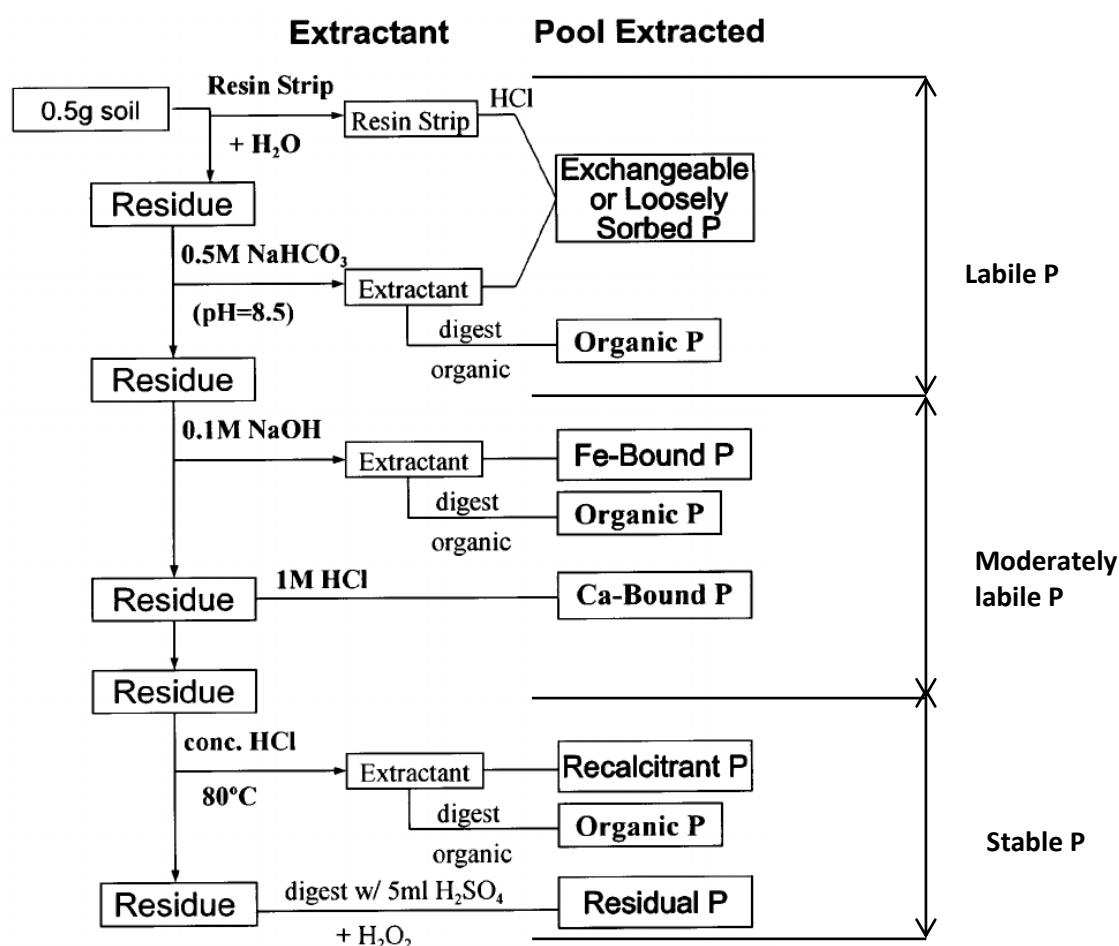

Figure 4 – soil P fractionation by Hedley protocol (Hedley et al., 1982).

Hedley's protocol and its modifications are successfully applied for soil P dynamics and cycling studies in various types of soils and is applicable to soils amended with commercial

