FACULTY OF
BIOSCIENCE ENGINEERING

# Animal manure derivatives as alternatives for synthetic nitrogen fertilizers

Ivona Sigurnjak

GHENT
UNIVERSITY

*‘It is not the strongest of the species that survives,*

*nor the most intelligent,*

*but the one most responsive to change.’*

- Charles Darwin

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# Animal manure derivatives as alternatives for synthetic nitrogen fertilizers

Thesis submitted in fulfillment of the requirements for the degree of Doctor

(PhD) in Applied Biological Sciences

**Dutch translation of the title:**
Derivaten van dierlijke mest als alternatieven voor synthetische stikstofmeststoffen

**Cover illustration:**
Maize field experiment in Wingene, Belgium

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Ghent 29/11/2017

Ivona Sigurnjak

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# List of Abbreviations

| | |
|---|---|
| AD | Anaerobic digestion |
| AM | Animal manure |
| ANR | Apparent nitrogen recovery |
| ASW | Air scrubber water |
| CAN | Calcium ammonium nitrate |
| C, N, P, K, S | Carbon, Nitrogen, Phosphorus, Potassium, Sulfur |
| Ca, Mg, Na | Calcium, Magnesium, Sodium |
| Cu, Zn | Copper, Zinc |
| DAF | Dissolved air flotation |
| DM | Dry matter |
| EC | European Commission |
| $EC_{1:5}$ or $EC_{2:1}$ | Electrical conductivity |
| EU | European Union |
| EoW | End-of-waste criteria |
| FE | Fertilizer Europe |
| FMD | Flemish Manure Decree |
| FRUE | Fertilizer replacement use efficiency |
| FSD | Flemish Soil Decree |
| FUE | Fertilizer use efficiency |
| FW | Fresh weight |
| ICP-OES | Inductively coupled plasma optical emission spectrometry |
| INAGRO | Provincial research and advice center for agriculture and horticulture |
| LF | Liquid fraction |
| MC | Mineral concentrate |
| NUE | Nitrogen use efficiency |
| NFRV | Nitrogen fertilizer replacement value |
| $NO_3^-$-N | Nitrate - nitrogen |
| $NH_4^+$-N | Ammonium - nitrogen |

| | |
|---|---|
| NVZ | Nitrate vulnerable zones |
| OM | Organic matter |
| PCG | Vegetable research center |
| RO | Reverse osmosis |
| SAR | Sodium adsorption ratio |
| VCM | Flemish coordination center for manure processing |
| VITO | Flemish institute for technological research |
| VLM | Flemish land agency |

# **CHAPTER 1:** GENERAL INTRODUCTION

## 1.1 Intensification of livestock production

Intensification of livestock production represents an increased use of inputs to increase the output quantity and/or value of livestock production per unit of inputs (Bebe *et al.*, 2002; Udo *et al.*, 2011). It has been widely advocated as a solution to meet the increasing demands for livestock products, which are expected to double in the next 10-15 years as a response to population growth, urbanization, economic progress and changing consumer preferences (Thornton, 2010; Udo *et al.*, 2011; Sakadevan and Nguyen, 2017). If not done in a sustainable manner, meeting the increased demand for livestock products places increased pressure on the environment. The livestock sector is currently responsible for 14.5% of all human-induced greenhouse gas (GHG) emissions (Sakadevan and Nguyen, 2017). Moreover, it generates large nutrient surpluses of on-farm nitrogen (N) and phosphorus (P) that may lead to a pollution of water bodies. With farmers managing almost half of the European Union (EU) land area, the EU agriculture is an example of how intensification can cause detrimental effects on the environment. Next to the environmental aspects, livestock intensification is also faced with i) the disturbed balance between number of animals and growing area for fodder production and ii) import of 'feed protein' from other regions.

## 1.2 The region of Flanders in a European context

After the World War II, European society was damaged by the crippled agriculture and insufficient food supplies. As a result, the European farmers were encouraged to maximize their yields through the increased use of fertilizers and imported animal feed. This action was also supported by Treaty of Rome in 1957 (De Clercq *et al.*, 2001; Huygens *et al.*, 2011), when six founding countries of the European Economic Community (EEC; among which was Belgium) introduced the Common Agricultural Policy (CAP). The objectives of the CAP were set at increasing agricultural productivity, stabilizing market and ensuring exchange of agricultural goods at reasonable prices. These objectives were followed by Flemish (Belgium) agriculture which quickly developed to intensive livestock farming, leading to a high animal manure generation and its use in crop production.

The first signals of unbalanced nutrient use in Flanders, and the consequent nitrate pollution of water bodies, were recognized in the early '80 of the last century (De Clercq *et al.*, 2001). The problem was, and still is in most of the European regions, the discrepancy between excess load of nutrients from livestock production and the possibility to apply these nutrients in an environmental way on agricultural land. On January 23, 1991, the Flemish government issued the first Manure Decree for the protection of waters against pollution by nitrates from agricultural sources (BS, 1991). The Decree was issued about one year prior the EU Nitrates Directive 91/676/EEC (December 12, 1991) whose enactment has led to i) identification of polluted waters; ii) designation of nitrate vulnerable zones (NVZs); iii) establishment of Codes of Good Agricultural Practice; iv) establishment of action programmes and v) obligatory national monitoring and reporting (EC, 1991). For the first time in history Flanders was confronted with restrictions on the use of nutrients from animal manure.

The most severe restrictions include a limitation on N application from animal manure up to maximum of 170 kg N $ha^{-1}$ $y^{-1}$ and limitation on presence of nitrates in water bodies up to maximum of 50 mg $NO_3^-$ $l^{-1}$ (Nitrates Directive 91/676/EEC; Water Framework Directive 2000/60/EC). To identify the nitrate polluted waters, the Flemish monitoring network of covering 750 monitoring stations in surface waters and 2100 monitoring wells in phreatic groundwater was introduced (De Clercq *et al.*, 2001). In the year 2000, 60% of all measuring points exceeded the European Union (EU) norm of 50 mg $NO_3^-$ $l^{-1}$ (De Clercq *et al.*, 2001), with most of the excesses in areas of intensive livestock farming. To tackle all of these issues, the Flemish Manure Decree, considered as the implementation of the EU Nitrates Directive (EC, 1991), has been adapted several times over the last 20 years as a result of different Manure Action Plans (MAP). After the first two MAPs the region of Flanders was condemned in 2005 by the European Court of Justice for not implementing the Nitrates Directive into Flemish legislation in a complete and correct way. The new MAP III (2007 - 2010) has resulted in designation of Flanders as a 100% NVZ and in imposing the obligation of processing animal manure surpluses. Currently MAP V (2015 - 2018) is on-going with the main objective to lower the percentage of all monitoring stations exceeding the threshold value of 50 mg $NO_3^-$ $l^{-1}$ from the current 16% to maximum of 5% (Donoso *et al.*, 2017).

The processing of animal manure, as one of the main measures of the Flemish Manure Decree, can be met by treating manure in such way that a) the derivatives are exported out of Flanders, b) the nutrients are removed from the manure (i.e. conversion of N to $N_2$ gas) or c) the nutrients are converted into a mineral fertilizer (Lebuf *et al.*, 2013b). The first two options are the most commonly used at the moment. In 2015, 9.1 million kg N was exported out of the Flanders (VLM, 2017) whereas 12.7 million kg N was converted to $N_2$ (VCM, 2016). Out of 118 operational installations for manure processing, the majority (81 installations) treat manure in a biological way involving the conversion of N to $N_2$ (VCM, 2016). At the same time, however, the intensive crop production systems require a high input of nutrients to optimize yields. Due to the imposed limitation on the application of N from animal manure on the arable land, the allowable application of N from animal manure is lower than the crop N demand. To fill the gap synthetic N fertilizers are used by farmers to satisfy the crop N requirements. As a result, Flanders is faced with a nutrient paradox where despite existing N excess from animal manure around 70 million kg of N is applied annually on Flemish soil by using synthetic N fertilizers (Lenders *et al.*, 2013). In this dissertation, synthetic fertilizers are defined as inorganic fertilizers that are synthesized via Haber-Bosch process (e.g. N fertilizers) or based on fossil ore deposits (e.g. P and K fertilizers).

## 1.3 Cradle-to-cradle concept

In livestock intensive regions the mineral input and output are not balanced, as illustrated in section 1.2. The crop production systems rely on synthetic fertilizers for fertilization, whereas livestock production systems face significant costs and problems in waste disposal. At the same time, the EU is aiming for a more sustainable agriculture in which available resources are used effectively by re-connecting crop and livestock production through recycling materials such as on-farm animal manure (Figure 1.1).

The processing of animal manure is recommended not only in Flanders, but also in other European regions with high livestock production (see Chapter 2). Different processing techniques will result in different bio-based materials which may have potential to substitute synthetic N fertilizers. However, their use is hampered by legal restrictions which categorize these bio-based materials as waste (i.e. animal manure) and as such limit their disposal on arable land.

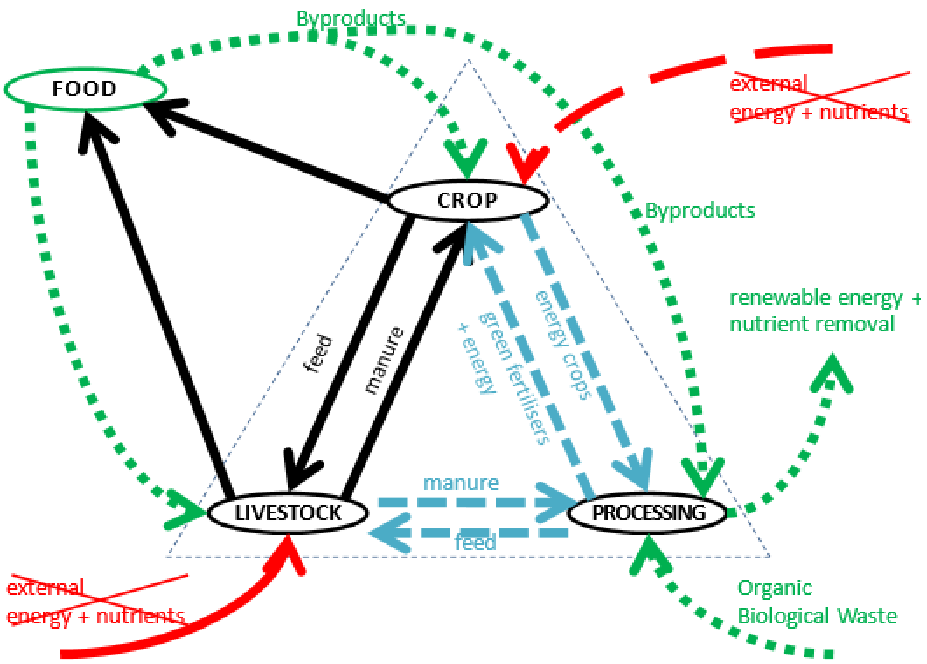


***Figure 1.1*** *Visualization of primary production nutrient flows and the cradle-to-cradle concept (Vaneeckhaute et al., 2013b).*

## 1.4 Objectives and dissertation outline

The EU has recently adopted an ambitious Circular Economy Package. One of the key principles of this package is the re-use of raw materials which are currently disposed as waste. This has placed bio-based materials from manure processing under the spotlight. To support EU legislation in considering these materials as products and not as waste, scientific research is needed to determine whether their use as bio-based fertilizer can be supported both from an environmental (i.e. risk for nutrient losses) and agronomic (i.e. effect on crop yield) perspective.

The overall aim of this PhD dissertation is to investigate the potential use of bio-based materials, derived from animal manure processing, as substitutes for synthetic N fertilizers. In particular, physicochemical characteristics of liquid fraction (LF) of digestate, air scrubber water (ASW) and mineral concentrate, and their impact on crop yield and soil properties will be addressed. The main hypothesis of the dissertation is that the use of LF of digestate, ASW and mineral concentrate will not cause significant differences in crop yield, nutrient uptake, soil N dynamics and soil properties as compared conventional synthetic N fertilizer. The following four research questions can be distinguished in this dissertation:

*i.* *Do bio-based materials behave similarly to animal manure or similarly to synthetic N fertilizer with respect to N dynamics?* **(Chapter 3)**

*ii.* *Does acidification increase N mineralization and N fertilizer replacement value of bio-based materials?* **(Chapter 4)**

*iii.* *Can bio-based materials be used as synthetic N substitutes in commercial greenhouse production of vegetables?* **(Chapter 5)**

*iv.* *What are single-year and multi-year effects of using bio-based materials on an open field scale production?* **(Chapters 6 and 7)**

The PhD dissertation consists of a general introduction (Chapter 1), literature review (Chapter 2), five research chapters (Chapters 3 – 7) and general conclusion which includes future research perspectives (Chapter 8). The interconnection of the different chapters is given in Figure 1.2.

**Chapter 2** presents the current status in utilization of bio-based materials as substitutes for synthetic N fertilizers. The chapter briefly introduces: a) the manure processing techniques that are currently available on the market, b) the relevant EU legislation that currently hampers the use of bio-based materials and c) the published scientific studies on utilization of LF of digestate, ASW and mineral concentrate.

**Chapter 3** deals with the first research question by assessing N dynamics (i.e. N release and N mineralization) of animal manure, digestate, LF of digestate derived from animal manure, LF of digestate derived from plant residues, mineral concentrate from LF of animal manure and mineral concentrate from LF of digestate. The chapter indicates which most commonly available bio-based materials behave more as synthetic N fertilizer and which tend to follow the trend of animal manure. ASW was not included in this experiment since it does not contain organic N, suggesting that from a perspective of N dynamics it will behave similarly as synthetic N fertilizer.

**Chapter 4** provides an answer on the second research question by evaluating the effect of acidification on: a) N dynamics via an incubation experiment, and b) marketable yield and N uptake of *Lactuca sativa* L. via a pot experiment. In Chapter 3 it is shown that animal manure, LF of animal manure, digestate and LF of digestate exhibit N dynamics that differ considerably from synthetic N fertilizer. Published studies suggest that acidification increases N mineralization and thus indirectly increases N fertilizer replacement value (NFRV) of bio-based materials. Therefore, these materials were subjected to acidification prior to the incubation and lettuce pot experiment, during which their performance was compared to synthetic fertilizers that are used in conventional horticulture.

**Chapter 5** examines the performance of LF of digestate and ASW as N fertilizers, struvite as P fertilizer and effluent from constructed wetlands (CW) as K fertilizer, in commercial greenhouse production of *Lactuca sativa* L. The performance of most commonly available bio-based materials was compared to their synthetic counterparts, with regard to crop growth, crop quality control and soil properties. In commercial greenhouse production synthetic N, P and K fertilizers are commonly used in the form of calcium ammonium nitrate (CAN), triple superphosphate and potassium sulfate, respectively. Hence LF of digestate and ASW, as N sources, were applied in combination with synthetic P and K fertilizer, but also in combination with struvite and effluent from CW.

**Chapter 6** evaluates the impact of LF of digestate and mineral concentrate, as a single source of N or in combination with animal manure, in a single-year cultivation of *Zea mays* L. These fertilization strategies were compared to the single use of CAN and conventional fertilization of using animal manure and synthetic fertilizers.

**Chapter 7** determines the effects of three-year field application of LF of digestate as a (partial) substitute of synthetic N fertilizer. LF of digestate was applied in combination with animal manure and digestate, respectively. The performance of these proposed fertilization strategies was tested in cultivation of *Zea mays* L. and compared to conventional fertilization of using animal manure and synthetic N fertilizer.

**Chapter 8** provides the general conclusion of the main findings of this work, and identifies the future research needs.

### Text Box: Agri-environmental indicators related to fertilizer performance

Agri-environmental indicators are a useful tool to determine fertilizer performance. The following agri-environmental indicators that consider the productivity level were introduced in this dissertation:

$$\text{Apparent N recovery (ANR)} = \frac{\text{N uptake}_{\text{TREATMENT}}\ (\text{kg ha}^{-1}) - \text{N uptake}_{\text{CONTROL}}\ (\text{kg ha}^{-1})}{\text{Total N applied}_{\text{TREATMENT}}\ (\text{kg ha}^{-1})} \quad \text{(Eq. 1)}$$

$$\text{N fertilizer replacement value (NFRV; \%)} = \frac{\text{ANR}_{\text{BIO-BASED TREATMENT}}}{\text{ANR}_{\text{REFERENCE}}} \times 100 \quad \text{(Eq. 2)}$$

$$\text{Fertilizer use efficiency (FUE)} = \frac{\text{Nutrient uptake (kg ha}^{-1})}{\text{Total nutrient applied (kg ha}^{-1})} \quad \text{(Eq. 3)}$$

$$\text{Fertilizer replacement use efficiency (FRUE; \%)} = \frac{\text{FUE}_{\text{BIO-BASED TREATMENT}}}{\text{FUE}_{\text{REFERENCE}}} \times 100 \quad \text{(Eq. 4)}$$

where, ‘Control’ is unfertilized treatment, ‘Bio-based treatment’ is a treatment containing one of the tested bio-based materials and ‘Reference’ is a conventional fertilization of using solely synthetic fertilizers (Chapters 4, 5 and 6) or combination of synthetic fertilizers and animal manure (Chapters 6 and 7).

As shown, there are similarities between ANR and FUE, and between NFRV and FRUE indicators. The main difference rises from the presence (Chapters 4 and 6) or absence (Chapters 5 and 7) of unfertilized treatment (i.e. control) in experimental design, and from the focus on assessed parameters. ANR and NFRV are solely related to N, whereas FUE and FRUE are associated also to phosphorus (P) and potassium (K).

In literature, the use of the control (unfertilized) treatment in agri-environmental indicators is inconsistent. In the policy context, the presence of unfertilized treatment is not practical because the approach of using control treatment is only valid for long-term field trials, whereas in short-term field trials the unfertilized treatment can still benefit from previous fertilizer application (Brentrup and Palliere, 2010).

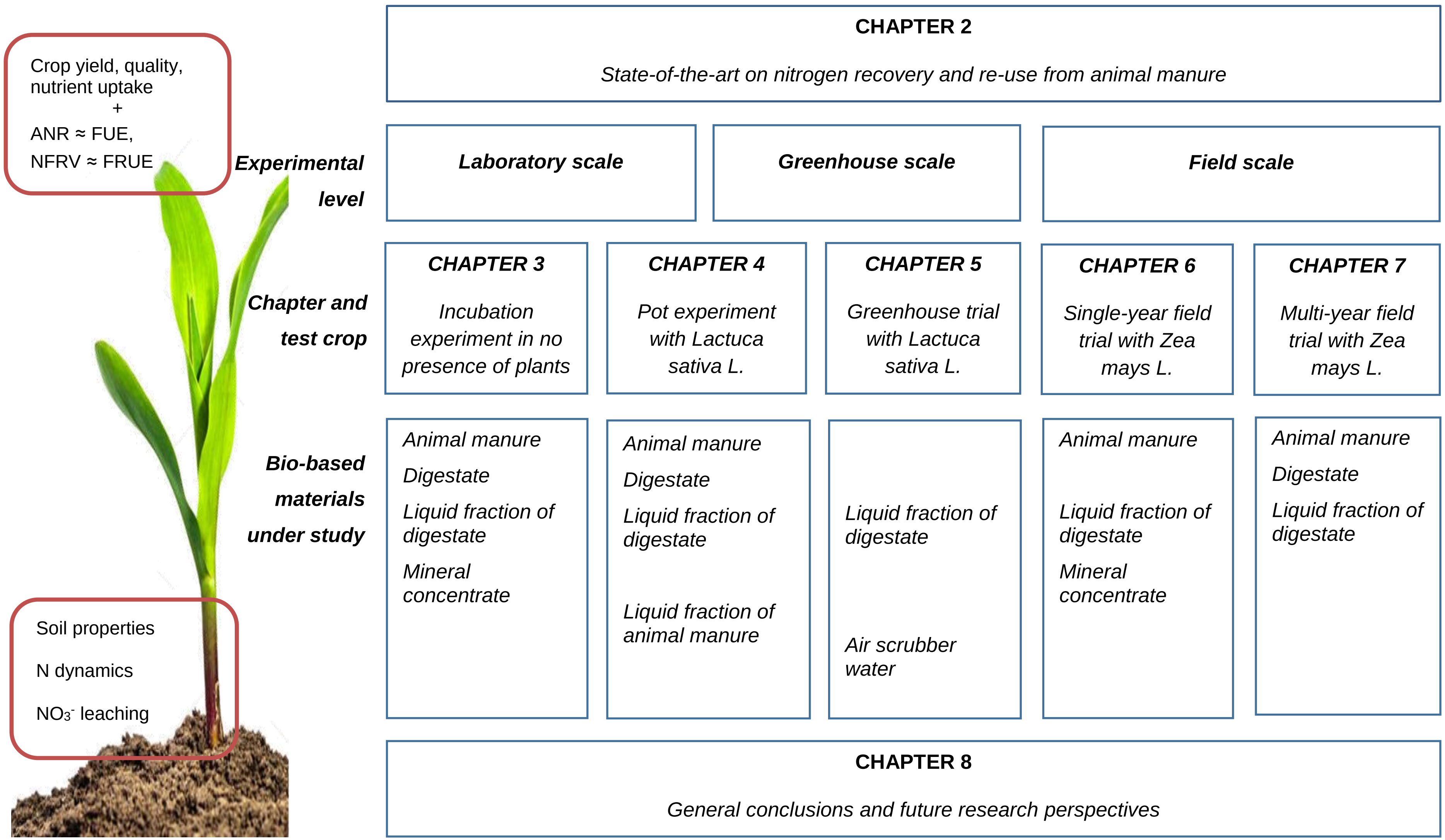


***Figure 1.2*** *Overview and interconnection of the different dissertation chapters including the main parameters of interest (FUE: Fertilizer use efficiency; ANR: Apparent nitrogen recovery; NFRV: Nitrogen fertilizer replacement; FRUE: Fertilizer replacement use efficiency). Maize picture © Alamy Stock Photo.*

# **CHAPTER 2:** STATE-OF-THE-ART ON NITROGEN RECOVERY AND RE-USE FROM ANIMAL MANURE

***This chapter has been redrafted after:***

Sigurnjak, I., Vaneeckhaute, C., Michels, E., Meers, E. xxxx. Manure as a resource for nutrients and energy. In Meers, E., and Velthof, G. (Eds.) Nutrient recovery book. Wiley Press. Under major revision.

## 2.1 Nitrogen cycle

Nitrogen (N) is a naturally occurring element that is essential for any life form on Earth. It is found in amino acids and nucleotides which are the building blocks of proteins and nucleic acids needed for the growth and reproduction of living organisms. Despite being one of the most abundant elements in the Earth's atmosphere (Sutton *et al.*, 2013), it is mostly present as rather inert di-nitrogen gas ($N_2$). Majority of plants and animals require reactive N ($N_r$) forms such as nitrate ($NO_3^-$) and ammonium ($NH_4^+$). In general, $N_r$ is defined as all N compounds except $N_2$.

The $N_r$ is scarce in the natural environment since it is provided by limited sources as biological N fixation (BNF) and lightning (Figure 2.1): prior to industrial and agricultural revolution, atmospheric deposition was considered as a relatively unimportant source (Bobbink *et al.*, 2010). The BNF is the primary non-anthropogenic input of $N_r$ that catalyzes the reduction of $N_2$ into $NH_4^+$ in the presence of the nitrogenase enzyme. The energy generated by lightning combines atmospheric $N_2$ and oxygen gas to N oxides ($NO_x$), which after reaction with rain form nitric acid ($HNO_3$) that is carried to the earth in the form of $NO_3^-$ (Fields, 2004). The global natural sources of $N_r$ (i.e. N fixation via lightning, terrestrial and marine BNF), prior to human influence on agricultural BNF and before the industrial revolution, are estimated at 203 Tg N yr$^{-1}$ (Fowler *et al.*, 2013).

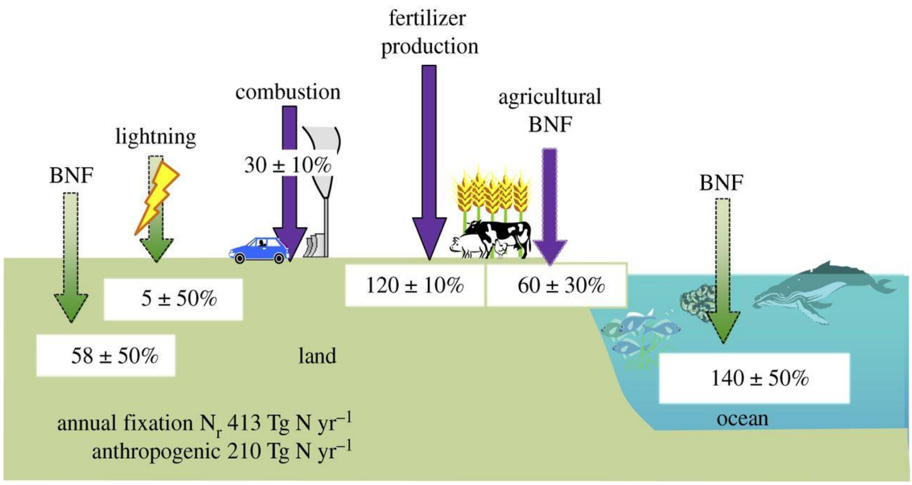


***Figure 2.1*** *Global nitrogen fixation, natural and anthropogenic in both oxidized and reduced forms through combustion, biological fixation, lightning and fertilizer and industrial production through the Haber-Bosch process for 2010. The arrows indicate a transfer from the atmospheric $N_2$ reservoir to terrestrial and marine ecosystems. Green arrows represent natural sources, purple arrows represent anthropogenic sources (Fowler et al., 2013).*

In response to the population growth, mankind has sought for additional sources of $N_r$ to sustain a global population by increasing the agricultural production. As a result, anthropogenic inputs of $N_r$ were introduced in 1908, when the Haber-Bosch process was patented as a catalytic combination of dihydrogen gas ($H_2$) with $N_2$ to form ammonia ($NH_3$) under high temperatures and pressures (Erisman *et al.*, 2008). Nowadays, the anthropogenic inputs of N (i.e. cultivated BNF in agriculture, $N_2$ fixation via

Haber-Bosch process, the burning of fossil fuels and forest fires) are estimated to be half (210 Tg N $yr^{-1}$) of the global (413 Tg N $yr^{-1}$; Figure 2.1) N fixation (Fowler *et al.*, 2013).