fertilizers (McLaughlin et al., 2011) or organic waste (Zohar et al., 2010), and for soils without previous fertilization history (Helfenstein et al., 2018). Interestingly, the study of the effect of animal manure on temperate soil in the UK with Hedley's extraction revealed that higher levels of readily extractable soil P in soils fertilized with animal manure comparing to soil which received conventional fertilizer (Colvan et al., 2001) indicating positive impact of recycled P impact on soil P status. The method is robust and informative, and is widely used on various soil types, however there are some disadvantages of the method. Firstly the method is based on a time consuming protocol, and secondly, despite the possibility of extraction of three P pool sand giving information about their composition, it offers very simplified speciation based on unproven chemical interaction between P and extraction reagents, which leads to over-simplifications of results (Negassa and Leinweber 2009). Ignition and provides very little information on structure and nature of the P species. More advanced analytical techniques have been developed for more precise and informative P speciation in soil, which can also show organic and inorganic P profile in soils. They are based on spectroscopy and include but are not limited by hey are 31P NMR, X-ray absorption near-edge structure (XANES). Combination of the techniques can allow detailed speciation of P species in soil samples amended with fertilizers and model chemical interactions between native soil P and P applied to soil as fertilizer or` returned to agricultural soil as a recycled waste. While XANES offers comprehensive method of direct characterisation of inorganic P species in samples, 31P NMR can provide detailed information on organic P species presented in samples. The method was successfully applied to temperate pasture soil studies completed in the UK and Ireland (Turner et al., 2003, Murphy et al., 2009) confirming that larger fraction of P in pasture soil is in form of organic P with the larger fraction of those being phosphomonoesters (up to 35% in the UK pastures and up to 24% in Irish grassland). These methods however have been mostly applied to native soil P studies, o tot P accumulation studies in agricultural soils, while they can offer tools for study of recycled fertilizers fate and transfer in agricultural soils providing detailed information on organic P fractions.

While soil speciation can provide insight to processes which occur in soil after application of fertilizers to soil, for supplying crops with sufficient amount of nutrients it is also important to evaluate the amount of plant available P in soils. In routine soil tests extraction methods such as Olsen P (Olsen et al., 1982), Morgan's P and Morgan's modified P (Morgan et al., 1941) are widely used to access soluble P pool in soil. The methods are based on chemical extraction of orthophosphate ions from soil followed by chemical reaction leading to formation of complex salt in the solution, which is then tested using spectroscopic techniques. The methods offer quick and comprehensive decision for soil fertility status estimation. However, since efficiency of these tests relies on chemical extraction process their efficiency depends on soil geology and pH. This was demonstrated in survey conducted on 199 temperate soils collected in the cross-border region of the Republic of Ireland and Northern Ireland showed pH impact on Olsen P- Morgan P relationship showing Olsen P, for given Morgan P values, decreasing with increasing soil pH (Foy et al., 1997).

More precise methods with application of P radioisotopes have been developed to estimate available and labile P. The methods are widely on different soil types and in a lack of standard protocol of the P availability determination can provide comparable results to previously reported in literature. Two radioactive phosphorus isotopes are used for isotopic study of available phosphorus: 32P and 33P. The isotopes emit beta particles and have half-

life of 14.6 and 25 days for 32P and 33P respectively, which allows using them in short-term trials for determination of residual effect of the applied fertilizers and plant uptake (Shipley & Clark, 1972) or organic P mineralization (Kellogg, Bridgham, & López-Hernández, 2006). There are three main groups of isotopic methods which is used for phosphorus cycling, availability, and pools studies they are tracer, dilution, and isotopic exchange methods: tracer method, isotopic dilution, and isotopic exchange (Fardeau et al., 1995; Shipley & Clark, 1972).

The tracer method is used for studying phosphorus flow and cycling in the studied system, and P uptake by crops. The method is based on application of radiolabeled P source which is then allowed to follow P fluxes in the system. The method allows to study P fluxes, plant uptake and fertilizer contribution to plant nutrition since it allows to trace P taken exclusively from the labelled fertilizer (Fardeau et al., 1995).

The isotopic dilution methods are methods in which application of radiolabeled P source is followed by studying available P pool size without extraction P from the system (Ziadi et al., 2013). This method is limited in its application as it can only be used on a single homogenous pool where constituent elements have same kinetics.

For more complex systems methods based on isotopic exchange are widely used. They can be applied for evaluation of P pools sizes and P dynamics in the system with various P forms (including organic P) and allow determining and modelling mineralization of organic P in soils or fertilizers. The methods are not limited by kinetic properties of the studied pools allowing to study impact of the applied product on the amount of P in soil solution and exchange kinetics between the pools (Fardeau et al., 1995; Shipley & Clark, 1972) and the only limitation factor for these methods is the need of separation of the exchangeable P species from bound P.