The soil N cycle is an integral part of the global N cycle. Once in the soil, the following N processes occur: mineralization, immobilization, nitrification, denitrification, ammonia volatilization, N uptake by plants, leaching, erosion and run-off (Salomez, 2004; Butterbach-Bahl *et al.*, 2011). **Mineralization** is the biological process by which microorganisms convert organic N to $NH_3$ that stabilizes in most soils (except alkaline soils) as $NH_4^+$. This occurs mostly during the decomposition of N rich substrates. The rates of mineralization vary with soil temperature, moisture and the amount of oxygen in the soil. In the presence of low soil temperature and limiting moisture, mineralization rates will be slower (Salomez, 2004). **Immobilization** is the opposite of mineralization where $NO_3^-$ and $NH_4^+$ are taken up by microorganisms and therefore become unavailable to crops. This occurs mostly during the decomposition of substrates that are poor in N. **Nitrification** is the process where relatively immobile $NH_4^+$ is oxidized via nitrite ($NO_2^-$) to highly mobile $NO_3^-$. This process is often considered as a key process in N cycling with regard to its relevance for N loss (Butterbach-Bahl *et al.*, 2011), because newly formed $NO_3^-$ is more susceptible to leaching than $NH_4^+$. **Denitrification** is the process where $NO_3^-$ is converted to gaseous forms such as nitric oxide, nitrous oxide and eventually to $N_2$. The process occurs in soil under anaerobic conditions when $NO_3^-$ replaces oxygen as the electron acceptor in soil microbial respiration. **Ammonia volatilization** is the loss of N through the conversion of $NH_4^+$ to $NH_3$ gas, which is released to the atmosphere. The volatilization losses increase at higher soil pH and conditions that favor evaporation, such as high temperature and wind. **Leaching** is the loss of water soluble nutrients from the soil that occurs due to heavy rainfall or excessive irrigation. In the context of N, leaching refers to the loss of $NO_3^-$ because anion exchange capacity of soils is lower than that for cations (Butterbach-Bahl *et al.*, 2011). As a result, $NO_3^-$ easily moves with water in the soil. **Erosion** is a process that results in the transfer of soil from arable land (mostly in hilly regions) to adjacent land. This process may transport large amounts of particulate N (i.e. N adsorbed on sediment particles), especially organic N and $NH_4^+$ that is adsorbed mainly on clay-sized particles (Salomez, 2004). Conversely, **run-off** is a process that results in the transfer of water from arable land to water courses. As compared to $NO_3^-$ leaching, small amounts of dissolved N are found in run-off water because during the heavy rainfall $NO_3^-$ is more prone to leaching (Salomez, 2004).

The soil N pool is continuously supplied with N inputs, but N is also leaving the system. In natural ecosystems the N cycle tends to be rather "closed" since inputs and outputs are very small as compared to the active N pool. In agricultural ecosystems the N cycle is however dominated by anthropogenic N inputs, making the inputs and outputs very significant compared to the active N pool, i.e. the N cycle is "open". As a result, agriculture is currently the largest sector driving $N_r$ creation.

## 2.2 Fertilizer production and consumption

The availability of $N_r$ via the Haber-Bosch process has led to increased crop production, intensification of livestock production and an increased world population. A recent estimate (Figure 2.2) showed that 48% of the current human population is supported by $N_r$ from Haber-Bosch process (Erisman *et al.*, 2008). Currently, the process is accountable for the production of 120 Tg N $y^{-1}$ (Sutton *et al.*, 2013). With a continuous growth of the global population, the demand for $N_r$ from the Haber-Bosch process is expected to increase to 165 Tg N $yr^{-1}$ by 2050 (Galloway *et al.*, 2004). As a result, the Haber-Bosch process seems to be indispensable for the global food security. The process is however energy intensive and fossil fuel dependent. Synthetic N production roughly consumes 1-2% of the world's annual primary energy supply and generates more than 300 Tg of fossil-derived $CO_2$ per year (Tanabe and Nishibayashi, 2013).

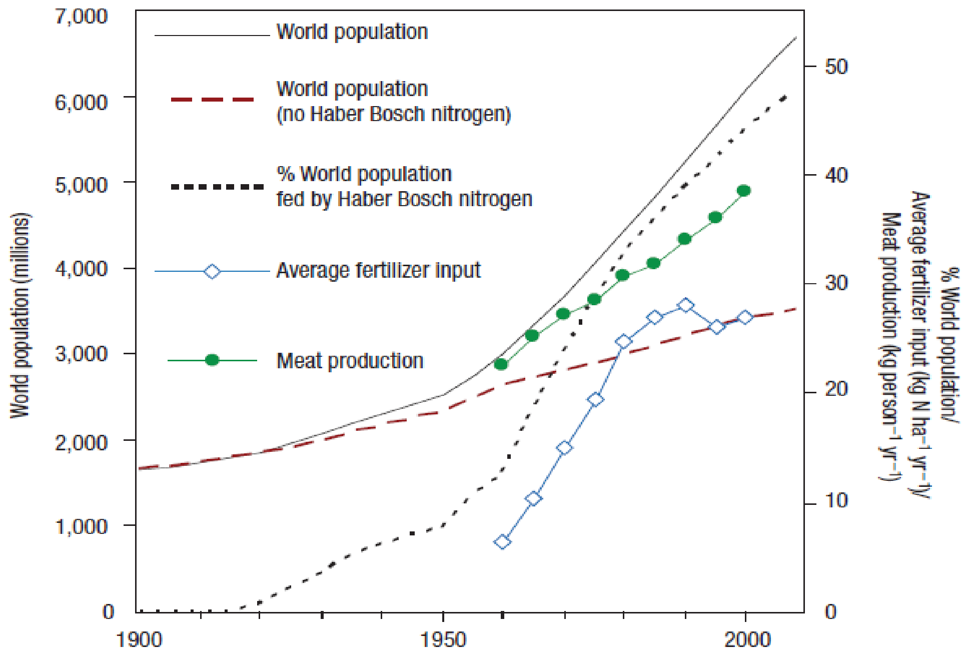


***Figure 2.2** Trends in world population (%), average fertilizer input (kg N $ha^{-1}$ $yr^{-1}$) and meat production (kg $person^{-1}$ $yr^{-1}$) throughout the twentieth century. From the total world population (millions), an estimate is made of the number of people that could be sustained without reactive nitrogen from the Haber–Bosch process, also expressed as a percentage of the global population (Erisman et al., 2008).*

At the same time, the intensification of the livestock production has resulted in local overproduction of animal manure. Animal manure is known as a source of $N_r$ whose unbalance redistribution may consequently lead to environmental problems. The estimated global amount of manure N ranges between 75 and 138 Tg N $y^{-1}$ (Oenema and Tamminga, 2005; Oenema, 2006; Liu *et al.*, 2016), and is as large as or larger than the synthetic N fertilizer use in the world, which ranges between 70 and 80 Tg N $y^{-1}$ (Oenema, 2006). However, N from animal manure is considered to be less effective than N from synthetic fertilizer, mostly due to the slow release of organically bound N and the lower concentration of readily available N (i.e. $NO_3^-$+$NH_4^+$). Therefore, N over-application often occurs in order to maximize the crop yield, whether through synthetic fertilizer or animal manure application. This occurs mostly in regions with intensive agriculture such as North America, Europe, South and South East Asia.

The increasing N inputs lead to losses of $N_r$, which have a detrimental effect on the environment and human health. The $NO_3^-$ leaching pollutes water courses, while the gaseous losses of $NH_3$ and NOx to the atmosphere reduce the quality of the air (i.e. particulate matter and smog). Animal excretion is the biggest contributor to these losses, accounting for 95 Tg N $y^{-1}$ of N loss via $NO_3^-$ leaching and runoff (Sutton *et al.*, 2013) and 45-75 Tg N $y^{-1}$ of gaseous N loss (Oenema, 2006).

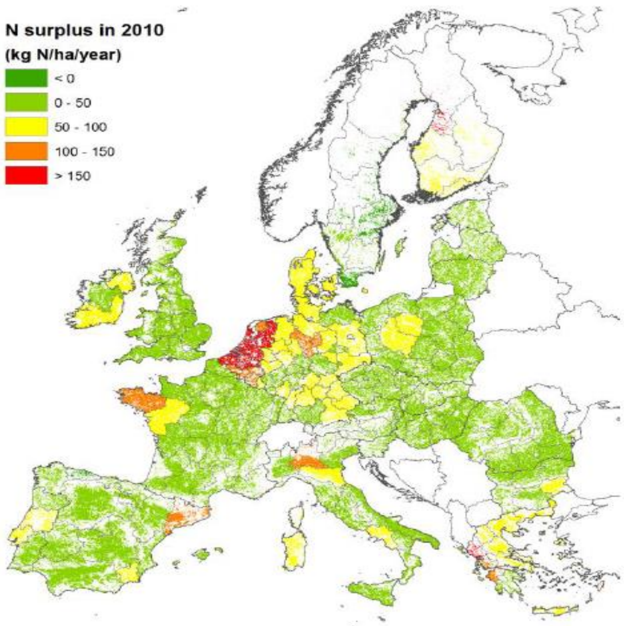


***Figure 2.3*** *Soil nitrogen (kg N $ha^{-1}$ $y^{-1}$ of utilized agricultural area) surplus in the EU-27 in 2010 according to MITERRA-Europe model. (Hou, 2016)*

As a one of the world's largest and most productive supplier of food and fibers, Europe was the first to recognize and deal with the issues of $N_r$ loss. In 1991, the Nitrates Directive (91/676/EEC) was implemented with the aim to protect water bodies by limiting the application of N from animal manure up to 170 kg N $ha^{-1}$ $y^{-1}$ in Nitrate Vulnerable Zones (NVZs) (EC, 1991). The NVZs are areas of land which drain into polluted waters (i.e. exceeding a concentration of 50 mg $NO_3^-$ $l^{-1}$ in surface waters or groundwater bodies) or waters at risk of pollution and which contribute to $NO_3^-$ pollution. These areas are mostly located in European regions known for the intensive livestock production (i.e. high livestock density), such as Flanders (Belgium), the Netherlands, Denmark, Brittany (France), Po Valley (Italy), Ireland, Aragon and Catalonia (Spain) (Figure 2.3).

As a result of the legal limitation on N application from animal manure, these regions need to (a) process their manure surplus and (b) improve the efficiency of N utilization from animal manure in order to minimize N losses.

## 2.3 Techniques for nutrient and energy recovery from animal manure

Animal manure is composed out of animal excreta dissolved in water or mixed with straw. It is a substance made up of organic matter (OM) and used as an organic fertilizer in agriculture, where it contributes to the fertility of the soil by adding plant nutrients and OM (Sommer, 2013). In the EU, any excess of animal manure should be transported to the N deficient areas or further treated. Transport of manure, which is often more than 90% water, is usually over considerably long distances and at a large financial and ecological cost. Processing of animal manure into bio-based materials with low water content facilitates transport, and additionally serves goals related to waste reduction and energy production from renewable wastes in the EU (Ehlert and Schoumans, 2015). Nowadays, the EU is trying to orient towards a circular economy that aims to "close the loop" of product lifecycles through greater recycling and re-use, and bring benefits for both the environment and economy (EC, 2015). Currently, this is impossible to achieve on the European level due to the continous import of nutrients from abroad, however, closing the loop on the smaller scale such as on farm level should be possible. This mindset has triggered the development of nutrient and bioenergy recovery techniques and their subsequent implementation into the manure management chain, where maximal amount of nutrients (NPK) and renewable energy is recovered. As such, livestock waste is reduced and renewable energy and minerals are produced. This section reviews current knowledge on the nutrient and bioenergy recovery techniques from livestock manure by providing an overview of most commonly used technologies and a classification of the resulting end-materials that can be used as bio-based fertilizers (Figure 2.4).

### 2.3.1 Classic processing of animal manure surplus

Composting is one of the oldest waste disposal methods that converts the biodegradable OM in the manure to oxidized end-products, primarily carbon dioxide ($CO_2$), water and compost with stabilized OM (Sweeten and Auvermann, 2008). As compare to earlier years, nowadays composting occurs under controlled temperature, moisture, nutrient and oxygen conditions. In regions with nutrient surpluses, this technique does not allow the reduction of the existing nutrient excess since it converts manure in more stable bio-based material containing N, P and potassium (K).

In order to reduce the costs of transporting the surplus of animal manure, farmers started to use mechanical separation as a first step in classic manure processing. The mechanical separation, by means of centrifuge, a screw press or a sieve band press, separates animal manure in NK-rich liquid and carbon (C) and P-rich solid fraction (SF) (Hjorth *et al.*, 2010). The SF can be composted or dried and exported as a soil enhancer, while the LF either is applied directly to the agricultural land or is subjected to biological treatment where it undergoes nitrification followed by denitrification. In high N pressure regions (Figure 2.3), application of the LF directly on the agricultural land might be seen as a competition with the application of animal manure, since both materials fall under the limitation of 170 kg N $ha^{-1}$ $y^{-1}$. Hence, some regions (eg. Flanders, Brittany and Po Valley) try to eliminate N via biological treatment involving nitrification and denitrification, where N is finally converted to atmospheric $N_2$. The resulting fraction can be used as a K-fertilizer (Figure 2.4).

Since the main goal of the biological treatment is a reduction of the N content and the biological oxygen demand (BOD) of the LF of manure (Lebuf *et al.*, 2012), this technique is usually complemented with constructed wetlands as a tertiary treatment. The remaining N, P and OM are removed through interactions between microorganisms, soil and plants, leaving dischargeable water with concentrations lower than Flemish discharge limits of 2 mg P $l^{-1}$, 15 mg N $l^{-1}$ and 125 mg COD $l^{-1}$ (Meers *et al.*, 2008). This "classical" manure processing is not a sustainable way of recovering nutrients present in manure, as N is converted to $N_2$ and lost to the atmosphere. In the bio-based economy it is important to fully recover nutrients present in manure. Next to nutrients, manure is also seen as a potential source for energy recovery. In that regard, bioenergy recovery techniques have been introduced in the classical manure management chain in the last decade (Figure 2.4).

### 2.3.2 Bioenergy recovery techniques

The energy content of animal manure can be estimated from its higher heat value (HHV): a total heat generated when a substance is combusted, including the latent heat which is released upon the water vapor condensation (Choi *et al.*, 2014). HHV of animal manure can differ depending on the type of livestock manure and its characteristics, for example, ranging from 7.9 MJ kg dry matter $(DM)^{-1}$ for soil surfaced feedlot manure to 18.2 MJ kg $DM^{-1}$ for flushed dairy manure (i.e. homogenized flushed manure liquid consisting of raw manure and rinsing water) (Cantrell *et al.*, 2007; Ro *et al.*, 2009). Ro *et al.* (2009) estimated the annual energy content of the 35 million dry tonnes of manure produced in USA to be c.0.43 EJ, providing renewable energy with an approximate worth of 0.7 billion US dollars per year. In EU-28, with an estimation of 104 million dry tonnes of manure being available for recycling and re-use (Liu *et al.*, 2017), the financial benefits could even be higher. These findings demonstrate that effective utilization of livestock waste as a renewable energy source can have significant impact on the country's agricultural energy budget and economy.

The renewable energy from animal manure can be extracted via biological and thermochemical conversion (TCC) processes. Biological processes convert manure for the production of methane ($CH_4$) by utilizing microorganisms, whereas TCC processes utilize heat at high temperature with or without the presence of air or oxidant. Both of these technologies have a dual function, to reduce the organic waste and to produce energy out of it. Only anaerobic digestion will be explained because TCC processes and their resulting end-materials are not within scope of this work.

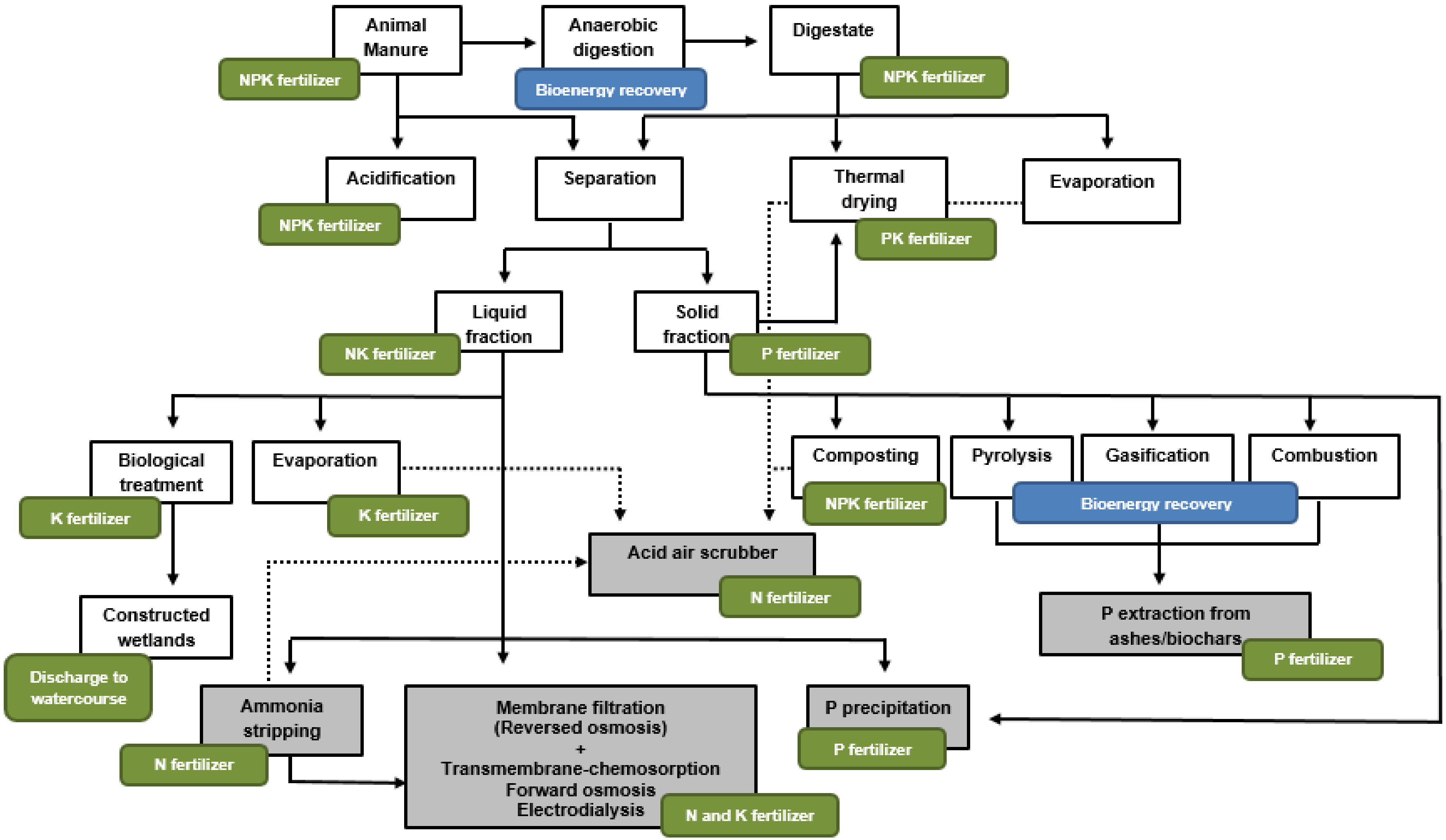


***Figure 2.4*** *Systematic overview of manure and digestate bioenergy and nutrient processing techniques. Nutrient recovery techniques are highlighted in grey. The green boxes present the by- or end-material of the respective technique that can be applied on the agricultural land. The blue boxes indicate techniques that generate bioenergy. The square dot dashes indicate the gaseous flow of N recovery via acid air scrubber.*

***Anaerobic digestion.*** Anaerobic digestion (AD) is a bioenergy recovery technique that involves the use of microorganisms or enzymes to convert animal manure into biogas and nutrient rich digestate. The digestion process starts with bacterial hydrolysis of the input materials, insoluble organic polymers such as carbohydrates are broken down to a range of organic compounds that are used by other bacteria. In a second phase, acidogenic bacteria convert the sugars and amino acids into $CO_2$, hydrogen ($H_2$), $NH_3$, and organic acids. In the third stage, acetogenic bacteria convert the resulting organic acids (i.e. the propionic acid and butyric acid) and alcohols into acetic acid, along with additional $NH_3$, $H_2$, and $CO_2$. Finally, methanogens convert the products of acetogenesis to methane ($CH_4$) and $CO_2$ (Bhatia, 2014). AD of animal manure, as a single substrate (mono-digestion), can inhibit the process of methanogens, due to the low organic loads and high $NH_4^+$-N concentration in animal manure. Adding one or more additional substrates (e.g. organic biological waste from food industry, energy maize, sludge, grass, etc.) in so called anaerobic co-digestion can overcome the limitations of mono-digestion, while improving the economic viability of AD plants due to higher $CH_4$ production (Mata-Alvarez *et al.*, 2014).

During AD about 20–95% of the feedstock OM is degraded (depending on feedstock composition) (Möller and Müller, 2012) and transformed into $CH_4$ and $CO_2$ (Möller and Müller, 2012). This implies that the OM and DM content decrease in the digestate. However, only easily degradable OM is decomposed while less degradable OM, such as lignin, remains in the digestate that retains soil improving qualities (Lebuf *et al.*, 2013a). Furthermore, the AD process converts a higher proportion of manure N into ammonium-N ($NH_4^+$-N) (Table 2.1), especially for feedstock with a high degradability, producing a digestate with high $NH_4^+$-N proportion in total N of above 80% (Möller and Müller, 2012; Sørensen and Jensen, 2013). The higher the share of $NH_4^+$-N is, the higher N fertilizer efficiency of digestate will be. Next, more than 90% of the volatile fatty acids (VFA) is decomposed, which leads to significantly lower odour emissions during the field application of digestate in comparison to pig slurry (Lebuf *et al.*, 2013a). On the other hand, decomposition of VFA results in a pH increase, which causes a higher risk for $NH_3$ volatilization. This volatilization during fertilization can be reduced by injection or incorporation of the digestate into the soil. The P content of the input streams is not changed during the AD process. Therefore, the P content of the digestate is entirely defined by the ingoing streams (Lebuf *et al.*, 2013a). Similarly, the AD does not alter the heavy metal content. However, during digestion DM content decreases which consequently increases the concentration of heavy metals in digestate. This is a particular attention point for zinc (Zn) and copper (Cu) during mono-digestion of pig slurry since the final levels in digestate can be above legally allowed limits. Impurities such as weed seeds and pathogens can be killed off during the digestion process. The extent to which this inactivation is sufficient depends entirely on temperature (mesophilic or thermophilic), residence time in the digester and the type of organism (Lebuf *et al.*, 2013a).

***Table 2.1*** *Range of main physicochemical characteristics of digestate (raw, liquid (LF) and solid (SF) fractions from mechanical separation) in comparison with undigested animal manure (raw, LF and SF from mechanical separation), and range of main characteristics of air scrubber water (obtained with $H_2SO_4$) and mineral concentrate (modified on the basis of EC, 2014).*

| Parameter | Digestate[a] | | LF of digestate[b] | | SF of digestate[b] | | Air scrubber water[c] | Mineral Concentrate[d] |
|---|---|---|---|---|---|---|---|---|
| | Absolute values | *Difference with manure* | Absolute values | *Difference with LF manure* | Absolute values | *Difference with SF manure* | Absolute values | Absolute values |
| DM (%) | 1.5-13.2 | *-1.5 to -5.5* | 1.6-6.6 | *-0.6 to -0.9* | 13.4-24.7 | *-0.3 to +0.3* | 26-36 | 1.4-5.2 |
| Total C (% DM) | 36-45 | *-2 to -3* | 33-48 | *-0.7 to -10.7* | 40-43 | *+0.8 to +1.0* | ND | ND |
| Total N (g $kg^{-1}$ FW) | 1.20-9.10 | *≈ 0* | 2.0-5.1 | *≈ 0* | 4.2-6.5 | *≈ 0* | 27-42 | 4.2-8.7 |
| $NH_4^+$-N/$N_{total}$ (%) | 44-81 | *+10 to +33* | 40-80 | *+6 to +13* | 26-49 | *+3 to +5* | 100 | 90-100 |
| C:N ratio | 3.0-8.5 | *-3 to -5* | 2.4-4.8 | *-1.6 to -3.1* | 11-19 | *-2.9 to +0.1* | ND | ND |
| Total P (g $kg^{-1}$ FW) | 0.4-2.6 | *≈ 0* | 0.2-1.0 | *-0.24* | 1.7-2.5 | *+0.4 to +0.8* | <0.05 | <0.3 |
| Total K (g $kg^{-1}$ FW) | 1.2-11.5 | *≈ 0* | 2.6-5.2 | *-0.13 to -0.17* | 2.4-4.8 | *+0.5 to +0.6* | <0.18 | 5.4-8.5 |
| pH | 7.3-9.0 | *+0.5 to +2* | 7.9-8.4 | *+0.66 to +1.19* | 8.5-8.7 | *+0.5 to +0.7* | 1.4-2.5 | 7.8-8.8 |
| EC (mS $cm^{-1}$) | 36-42 | *ND* | 34-47 | *ND* | ND | *ND* | 157-297 | 19-63 |

*DM: dry matter; FW: fresh weight; EC: electrical conductivity; ND: not determined*

[a] *Data from Möller and Müller (2012)*

[b] *Data from Möller and Müller (2012), Monaco et al. (2010; cited in EC 2014) and unpublished data on EC from Ghent University*

[c] *Data from Vaneeckhaute et al. (2014) and unpublished data from Ghent University*

[d] *Data from Schröder et al. (2014) and unpublished data from Ghent University*

### 2.3.3 Nutrient recovery techniques

As described before, AD results in nutrient rich by-material called digestate, next to the energy as a main output. The digestate, that is a result of animal manure processing, competes with animal manure for the disposal on arable land because both materials are considered as animal manure and as such currently fall under the legislative limit of 170 kg N $ha^{-1}$ $y^{-1}$. As a result of legislation, the N applied from animal manure and digestate is often lower than the crop N requirements. This is usually corrected by additional supply of synthetic N fertilizer that does not fall under the imposed legislative limit. Moreover, the Nitrates Directive indirectly also limits P fertilization rates. For some European regions (e.g. Flanders, Estonia, Brittany, Germany, Ireland, Northern Ireland, Norway, Sweden and the Netherlands) at the risk of soil with a high P status, this P limitation is not sufficient. Depending on the crop type and the soil P, the total P fertilization rates in these regions can be additionally imposed in range from 0-125 kg P $ha^{-1}$ $y^{-1}$ (Amery and Schoumans, 2014). In order to tackle the P issue, digestate is often separated into LF and SF. Due to its high P content, SF is usually composted and exported out of NVZs, or dried and subjected to TCC process. LF of digestate can be subjected to the similar valorization pathways as LF of animal manure.

In order to replace synthetic fertilizers, we need to tailor products that have similarities with synthetic fertilizer characteristics, where the most of N is present in mineral form (e.g. calcium ammonium nitrate, ammonium nitrate). Figure 2.4 shows a range of techniques suitable for manure and digestate processing, but not all of them can be considered as nutrient recovery techniques. Nutrient recovery techniques are defined as techniques that (a) create an end-product with higher nutrient concentrations than the raw digestate/manure or (b) separate the envisaged nutrients from organic compounds, with the aim to produce an end-product that is fit for use in chemical or fertilizer industry or as a mineral fertilizer replacement (Lebuf *et al.*, 2013b). These techniques make it possible to re-use the nutrients and close the nutrient cycle. Since the dissertation focuses on N recovery, only nutrient recovery techniques (i.e. ammonia stripping/scrubbing and membrane filtration) that generate N-rich bio-based materials are discussed (Figure 2.4).

***Ammonia stripping and scrubbing.*** $NH_3$ removal from N-rich waste streams (eg. LF of digestate or LF of animal manure) usually involves two steps: stripping and scrubbing. First, $NH_3$ is stripped (i.e. removed) by blowing air or steam through the waste stream in a packed bed tower (Figure 2.5A). As a result, $NH_3$ is transferred from the aqueous phase to a gas phase (Guštin and Marinšek-Logar, 2011). The released $NH_3$ is removed in a chemical air scrubber by washing it with a strong acidic solution (Figure 2.5B). To obtain optimal removal, often pH of the waste stream and temperature are adjusted to 10 and 70°C, respectively (Lemmens *et al.*, 2007). This technique can reach $NH_3$ removal efficiency of 99% (Melse and Ogink, 2005; Van der Heyden *et al.*, 2015).

Because of the low price, most often sulfuric acid ($H_2SO_4$) is used as an acidic solution. Ammonia can also be removed with hydrochloric (HCl), nitric ($HNO_3$) and phosphoric ($H_3PO_4$) acid. The reaction of $NH_3$ with $H_2SO_4$ results in ammonium sulfate $(NH_4)_2SO_4$ (also known as air scrubber water (ASW): the term includes all $NH_4^+$-N rich waters obtained after scrubbing $NH_3$ saturated air) that can be used as a NS-fertilizer. As a NS-fertilizer, ASW is characterized by acidic pH and a high salt content (Table 2.1).

In the system, deposition of the formed $(NH_4)_2SO_4$ can take place when the maximum solubility of the salt is exceeded. This leads to clogging and subsequently increases energy requirement during the production phase. To prevent this effect, in Flanders (Belgium) and the Netherlands, the N content in the ASW is not legally allowed to exceed 58.8 g N $l^{-1}$, which is about three times lower than the maximum solubility of the salt (164 g N $l^{-1}$; Van der Heyden *et al.*, 2015). Next to the removal of $NH_3$ from waste streams, $NH_3$ can also be recovered from livestock or manure operations such as housing, separation, composting and drying units. The ASW from livestock or manure operations contains N completely in mineral N form and as such is recognized in Flanders (VLM, 2014a) via national derogation as a substitute for synthetic N fertilizer. On the other hand, the ASW obtained by stripping and scrubbing ammonia from animal waste streams is still seen as animal manure despite having same product characteristics. The 'animal manure' status is currently assigned to this material because its use has not been regulated on the European level (VCM, personal communication).

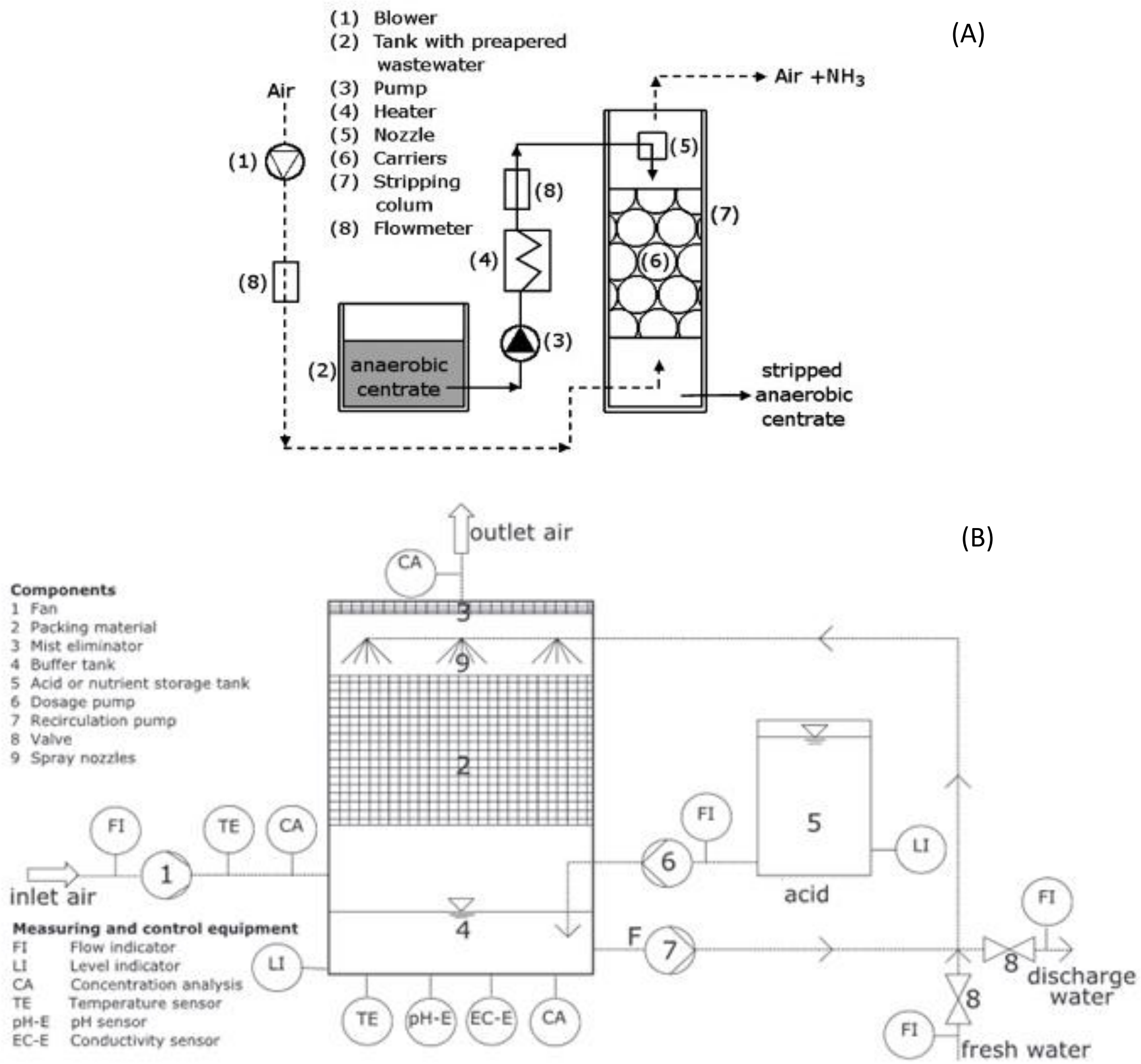


***Figure 2.5*** *Ammonia stripping (A; Guštin and Marinšek-Logar, 2011) and scrubbing (B; Van der Heyden et al., 2015) tower.*

***Membrane filtration.*** Membrane filtration is seen as an attractive supplement to mechanical separation by concentrating N present in LF of digestate and/or LF of animal manure in a more mineral N form. This can be achieved by subjecting LF to membrane filtration process where the waste stream is forced through the membrane by means of pressure (Figure 2.6). The material that is retained on the membrane is called retentate or mineral concentrate (Table 2.1), and is known for containing N ($NH_4^+$-N/$N_{total}$ = 0.9-1.0) almost completely in $NH_4^+$-N form (Schröder *et al.*, 2014; Velthof, 2015). There are several types of membranes used in manure/digestate processing: microfiltration (MF; pores >0,1 µm, 0,1-3 bar), ultrafiltration (UF; pores 5-200 nm, 2-10 bar) and reverse osmosis (RO; no pores, 10-100 bar) membranes (Christensen *et al.*, 2013).

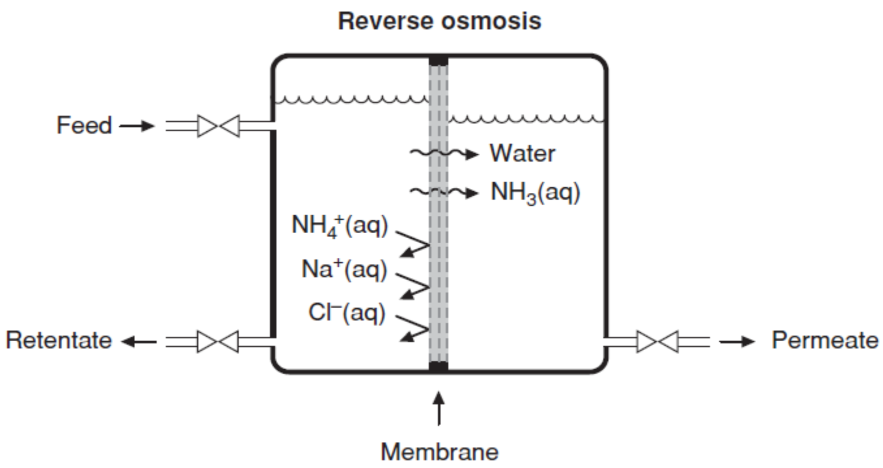


***Figure 2.6*** *Reverse osmosis filtration for concentration of ions in liquid manure (Christensen et al., 2013).*

In a MF-concentrate suspended solids are retained, while in a UF-concentrate also macromolecules are retained. Both filtration steps can be used as a pre-treatment for RO, in order to prevent that either suspended solids or macromolecules block the RO-membrane (Lebuf *et al.*, 2013a). Another technique that can be used prior to RO is dissolved air flotation (DAF), a technique in which small air bubbles are blown through the LF, entraining suspended solids to the surface where they form a crust. This crust is then scraped off (Vaneeckhaute, 2015). When using DAF coagulants (eg. Fe(III)$Cl_3$, PAC (polyaluminium chloride)) and flocculants (eg. Chitosan) are often added. The permeate of RO, which consists mainly of water and small ions, can be discharged or used as a process water. The biggest problem reported in membrane filtration is the blocking of the membrane. During MF and UF, this is mainly caused by suspended solids that form a cake on the surface of the membrane. Most of installations reduce the blocking of the membrane pores by continuously dosing acid solution to the RO-system, which is the most efficient way to reduce scaling and fouling (Lebuf *et al.*, 2013a).

## 2.4 Legal framework of utilizing bio-based materials as N fertilizers

As indicated before, animal manure processing leads to a variety of bio-based materials (e.g. digestate, LF of digestate, ASW and mineral concentrate) that might have potential to substitute synthetic N fertilizer (Figure 2.4). Their use and trade is currently governed by different European regulations. If European regulations do not apply, national regulations are in effect (Ehlert and Schoumans, 2015). As such, the legal framework for utilizing bio-based materials as N fertilizer is a complex combination of European Directives and National regulations. For example, ASW obtained from livestock or manure operations (i.e. housing, separation, composting and drying units) is currently accepted as a mineral fertilizer in the Flemish and Dutch national fertilizer regulation, but not on the European level. The European regulations that apply to end- and by-materials of animal manure processing are the Waste Framework Directive (2008/98/EC), the Animal By-Products regulation (EC/1069/2009), the Fertilizer regulation (EC/2003/2003), the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation (EC/1907/2006) and the Nitrates Directive (91/676/EEC).

### 2.4.1 Waste Framework Directive

The Waste Framework Directive (WFD) regulates waste management in EU, and explains when waste ceases to be waste and becomes a secondary raw material (EC, 2008). According to Article 6 (1) and (2), a certain specified waste shall cease to be waste when it has undergone a recovery operation (including recycling) and complies with specific criteria to be developed in line with certain legal conditions, in particular:

(a) the substance or object is commonly used for specific purposes;
(b) there is an existing market or demand for the substance or object;
(c) the use is lawful (substance or object fulfils the technical requirements for the specific purposes and meets the existing legislation and standards applicable to products);
(d) the use will not lead to overall adverse environmental or human health impacts.

These requirements are generally met by animal manure and bio-based materials of animal manure processing, if an environmental and agronomic sound application is possible (Ehlert and Schoumans, 2015). However, the WFD distinguishes between the animal manure and bio-waste (eg. waste from food industry) category. Since digestate is usually obtained via co-digestion of animal manure and bio-waste, it is categorized as biodegradable waste and as such must comply with End-of-waste (EoW) criteria. These criteria have not yet been implemented in EU regulations, but are currently being developed and proposed for compost and digestate. For other derivatives of animal manure (digestate) processing, these criteria are still missing.

### 2.4.2 Animal By-products regulation

The Animal By-products regulation prevents pathogen transmission from animals to humans by stipulating certain conditions towards the production, collection, transport, storage, use and disposal of animal by-products (EC, 2009). In the case of exporting animal by-products in the EU, exports need to be certified under this regulation by their National responsible entity. One of the preconditions for the export of products is pasteurization (i.e. heating products for minimum 1h at 70°C).

### 2.4.3 Fertilizer regulation

The Fertilizer regulation (2003/2003) ensures free movement in the single market for conventional synthetic fertilizers from primary raw materials by regulating the quality of the fertilizing material (EC, 2003). The regulation currently excludes products originating from organic or secondary raw materials, and as such conditions for the free movement of materials from nutrient recovery techniques have not yet been adapted. This regulation is currently under revision and it seems that new regulation will be imposed in future with the aim to broaden the scope by including organic fertilizers, biostimulants, soil improvers, growing medium, etc.

### 2.4.4 REACH regulation

The REACH regulation ensures a high level of protection for human health and the environment by requesting registration, evaluation, authorization and restriction of the properties of chemical substances (EC, 2006). All EU synthetic fertilizers are registered in REACH. Waste is excluded from REACH, but the REACH obligation comes into force once the EoW status is reached (Ehlert and Schoumans, 2015). Compost and biogas are exempted from the obligation to register. However, there is still confusion which materials from animal manure processing should be registered as in the case of digestate which currently is not exempt from REACH. The article 2(7)(d) of REACH states that a conditional exemption is provided for substances "which are recovered" in the EU from certain REACH requirements. In order to benefit from this exemption, a recovery operator must be able to demonstrate 1) that his recovered substance is the same as a substance that has already been registered under REACH and 2) that safety information on that substance is available to the recovery operator (EC, 2006). With that regard, products from manure processing (air scrubber water, struvite, etc.) could possibly be seen in the future as ‘recovered substances’ (VCM personal communication).

### 2.4.5 Nitrates Directive

The Nitrates Directive is the most crucial legislation on the utilization of N from animal manure. Currently, the Directive limits the use of N from animal manure and defines materials from animal manure processing as a waste rather than a product: ‘*livestock manure means waste products excreted by livestock or a mixture of litter and waste products excreted by livestock, even in processed form*’ (Article 2(g) of the Nitrates Directive 91/676/EC). This hinders the utilization of materials from animal manure processing. Currently, there is an indication that beginning of 2018 the revision process of the Nitrates Directive might take place.

In general, EU legislation on manure utilization should develop further by taking into consideration the last developments in manure processing. However, for this to occur scientific research on bio-based fertilizer utilization and performance is needed to prove the environmental and agronomic sound utilization of these bio-based materials.

## 2.5 Agricultural use of bio-based materials as substitutes for synthetic N fertilizers

The recognition of bio-based materials as potential substitutes for synthetic N fertilizers will only take place once their environmental and agronomic sound application is scientifically proven. In order to achieve this aim, the performance of materials from animal manure processing should be compared to the performance of synthetic N fertilizers, with respect to plant availability, efficiency and risk of losses. This type of investigations are quite recent and still relatively few, especially if compared with those on animal manure (Nkoa, 2014). In the following sections an overview of the current knowledge on the utilization of digestate, LF of digestate, ASW and mineral concentrate, as a potential substitute for synthetic N fertilizer, is given.

### 2.5.1 Digestate

Current research on bio-based fertilizer utilization aimed mostly at evaluating the fertilization value of digestate as compared to animal manure and mineral N fertilizers as a reference. Findings across a wide range of studies (EC, 2014b) have indicated that a higher availability of N can be expected in digestate as compared to animal manure. This is attributed to the higher presence of N in $NH_4^+$-N form as a result of the AD process (see section 2.3.2). Next to the $NH_4^+$-N increase, AD increases the pH of digestate. Thus, a higher risk of $NH_3$ volatilization should be anticipated when using digestate as a fertilizer. With regard to $NO_3^-$ losses, it is expected that the reduced content of organic N in the digestate results in reduced potential for long-term $NO_3^-$ leaching as compared to animal manure. Current findings indicate that in the short-term digestate has a similar $NO_3^-$ leaching potential as animal manure (Svoboda *et al.*, 2013). With regard to $NH_3$ losses, they can be prevented by utilizing a proper method of digestate application (e.g. injection or incorporation). Moreover, it was reported that by injecting or incorporating digestate a higher crop N uptake can be observed as compared to animal manure (Webb *et al.*, 2013). Experiments in which digestate performance was compared to the use of synthetic N fertilizers, point out that sometimes similar crop N use efficiencies can be achieved with digestate and synthetic N fertilizer (EC, 2014b; Tampio *et al.*, 2016). These results were observed mostly in the first-year of application. Experimental results on N use efficiency of long-term digestate applications are lacking thus far. In general, the potential environmental problems that are associated with digestate utilization as a fertilizer are similar to the ones caused by utilizing animal manure. In order to create products which resemble more to synthetic N fertilizers, digestate should be further processed in, for example, LF of digestate, ASW and mineral concentrate.

### 2.5.2 Liquid fraction of digestate

In the last decade, only few field scale studies investigated the agronomic and environmental performance of LF of digestate as a potential synthetic N substitute. Literature findings indicate that LF of digestate can result in equal (Cavalli *et al.*, 2014; Cavalli *et al.*, 2016; Riva *et al.*, 2016) or even higher (Walsh *et al.*, 2012; Vaneeckhaute *et al.*, 2014) grass, grass-clover and maize yields as compared to the use of synthetic N fertilizer. However, only in two studies the N fertilizer value of LF of digestate was reported (Vaneeckhaute *et al.*, 2014; Cavalli *et al.*, 2016).

The N fertilizer value of the certain material can be determined by calculating the apparent N recovery (ANR) and N fertilizer replacement value (NFRV). The ANR (Eq. 1; Chapter 1), also known as N use efficiency (NUE), is defined as the amount of applied total N that can be taken up by the crop on top of what is taken up by an unfertilized control in a single season of fertilizer application (Schröder *et al.*, 2013; Cavalli *et al.*, 2016; Tampio *et al.*, 2016). The NFRV (Eq. 2; Chapter 1) or mineral fertilizer equivalent (MFE) is the ratio between the ANR of the bio-based fertilizer and that of the synthetic fertilizer ANR expressed as percentage (Schröder *et al.*, 2013; Cavalli *et al.*, 2016). In a two-year field maize experiment (Montanaso Lombardo, Italy) by Cavalli *et al.* (2014), the ANR of LF of digestate (≈ 20% in 2011 and ≈ 25% in 2012) was lower as compared to the ANR (68% in 2011 and 82% in 2012) of ammonium sulfate that was used as a synthetic N fertilizer, resulting in NFRV of 30% for LF of digestate. This study was conducted on loam soil (0-30 cm soil layer: sand 47%, silt 39%, clay 14%, pH ($H_2O$) 5.8, total N and organic carbon (C) (% DM) 0.10 and 0.84, extractable phosphorus (P) (Bray and Kurtz method) 61 mg $kg^{-1}$, exchangeable potassium (K) 167 mg $kg^{-1}$, and bulk density 1.49 g $cm^{-3}$) and in region characterized by an annual rainfall of about 800 mm and an average annual mean air temperature of 12.5 °C. In the same time period, a two-year field experiment was conducted in Flanders (Belgium: soil characteristics and weather data available in Chapter 7) in which LF of digestate was applied in maize cultivation (Vaneeckhaute *et al.*, 2014). The LF of digestate was applied on top of a) animal manure with (35% of total N applied came from LF of digestate in 2011) or without (60% of total N applied came from LF of digestate in 2012) start synthetic N addition and b) in combination with digestate (24% and 31% of total N applied came respectively from LF of digestate in 2011 and 2012) (Vaneeckhaute *et al.*, 2014). The performance of both treatments was compared to the conventional fertilization strategy where animal manure is applied in combination with synthetic N fertilizer. The reported NUE (i.e. in this case, crop N uptake/total N applied; in Chapter 5 and 7 introduced as N fertilizer use efficiency (FUE)) of conventional and newly proposed fertilization strategies did not differ from each other. Moreover, NUE values for all treatments tended to exceed 100%, indicating that N uptake by the plant was higher than the available amount brought via bio-based fertilizer application (Vaneeckhaute *et al.*, 2014). The determination method of N fertilizer value of LF of digestate from these two studies differed with regard to unfertilized control treatment that was present in study by Cavalli *et al.* (2014), but not in Vaneeckhaute *et al*. (2014). As a result, the percent of applied N that was exported by maize varied from c.20% (Cavalli *et al.*, 2014) to above 100% (Vaneeckhaute *et al.*, 2014). As such, it is difficult to draw conclusions from these studies due to the differences in methodology.

Next to the agronomic performance, an environmental impact of using LF of digestate was determined with respect to $NH_3$ emissions and $NO_3^-$ losses. $NH_3$ emissions from LF of digestate (cattle manure + energy crops) applied via injection were similar on average to those measured for urea application (Riva *et al.*, 2016). As expected, the surface application of LF of digestate led to serious $NH_3$ losses as compared to urea utilization that is the common fertilization practice used in Italy (Riva *et al.*, 2016). In general, $NH_3$ reduction can be obtained by: a) slurry/digested injection, b) anaerobic digestion of slurries and c) mechanical separation of digestate (Riva *et al.*, 2016). With regard to potential risk of $NO_3^-$ losses, the soil mineral N content at harvest time in maize cultivation did not differ between the LF of digestate and synthetic N fertilizer treatment (Cavalli *et al.*, 2014; Vaneeckhaute *et al.*, 2014; Cavalli *et al.*, 2016). The only difference was reported in a grassland pot experiment where it was observed that application of inorganic fertilizer, rather than LF of digestate, could lead to higher $NO_3^-$ concentration in the soil (Walsh *et al.*, 2012). This experiment was conducted in controlled environment, while observed results could differ on the field scale due to the weather conditions, application method, timing and load of fertilizer application (Walsh *et al.*, 2012).