Over the years there were developed a number of protocols based on isotopic dilution which allow studying P cycling and dynamics both on the bench and field scale. In general, there are three parameters which can be determined in the experiments: available, exchangeable, and labile phosphorus which are described by such parameters as A-, E- and L-values respectively. All three values can be determined for system of different scales: from bench top (Achat et al., 2014) to field trials (van der Bom et al., 2019) and can involve interaction between soil P pool and plants (A-value and L-value). The effect of fertilizers application to the soil can be evaluated by tracing changes in the exchangeable phosphorus pool which reflects bioavailability of phosphorus for crops and relative agronomic effectiveness of the products applied to the studied soils. E-value procedure does not require application of additional P source therefore it can also be used as a baseline experiment showing initial P pool size in studied soil, or can be utilized for studies of chemical soil cycling including microbial activity (Wanek et al., 2019).

Regardless scale of the experimental set up and samples treatment A-, E- and L- value determination are based on isotopic dilution equation (1). Visual representation of the method is shown on Figure 5: studied system is comprised of multiple pools (e.g. soil and fertilizer), and the aim of the study is determination of the targeted P pool size (X). For determination of the pool size 32/33P (tracer) of known radioactivity R is added to the system (1). As isotopic exchange between soil exchangeable P and radioactive P introduced to the system occurs, specific activity (32P/31P or 33P/31P) decreases, and sufficient time

must be allowed on this stage for system to reach isotopic equilibrium (2). To achieve this system is carefully mixed to make the system homogenous. After this the sample is then sampled (3), filtered, and analysed. Two main parameters are determined on this stage (4): soil solution radioactivity (r) and P content (q).
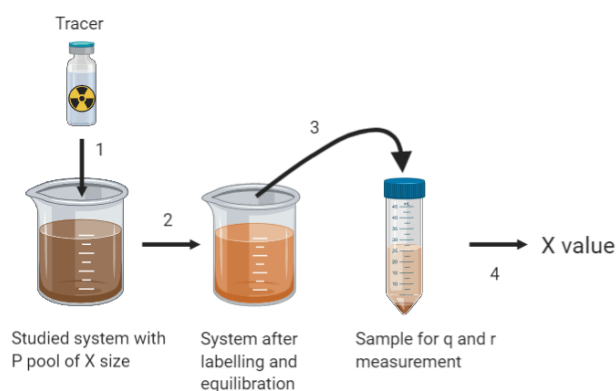
Figure 5 – Isotopic dilution method visualisation.

In homogenous system specific the following equation is true:

$$R/X = r/q \qquad (1)$$

Therefore isotopically exchangeable P pool size can be determined as follows:

$$X = R\left(\frac{q}{r}\right) \qquad (2)$$

where X is determined pool size, R – radioactivity introduced to the studied system, MBq, q – P concentration in tested sample, r – radioactivity of the sample.

Depending of the protocol followed, the system may contain one more component: plants (A-value and L-value determination), in which case sample is not taken by syringing, but "sampled" by plants, and plants material is then tested for radioactivity and P content in plant tissues.

A-value determination is based on plants growth experiments and can be run both on bench and field scale. Briefly the procedure of A value determination is as follows; 32/33P solution is used as a tracer added to a fertilizer. Radiolabeled fertilizer is applied at the rate M and radioactivity R to the soil localized and bundled (for example placed in a filter paper bag). Plants are them sown to the soil and allowed to grow sampling P from two nutrient sources: soil soluble P and P derived from the applied fertilizer. At the end of experiment plants are sampled and analysed for tissues radioactivity (r) and phosphorus content (q), which with known R and M allows to determine A-value based on isotopic dilution equation (1) (Fardeau et al., 1995):

$$a = R\left(\frac{q}{r}\right) - M \qquad (3)$$

The main concern of this method is that the calculations of the value are based on isotopic dilution method, when in fact there were no isotopic dilution in the system, since the pools were not mixed and there was no equilibration between the pools. Moreover, chemical

processes leading to fertilizer-delivered P transfer to roots of the plants and are not considered in the study, and only unknown fraction of P delivered is "sampled" by plants. Theoretical concerns of the methods application can be supported by data published on the subject before A-value determined for the same soil and fertilizer samples was reported to overestimate the amount of available phosphorus comparing to data collected for same samples in course of determination E-, and L-value (Fardeau&Jappe, 1979). The method of the fertilizer application and plant sowing must be carefully validated prior to starting the experiment, as localization of the fertilizer and method of it "bundling" may also have impact on P transfer to plant's roots, as well the root development may have an impact on P uptake which is not related to form and chemical composition of the fertilizer, which makes method less robust comparing to other isotopic methods.