### 2.5.3 Air scrubber water

To date, only Vaneeckhaute *et al*. (2013b; 2014) investigated the use of ASW as a potential substitute for synthetic N fertilizer. In a two-year field experiment, ASW was applied in maize cultivation on top of a) animal manure and start synthetic N (19% and 25% of total N applied came respectively from ASW in 2011 and 2012), b) animal manure (35% and 51% of total N applied came respectively from ASW in 2011 and 2012), c) mixture of LF of digestate and raw digestate (29% of total N applied came from ASW in 2011) and d) raw digestate (19% of total N applied came from ASW in 2011). The performance of these treatments was compared with the conventional fertilization strategy where animal manure is applied in combination with synthetic N fertilizer. With respect to crop yield no differences were reported between the conventional and newly proposed fertilization strategies. The NUE, as in the case of LF of digestate (see section 2.5.2), tended to exceed 100%. Finally, there were no differences among the treatments at harvest time with regard to soil $NO_3^-$ content.

### 2.5.4 Mineral concentrate

The currently available studies on agronomic and environmental performance of MC from LF of animal manure were conducted in the Netherlands (Klop *et al.*, 2012; Schröder *et al.*, 2013; Schröder *et al.*, 2014). It was found that the effect of MC on crop yield is highly dependent on the method of fertilizer application. Klop *et al.* (2012) observed that surface application of MC, in a 26-day greenhouse experiment, lead to lower ryegrass yield as compared to CAN performance. If MC was injected, not only an increase in crop yield was observed but also an increase in ANR. The ANR of MC increased from 38% (surface application) to 59% (injection application) as compared to observed ANR of CAN which amounted in the same conditions to 62% and 64%, respectively. During a 4-year period (2009-2012), the performance of MC was investigated in six field experiments where potato and maize were used as test crops by Schröder *et al.* (2013; 2014). In all experiments, similar amounts of MC-derived N and CAN derived-N resulted in comparable yields. The NFRV values of injected MC in all six experiments ranged between 72-84% (Schröder *et al.*, 2014). If the $NH_4^+$-N/$N_{total}$ ratio (0.90 – 1.00) of MCs is taken into consideration, the NFRV is relatively small (Schröder *et al.*, 2013).The observed reduction in NFRV was seen as the result of enhanced $NH_3$ losses despite of injecting the bio-based material, which was also confirmed in this study by reduced amounts of residual soil mineral N after using MCs. It seems even when injected, MCs may lose $NH_3$ from the injection slots due to the high pH and high $NH_4^+$-N concentration (Table 2.1; Schröder *et al.,* 2013; Schröder *et al.,* 2014).

# **CHAPTER 3:** NITROGEN RELEASE AND MINERALIZATION POTENTIAL OF BIO-BASED MATERIALS UNDER CONTROLLED CONDITIONS

*PVC tubes, containing mixture of soil and bio-based materials, incubated in 120-day incubation experiment (Pictures: Sigurnjak I.)*

***This chapter has been redrafted after:***

Sigurnjak, I., De Waele, J., Michels, E., Tack, F.M.G., Meers, E., De Neve, S. 2017. Nitrogen release and mineralization potential of derivatives from nutrient recovery processes as substitutes for fossil fuel based nitrogen fertilizers. Soil Use and Management 33, 437–446.

## Abstract

The need to meet rapidly-increasing demands for synthetic nitrogen (N) while reducing dependence on fossil fuels has been driving widespread attention to the recuperation and reuse of nutrients present in digestate and animal manure. The N release and mineralization potential of animal manure (AM), digestate (DIG), liquid fraction of digestate (LFDIG) and mineral concentrate (MC) were assessed in comparison with N availability from calcium ammonium nitrate (CAN) as reference. The release was highly dependent on the material $NH_4^+$-N/$N_{total}$ ratio, while mineralization occurred only for bio-based materials containing more than 5 % of organic N. The magnitude of the released N, on average after 120 days of an incubation experiment, was in the order: CAN > MC > LFDIG > DIG ≥ AM. These results indicate that only the N release from MC exhibited patterns similar to CAN, suggesting that this material will provide plant available N in a similar fashion as synthetic fertilizers. The N release from LFDIG was higher than AM, but did not closely follow the pattern of CAN. The N availability in LFDIG may be increased by using substrates richer in N, such as animal manure or waste food and not only plant residues.


## 3.1 Introduction

European agriculture has evolved towards increasingly intensive plant production systems, resulting in increasing demand for mineral fertilizers. In 2014, mineral fertilizer use in Europe (EU-27) amounted to 10.7 million tonnes of N, 2.5 million tonnes of phosphate ($P_2O_5$) and 2.7 million tonnes of potash ($K_2O$) (FE, 2015). European (EU-27) livestock production generates around 1,400 million tonnes of manure each year (Flotats *et al.*, 2013). Currently, 7.8 % of all livestock manure in Europe is processed. This involves 108 million tonnes of animal manure containing 556,000 tonnes N and 139,000 tonnes phosphorus (P) (Flotats *et al.*, 2013).

Even though processing of animal manure is meant to increase the overall agronomic nutrient use efficiency, bio-based materials such as digestate, liquid fraction of digestate and mineral concentrate derived from it continue to be subject to the legal definition of livestock manure in the Nitrates Directive. Consequently, these materials are subject to the same restrictions on N application as animal manure, which hinders the profitable development of the European biogas sector (Lebuf *et al.*, 2012; EC, 2013).

In order to enhance the production of renewable energy and the sustainability of agriculture, it has become crucial to evaluate the performance of these materials in order to determine their potential as bio-based N fertilizer. The information gained from N mineralization studies is key in assessing N availability from bio-based materials and efficiently predicting the need for N fertilization (De Neve and Hofman, 1996; Alburquerque *et al.*, 2012a). To date, research has mostly focused on N availability and N efficiency of digestate and animal manure (Azeez and Van Averbeke, 2010; Grigatti *et al.*, 2011; Abubaker *et al.*, 2012; Alburquerque *et al.*, 2012a; Rigby and Smith, 2013). Less is known about the composition and N fertilizer properties of digestate derivatives such as the liquid fraction of digestate and mineral concentrate, compared to synthetic N fertilizers. Determining the main properties affecting

N dynamics in soil upon addition of these processed materials can help to better and more sustainably valorize these materials as synthetic N substitutes. Moreover, these results can indicate how digestate, liquid fraction of digestate and mineral concentrate behave compared to “reference” materials (either animal manure or synthetic N fertilizer). Thus, this chapter aimed to (1) characterize the physicochemical composition of animal manure, digestate, liquid fraction of digestate and mineral concentrate, (2) determine their N release and mineralization potential and (3) assess their potential as N source with reference to the conventional mineral fertilizer calcium ammonium nitrate (CAN). It was hypothesized that (1) the N release and mineralization dynamics will differ depending on the type of processed material added and (2) the processed materials such as liquid fraction of digestate and mineral concentrate will behave more similarly to synthetic N fertilizer than animal manure.

## 3.2 Materials and methods

### 3.2.1 Soil collection and analysis

The soil used for the incubation experiment was collected from the surface layer (0-30 cm) of an arable field in Roeselare, Belgium. The soil is an Eutric Retisol loamic (WRB classification; Dondeyne *et al.*, 2014) and contains 9% clay, 32% loam and 59% sand fraction. Its texture is classified as sandy-loam (USDA texture triangle). Maize and chicory were cultivated in 2012 and 2013, respectively. The soil sample was obtained on April 1 2014 before the fertilizer application and sowing of a new maize crop. A subsample of field-moist soil was taken for the determination of the moisture content, organic carbon (OC), pH-KCl, pH-$H_2O$ and mineral N ($NO_3^-$-N and $NH_4^+$-N). The moisture content was determined by weight loss after drying the soil sample to constant weight at 105 °C for at least 24 h. OC was determined in two steps: first organic matter (OM) was measured using a muffle furnace for 4 h at 550 °C, and secondly the calculated OM was divided by factor 2 to obtain the OC level in the soil samples (Sleutel *et al.*, 2007). Soil potential acidity (pH-KCl) was measured using an Orion-520A (USA) pH-meter after adding 50 ml of 1M KCl to 10 g of soil and allowing it to equilibrate for 10 minutes (Van Ranst *et al.*, 1999). Soil actual acidity (pH-$H_2O$) was measured using the same device (i.e. Orion-520A USA) after 10 g of soil was allowed to equilibrate in 50 ml demineralized water for 16 h (Van Ranst *et al.*, 1999). Total N content in soil was determined using the Kjeldahl destruction method, while nitrate N ($NO_3^-$-N) (ISO 13395:1996) and ammonium N ($NH_4^+$-N) (ISO 11732:1997) in soil were analyzed from 1M KCl extract using a continuous flow auto-analyzer (Chemlab System 4, Skalar, the Netherlands). The soil used for the N incubation experiment was air-dried and stored in the laboratory before use.

### 3.2.2 Collection and analysis of bio-based materials

Six bio-based materials were investigated: (1) animal manure (AM); (2) digestate (DIG); (3) liquid fraction of digestate from animal origin (LFDIG_AM); (4) liquid fraction of digestate from non-animal (plant) origin (LFDIG_PLT); (5) mineral concentrate (MC_LFDIG) obtained after reverse osmosis (RO) treatment of liquid fraction of digestate; (6) mineral concentrate (MC_LFAM) obtained after RO treatment of liquid fraction of animal manure. AM used in the trial was collected at a local pig farm in Beitem, Belgium. DIG and LFDIG_AM were sampled from Bioelectric (Beernem, Belgium), a thermophilic (54°C) anaerobic co-digestion plant (capacity: 60,000 tonnes $y^{-1}$, 2.46 $MW_{el}$) with a retention time of 45 – 60 days, and with an input feed consisting of 16 % animal manure, 12 % energy maize and 72 % organic biological waste originating from the food industry (e.g. starch from potatoes, biological sludge, glycerin, unpacked products from supermarket). LFDIG_AM was obtained after the decanter centrifuge of the DIG. LFDIG_PLT was sampled from Agrogas (Geel, Belgium), a mesophilic (38°C) anaerobic co-digestion plant (capacity: 60,000 tonnes $y^{-1}$, 2.98 $MW_{el}$) with a retention time of 50 days, and with an input feed consisting of 50 % energy maize and 50 % organic biological waste originating from the food industry (e.g. leek leaves, onion and starch from potatoes). LFDIG_PLT was obtained after the centrifuge of a digestate. MC_LFDIG was sampled at the site of Ampower (Pittem, Belgium), a thermophilic (54°C) anaerobic co-digestion plant (capacity: 180.000 tonnes $y^{-1}$, 7.44 $MW_{el}$) with a retention time of 45 – 60 days, and with an input feed consisting of 10 % animal manure and 90 % organic biological waste originating from the food industry (e.g. starch from potatoes, carrots, biological sludge, glycerin, unpacked products from supermarket). The digestate from this installation was subjected to decanter centrifuge to obtain LF of digestate that was subsequently sent to a dissolved air flotation (DAF) unit. The DAF unit clarifies the LF of digestate by removing suspended organic material using micro bubbles which force particles to float on the surface and form a layer of sludge that is scraped off and sent back to the centrifuge. The LF of digestate after DAF is subjected to a RO system, resulting in MC_LFDIG. MC_LFAM was obtained from a pig farm Houbraken (Bergeijk, the Netherlands) whose system subjects pig manure to DAF unit from where sludge is subsequently separated by means of a sieve band press. The LF of animal manure from separation and LF of animal manure from the DAF unit are subjected to microfiltration (50 µm pore size) and afterwards to RO, resulting in MC_LFAM.

All bio-based materials were collected in polyethylene sampling bottles (2 L), stored (4 °C) and characterized (Table 3.1) to determine the required fertilizer dosage. Dry matter (DM) content was determined as the residual weight after 72 h drying at 80°C. OM was measured after incineration (loss on ignition) of the samples during 4 h at 550°C in a muffle furnace. The loss of mass on ignition was considered as the OM and subsequently used to determine OC content according to CMA/2/IV/3 method (VITO, 2012). Electrical conductivity (EC) and pH were determined on fresh sample using a WTW-LF537 (GE) conductivity electrode and an Orion-520A pH-meter (USA), respectively. Total N was determined using Kjeldahl destruction, and $NH_4^+$-N was determined using a Kjeltec-1002 distilling unit (Gerhardt Vapodest, GE) after addition of MgO to the sample, and subsequent titration (Van Ranst *et al.*, 1999). $NO_3^-$-N was determined by using a continuous flow auto-analyzer (Chemlab System 4, Skalar, the Netherlands) from a 1M KCl extract. Organic N was calculated as a difference in the values of total and mineral ($NH_4^+$-N + $NO_3^-$-N) N. After wet digestion (2 ml $HNO_3$ and 1 ml $H_2O_2$), total P was analyzed using the colorimetric Scheel method (Van Ranst *et al.*, 1999), while total K, Ca, Mg and Na were analyzed using Inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista MPX, USA).

***Table 3.1*** *Characterization of bio-based materials on fresh weight basis.*

| Parameters | AM | DIG | LFDIG_AM | LFDIG_PLT | MC_LFDIG | MC_LFAM |
|---|---|---|---|---|---|---|
| Dry matter (g $kg^{-1}$) | 85 | 70 | 23 | 63 | 49 | 35 |
| Organic matter (g $kg^{-1}$) | 58 | 48 | 13 | 35 | 31 | 17 |
| Organic carbon (g $kg^{-1}$) | 32 | 27 | 7.1 | 19 | 17 | 9.2 |
| pH | 7.9 | 8.0 | 8.7 | 8.1 | 8.2 | 8.1 |
| EC (mS $cm^{-1}$) | 28 | 38 | 20 | 33 | 59 | 55 |
| $N_{total}$ (g $kg^{-1}$) | 5.6 | 4.3 | 4.7 | 6.6 | 4.0 | 7.3 |
| $NH_4^+$-N (g $kg^{-1}$) | 3.3 | 2.2 | 3.6 | 4.1 | 3.8 | 5.9 |
| $NO_3^-$-N (g $kg^{-1}$) | <0.002 | <0.002 | <0.002 | <0.002 | <0.002 | <0.002 |
| $N_{organic}$ (g $kg^{-1}$) | 2.3 | 2.1 | 1.1 | 2.5 | 0.2 | 1.4 |
| $P_{total}$ (g $kg^{-1}$) | 2.1 | 1.1 | 0.027 | 1.2 | 0.021 | 0.31 |
| $K_{total}$ (g $kg^{-1}$) | 3.3 | 2.2 | 3.0 | 4.4 | 3.4 | 7.3 |
| $Ca_{total}$ (g $kg^{-1}$) | 4.9 | 2.0 | 0.10 | 2.1 | 0.10 | 0.13 |
| $Mg_{total}$ (g $kg^{-1}$) | 1.6 | 0.7 | 0.002 | 0.21 | 0.17 | 0.023 |
| $Na_{total}$ (g $kg^{-1}$) | 1.0 | 0.72 | 1.8 | 3.8 | 3.0 | 2.2 |
| $C/N_{total}$ | 5.7 | 6.2 | 1.5 | 2.9 | 4.3 | 1.3 |
| $C/N_{organic}$ | 13.9 | 12.9 | 6.5 | 7.6 | 85 | 6.6 |
| N/P | 2.7 | 4.1 | 168 | 5.6 | 190 | 24 |
| $NH_4^+$-N/$N_{total}$ | 0.59 | 0.51 | 0.76 | 0.62 | 0.95 | 0.80 |
| $N_{organic}$ /$N_{total}$ | 0.41 | 0.49 | 0.24 | 0.38 | 0.05 | 0.20 |

*AM: animal manure; DIG: digestate; LFDIG_AM: liquid fraction of digestate from animal origin; LFDIG_PLT: liquid fraction of digestate from non-animal (plant) origin; MC_LFDIG: mineral concentrate from liquid fraction of digestate; MC_LFAM: mineral concentrate from liquid fraction of animal manure.*

### 3.2.3 N incubation experiment

The air-dried soil was pre-incubated at 35 % water filled pore space (WFPS) for one week at 25 °C in the dark. At the start of the incubation experiment, liquid fractions, mineral concentrates and CAN were thoroughly mixed with 260 g of pre-incubated soil (equivalent to 237 g of air-dried soil) at a rate of 150 kg of effective N $ha^{-1}$ (Table 3.2), in agreement with the Flemish manure regulation for the cultivation of maize on non-sandy soils (FMD, 2011) where the maximum allowable rate of 80 kg $P_2O_5$ $ha^{-1}$ was respected (FMD, 2011). Due to a low N/P ratio in AM and DIG, the N application rate had to be limited to 56 and 86 kg effective N $ha^{-1}$, respectively (instead of 150 kg effective N $ha^{-1}$) in order to comply with the maximum application rate of 80 kg $P_2O_5$ $ha^{-1}$ (Table 3.2). The effective N is the amount of N from applied bio-based material that is expected to be available for crop uptake in the season of application (Webb *et al.*, 2010). According to Flemish manure regulation the amount of effective N in animal manure and digestate was legally accepted to be 60 % of the total N content. For liquid fractions of digestate and mineral concentrates it was hypothesized that 100 % of total N present in these materials would be available during the experiment, which is similar to that expected from the application of synthetic N fertilizer.

***Table 3.2*** *Application rates of bio-based materials, on fresh weight basis, used in 120-day incubation experiment.*

| Material | tonnes $ha^{-1}$ | total N (kg $ha^{-1}$) | effective N (kg $ha^{-1}$) | $P_2O_5$ (kg $ha^{-1}$) | OC (kg $ha^{-1}$) |
|---|---|---|---|---|---|
| AM | 16.7 | 93 | 56[a] | 79.9 | 534 |
| DIG | 33.3 | 143 | 86[a] | 79.9 | 899 |
| LFDIG_AM | 31.9 | 150 | 150 | 2.0 | 226 |
| LFDIG_PLT | 22.7 | 150 | 150 | 61.4 | 431 |
| MC_LFDIG | 37.5 | 150 | 150 | 1.8 | 637 |
| MC_LFAM | 20.6 | 150 | 150 | 14.6 | 189 |
| CAN | 0.56 | 150 | 150 | 0 | 0 |

*OC: organic carbon; AM: animal manure; DIG: digestate; LFDIG_AM: liquid fraction of digestate from animal origin; LFDIG_PLT: liquid fraction of digestate from non-animal (plant) origin; MC_LFDIG: mineral concentrate from liquid fraction of digestate; MC_LFAM: mineral concentrate from liquid fraction of animal manure; CAN: calcium ammonium nitrate.*

*[a] N application rate of AM and DIG had to be limited to 56 and 86 kg effective N $ha^{-1}$, respectively (instead of 150 kg effective N $ha^{-1}$) in order to comply with the Flemish manure regulation for cultivation of maize on non-sandy soils where maximum application rate of 80 kg $P_2O_5$ $ha^{-1}$ needs to be respected (FMD, 2011).*

The homogenous mixture of soil and bio-based material was placed in PVC tubes with a diameter of 4.6 cm and 18 cm in length (Figure 3.1). The soil was brought to a bulk density of 1.4 Mg $m^{-3}$ by compacting the mixture to a height of 10 cm. Next to the six treatments where bio-based materials were tested as a potential synthetic N fertilizer replacement, a treatment with CAN (27% of mineral N) was added and used as a reference for the conventional fertilizer. For the control, bare soil was used which went through the same procedure as the soil in the amended treatments. The moisture content of the soil for the incubations was adjusted to 50 % of WFPS, and the tubes were covered with a single layer of pin-holed gas permeable parafilm to minimize water loss whilst allowing air exchange. The total weight of the tubes was recorded and subsequently incubated at 15°C. The moisture content was monitored weekly during the incubation period by weighing the tubes and maintaining them at 50 % WFPS by adding demineralized water when needed. Four separate replicates of seven treatments and the control were analyzed at day 20, 40, 60, 80, 100 and 120 by removing intact tubes. The mineral N in the control treatment was determined again at day 0 in order to include any effects of soil air-drying and re-wetting. The soil was removed from the tubes, mixed thoroughly, and analyzed for soil $NO_3^-$-N and $NH_4^+$-N (see section 3.2.1. for the description of the analysis). Note that in these experimental conditions ammonia volatilization is considered to be negligible due to the homogenous mixing of materials and soil, and the lack of airflow at the soil surface during the incubation (de la Fuente *et al.*, 2010; Alburquerque *et al.*, 2012a).

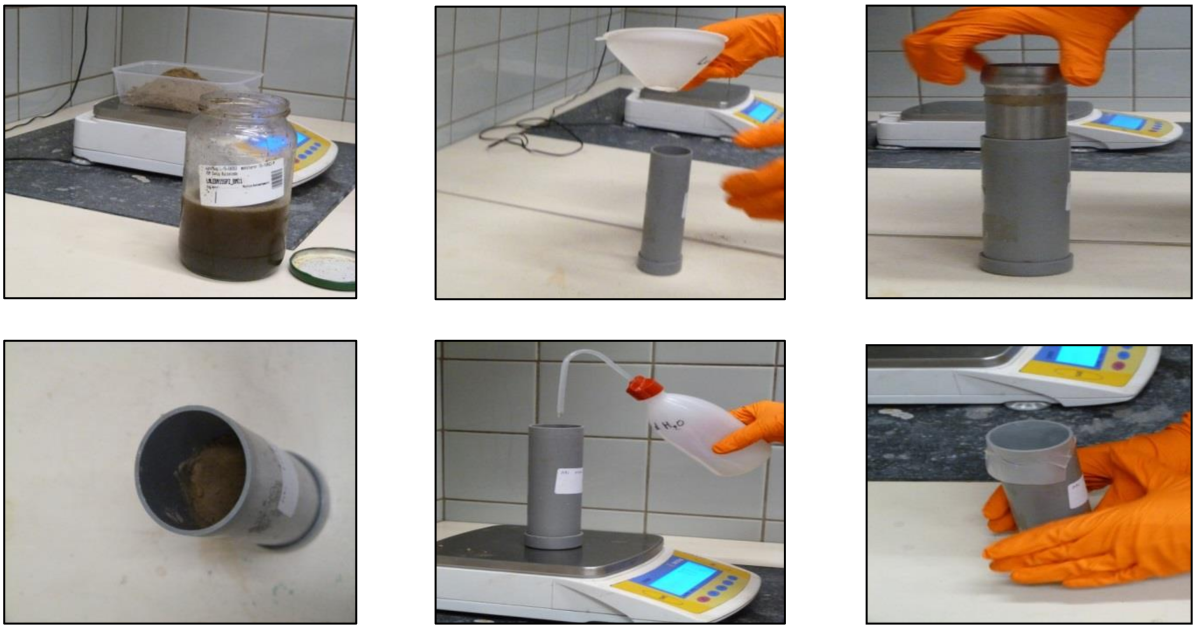

***Figure 3.1*** *Experimental setup of N incubation experiment for determination of N release and N mineralization (photo's: Sigurnjak, I.).*

### 3.2.4 Data analysis

Both the net N release ($N_{rel,net}$) from the added materials and the net N mineralization ($N_{min,net}$) were calculated. The $N_{rel,net}$ is the difference between the mineral N measured in the amended soil minus the mineral N measured in the control (i.e. unamended soil), calculated according to De Neve and Hofman (1996), as follows:

$$N_{rel,net}\,(\%) = \frac{([NO3^{-}-N,treatment]-[NO3^{-}-N,control])+([NH4^{+}-N,treatment]-[NH4^{+}-N,control])}{Ntotal\ applied} \times 100 \qquad \text{(Eq. 5)}$$

At t=0, the $N_{rel,net}$ (%) equals the product $N_{mineral}/N_{total}$ ratio x 100. $N_{min,net}$ (%) is the N mineralized from the organic fraction of the product (expressed as a percentage of total N in the product), and is calculated by subtracting the amount of mineral N already present in the products at t=0, as follows:

$$N_{min,net}\,(t;\ \%\ total\ N) = (N_{rel,net}\,(t) - N_{rel,net}\,(t=0)) \qquad \text{(Eq. 6)}$$

A positive $N_{min,net}$ value indicates net mineralization, whereas a negative $N_{min,net}$ value indicates net N immobilization.

Treatment effect during the incubation period on $N_{rel,net}$ and $N_{min,net}$ was assessed by one-way ANOVA and Tukey's post-hoc test. The condition of normality was checked using the Shapiro-Wilk test, whereas the homogeneity was tested with the Levene Test. The $N_{rel,net}$ kinetics was estimated by linear regression. Pearson's correlation analysis was performed to test relationship between $N_{rel,net}$, $N_{min,net}$ and physicochemical properties of applied bio-based (organic) materials. All statistical analyses were performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL).

## 3.3 Results

### 3.3.1 Soil characteristics

The soil characteristics of the 0-30 cm soil layer prior to the experiment were pH-KCl = 6.6; pH-$H_2O$ = 7.3; OC = 1.2 %; $NO_3^-$-N = 0.95 mg kg$^{-1}$; $NH_4^+$-N = 5.1 mg kg$^{-1}$; DM = 83 %. The mineral N in the soil was low at the time of sampling and mainly in the form of $NH_4^+$-N. As such, the soil was suited for the mineralization experiment since it did not exceed recommended criteria ($N_{mineral}$ < 20 mg $NO_3^-$-N kg$^{-1}$ soil, pH-KCl between 5.0 - 7.5 and OC < 1.5 % (VITO, 2010b) to be used in this type of trial.

### 3.3.2 Characteristics of bio-based materials and application rates

Contents of DM, OM, OC, P, Ca and Mg were higher in AM compared to end- and by-materials obtained from animal manure processing (Table 3.1). Animal manure processing techniques such as anaerobic digestion, mechanical separation and reverse osmosis generally result in a reduction of the DM content of the input stream. The DM content of the tested materials varied between 23 - 85 g kg$^{-1}$, with AM exhibiting the highest concentration. A similar trend was observed for OM and OC content. The pH-values were in the same range for all bio-based materials, while EC values increased with processing and the highest values were measured in mineral concentrates. EC is an indication of soluble salts, which include all ions that are contained in the liquid part of tested materials. Because K and Na salts are most soluble, they remain in higher concentrations in liquid fractions of digestate and mineral concentrates as compare to AM and DIG. On the other hand, Ca and Mg concentrations decrease with further treatment since they are mostly present in crystalline form with P or adsorbed onto particles (Bachmann *et al.*, 2016). Thus, after mechanical separation, P, Ca and Mg will remain in higher proportions in the solid fraction of separated animal manure or raw digestate.

In general, end- and by-materials of animal manure processing are richer in $N_{total}$, $NH_4^+$-N, K and Na. As a first step, anaerobic digestion releases some organically bound N as $NH_4^+$-N. Mechanical separation and reverse osmosis remove additional organic N that is still present in their input streams. This results in higher efficiency of liquid fractions of digestate and mineral concentrates as a N fertilizer by increasing their $NH_4^+$-N/$N_{total}$ ratio, which in this chapter ranged from 0.62 - 0.76 and 0.80 - 0.95, respectively. DIG and AM had a lower $NH_4^+$-N/$N_{total}$ ratios of 0.51 and 0.59, respectively. Moreover, these materials had a lower N/P ratio. Since Flemish soil is quite P rich, the high P content is the limiting factor for the amounts of bio-based materials that can be applied under current nutrient legislation. The N application rate of AM and DIG had to be limited to 56 and 86 kg effective N ha$^{-1}$, respectively (instead of 150 kg effective N ha$^{-1}$) in order to comply with the maximum application rate of 80 kg $P_2O_5$ ha$^{-1}$ (Table 3.2). This led to a N application lower than the crop N requirements, which in practice would be corrected with an additional application of synthetic N. However, liquid fractions of digestate and mineral concentrates can satisfy crop N requirements without exceeding imposed legal limits for P. As such they have a potential to be used as a replacement for synthetic N.

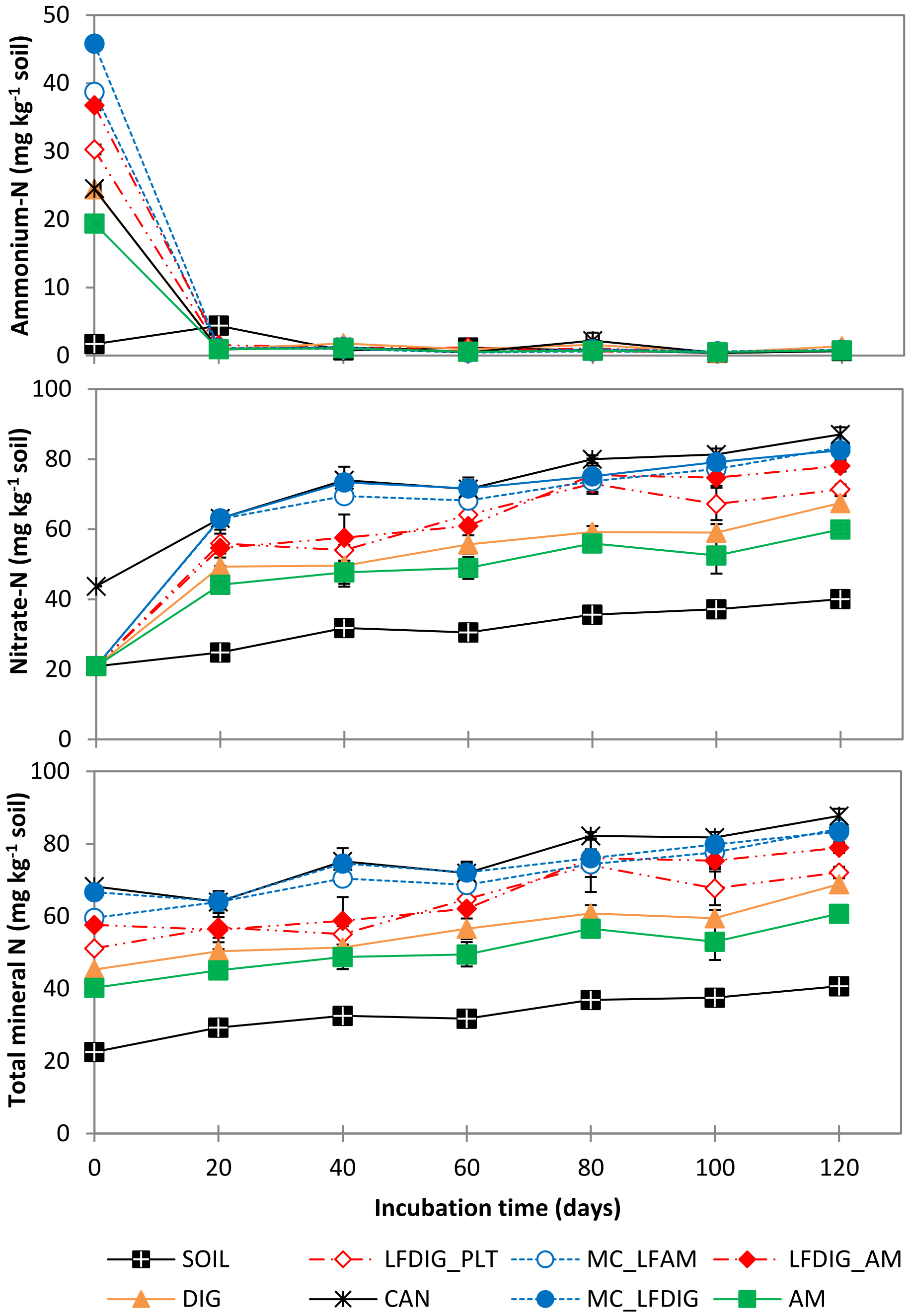


***Figure 3.2*** *Evolution of mineral N (mg kg$^{-1}$) in an unamended soil and soil treated with bio-based and synthetic fertilizers (n=4) during 120-day incubation experiment (mean ± standard deviation; where absent, error bars fall within symbols). AM: animal manure; DIG: digestate; LFDIG_AM: liquid fraction of digestate from animal origin; LFDIG_PLT: liquid fraction of digestate from non-animal (plant) origin; MC_LFDIG: mineral concentrate from liquid fraction of digestate; MC_LFAM: mineral concentrate from liquid fraction of animal manure; CAN: calcium ammonium nitrate.*

### 3.3.3 N dynamics in the soil

At day 0 all bio-based materials supplied significant amounts of N to the soil in the form of $NH_4^+$-N (Figure 3.2). During the initial stage of the incubation experiment, there was a slightly larger build-up of $NH_4^+$-N in the unamended soil up to day 20 (4.4 ± 0.7 mg $NH_4^+$-N $kg^{-1}$) compared to the amended treatments (0.90 - 1.55 mg $NH_4^+$-N $kg^{-1}$). This suggests that the addition of these materials stimulated nitrification, or that mineralization of native soil organic N was faster in the unamended soil up to day 20. From 40 days of incubation onwards, the $NH_4^+$-N content of the soil with and without amendments was at a similar level, between 0.5 – 1.2 mg $NH_4^+$-N $kg^{-1}$. These very small $NH_4^+$-N concentrations indicate that there were no anaerobic conditions during the incubation period (De Neve and Hofman, 1996). The nitrification of $NH_4^+$-N added via the materials was complete within 20 days as the $NH_4^+$-N content had become almost negligible, while the $NO_3^-$-N content in the soil had more than doubled or tripled. The $NO_3^-$-N content continued to increase for all treatments throughout the duration of the experiment (Figure 3.2). The pattern of nitrification was similar for all treatments to that of total mineral N, indicating that any $NH_4^+$-N produced was quickly nitrified (De Neve *et al.*, 2004).

### 3.3.4 N release

The $N_{rel,net}$ from the bio-based and synthetic fertilizers as a percent of added total N is shown in Figure 3.3. During the initial stage of the incubation experiment, reduction in $N_{rel,net}$ was observed at day 20. This temporary immobilization can be seen as a transient phenomenon probably associated with the soil manipulations (drying and rewetting) prior to the start of the incubation experiment (De Neve *et al.*, 2003). From day 20 to the end of the experiment, $N_{rel,net}$ increased again from this minimum value for all treatments. The highest $N_{rel,net}$ (%) was measured in CAN treatment amounting to 103 ± 4 at day 120. There was no significant difference between mineral concentrate obtained from liquid fraction of digestate and the one obtained from liquid fraction of animal manure. At day 120, these treatments exhibited on average only a 10 % lower $N_{rel,net}$ compared to CAN (Figure 3.3). A significant difference was detected in $N_{rel,net}$ between liquid fractions of digestate. The lowest $N_{rel,net}$ was observed for DIG and AM treatment, where 61 ± 1 and 66 ± 4 % of applied N was released at day 120, respectively.

In order to compare $N_{rel,net}$ kinetics of amended treatments, a linear regression was fitted to the $N_{rel,net}$ (%) data. Significance of the regression ($p < 0.05$) was used as the criterion for fit. The linear regression was only significant ($p = 0.026$) in MC_LFAM treatment, and indicated that 89 % of applied total N (equals 134 kg N $ha^{-1}$) from MC_LFAM was available after 120 days ($y = 0.114x+76$). For other treatments the regression was not significant ($p > 0.05$), and for these treatments we estimate potential $N_{rel,net}$ (%) by averaging all $N_{rel,net}$ (%) values over the entire incubation period (including t=0). According to the proposed estimation, CAN, MC_LFDIG, LFDIG_AM and LFDIG_PLT with the potential $N_{rel,net}$ of 94 ± 9, 88 ± 6, 73 ± 12 and 65 ± 10%, will on average release 141, 132, 110 and 98 kg of N $ha^{-1}$, respectively, under the conditions of the incubation experiment. In the DIG treatment, P was a limiting factor for the N application rate, which led to the reduction of N dosage from 150 to 86 kg effective N $ha^{-1}$. On the basis of total N applied (Table 3.2), it means that with potential $N_{rel,net}$ of 52 ± 6% only 74 kg of

N ha$^{-1}$ will be released on average from 143 kg of applied total N ha$^{-1}$. P also limited the amount of N applied in the AM treatment, with an average potential $N_{rel,net}$ (%) of 59 ± 6 (equals 55 kg N ha$^{-1}$).

### 3.3.5 N mineralization

The $N_{min,net}$ (expressed as % of organic N applied) from amended treatments on 120 day was 0%, 16%, 28% 19%, 25% and 69% for MC_LFDIG, LFDIG_PLT, LFDIG_AM, AM, DIG and MC_LFAM, respectively (graph not shown). Obviously the amount of applied organic N differed greatly between tested materials and was mostly low, which leads to large variabilities in the estimation of $N_{min,net}$ data. Alternatively, $N_{min,net}$ can also be obtained as the difference between $N_{rel,net}$ (%) at t=0 and $N_{rel,net}$ (%) observed at a given sampling moment (Figure 3.3; section 3.2.4). A negative difference thus implies net N immobilization which occurred in all amended treatments from the start, but was less pronounced with LFDIG_PLT, MC_LFAM, DIG and AM. The net N immobilization in LFDIG_PLT, LFDIG_AM, MC_LFAM, DIG and AM remained relatively constant until half way through (60 – 80 days) the experiment when remineralization of N started towards the end of the incubation period. The $N_{min,net}$ (when expressed as % of total N applied) from amended treatments on 120 day was 3±4%, -3±3%, 6±2%, 7±3% 8±4%, 12±1% and 14±2% for CAN, MC_LFDIG, LFDIG_PLT, LFDIG_AM, AM, DIG and MC_LFAM, respectively.

The effect of bio-based material characterization on N dynamics is presented in Table 3.3. The effect of $C/N_{total}$ (r = -0.675, p = 0.142), $C/N_{organic}$ (r = 0.284, p = 0.585) and $NH_4^+$-N/$N_{total}$ (r = 0.693, p = 0.127) on $N_{rel,net}$ was not significant. The strongest correlation was found between the $N_{rel,net}$ (%) and the amount of applied $NH_4^+$-N (r = 0.898, p = 0.015) and $N_{total}$ (r = 0.929, p = 0.007). On the contrary, $N_{min,net}$ (%) exhibited the strongest correlation with the $C/N_{organic}$ (r = -0.847, p = 0.033) ratio of applied bio-based materials.

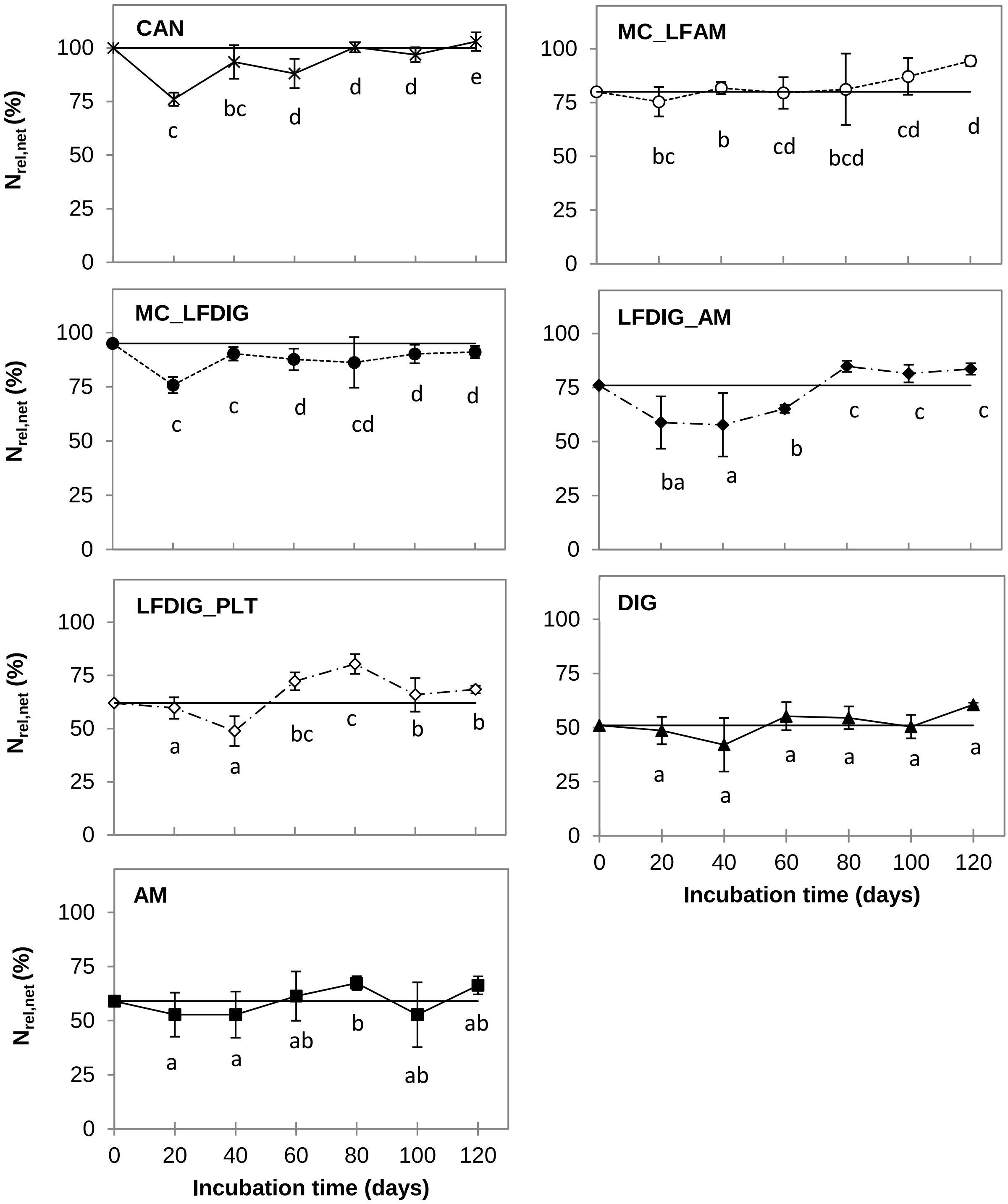


***Figure 3.3*** *N release ($N_{rel,net}$; %) relative to the N input of added materials in 120-day incubation experiment. Value plotted at t=0 indicates the percentage of mineral N in applied material and is presented with straight line throughout 120 days of incubation time. Values observed above the line indicate net N mineralization, while values below the line indicate net N immobilization. Error bars indicate standard deviations (n=4). Lower-case letters indicate significant different means (Tukey's Test ($p<0.05$)) between materials per each sampling time (t=20, 40, 60,…,120). AM: animal manure; DIG: digestate; LFDIG_AM: liquid fraction of digestate from animal origin; LFDIG_PLT: liquid fraction of digestate from non-animal (plant) origin; MC_LFDIG: mineral concentrate from liquid fraction of digestate; MC_LFAM: mineral concentrate from liquid fraction of animal manure; CAN: calcium ammonium nitrate.*

***Table 3.3*** *Significant correlations among parameters related to bio-based material composition and N release and mineralization dynamics (n=6)*

| Parameters | OC | $N_{total}$ | $NH_4^+$-N | C/$N_{total}$ | C/$N_{organic}$ | $NH_4^+$-N/$N_{total}$ | $N_{organic}$/$N_{total}$ | $N_{rel,net}$ | $N_{min,net}$ | $N_{total}$ added | $NH_4^+$-N added | OC added |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OC | 1 | | | | | | | | | | | |
| $N_{total}$ | -0.212 | 1 | | | | | | | | | | |
| $NH_4^+$-N | -0.635 | 0.767 | 1 | | | | | | | | | |
| C/$N_{total}$ | 0.928** | -0.518 | -0.776 | 1 | | | | | | | | |
| C/$N_{organic}$ | 0.021 | -0.564 | -0.080 | 0.252 | 1 | | | | | | | |
| $NH_4^+$-N/$N_{total}$ | -0.653 | -0.104 | 0.544 | -0.504 | 0.681 | 1 | | | | | | |
| $N_{organic}$/$N_{total}$ | 0.653 | 0.104 | -0.544 | 0.504 | -0.681 | 1.000** | 1 | | | | | |
| $N_{rel,net}$ | -0.863* | 0.016 | 0.500 | -0.675 | 0.284 | 0.693 | -0.693 | 1 | | | | |
| $N_{min,net}$ | 0.018 | 0.053 | 0.163 | -0.120 | -0.847* | -0.583 | 0.583 | -0.158 | 1 | | | |
| $N_{total}$ added | -0.730 | -0.032 | 0.281 | -0.545 | 0.160 | 0.425 | -0.425 | 0.929** | -0.103 | 1 | | |
| $NH_4^+$-N added | -0.786 | -0.096 | 0.525 | -0.598 | 0.587 | 0.933** | -0.933** | 0.898** | -0.488 | 0.722 | 1 | |
| OC added | 0.762 | -0.625 | -0.792 | 0.929** | 0.356 | -0.409 | 0.409 | -0.381 | -0.179 | -0.196 | -0.386 | 1 |

*OC: organic carbon (g $kg^{-1}$); $N_{total}$: total N (g $kg^{-1}$); $N_{organic}$: organic N (g $kg^{-1}$); $NH_4^+$-N: ammonium nitrogen (g $kg^{-1}$); $N_{rel,net}$ (%): mineral N released after 120 days of incubation (% of $N_{total}$ applied); $N_{min,net}$ (%): mineral N mineralized after 120 days of incubation (% of $N_{total}$ applied); $N_{total}$ added: total N amount added via material application (mg); $NH_4^+$-N added: $NH_4^+$-N amount added via material application (mg); OC added: OC amount added via material application (mg).*

** Significant at probability level $p < 0.05$*

*** Significant at probability level $p < 0.01$*

## 3.4 Discussion

As expected, the highest $N_{rel,net}$ (%) was observed in the CAN treatment, given that all N in CAN is present as mineral N. The $N_{rel,net}$ pattern of mineral concentrates was similar to CAN. These observations are in line with our hypothesis that the $N_{rel,net}$ of mineral concentrates will more closely follow the pattern of synthetic N fertilizer, and as such is not equal to the ones of animal manure. The average potential $N_{rel,net}$ from mineral concentrates was higher as compared to other bio-based fertilizers. In the case of liquid fractions of digestate, the $N_{rel,net}$ (%) was higher as compared to AM, but did not closely follow the pattern of synthetic N fertilizer. Moreover, a significant difference that was detected between $N_{rel,net}$ (%) of liquid fractions of digestate, was probably caused by the input streams that were fed to the digester. LFDIG_AM contained 16% animal manure and 72% food waste compared to LFDIG_PLT which was the product of only plant (vegetable) inputs (50% energy maize and 50% food waste). Both animal manure and food waste are input streams known to increase $NH_4^+$-N/$N_{total}$ ratio of digestate. The food-based digestate can contain c.80% of total N in the form of $NH_4^+$-N (WRAP, 2016; Nicholson *et al.*, 2017). This could probably explain the higher amount of available N in LFDIG_AM compared to LFDIG_PLT (Table 3.1), which at the end led to higher $N_{rel,net}$. With $N_{rel,net}$ (%) of 84 ± 3 and 68 ± 2, LFDIG_AM and LFDIG_PLT scored respectively 19 and 35 % lower $N_{rel,net}$ as compared to CAN (Figure 3.3). The N availability in liquid fractions of digestate may be increased by using animal manure or food waste as one of the feeds for anaerobic digestion, and not only plant (vegetable) residues. The $N_{rel,net}$ from DIG was within the range reported by Alburquerque *et al.* (2012), where 44 – 84 % of total N present in four digestates was converted in $NO_3^-$-N after 56 days of incubation. Finally, the $N_{rel,net}$ from AM and DIG was in accordance with Flemish and European legislation that assumes 60 % of applied total N from these materials is available for the crop in the growing season (Webb *et al.*, 2010; FMD, 2011).

Along with the mineral N, bio-based materials may contain significant amounts of organic N which upon mineralization may also contribute to plant N availability. Addition of mineral N fertilizer to the soil has been reported to lead to a priming effect (Kuzyakov *et al.*, 2000), resulting in increased N mineralization from soil organic matter as compared to unamended soil (Raun *et al.*, 1998; Mulvaney *et al.*, 2009). In the CAN treatment a maximum $N_{min,net}$ (%) of 3 ± 4 was recorded on day 120, i.e. no additional mineralization from SOM as compared to the control treatment, indicating throughout entire duration of the experiment that CAN application did not lead to a priming effect. In treatment MC_LFDIG, where 5 % of applied total N was organic N, there was no $N_{min,net.}$ Similar behaviour was reported by Velthof (2015), where no clear difference was detected in N availability between the mineral concentrate (containing 5 - 10% organic N) and CAN during an incubation period of 54 days, suggesting that addition of mineral concentrate did not affect immobilization or mineralization of N in the soil. In the current study, MC_LFAM (20 % of organic N) reached the highest $N_{min,net}$ (% of total N applied) on day 120, amounting to 14 ± 1%. This can be seen as a result of applied treatment techniques. In the case of MC_LFDIG, anaerobic digestion was introduced in the treatment chain. This allowed conversion of organic N to $NH_4^+$-N, subsequently reducing $N_{organic}$/$N_{total}$ ratio in MC_LFDIG. However, this was not the case for MC_LFAM were anaerobic digestion was absent, leading to higher $N_{organic}$/$N_{total}$ ratio and consequent

$N_{min,net}$ of the present organic N. Next, LFDIG_AM and LFDIG_PLT reached $N_{min,net}$ (%) of 6.8 ± 1.5 and 6.1 ± 1.6, respectively, on day 120. The $N_{min,net}$ was faster in LFDIG_PLT treatment where already from day 60 onwards $N_{min,net}$ was detected. This can be attributed to a high $N_{organic}/N_{total}$ and low $NH_4^+$-$N/N_{total}$ ratio in LFDIG_PLT compared to LFDIG_AM. Similar mineralization patterns were observed in AM and DIG treatments where $N_{min,net}$ (%) of 7.7 ± 1.5 and 12.2 ± 2.1, respectively, were reached on day 120.

It is known that the $N_{rel,net}$ and $N_{min,net}$ (%) are usually influenced by C/N and $NH_4^+$-$N/N_{total}$ ratio of applied bio-based materials (Azeez and Van Averbeke, 2010; Grigatti *et al.*, 2011; Abubaker *et al.*, 2012). In this chapter, we distinguished between the $C/N_{total}$ and the $C/N_{organic}$ ratio. According to the observed correlations, bio-based materials with relatively low $C/N_{organic}$ and high $NH_4^+$-$N/N_{total}$ ratio would likely result in similar performance as synthetic N fertilizers. Finally, it is important to highlight that this experiment was conducted in the absence of plants whose presence might affect N mineralization and immobilization processes in soil. Thus, it is important to test these materials on a field scale with respect to their impact on crop yield and potential nitrate leaching.

## 3.5 Conclusion

The selected bio-based materials differed in their ability to release and mineralize N, depending on their feed characteristics and applied processing technique(s). The N release from MC was similar to CAN performance due to the high $NH_4^+$-$N/N_{total}$ ratio of these materials. Net N mineralization was observed for all materials containing more than 5% of organic N. However, additional release by mineralization contributed only to a limited extent (6-14%) on top of mineral N initially present in the materials. Overall, materials with relatively low $C/N_{organic}$ and high $NH_4^+$-$N/N_{total}$ ratio might exhibit similar performance as synthetic N fertilizers.