As opposite to the A-value, E-value is determined after system was homogenised and equilibrated. This procedure is widely used in environmental and agricultural study for evaluation of exchangeable P pool size, which is associated with immediately available for plants (soluble P). When used to evaluate the impact of fertilizer application to the studied soil the procedure is simple and can be performed on bench scale. Soil samples must be sourced, air-dried, and sieved prior to the beginning of the experiment. Soil is weighted into bottles and mixed with deionized water and shaken to equilibrate the system. After system is equilibrated radiolabeled P source is added at rate M with known radioactivity R to the system and mixed (Figure 6a).
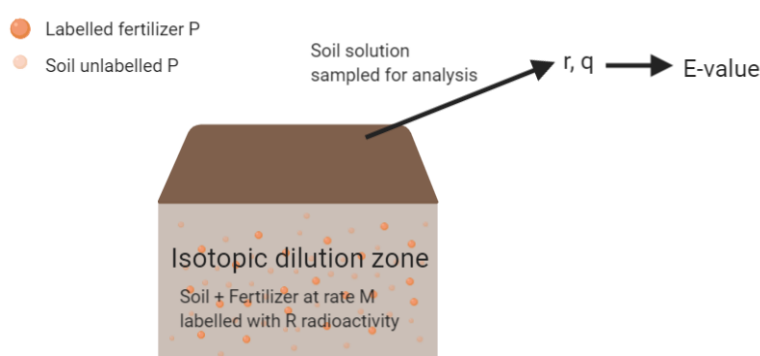
Figure 6a – E-value determination procedure.

Isotopic dilution occurs within the system and P pools now consists of soil unlabeled P and radiolabeled P delivered from radiolabeled source. When the parameter to be determined is exchangeable P pool (E-value) further procedure is simple and rapid: soil solution is sampled for analysis and filtered. Radioactivity and concentration of phosphate ions are measured in the solution and E-value is calculated from using the isotopic dilution equation (1).

One of the main advantages of the method for current study is possibility of determination E-value of soil not amended with DPW providing baseline point and allowing to evaluate changes in exchangeable P pool. This can simply be achieved for running blank experiment – when the tested soil does not receive any additional P source and isotope is added to soil solution as pure 32P with known radioactivity. The laboratory set up also develops interested as it was successfully used for determination of mineralization rate of the

organic P pool and P fluxes in soil amended and not amended with fertilizers et al., 2004; Wanek et al., 2019). The laboratory set up and samples treatment procedure remain identical to original method for the E-value determination with multiply sampling of soil solution e.g. three sampling at 0, 4, and 24h as reported by (Wanek et al., 2019) in their study of gross rates, biotic and abiotic processes in the soil cycle. Additional treatments of soil samples, such as sterilization and fumigation, will allow to investigate role of microbiological factors in soil P cycling. The main concern when working with this protocol is determination of the orthophosphate ions: when working with P deficient soil, the method limits for orthophosphate measurement must be carefully evaluated to avoid errors in measurement of low P samples.