**Acknowledgments**

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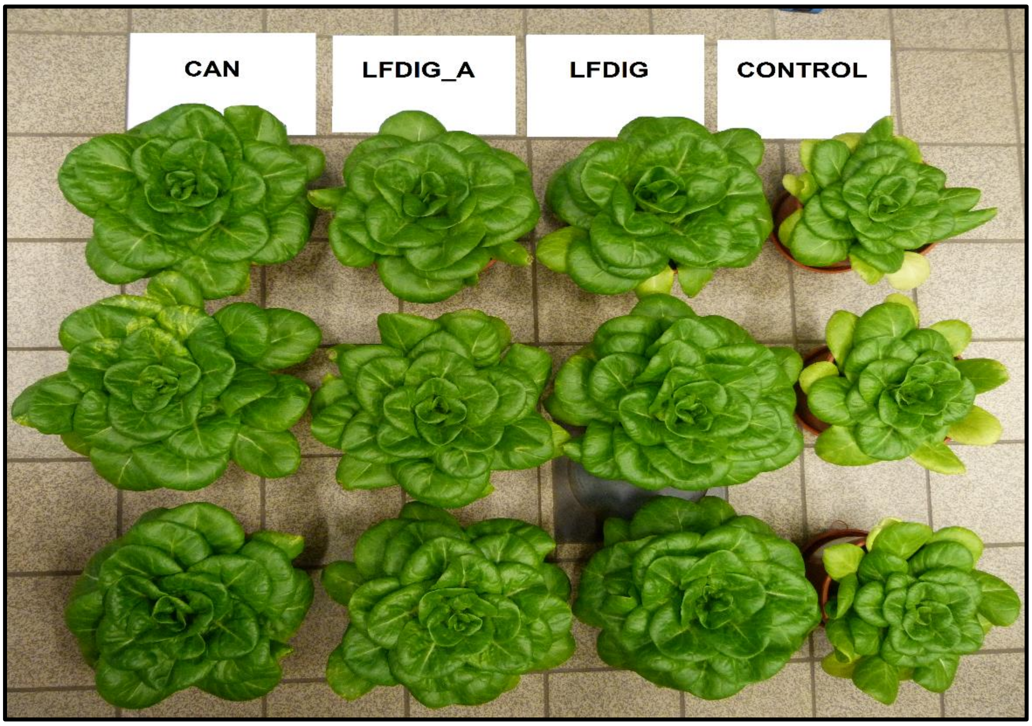
# **CHAPTER 4:** EFFECT OF ACIDIFICATION ON NITROGEN FERTILIZER REPLACEMENT VALUE OF BIO-BASED MATERIALS IN A POT CULTIVATION OF *LACTUCA SATIVA* L.




*Lettuce fertilized with synthetic N (CAN), acidified liquid fraction of digestate (LFDIG_A), non-acidified liquid fraction of digestate (LFDIG) and without fertilization (control) at harvest time (Picture: Mosaso Egbe, S.)*

***This chapter has been redrafted after:***

Sigurnjak, I., Michels, E., Crappé, S., Buysens, S., Biswas, J.K., Tack, F.M.G., De Neve, S., Meers, E. 2017. Does acidification increase the nitrogen fertilizer replacement value of bio-based fertilizers in *Lactuca sativa* L. cultivation? Journal of Plant Nutrition and Soil Science 180, 800-810.

## Abstract

Acidification of manure, digestate and their processed derivatives has been proposed as a technique to, amongst others, mitigate ammonia ($NH_3$) emissions related to application in the field. The current study investigated whether acidification of (i) animal manure (AM), (ii) liquid fraction of animal manure (LFAM), (iii) digestate (DIG) and (iv) liquid fraction of digestate (LFDIG) increases their nitrogen (N) fertilizer replacement value (NFRV) as compared to non-acidified counterparts, a mineral fertilizer control (calcium ammonium nitrate; CAN) and an unfertilized control. Performance of materials was evaluated from the perspective of (I) crop growth (yield, nutrient uptake and crop quality assessment) via a pot experiment with *Lactuca sativa* L. and (II) soil N dynamics (net N release ($N_{rel,net}$) and net N mineralization) via a soil incubation experiment. Crop yield of pots receiving bio-based materials performed 'on par' with CAN as compared to unfertilized control. This implies that mineral fertilizer substitutes derived from digestate or manure could potentially play a role in replacing fossil fuel based fertilizers, also in horticultural applications. However, our findings also suggest that acidification did not result in an increased use efficiency of applied N: NFRV of acidified materials were below those of non-acidified materials and CAN control alike with crop yield on average 6-13% and 11-18% lower compared to non-acidified materials and the CAN treatment, respectively. A possible explanation for lower performance as compared to non-acidified materials could be an inhibitory delay in the $N_{rel,net}$, which in our experimental design proved to be negative for crops with short production cycles. This pattern was revealed in the incubation experiments in which $N_{rel,net}$ in acidified materials remained below that of non-acidified, in this study tentatively attributed to immobilization of mineral N. However, this negative effect on N availability should be reaffirmed in crops with longer production cycles. Finally, some interesting findings in regards with plant composition also warrant further in-depth investigation – e.g. crop Zn concentration in acidified treatments was significantly higher than that of non-acidified treatments. This implies that material pre-treatment may play a future role in biofortification and amelioration of (trace) element composition of crops (arguably for crops with longer production cycles).


## 4.1 Introduction

During and after application of animal manure to the soil, more than 50% of the applied N can be lost by $NH_3$ emissions during the first 24h (Fangueiro *et al.*, 2015; Insam *et al.*, 2015). In the case of digestate and LF of digestate, the risk for $NH_3$ volatilization may even be higher because anaerobic digestion increases the ammonium ($NH_4^+$) concentration and the pH (Webb *et al.*, 2013; Insam *et al.*, 2015) as compared to untreated animal manure. Furthermore, a large proportion of N present in these materials is organically bound and needs to be mineralized before becoming available to plants. However, the synchronization of organic N mineralization and crop N demand is not always optimal (Schröder *et al.*, 2014). Potential losses of $NH_4^+$ and nitrate ($NO_3^-$) can reduce the apparent N recovery (ANR) and the N fertilizer replacement value (NFRV) of LF of animal manure, digestate and LF of digestate. The NFRV is highly dependent on the ratio of mineral and organic N. Hence, the NFRV of LF of animal manure, digestate and LF of digestate is generally lower and more variable compared to synthetic N fertilizers (Schröder *et al.*, 2014) which contain N only in mineral form. Moreover, as shown in Chapter 3, bio-based materials with lower $NH_4^+$-N/$N_{total}$ ratio might result in lower N release.

Acidification of animal slurry reduces the $NH_3$ concentration relative to $NH_4^+$ and thus may reduce $NH_3$ volatilization and subsequently increase NFRV (Kai *et al.*, 2008; Sørensen and Eriksen, 2009; Fangueiro *et al.*, 2015). Previous studies have shown that incorporation of acidified slurry can affect N dynamics in soil by stimulating N mineralization, decreasing potential N immobilization and delaying or inhibiting nitrification (Fangueiro *et al.*, 2009; Fangueiro *et al.*, 2010; Fangueiro *et al.*, 2013). The mechanism behind these observations, however, is not yet clear (Fangueiro *et al.*, 2015). Moreover, there is still a lack of knowledge on how acidification affects the nutrient availability and crop yield as pH reduction does not only affect changes in N dynamics, but also plant availability of other nutrients, including sulfur (S), phosphorus (P) and heavy metals. Current studies mostly focused on efficacy assessment of slurry acidification towards $NH_3$ losses (Fangueiro *et al.*, 2015). Less is known about the impact on NFRV, crop yield, N dynamics (i.e. N availability) in soil and plant availability of P, S, copper (Cu) and zinc (Zn).

The aim of the chapter is to evaluate if acidification can increase the NFRV of (i) animal manure (AM), (ii) LF of animal manure (LFAM), (iii) digestate (DIG) and (iv) LF of digestate (LFDIG) in lettuce (*Lactuca sativa* L.) cultivation compared to the performance of the original materials (i.e. non-acidified) and calcium ammonium nitrate (CAN). This was done through the assessment of (i) lettuce growth (i.e. crop yield, crop nutrient uptake and crop quality assessment) and (ii) N dynamics in soil via a pot and an incubation experiment. In the pot experiment, lettuce was chosen as a test crop because of its high demand for N over a short time period (Leogrande *et al.*, 2013) shallow rooting system, salt-sensitivity, high potential for metal absorption and the lack of knowledge on the effects of bio-based fertilizers on horticultural crops in particular (Montemurro *et al.*, 2010; Leogrande *et al.*, 2013; Montemurro *et al.*, 2015). The performance of selected materials was evaluated and compared to the conventional use of CAN and a control (i.e. no fertilization) treatment. It was hypothesized that acidified materials would have: i) higher NFRV, ii) higher yield, iii) lower potential for N immobilization and iv) higher crop S, P, Cu and Zn concentration, as compared to non-acidified materials.

## 4.2 Materials and methods

### 4.2.1 Bio-based material collection, analysis and acidification

Raw and LF of AM were collected at a pig farm (Pittem, Belgium), whereas DIG and its LF were obtained from an anaerobic co-digestion plant (Deinze, Belgium). The feed of the installation (capacity: 60,000 tonnes $y^{-1}$, 3.51 $MW_{el}$) consisted of agricultural waste (20% animal manure and 30% other agricultural residues) and waste from the food industry (50%). The LFAM and LFDIG were obtained through a centrifuge. All bio-based materials were collected in polyethylene sampling bottles (2 L), stored (4 °C) and analyzed to determine their physicochemical characteristics (Table 4.1).

Product DM, OM, OC, EC, pH, total N, $NH_4^+$-N and $NO_3^-$-N were determined as described in section 3.2.2 (Chapter 3). After determination of OM, samples were subjected to hot plate mineralization digestion (5 ml 6N $HNO_3$ and 5 ml 3N $HNO_3$) and filtered (Van Ranst *et al.*, 1999). From filtered suspension total P was analyzed using the colorimetric Scheel method (Van Ranst *et al.*, 1999), while total K, S, Cu and Zn were analyzed using Inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista MPX, USA).

One part of the organic material was preserved at the original pH, while another part was acidified to pH 5.5. This pH value is considered as a target pH for commercial in-house acidification systems (Fangueiro *et al.*, 2015) and as has also been selected in previously published studies on slurry acidification (Sørensen and Eriksen, 2009; Fangueiro *et al.*, 2016). To this end, ± 250 ml of fresh sample was transferred to a 500 ml media storage bottle, and acidified by the addition of 5M $H_2SO_4$ (96% $H_2SO_4$) under continuous stirring and pH monitoring using a Metrohm titration device. Titration was done one day prior to the experiment at low addition rate to avoid foaming and allow sufficient time for pH stabilization. Acidified materials were stored at 4°C and approximately one hour prior to the experiment pH was measured to determine any potential pH increase due the buffer capacity of the materials. The pH increase was observed by c. 0.5 units and subsequently adjusted to pH 5.5. The non-acidified treatments were treated in exactly the same way, with the exception of acid addition. To reduce the initial pH (≈ 8) of materials to the pH level of 5.5, 18 g $H_2SO_4$ $l^{-1}$ material$^{-1}$ was required for AM and its LF, while 27 g $H_2SO_4$ $l^{-1}$ material$^{-1}$ was added for acidification of DIG and LF of digestate. These quantities are in agreement with the ones reported by Schoumans *et al.* (2014), but higher than the ones reported by Kai *et al.* (2008) and Sørensen and Eriksen (2009). The required amount of acid can differ for different types of manure, because animal type, feed type and duration of storage influences the composition and hence the buffer capacity of manure (Schoumans *et al.*, 2014). In co-digestion, the type of used substrates next to the animal manure influences additionally the buffer capacity of digestate.

After titration, the media storage bottles were closed with screw caps that had an opening hole through which a rubber tube was connected to perform $NH_3$ measurements. The bottles were left for half an hour to stabilize after the titration. During this stabilization period the rubber tubes were closed with tubing pinch plastic clamps to prevent loss of $NH_3$. After the stabilization period $NH_3$ was measured by using gas aspirating pump connected to a $NH_3$ gas detector tube with a 0.2-20 ppm sampling range (Kitagawa, Japan), where $NH_3$ concentration can be read directly in ppm using the detection tube calibrated scale. For non-acidified materials measured $NH_3$ concentrations were above (> 20 ppm) the reagent tube detection limit, while for acidified materials they fell below the detection limit of 0.2 ppm.

***Table 4.1*** *Characterization of bio-based materials (n=2) on fresh weight (FW) basis.*

| Parameter | AM | AM_A | LFAM | LFAM_A | DIG | DIG_A | LFDIG | LFDIG_A |
|---|---|---|---|---|---|---|---|---|
| DM (g $kg^{-1}$) | 32 ± 0 | ND | 19 ± 0 | ND | 139 ± 1 | ND | 92 ± 1 | ND |
| OM (g $kg^{-1}$) | 17 ± 0 | ND | 8.9 ± 0.3 | ND | 85 ± 0 | ND | 53 ± 0 | ND |
| OC (g $kg^{-1}$) | 9.5 ± 0.1 | ND | 4.9 ± 0.2 | ND | 47 ± 0 | ND | 30 ± 0 | ND |
| pH | 7.9 | 5.6 | 8.1 | 5.5 | 8.1 | 5.4 | 8.4 | 5.5 |
| EC (dS $m^{-1}$) | 26 | ND | 29 | ND | 36 | ND | 42 | ND |
| $N_{total}$ (g $kg^{-1}$) | 4.96 ± 0.14 | 5.13 ± 0.13 | 4.37 ± 0.09 | 4.39 ± 0.04 | 5.66 ± 0.23 | 5.50 ± 0.34 | 4.10 ± 0.37 | 4.10 ± 0.29 |
| $NH_4^+$-N (g $kg^{-1}$) | 2.79 ± 0.25 | 2.96 ± 0.05 | 2.80 ± 0.08 | 2.71 ± 0.06 | 3.25 ± 0.11 | 3.11 ± 0.10 | 3.35 ± 0.05 | 3.27 ± 0.08 |
| $NO_3^-$-N (g $kg^{-1}$) | <0.002 | <0.002 | <0.002 | <0.002 | <0.002 | <0.002 | <0.002 | <0.002 |
| $NH_4^+$-N/ $N_{total}$ | 0.56 | 0.58 | 0.64 | 0.62 | 0.57 | 0.56 | 0.82 | 0.80 |
| $P_{total}$ (g $kg^{-1}$) | 0.5 ± 0.0 | ND | 0.1 ± 0.0 | ND | 2.4 ± 0.1 | ND | 1.6 ± 0.1 | ND |
| $K_{total}$ (g $kg^{-1}$) | 3.0 ± 0.1 | ND | 3.1 ± 0.1 | ND | 3.5 ± 0.2 | ND | 3.7 ± 0.3 | ND |
| $S_{total}$ (g $kg^{-1}$) | 0.2 ± 0.0 | ND | 0.3 ± 0.0 | ND | 0.9 ± 0.0 | ND | 0.7 ± 0.0 | ND |
| $Cu_{total}$ (mg $kg^{-1}$) | 31 ± 1 | ND | 24 ± 2 | ND | 17 ± 0 | ND | 15 ± 1 | ND |
| $Zn_{total}$ (mg $kg^{-1}$) | 46 ± 3 | ND | 24 ± 8 | ND | 32 ± 0 | ND | 26 ± 4 | ND |

*DM: dry matter; OM: organic matter; OC: organic carbon; EC: electrical conductivity; ND: not determined; AM: animal manure; LFAM: liquid fraction of animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; _A: acidified.*

### 4.2.2 Soil collection and analysis

All experiments involved a loamy sand (USDA texture triangle: 4% clay, 75% sand and 21% loam fraction) soil, randomly collected from the surface layer (0-30 cm) of an arable field in Beernem, Belgium. The preceding crop was maize which was harvested three months before soil sampling. Subsamples of field-moist soil were taken for the physicochemical determination. The soil moisture content, OM, OC, pH-KCl, total N, $NO_3^-$-N and $NH_4^+$-N were determined as described in section 3.2.1 (Chapter 3). Soil EC was measured with a WTW-LF537 (Germany) electrode after equilibration for 30 min in deionized water at a 5:1 liquid to dry sample ratio and subsequent filtration (MN 640 m, Macherey–Nagel, Germany). After aqua regia digestion (1 g sample + 7.5 ml HCl, 2.5 ml $HNO_3$ and 2.5 ml demineralized water), at harvest time, total P was analyzed using the colorimetric Scheel method (Van Ranst *et al.*, 1999), while total K, S, Cu and Zn were analyzed using ICP-OES. The soil to be used for the pot trial and the incubation experiment was air-dried, sieved to 2 mm and stored before use.

### 4.2.3 Pot experiment and plant analysis

A pot experiment with lettuce (*Lactuca sativa L., cv. Cosmopolia)* was conducted in 1.7 l conical pots of 13 cm height with bottom and top diameters of 12 cm and 16 cm, respectively. Each pot contained 1.7 kg of air-dried soil, corresponding to 10 cm of pot height. One day prior to the pot experiment, 100 ml of demineralized water was added per 1 kg of air-dried soil in order to assure homogenous mixing with materials. The pots were filled in two steps. First 500 g of soil was added directly to the pots. The remaining soil was mixed with the respective fertilizer materials (Table 4.2) and subsequently added to the pots. In total, 10 different fertilization treatments in triplicate pots were tested: control (i.e. not fertilization), CAN, non-acidified (AM) and acidified (AM_A) AM, non-acidified (LFAM) and acidified (LFAM_A) LFAM, non-acidified (DIG) and acidified (DIG_A) DIG, non-acidified (LFDIG) and acidified (LFDIG_A) LFDIG. The fertilization advice on a per-hectare basis was recalculated on a weight basis (equivalent to the weight of soil per pot). The material application rate was calculated (Table 4.2) according to the nutrient requirements for lettuce (210 N, 125 $P_2O_5$ and 240 $K_2O$ kg $ha^{-1}$; personal communication PCG) by taking into consideration the nutrient value of fertilizers (Table 4.1). In each pot an equal amount of $N_{total}$ was applied amounting to 77 mg N $pot^{-1}$. Due to a technical error, 72 mg N $pot^{-1}$ was added in LFDIG and LFDIG_A treatment. This was taken into consideration while calculating ANR. In order to achieve an equal application of P and K in all treatments, triple superphosphate (TSP; 46 % $P_2O_5$) and potassium sulfate (PAT; 30 % $K_2O$, 10 % MgO and 42.5 % $SO_3$) were added as additional sources of P and K, respectively (Table 4.2). It should be noted that while satisfying the $N_{total}$ requirements of lettuce in DIG, DIG_A, LFDIG and LFDIG_A treatments, P was applied in excess via material application (i.e. no need for additional TSP application). After fertilization, one lettuce plant with a 5 cm soil block was transplanted in each pot and additional water was added to reach 60 % water holding capacity (WHC). The WHC was measured according to Meers *et al.* (2006). Pots were placed on a metal shelf where they were exposed to artificial light (Brite-grow bio-growth light) of 2000 lx for 12 h $day^{-1}$. The pots were kept at 20°C for 54 days. On daily basis water was added to maintain soil moisture at 60 % of WHC. The pots were randomized once a week.

***Table 4.2*** *Material (g pot[-1]; pot = 1.7 kg of soil) and total nutrient (mg pot[-1]) application per pot for tested treatments (n=3). The same amount of the material was added in acidified and non-acidified treatments*.

| Treatment | Material amount (g pot$^{-1}$)[b] | | | | | | | Total applied (mg pot$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | CAN | TSP | PAT | AM | LFAM | DIG | LFDIG | $N_{total}$ | Mineral N | $P_2O_5$ | $K_2O$ |
| Control | - | - | - | - | - | - | - | - | - | - | - |
| CAN | 0.28 | 0.10 | 0.29 | - | - | - | - | 77 | 77 | 46 | 87 |
| AM / AM_A | - | 0.06 | 0.11 | 15.43 | - | - | - | 77 | 43 | 46 | 87 |
| LFAM / LFAMA | - | 0.09 | 0.07 | - | 17.54 | - | - | 77 | 49 | 46 | 87 |
| DIG / DIG_A | - | - | 0.10 | - | - | 13.53 | - | 77 | 44 | 73 | 87 |
| LFDIG / LFDIG_A | - | - | 0.03 | - | - | - | 17.54[a] | 72 [a] | 59 [a] | 63 [a] | 82 [a] |

*CAN: calcium ammonium nitrate; TSP: triple superphosphate; PAT: potassium sulfate; AM: animal manure; LFAM: liquid fraction of animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; _A: acidified.*

*[a] Due to the technical error, 17.54 g of material was applied instead of 18.67 g. Hence, fewer nutrients were applied as compared to other treatments.*

*[b] Material amount on hectare basis corresponds to 0.78, 42, 48, 37 and 51 tonnes ha$^{-1}$ for CAN, AM / AM_A, LFAM / LFAM_A, DIG / DIG_A, LFDIG / LFDIG_A, respectively.*

After 54 days, the lettuce was harvested. Prior to harvest, crop quality control was conducted with respect to tipburn, basal rot, yellow leaves, presence of bremia, crop volume and uniformity as described in Sigurnjak *et al.* (2016; Chapter 5, section 5.2.3). At harvest, the plants were clipped from the root with a knife and cleaned with demineralized water from soil particles where needed. The fresh weight (FW) was recorded and DM was determined by oven drying at 60ºC for 48 h. The dried samples were ground and sieved to <1 mm using a Culatti DCFH 48 grinder (GE), and subsequently incinerated for 4 h at 550°C to determine the ash content. The measured macro- and micronutrients were analyzed after hot plate mineralization digestion as described in section 4.2.1, except for total S content for which 0.2 g of plant material mixed with 2.5 ml $H_2O_2$ and 2.5 ml $HNO_3$ was allowed to stand for 12 h followed by microwave heating (CEM MARS 5, BE) at 600W for 10 min at 55°C, 10 min at 75°C and 30 min at 100°C (Van Ranst *et al.*, 1999). The $NO_3^-$ in lettuce was determined according to Anderson and Case (1999). Finally, in order to evaluate the fertilizer value of tested materials in terms of N, ANR and NFRV were determined as described in Chapter 1.

### 4.2.4 N incubation experiment

The same soil as in the pot experiment was used for the incubation experiment, but was first pre-incubated at 35 % water filled pore space (WFPS) for one week at 25°C in the dark. At the start of the incubation experiment, materials were thoroughly mixed with 260 g of pre-incubated soil (equivalent to 234 g of air-dried soil) at a rate of 210 kg N ha$^{-1}$, calculated on a weight basis as in the pot experiment, and the mixture of soil and material was placed in PVC tubes with a diameter of 4.6 cm and 18 cm in length. The soil was brought to a bulk density of 1.4 Mg m$^{-3}$, by compacting the mixture to a height of 10 cm. The same ten treatments were used as in the pot experiment, but no additional P and K was added. The moisture content of the soil for the incubations was adjusted to 50 % of WFPS, and the tubes were covered with a single layer of pin-holed gas permeable parafilm to minimize water loss whilst allowing air exchange. The total weight of the tubes was recorded and subsequently incubated at 15°C. The moisture content was monitored weekly during the incubation period by weighing the tubes and maintaining them at 50 % WFPS by adding demineralized water when needed. Three separate replicates of the nine treatments and the control were analyzed at day 20, 40, 60, 80, 100 and 120 by removing intact tubes. The soil was removed from the tubes, mixed thoroughly, and analyzed for soil $NO_3^-$-N and $NH_4^+$-N as described in section 3.2.1 (Chapter 3).

### 4.2.5 Data analysis

The net N release ($N_{rel,net}$) from the added materials and the net N mineralization ($N_{min,net}$) were calculated according to Eq. 5 and Eq. 6 (section 3.2.4, Chapter 3). Statistical analysis was performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL). One-way ANOVA was used to determine the effect of the applied fertilizers on soil properties along with the effect on crop yield and crop nutrient concentration, based on the obtained physicochemical data. When significant differences between means were observed, additional post hoc assessment was performed using Tukey's Test ($p < 0.05$, n=3). The condition of normality was checked using the Shapiro-Wilk test, whereas the homogeneity was tested with the Levene Test. Significant parameter correlations were determined using the Pearson correlation coefficient (r).

## 4.3 Results

### 4.3.1 Initial properties of soil and materials

The soil characteristics of the 0-30 cm soil layer prior the experiment were pH-KCl = 4.9; EC = 0.092 dS $m^{-1}$; OC = 1.3%; $NO_3^-$-N = 0.65 mg $kg^{-1}$; $NH_4^+$-N = 1.05 mg $kg^{-1}$; $N_{total}$ = 1.1 g $kg^{-1}$. The relatively low mineral N levels of the test soil allowed for the impact of applied materials on N dynamics to be more clearly observed.

With respect to material properties, acidification did not influence $N_{total}$, $NH_4^+$-N and $NO_3^-$-N concentration of the materials (Table 4.1). This observation is in agreement with other studies on acidification of pig slurry (Sørensen and Eriksen, 2009; Fangueiro *et al.*, 2016). From the $NH_4^+$-N/$N_{total}$ ratio of tested materials, it is clear that mechanical separation up-concentrates mineral N. Hence, LFAM and LFDIG had 12% and 30% higher $NH_4^+$-N/$N_{total}$ ratio than their corresponding raw fractions.