While P uptake by plants depends on P concentration at roots surface, the quantity of P in soil solution may not represent available P and can be as low as 1% from P taken up by plants meaning up to 99% of P taken up by plants is delivered from adsorbed P (Grant, Bittman et al., 2005). Therefore to soil-P plant interaction included to the studied system it may offer in-depth information on soil P availability in the system. Advanced procedure for this which provides satisfies isotopic dilution conditions is L value (Figure 6b).
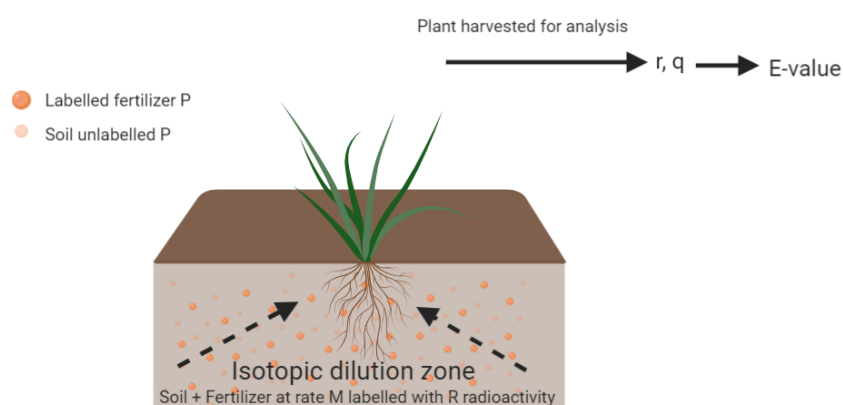
Figure 6b – L-value determination procedure.

The laboratory set up is similar to A-value determination procedure; however radiolabeled fertilizer is added to soil prior to sowing, and thoroughly mixed. This allows achieving homogenisation required, and after this plants are sown to soil containing P radioisotopes. P is taken up ("sampled") by plants. The plants' shoots are harvester for r and q determination in plant digest. L-value is calculated based on isotopic dilution.

Due to increasing interest to development of alternative P sources such as municipal waste, animal manure and wastes from food industry, effective tools for P availability study are in high demand. There were a number of papers published over the past decades with successful application of isotopic techniques for determination of soil P availability. Solubility and mobility of P in soils were evaluated based on E value of soil amended with recycled dairy effluents and pig manures (Achat et al.,2014). E-value and soil soluble concentration P were successfully determined for all soil samples. The tests were run on soil samples after various incubation times which were proved to impact results (maximum

E value reached on the 28th day of incubation) proving dependence of the P dynamics on microbiological activity in the tested soil. For further utilizations of the method this means soil preparation methods must be validated and standardized to avoid errors. Another valuable information which can be gained with application of simple isotopic dilution technique in plant growth experiment (L-value) is P recovery from below ground residues (Foyjunnessa et al., 2018). Plant growth experiment can be successfully used for soil fertility potential based on soil-plant interaction and estimate labile P pool which can be hidden when using traditional extraction (Olsen or Morgan) protocols, and single E-value. In addition to P availability data, plant growth experiments involving isotopic technics can be an effective tool to estimate P distribution in plant tissues showing plant response to various application rates and allow reducing P losses accumulated in below-ground residue after harvest.

5   Conclusions

Due to high P demand in agriculture and no significant opportunities for rock phosphate mining in the region, rock phosphate has been added to the list of EU critical raw materials therefore alternative sources of P are in high demand. New promising sources of P which have potential to meet agricultural demands and comply with a circular economy approach by returning consumed P to agricultural soils. A P-rich effluent which can potentially substitute large amount of commercial fertilizer is DPW. However, despite EU policy embracing reusing and recycling waste and using it as alternative nutrients source in agriculture, there are challenges in evaluation of their effect on agricultural soils, P release from DPW, and prediction on P uptake from such fertilizers is not well understood.

To provide clear guidelines for farmers on the recycled DPW application rate and timing of fertilization, nature of DPW P, its behaviour, dynamics and transport in agricultural soils must be studied first. To develop a model of possible chemical interactions between native soil P and DPW P chemical nature of P compounds, and their transformation needs to be investigated. There is no standard protocol of soil P speciation and P bioavailability, and effectiveness of most methods depends on soil properties and P forms in soil and fertilizer. Since the main focus of the study is investigating P dynamics and transport in pasture soils potentially amended with organic waste, 31 NMR spectroscopy can be applied for investigation of chemical interactions between P species in pasture soils, with potentially high amount of organic P, amended with organic waste. Combining 31NMR spectroscopy with isotope dilution method can also provide information about P pools sizes, and potentially, will allow to evaluate P release patterns from organic fraction of DPW and soils. Information on soil P speciation, P release from organic P pool will allow to address concerns on DPW P efficiency as well as will allow to create clear guidelines on recycled DPW application to soil to provide P supply to crops and avoid P leaching.

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