### 4.3.2 Pot experiment

***Crop yield, nutrient concentration and NFRV***

The lowest crop FW yield of 38 g $pot^{-1}$ was recorded in the control (Table 4.3). Non-acidified treatments had similar crop yields as the CAN treatment. The only exception was LFDIG treatment ($p < 0.05$) which had a 12%, 9%, 11% and 9% higher crop yield as compared to CAN, AM, LFAM and DIG treatments, respectively. On average, lower crop yields were recorded in acidified treatments. Treatments LFAM_A, DIG_A and LFDIG_A had 13%, 10% and 12% lower crop yield ($p < 0.05$), respectively, as compared to their non-acidified fractions (i.e. non-acidified treatments). Only in AM_A the FW yield was not significantly ($p > 0.05$) lower from the FW yield in the AM treatment. The lettuce DM yield was lower in DIG, DIG_A, LFDIG and LFDIG_A treatments compared to other fertilization scenarios, including the control (Table 4.3). With regard to the observed influence of tested treatments on crop yield and crop N uptake, the lowest ANR values, and consequently NFRV values, were obtained for acidified treatments (Table 4.3). The highest ANR value was observed for CAN, followed by untreated materials.

The highest $N_{total}$ concentration on average was measured in CAN and non-acidified treatments (Table 4.3). Even though there were no significant differences between acidified and non-acidified fraction of the tested materials with respect to crop $N_{total}$ concentration, the measured crop $N_{total}$ concentration in acidified treatments was significantly lower ($p<0.05$) compared to the CAN treatment. With respect to crop $P_{total}$ and $K_{total}$ concentration, there were no differences between acidified and non-acidified fraction of the respective materials. Fertilization with CAN and acidified materials led to similar $S_{total}$ concentration in lettuce. These concentrations of $S_{total}$ were significantly higher ($p<0.05$) than the ones detected in non-acidified treatments. Significantly higher $Zn_{total}$ concentrations were found in lettuce that was fertilized with acidified materials, but there was no influence of treatments on crop $Cu_{total}$ concentration. The former may be relevant for biofortification and amelioration of (trace) elements of crops.

No differences were observed between the fertilizer treatments with respect to tipburn (average score: 6.8 ± 0.6), basal rot (average score: 6.2 ± 0.6), yellow leaves (average score: 6.4 ± 0.6), presence of bremia (average score 9 ± 0), crop volume (average score: 6.1 ± 0.6) and uniformity (average score: 6.5 ± 0.5). Quality parameters with a high score such as those observed for bremia indicate complete absence of the disease. The control treatment scored lower with respect to head volume and yellow leaves (Table 4.4) as a result of N deficiency. The $NO_3^-$ concentration in lettuce was in the order: CAN > non-acidified treatments > acidified treatments > control.

***Table 4.3*** *Mean values ± standard deviation of plant fresh weight (FW), dry matter (DM), plant nutrient concentration (on DM basis), apparent nitrogen recovery (ANR) and nitrogen fertilizer replacement value (NFRV) for ten different treatments (n=3) at harvest.*

| Parameter | Control | CAN | AM | AM_A | LFAM | LFAM_A | DIG | DIG_A | LFDIG | LFDIG_A |
|---|---|---|---|---|---|---|---|---|---|---|
| FW (g pot$^{-1}$) | 38 ± 4a | 69 ± 2c | 71 ± 2c | 67 ± 3c | 69 ± 5c | 60 ± 2b | 71± 2c | 64 ± 2b | 78 ± 0d | 69 ± 4c |
| DM (%) | 8.5 ± 0.6c | 7.8 ± 0.7bc | 7.5 ± 0.6c | 7.3 ± 0.7c | 7.5 ± 0.6c | 8.5 ± 0.5c | 6.3 ± 0.5a | 7.1 ± 0.4ab | 6.2 ± 0.4a | 6.6 ± 0.3a |
| $N_{total}$ (g kg$^{-1}$) | 15.2 ± 1.2a | 22.7 ± 1.6c | 21.2 ± 2.0bc | 20.3 ± 0.7b | 20.7 ± 0.7bc | 18.7 ± 1.3b | 22.1 ± 1.8bc | 20.0 ± 0.7b | 22.1 ± 1.7bc | 19.8 ± 0.7b |
| $P_{total}$ (g kg$^{-1}$) | 4.0 ± 0.1a | 5.2 ± 0.4c | 4.7 ± 0.2bc | 4.7 ± 0.3bc | 4.2 ± 0.2a | 4.0 ± 0.0a | 4.4 ± 0.1ab | 4.5 ± 0.3bc | 4.8 ± 0.3bc | 4.4 ± 0.1a |
| $K_{total}$ (g kg$^{-1}$) | 35 ± 2a | 32 ± 1a | 35 ± 3ab | 39 ± 1b | 38 ± 1ba | 39 ± 1b | 39 ± 1b | 40 ± 5ba | 34 ± 4ba | 42 ± 4b |
| $S_{total}$ (g kg$^{-1}$) | 1.7 ± 0.1a | 2.8 ± 0.2c | 2.4 ± 0.1b | 2.7 ± 0.1c | 2.4 ± 0.1b | 2.8 ± 0.2c | 2.2 ± 0.1b | 2.6 ± 0.0c | 2.2 ± 0.1b | 2.6 ± 0.1c |
| $Cu_{total}$ (mg kg$^{-1}$) | 8.3 ± 2.0ab | 8.4 ± 1.2b | 7.0 ± 1.3ab | 6.5 ± 0.3a | 6.1 ± 0.2a | 8.3 ± 0.7b | 7.3 ± 1.1b | 7.2 ± 0.7b | 7.3 ± 1.3b | 6.8 ± 0.4b |
| $Zn_{total}$ (mg kg$^{-1}$) | 43 ± 4a | 51 ± 4ab | 44 ± 4a | 61 ± 6b | 53 ± 6a | 75 ± 10c | 46 ± 3a | 77 ± 8c | 54 ± 5b | 83 ± 8c |
| ANR | - | 0.94 ± 0.00c | 0.82 ± 0.03a | 0.65 ± 0.15a | 0.74 ± 0.04b | 0.61 ± 0.08a | 0.65 ± 0.05a | 0.55 ± 0.05a | 0.79 ± 0.03b | 0.58 ± 0.07a |
| NFRV (%) | - | 100[a] | 87 ± 4b | 69 ± 16b | 78 ± 4b | 64 ± 9a | 69 ± 5a | 58 ± 6a | 84 ± 3b | 61 ± 7a |

*Control: no fertilization; CAN: calcium ammonium nitrate; AM: animal manure; LFAM: liquid fraction of animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; _A: acidified. Mean values denoted by the same letter in a row are not statistically different according to Tukey's test at the 5% probability level. The difference is denoted only by comparing the acidified and non-acidified fraction of the tested material with (or without in the case of NFRV) the control and CAN treatment. CAN treatment as a reference was considered to be 100% efficient.*

***Table 4.4*** *Observation score for lettuce volume (1 = small volume, 9 = voluminous) and yellow leaves (1 = much and 9 = absent) during the growing period (day 34) and at harvest, including crop nitrate concentration (mg $NO_3$ $kg^{-1}$ FW), as a part of crop quality assessment for ten different fertilization treatments (n=3).*

| Treatment | During growing period | | Harvest | | |
|---|---|---|---|---|---|
| | Volume | Yellow leaves | Volume | Yellow leaves | mg $NO_3$ $kg^{-1}$ FW |
| Control | 4.67 a | 6.33 a | 2.67 a | 4.33 a | 11 ± 3 a |
| CAN | 7.67 b | 8.33 b | 6.63 b | 6.67 b | 295 ± 32 d |
| AM | 7.33 b | 7.67 b | 6.67 b | 5.67 b | 114 ± 26 c |
| AM_A | 7.33 b | 7.67 b | 5.67 b | 6.33 b | 40 ± 13 b |
| LFAM | 7.67 b | 8.00 b | 6.67 b | 6.33 b | 126 ± 31 c |
| LFAM_A | 7.33 b | 8.00 b | 6.00 b | 7.00 b | 53 ± 4 b |
| DIG | 6.67 b | 8.00 b | 6.00 b | 6.67 b | 145 ± 31 c |
| DIG_A | 7.33 b | 7.67 b | 5.33 b | 6.00 b | 61 ± 10 b |
| LFDIG | 7.67 b | 8.00 b | 6.67 b | 6.67 b | 128 ± 28 c |
| LFDIG_A | 7.00 b | 8.33 b | 6.00 b | 6.63 b | 51 ± 20 b |

*Control: no fertilization; CAN: calcium ammonium nitrate; AM: animal manure; LFAM: liquid fraction of animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; _A: acidified. Mean values denoted by the same letter in a column are not statistically different according to Tukey's test at the 5% probability level.*

***Soil properties at harvest***

There was no difference in soil pH between acidified bio-based materials and their respective non-acidified fraction, except for DIG and DIG_A (Table 4.5). The lowest concentrations of soil $EC_{5:1}$ and $S_{total}$ were measured in the control, while the highest concentrations were detected in acidified treatments. There were no significant differences in soil $P_{total}$ and $K_{total}$ concentrations observed among the ten treatments. With regard to $Cu_{total}$ and $Zn_{total}$ concentration, small variations were observed for $Zn_{total}$ concentration in soil, but they were not significant (p>0.05).

### 4.3.3 N release and mineralization

Application of acidified or non-acidified materials led to significant increases in soil $NH_4^+$-N concentration, which decreased over time and from day 20 reached values lower than 5 mg $NH_4^+$-N $kg^{-1}$ (Figure 4.1). The $NO_3^-$-N content of used materials was negligible (except for CAN), and as such $NO_3^-$-N concentration of amended treatments at day 0 equals to that of unfertilized treatment. The concentration of $NO_3^-$-N in non-acidified treatments was always significantly ($p < 0.05$) higher than in soils amended with acidified materials (Figure 4.1).

***Table 4.5*** *Soil characterization as mean ± standard deviation of three independent samples at harvest. Soil nutrient concentrations are expressed on dry matter (DM) basis.*

| Parameter | Control | CAN | AM | AM_A | LFAM | LFAM_A | DIG | DIG_A | LFDIG | LFDIG_A |
|---|---|---|---|---|---|---|---|---|---|---|
| Moisture (%) | 18 ± 1a | 15 ± 0c | 17 ± 1ab | 16 ± 0b | 15 ± 1c | 16 ± 1ac | 15 ± 0c | 15 ± 0c | 14 ± 1c | 16 ± 1ac |
| OM (%) | 2.6 ± 0.0a | 2.5 ± 0.1a | 3.0 ± 0.4a | 2.6 ± 0.0a | 2.8 ± 0.0b | 2.8 ± 0.1b | 2.7 ± 0.0b | 2.7 ± 0.1ab | 2.8 ± 0.2ba | 3.0 ± 0.3b |
| pH-KCl | 4.8 ± 0.1c | 4.6 ± 0.0b | 4.7 ± 0.0c | 4.6 ± 0.1bc | 4.6 ± 0.1abc | 4.5 ± 0.0a | 4.7 ± 0.1c | 4.4 ± 0.0a | 4.6 ± 0.0b | 4.5 ± 0.1b |
| $EC_{5:1}$ ($dS\ m^{-1}$) | 0.05 ± 0.00a | 0.09 ± 0.00b | 0.08 ± 0.01b | 0.15 ± 0.02d | 0.06 ± 0.00a | 0.13 ± 0.00d | 0.07 ± 0.01c | 0.16 ± 0.01d | 0.06 ± 0.00a | 0.17 ± 0.01d |
| $N_{total}$ ($g\ kg^{-1}$) | 1.1 ± 0.1 | 1.0 ± 0.1 | 1.1 ± 0.0 | 1.0 ± 0.1 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.0 | 1.0 ± 0.1 |
| $P_{total}$ ($mg\ kg^{-1}$) | 775 ± 40 | 755 ± 29 | 772 ± 22 | 783 ± 27 | 769 ± 38 | 799 ± 14 | 753 ± 41 | 742 ± 24 | 753 ± 24 | 770 ± 51 |
| $K_{total}$ ($mg\ kg^{-1}$) | 609 ± 77 | 658 ± 22 | 692 ± 92 | 670 ± 13 | 664 ± 29 | 620 ± 63 | 685 ± 11 | 702 ± 40 | 656 ± 36 | 684 ± 90 |
| $S_{total}$ ($mg\ kg^{-1}$) | 143 ± 3a | 159 ± 9b | 189 ± 8c | 253 ± 10d | 212 ± 7c | 255 ± 4d | 193 ± 18c | 236 ± 10d | 167 ± 9bc | 236 ± 16d |
| $Cu_{total}$ ($mg\ kg^{-1}$) | 16 ± 0 | 16 ± 2 | 16 ± 0 | 16 ± 0 | 15 ± 1 | 15 ± 1 | 16 ± 0 | 15 ± 1 | 16 ± 0 | 15 ± 1 |
| $Zn_{total}$ ($mg\ kg^{-1}$) | 23 ± 1b | 26 ± 2bc | 25 ± 0c | 24 ± 1bc | 21 ± 2b | 21 ± 1b | 20 ± 1a | 20 ± 1a | 21 ± 1a | 21 ± 2ab |

*OM: organic matter; $EC_{5:1}$: electrical conductivity (L:S ratio = 5:1); Control: no fertilization; CAN: calcium ammonium nitrate; AM: animal manure; LFAM: liquid fraction of animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; _A: acidified. Mean values denoted by the same letter in a row are not statistically different according to Tukey's test at the 5% probability level. The difference is denoted only by comparing the acidified and non-acidified fraction of the tested material with the control and CAN treatment.*

The average N release over the entire incubation experiment, was in the order: CAN > non-acidified treatments > acidified treatments (Figure 4.2). In the non-acidified treatments the highest $N_{rel,net}$ was observed for LFDIG (88±2%), followed by AM (81±2%), LFAM (76±2%) and DIG (76±8%). The average $N_{min,net}$ (% of added total N) measured in LFDIG, AM, LFAM and DIG at day 120 was 6±2%, 25±2%, 12±2% and 19±8%, respectively. For acidified treatments N immobilization occurred from the start of the incubation experiment, and no net N mineralization was observed throughout the incubation experiment. After 60 to 80 days, levels of soil mineral N in LFAM_A, AM_A and DIG_A reached approximately the initial mineral N level present at t=0. Only in LFDIG_A, mineral N continued to be immobilized, and at the end (t=120) was 34±5 % lower compared to the initial mineral N concentration.

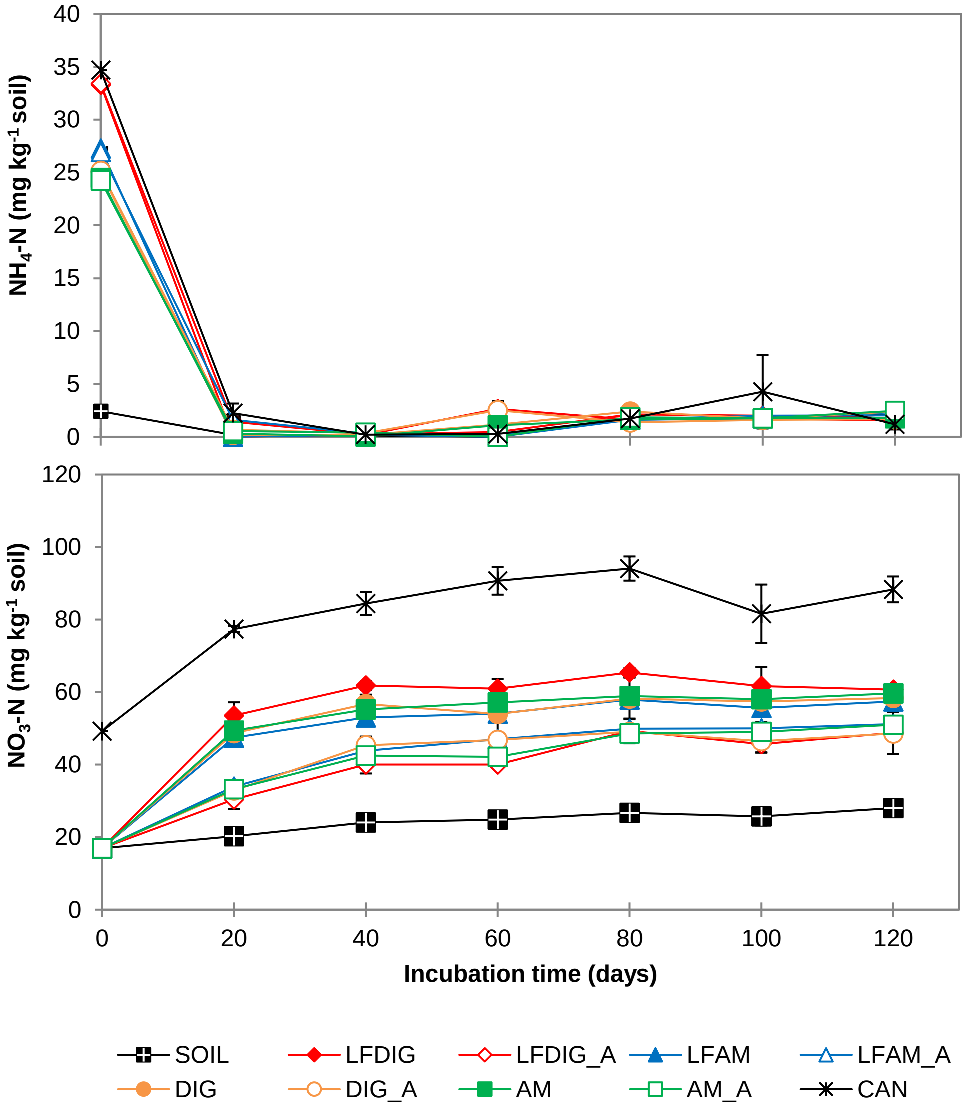



***Figure 4.1*** *Evolution of mineral N (mg kg$^{-1}$ soil) in an unamended soil and soil treated with bio-based and synthetic fertilizers (n=3) during 120-day incubation experiment (mean ± standard deviation; where absent, error bars fall within symbols). AM: animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; LFAM: liquid fraction of animal manure; CAN: calcium ammonium nitrate; _A: acidified.*

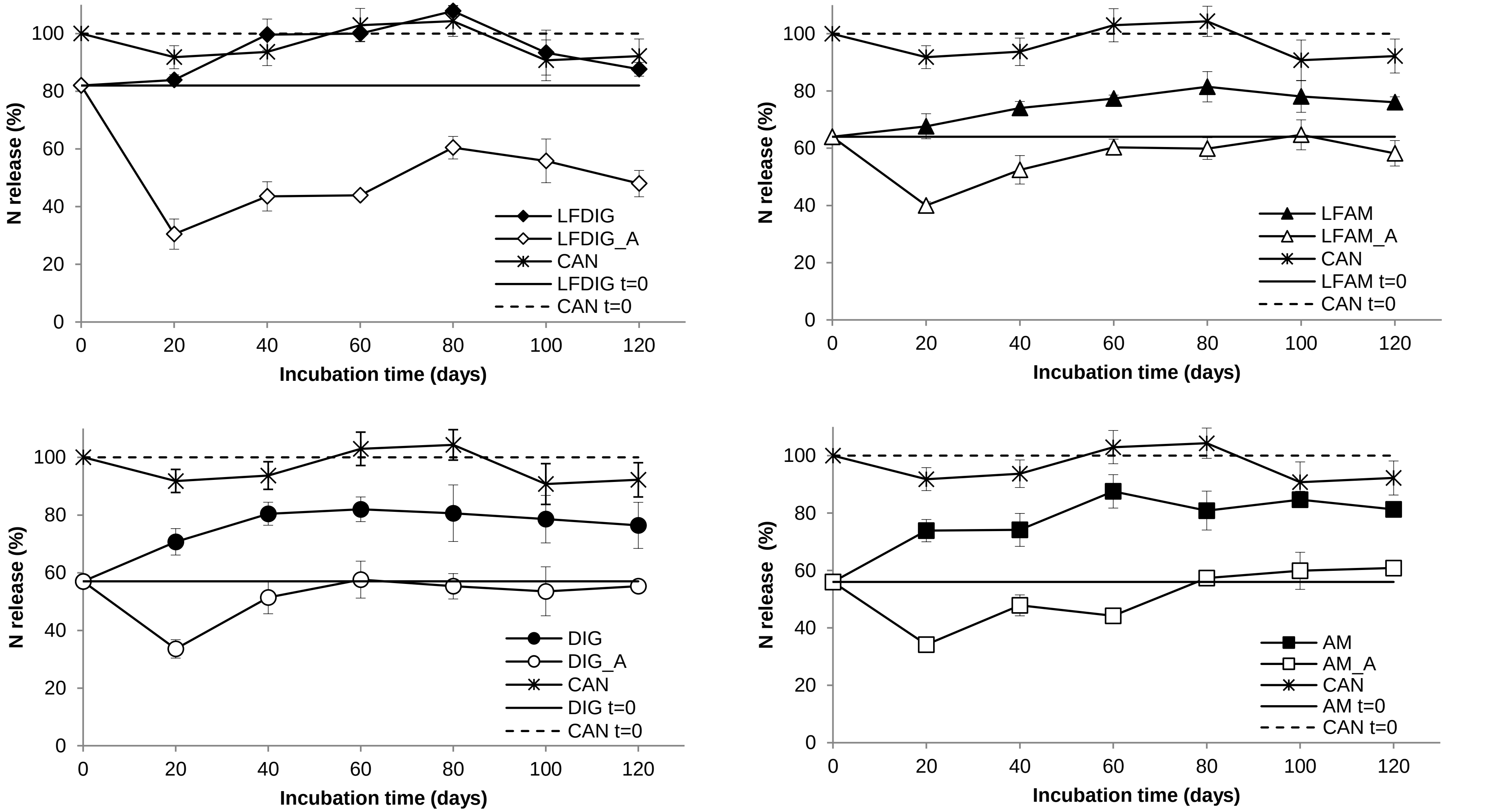



***Figure 4.2** N release ($N_{rel,net}$; %) relative to the N input during the incubation experiment. The $N_{rel,net}$ at t=0 is the percentage of mineral N initially present in the material (dashed line for CAN and solid line for tested material). Values observed above the line indicate net N mineralization, while values below the line indicate net N immobilization. Error bars indicate standard deviation (n=3). AM: animal manure; DIG: digestate; LFDIG: liquid fraction of digestate; LFAM: liquid fraction of animal manure; CAN: calcium ammonium nitrate; _A: acidified.*

## 4.4 Discussion

### 4.4.1 Effect of acidification on N release and mineralization

Acidification is primarily used as a tool to reduce $NH_3$ volatilization from the manure/digestate management chain, which might indirectly induce changes in soil N dynamics and NFRV upon land application of acidified materials. Findings from previous studies with regard to the impact of acidified pig slurry on N dynamics relative to untreated slurry have been variable. While Sørensen and Eriksen (2009) found no particular effects, Fangueiro *et al.* (2009; 2010; 2013) observed a stimulation of N mineralization and a delay or inhibition of nitrification with acidified pig and cattle slurry on sandy soil. In the present study on loamy sand soil, acidification led to N immobilization in all acidified treatments, while in non-acidified treatments N mineralization was detected (Figure 4.2). This is in contrast to our third research hypothesis and to previously reported observations (Fangueiro *et al.*, 2009; Fangueiro *et al.*, 2010; Fangueiro *et al.*, 2013). However, a recent study by Fangueiro *et al*. (2016) reported that the application of acidified slurry can lead to a more significant immobilization and as such have little effect on N mineralization, depending on soli characteristics. It has been proposed that the effect of slurry acidification might be mitigated in soils with a high buffer capacity, high carbonate content and in which no change in pH occurs following the application of acidified materials (Fangueiro *et al.*, 2016). Moreover, we cannot exclude the possibility that acidification might have triggered the release of substances that inhibited the activity of microorganisms or their enzymes, consequently causing the observed N immobilization in acidified treatments. This type of negative priming effect was reported by Kuzyakov *et al.* (2000) and was attributed to the release of substances that are toxic for microbial community. Currently, there is a significant lack of knowledge regarding the effect of acidification on the microbial community (Fangueiro *et al.*, 2015), which should be definitely tackled in the future to clarify the mechanisms behind these observations.

Acidification resulted in a significant and consistent decrease in $N_{rel,net}$ (%) as compared to non-acidified materials. While in most of the acidified treatments the initial mineral N level present in materials at t=0 was reached from day 60, in LFDIG_A treatment strong N immobilization was detected throughout the entire incubation experiment. The reason probably lies in the amount of applied $NH_4^+$-N, which was on average 20-30% higher in LFDIG_A treatment as compared to other acidified treatments. Moreover, a negative correlation ($r = -0.989$, $p<0.05$) was found between applied $NH_4^+$-N and $N_{min,net}$ (%) among acidified treatments, meaning the lower amount of $NH_4^+$-N is applied the higher $N_{min,net}$ (%) will be.

As expected, the highest $N_{rel,net}$ (%) on average at day 120 was observed with CAN application. During the 120 days (with an exception on the day 100) of the incubation experiment, the $N_{rel,net}$ (%) in CAN treatment was significantly higher as compared to DIG, AM and LFAM treatments. A significant difference between LFDIG and CAN treatment was observed only during the first 20 days. Afterwards, there was no significant difference in $N_{rel,net}$ (%) between these two treatments. The high $N_{rel,net}$ (%) in LFDIG treatment was probably influenced by the initially present amount of mineral N (82% of total N), which was the highest of all non-acidified treatments.

The inhibition or in this case the reduction of the $N_{rel,net}$ (%) in acidified treatments is of high importance considering the potential risk of $NO_3^-$ leaching that might occur by applying bio-based fertilizers in an open field cultivation. This is especially relevant at the beginning of the growing season when plants are too small to take up large amounts of N and excess mineral N might be leached in case of excess precipitation. However, N leaching losses may be increased if $N_{rel,net}$ is postponed too long after the peak in crop N uptake.

### 4.4.2 Effect of acidification on crop growth and soil properties

In contrast to our hypothesis, acidification did not indirectly lead to an increase of lettuce FW yields, but rather had a tendency towards moderate decrease of the marketable yield (Table 4.3). The reduction of $NH_3$ losses via acidification may not necessarily be reflected in an increase of the crop yield. The impact of other parameters on lettuce yield should be investigated.

As a salt sensitive crop, lettuce can react negatively on the increase of soil salt content. In this study, by the direct addition of $H_2SO_4$, soil $EC_{5:1}$ (Table 4.5) and consequently crop $S_{total}$ concentration (Table 4.3) increased by 46-68% and 15%, respectively, compared to non-acidified treatments. Nevertheless, no correlations were found between crop FW yield and soil $EC_{5:1}$ content. Also, the measured $EC_{5:1}$ values remain far below the upper limit value of 1.8 dS $m^{-1}$ set for the lettuce (Sigurnjak *et al*., 2016; Chapter 5). The lettuce FW yield in fertilized treatments was positively correlated with the crop $N_{total}$ concentration ($r=0.513$, $p<0.01$) which on average was lower with acidified materials (Table 4.3). This can be attributed to the decrease of $N_{rel,net}$ (%; $r=0.629$, $p<0.01$) as observed after the application of acidified materials (Figure 4.2). This was especially evident in the N incubation test where for all acidified materials fast N immobilization occurred within the first 20 days after application, followed by a period of decrease in net N immobilization. Nevertheless, the $N_{rel,net}$ (%) of acidified materials did not reach the initial (t=0) level of mineral N before day 60. Taking into consideration that the crop cultivation cycle was 54 days, it seems that lettuce in acidified treatments did not receive an equal amount of plant available N despite equal mineral N application rate as in non-acidified treatments (Table 4.2). This occurred probably due to the reduced N availability, as also evident in the crop $NO_3^-$ concentration which was the lowest in acidified treatments and the control (Table 4.4). Therefore, acidification might not indirectly lead to a higher NFRV of bio-based fertilizers applied in crops with a short cultivation cycle such as lettuce.

On the other hand, when comparing the performance of non-acidified materials with CAN, only the application of LFDIG increased the lettuce FW yield on average by 10% as compared to CAN application (Table 4.3). LFDIG is a non-acidified material with the highest amount of initially present mineral N ($NH_4$-N/$N_{total}$ ratio = 82%) and also a material whose P contribution exceeded the crop P requirements (i.e. 125 kg $P_2O_5$ $ha^{-1}$). The impact of P over-fertilization on crop FW yield can be excluded since DIG treatment with similar P levels exhibited lower FW yield as compared to LFDIG treatment. Note that LFDIG was also the material whose application brought the amount of mineral N comparable to the one applied via CAN application. On top of that, $N_{min,net}$ (%) from organic N in LFDIG might additionally increase the $N_{rel,net}$ (%) from this material. In the N incubation test it can be seen that between day 40 – 60 (i.e. the time that corresponds to the end of lettuce growth (54 days), there was no significant difference between the $N_{rel,net}$ (%) of CAN and LFDIG (Figure 4.2). Hence, it is possible that application of LFDIG with high mineral N content slightly increase the lettuce FW yield. These results are supported by Tampio *et al.* (2016) where in a pot experiment 5 different urban digestates produced 5-30% higher ryegrass yields compared to synthetic N fertilizer ($NH_4NO_3$) with a similar N concentration, and equal P and K levels to maintain N as the only responsive nutrient. In order to calculate the ANR and NFRV values, the crop N concentration and yield data were taken into consideration. The lower lettuce FW yield and crop N uptake in acidified treatments led on average to a lower ANR of acidified materials as compared to untreated materials and CAN. Consequently, untreated materials resulted in higher NFRV.

Furthermore, the pH decrease of bio-based fertilizers might lead to higher plant availability of heavy metals as Cu and Zn (Tampio *et al.*, 2016). In our study only an increase of $Zn_{total}$ concentration in the crop was observed. Lettuce in acidified treatments AM_A, LFAM_A, DIG_A and LFDIG_A had on average 28%, 29%, 40% and 30% higher $Zn_{total}$ concentration than the lettuce fertilized with untreated materials, respectively (Table 4.3). There were no differences observed in $Cu_{total}$ concentration of the lettuce. These observations can be explained by the bioavailability of Zn that is strongly dependent on the low pH of soil or in this case on the low pH of acidified materials. Even though Cu bioavailability is also regulated by pH, in soils rich in OM, bioavailability of Cu will be more dependent on the OM content rather than on pH itself (Reichman, 2002). In this study, both Cu and Zn concentrations of lettuce were below the critical threshold of 10-30 mg Cu $kg^{-1}$ DM and 100-500 mg Zn $kg^{-1}$ DM, respectively, that may lead to growth depression of the crop (Kabata-Pendias and Pendias, 2011). The soil Zn concentrations were below the Flemish soil environmental quality standard of 62 mg Zn $kg^{-1}$ DM, while the soil Cu concentrations, including the control, were close to the imposed threshold of 17 mg Cu $kg^{-1}$ DM (FSD, 2006).The relatively high Cu concentration in the soil is attributed to the long-term application of animal manure and inorganic fertilizers. The observed increase of crop Zn concentration in the acidified treatments might be of interest for Zn biofortification.

Finally, crop quality assessment showed no negative impact of applied fertilizer treatments with respect to tipburn, basal rot, yellow leaves, presence of bremia, crop volume and uniformity. The crop $NO_3^-$ concentration as an important crop quality criterion was in all treatments below the maximum levels for $NO_3^-$ as laid down in European Commission Regulation No 1881/2006 (EC, 2006).

## 4.5 Conclusion

Acidification did not increase the NFRV of bio-based fertilizers applied in the cultivation of lettuce as a consequence of FW yield and crop N uptake reduction that was caused by an observed delay in $N_{rel,net}$ (%). Of course, we cannot generalize on effects of acidification on crop yield and NFRV based on this single investigation. Yet, current findings suggest that acidified bio-based fertilizers might be less suitable for crops with short growth cycle. Thus, experiment should be done on other horticultural crops with a longer cultivation period and on plot level. Crops that might be of interest are the one with higher demand for sulphur such as cauliflower. In the current setup, acidification did affect N dynamics of applied materials by delaying or reducing $N_{rel,net}$ (%). The negative correlation between applied amount of $NH_4^+$-N amount and $N_{min,net}$ (%) among acidified treatments, indicates that acidification of materials with high $NH_4^+$-N content will lead to a longer delay or more profound reduction of $N_{rel,net}$ (%). Finally, acidification increased the crop Zn concentration, implying that material pre-treatment may play a role in biofortification and melioration of (trace) element composition of crops (arguably for crops with longer production cycles).

**Acknowledgments**

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# **CHAPTER 5:** LIQUID FRACTION OF DIGESTATE AND AIR SCRUBBER WATER AS NITROGEN FERTILIZERS IN COMMERCIAL GREENHOUSE PRODUCTION OF *LACTUCA SATIVA* L.

*Struvite (top left), liquid fraction of digestate (top right), effluent from constructed wetlands (bottom left) and air scrubber water (bottom right) (Pictures: Sigurnjak, I.)*

***This chapter has been redrafted after:***

Sigurnjak, I., Michels, E., Crappé, S., Buysens, S., Tack, F.M.G., Meers, E. 2016. Utilization of derivatives from nutrient recovery processes as alternatives for fossil-based mineral fertilizers in commercial greenhouse production of *Lactuca sativa* L. Scientia Horticulturae 198, 267-276.

## Abstract

The production of vegetables relies on the use of mineral fertilizers which are based either on fossil fuel (e.g. nitrogen fertilizers based on the Haber-Bosch process) or on fossil ore deposits (phosphate rock). On the other hand, nutrient recovery processes generate various end and side materials that can provide a sustainable alternative for fossil-based fertilizers. Their use however, is currently limited by insufficient knowledge about the properties and the impact of these materials on soil properties and crop yield. This research was aimed to evaluate the use of the liquid fraction of digestate, effluent from constructed wetlands originating from a manure/digestate treatment facility, air scrubber water and struvite in the cultivation of lettuce *(Lactuca sativa L.)* compared to their conventional fossil-based counterparts. In accordance with the crop nutrient requirements, different fertilization treatments were set up in which individual bio-based materials and combinations were tested. At harvest time, assessment of soil properties was conducted, along with crop quality control. Fertilizer use efficiencies (FUE) were determined and an economic assessment was done. There were no significant differences in crop yield and soil properties at harvest time between conventional fossil-based mineral fertilizers and selected bio-based mineral alternatives. Nitrogen and potassium FUE were slightly lower in treatments with bio-based fertilizers. Moreover, economic assessment showed bio-based alternatives to be more beneficial for the farmer compared to the use of synthetic fertilizers only.


## 5.1 Introduction

Increasing demand to reduce our dependence on fossil fuels and currently depleting nutrient resources has been driving widespread attention to valorization and nutrient recovery from biomass (Gonzáles-Ponce *et al.*, 2009; Leogrande *et al.*, 2013; Vaneeckhaute *et al.*, 2013a). Animal waste offers potential as both, an input to produce renewable energy and as a fermentation residue, called digestate, to be used as a soil amendment. Many studies have investigated the fertilizer potential of digestate (Fuchs *et al.*, 2008; Alburquerque *et al.*, 2012a; Leogrande *et al.*, 2013; Vaneeckhaute *et al.*, 2013b; Nicoletto *et al.*, 2014). Far less is known about the fertilizer potential of manure and digestate derivatives obtained via nutrient recovery processes that could be used as substitutes for synthetic fertilizers, potentially entailing significant economic benefits (Vaneeckhaute *et al.*, 2013a; Nicoletto *et al.*, 2014).

In previous chapters (Chapter 3 and 4), bio-based materials were tested on a laboratory scale. In order to investigate their environmental and agronomic sound application in practice, the experimental scale needed to be expanded to a commercial level. This chapter aimed to determine the potential use of liquid fraction (LF) of digestate, effluent from constructed wetlands (CW), air scrubber water (ASW) and struvite as substitutes for fossil-based mineral fertilizer in commercial cultivation of lettuce. Air scrubber water, LF of digestate and effluent from CW are end and side bio-based materials from manure and digestate processing. ASW results from the treatment of indoor air from animal housing, where $NH_3$ in the air is captured in sulfuric acid. LF of digestate is a P-poor and NK-rich material obtained by mechanical separation of raw digestate. Effluent from CW is the end-material of tertiary treatment of livestock wastewater. In Flanders it assumes the treatment of liquid fraction of pig manure after mechanical separation and subsequent biological treatment (an activated sludge reactor with nitrification and denitrification processes). As a last step in manure treatment, effluent from CW complies with the Flemish legal discharge criteria of 2 mg P $l^{-1}$, 15 mg N $l^{-1}$ and 125 mg COD $l^{-1}$ (Meers *et al.*, 2008). This end-material is colorless, odorless and although characterized by reduced nutrient concentrations, still can potentially be used as a K-source. Finally, struvite is currently obtained as an end-material of phosphorus recovery from industrial or agricultural wastewater (Gonzáles-Ponce *et al.*, 2009; Antonini *et al.*, 2012). It can be considered as a slow release granular P-fertilizer. There is currently not yet a full-scale struvite crystallization plant as part of manure treatment in the region of Flanders (Belgium).

These bio-based materials were evaluated with respect to their effect on soil properties, crop yield quality and nutrient concentration as compared to mineral fertilizers including calcium ammonium nitrate (CAN; 27% N), triple superphosphate (TSP; 46% $P_2O_5$) and potassium sulfate (PAT; 30% $K_2O$, 10% MgO and 42% $SO_3$). We hypothesized that the use of these bio-based materials will not cause significant differences in crop yield, crop quality, nutrient concentration and soil properties compared to the use of conventional synthetic fertilizers.

## 5.2 Materials and Methods

### 5.2.1 Soil and bio-based materials

The study was carried out in two experimental greenhouses at the Vegetable Research Centre (PCG) in Kruishoutem, Belgium. Prior to the experiment, soil flushing was performed as a common practice by farmers. Soil flushing is usually done between cropping cycles during the summer months to avoid build-up of salt concentrations, or after soil disinfection. Six hours of irrigation were spread over a period of 8 days: 1 min of irrigation equals 180 ml/$m^2$. As such a homogenous nitrate and salt poor sand soil was obtained from the plow layer (0-30 cm) in greenhouse 1 (pH-$H_2O$, 6.3; EC, 0.4 dS $m^{-1}$; $NH_4^+$-N, <1.5 mg $kg^{-1}$; $NO_3^-$-N, 1.7 mg $kg^{-1}$; K, 11 mg $kg^{-1}$; P, 1.7 mg $kg^{-1}$; S, 56 mg $kg^{-1}$; Cu, <0.02 mg $kg^{-1}$ and Zn, <0.01 mg $kg^{-1}$) and greenhouse 2 (pH-$H_2O$, 6.2; EC, 0.5 dS $m^{-1}$; $NH_4^+$-N, <1.5 mg $kg^{-1}$; $NO_3^-$-N, 5.2 mg $kg^{-1}$; K, 11 mg $kg^{-1}$; P, 1.3 mg $kg^{-1}$; S, 65 mg $kg^{-1}$; Cu, <0.02 mg $kg^{-1}$ and Zn, <0.01 mg $kg^{-1}$). These analyses were performed on fresh soil in 1:2 (v/v) water extract and analyzed by an external laboratory.

The LF of digestate (LFDIG) was sampled at the site of Sap Eneco Energy (Merkem, Belgium), a mesophilic (37°C) anaerobic co-digestion plant (capacity: 60,000 tonnes $y^{-1}$, 2.83 $MW_{el}$) with an input feed consisting of 30 % pig manure, 30 % energy maize and 40 % organic waste originating from the food industry (i.e. carrots, starch from potatoes, unpacked products from supermarket, etc.). The digester is a continuously stirred reactor tank (CSTR) with a hydraulic retention time of 35 days. The LF of digestate underwent an obligatory hygenization step (1h at 70°C) and separation step (sieve band press). Struvite was collected at the site of the potato factory Agristo (Harelbeke, Belgium), whereas the effluent from CW was collected at a pig farm in Langemark (Belgium) and ASW from a stable air washing installation at a pig farm in Merkem (Belgium). All bio-based materials were collected in polyethylene bottles (2L), stored (<4°C) and characterized to determine the required total N application rate for the different cultivation treatments based on the total N demand of lettuce. Physicochemical characterization of the products showed that LF of digestate, effluent from CW, struvite and ASW have a high fertilizer potential as NK, K, P and NS-fertilizer, respectively (Table 5.1).

Their potential applications as substitutes for fossil-based fertilizers were evaluated by means of a full scale greenhouse experiment for commercial production. The greenhouse is a VENLO type glass greenhouse with a column height of 4 m, truss size of 3.2 m with a column every 6.4 m. It is covered with 4 mm thick floating glass. Ventilation of the greenhouse is regulated through windows in the roof of which opening and closing is managed by the climate computer. Heating of the greenhouse is done by a gas burner that is fixed to the roof construction. There is no artificial lightning in the greenhouse. Through sensors (wet bulb temperature) that are installed in the greenhouse and that are connected with the climate computer, temperature and relative hydration are monitored.

***Table 5.1*** *Physicochemical characterization of bio-based materials applied as fossil-based mineral fertilizer replacements for lettuce cultivation. Results are expressed on fresh weight (FW) basis as means ± standard deviation of two independent samples.*

| Parameter | LFDIG | CW effluent | Struvite | ASW |
|---|---|---|---|---|
| Dry matter (%) | 3.27 ± 0.05 | 0.46 ± 0.03 | 92 ± 0 | 32.7 ± 0.4 |
| Organic matter [a] (%) | 47 ± 0 | 6.4 ± 0.0 | 48 ± 0 | 100 ± 0 |
| pH | 8.6 ± 0.0 | 7.8 ± 0.0 | 7.3 ± 0.0 [b] | 2.4 ± 0.0 |
| EC (dS $m^{-1}$) | 41 ± 0 | 7.1 ± 0.0 | 0.93 ± 0.00 [b] | 262 ± 0 |
| Total N (g $kg^{-1}$) | 5.3 ± 0.1 | 0.02 ± 0.00 | 52 ± 4 | 86 ± 3 |
| $NH_4^+$-N (g $kg^{-1}$) | 4.56 ± 0.04 | 0.002 ± 0.000 | 0.97 ± 0.02 | 86 ± 3 |
| $NO_3^-$-N (g $kg^{-1}$) | 0.002 ± 0.00 | 0.001 ± 0.000 | 0.24 ± 0.00 | - |
| Total P (g $kg^{-1}$) | 0.38 ± 0.01 | 0.002 ± 0.000 | 93 ± 2 | 0.05 ± 0.00 |
| Extractable P [c] (%) | 100 ± 0 | 8 ± 0 | 100 ± 0 | 17 ± 0 |
| Total K (g $kg^{-1}$) | 3.36 ± 0.07 | 1.2 ± 0.0 | 9.1 ± 0.8 | 0.2 ± 0.0 |
| Extractable K [d] (%) | 100 ± 0 | 100 ± 0 | 10 ± 0 | 100 ± 0 |
| Total S (g $kg^{-1}$) | 0.45 ± 0.01 | 0.04 ± 0.00 | 0.07 ± 0.01 | 53 ± 2 |
| Total Cu (mg $kg^{-1}$) | 0.28 ± 0.01 | 0.01 ± 0.00 | 1.6 ± 0.4 | 0.3 ± 0.0 |
| Total Zn (mg $kg^{-1}$) | 1.26 ± 0.04 | 0.02 ± 0.00 | 9 ± 1 | 2.9 ± 0.1 |

*EC: electrical conductivity; LFDIG: liquid fraction of digestate; CW: constructed wetlands; ASW: air scrubber water. [a] Organic matter (OM) calculated on a dry matter basis. [b] pH and EC of struvite was measured at a 5:1 liquid to dry sample ratio, other (liquid) products were analyzed directly. [c] Ammonium lactate extractable P (%): percent of total P that is plant available. [d] 0.01 M $CaCl_2$ extractable K (%): percent of total K that is plant available.*

### 5.2.2 Experimental design

The experiment was set-up according to a fully randomized block design with four replicate plots of 10 $m^2$ (4 m x 2.5 m) per treatment. Eight different fertilization treatments were established over the two greenhouses (Table 5.2). In greenhouse 1, single replacement treatments were conducted. In each treatment synthetic fertilizer was replaced by one bio-based material with similar characteristics. In greenhouse 2, synthetic fertilizer was partially or completely replaced by a combination of different bio-based material. In each greenhouse, a treatment with conventional fossil-based fertilizer (treatments Ref G1 and Ref G2) was included as a reference to eliminate effects of the greenhouse. The required total application dosage was calculated (Table 5.2) according to the nutrient requirements for lettuce by taking into consideration the nutrient content of fertilizers (Table 5.1). The nutrient requirements of 210 N, 125 $P_2O_5$ and 240 $K_2O$ kg $ha^{-1}$ for lettuce were based on the practical experience of PCG.

On June 13 2013, one day before transplanting the lettuce, the liquid bio-based materials were manually applied by using a watering can or a sprayer depending on the necessary amount of fertilizers according to the application rate (Table 5.2). The liquid materials were always applied first to avoid any detrimental effects on lettuce (e.g. burning of leaves) associated to the low pH of ASW and higher salt content. On June 14 2013, struvite and synthetic fertilizer (CAN, TSP and PAT) were applied by hand to ensure high precision of the applied dosage. To facilitate homogeneous application of liquid materials in low amount, ASW was diluted by adding tap water. The incorporation of liquid and solid materials was done manually in the soil upper level (depth 10 cm) to ensure their uniform distribution and avoid mixing soil of one plot with the other. Finally, lettuce (*Lactuca sativa L.* cv. Cosmopolia) was transplanted with a 5 cm soil block using a planting distance of 27 x 27 cm, leading to a plant density of 126 plants per plot (10 $m^2$). During the entire growing period water supply was provided by means of irrigation (Figure 5.1).

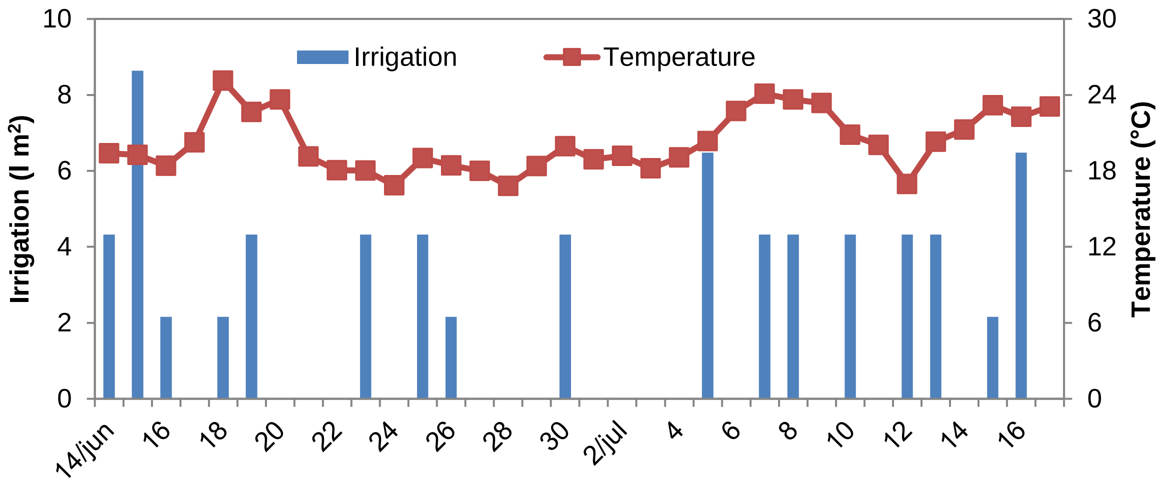


***Figure 5.1*** *Temperature and irrigation events during the growing period (14/06/2013 – 17/07/2013).*

***Table 5.2** Fertilizer and macronutrient dosage per ha applied for the eight different fertilization treatments (n=4, plot size = 10m$^2$) in greenhouse 1 (G1) and greenhouse 2 (G2). Treatments Ref G1 and Ref G2 present conventional fertilization with synthetic fertilizers (CAN+TSP+PAT) as a reference of the respective greenhouse. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO ad 42% $SO_3$); STR: struvite; ASW: air scrubber water; CW: effluent from constructed wetlands; LFDIG: liquid fraction of digestate.*

| | TRT | Synthetic fertilizer | | | Bio-based fertilizer | | | | | | | | | | | | | | | | Total applied | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | CAN | TSP | PAT | Struvite | | | | Air scrubber water | | | | Effluent from CW | | | | LFDIG | | | | | | | |
| | | kg N | kg $P_2O_5$ | kg $K_2O$ | kg | kg N | kg $P_2O_5$ | kg $K_2O$ | tonnes | kg N | kg $P_2O_5$ | kg $K_2O$ | tonnes | kg N | kg $P_2O_5$ | kg $K_2O$ | tonnes | kg N | kg $P_2O_5$ | kg $K_2O$ | kg N | kg $P_2O_5$ | kg $K_2O$ | kg S |
| Greenhouse 1 | Ref G1 | 211 | 124 | 240 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 211 | 124 | 240 | 116 |
| | CAN+STR+PAT | 176 | - | 234 | 587 | 30 | 126 | 6.4 | - | - | - | - | - | - | - | - | - | - | - | - | 206 | 126 | 240 | 111 |
| | ASW+TSP+PAT | - | 124 | 240 | - | - | - | - | 2.4 | 210 | 0.27 | 0.4 | - | - | - | - | - | - | - | - | 210 | 124 | 240 | 246 |
| | CAN+TSP+CW | 211 | 124 | - | - | - | - | - | - | - | - | - | 161 | 3.2 | 0.64 | 240 | - | - | - | - | 214 | 125 | 240 | 8.8 |
| | LFDIG+TSP+PAT | - | 97 | 75 | - | - | - | - | - | - | - | - | - | - | - | - | 39 | 210 | 34 | 173 | 210 | 131 | 248 | 55 |
| Greenhouse 2 | Ref G2 | 211 | 124 | 240 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 211 | 124 | 240 | 116 |
| | ASW+STR+CW | - | - | - | 587 | 30 | 126 | 6.4 | 2.0 | 172 | 0.22 | 0.4 | 157 | 3.1 | 0.63 | 233 | - | - | - | - | 205 | 127 | 240 | 113 |
| | LFDIG+STR+PAT | - | - | 81 | 450 | 23 | 96 | 4.9 | - | - | - | - | - | - | - | - | 35 | 187 | 30 | 154 | 210 | 126 | 240 | 54 |
| | ASW+STR+PAT | - | - | 225 | 587 | 30 | 126 | 6.4 | 2.0 | 172 | 0.22 | 0.4 | - | - | - | - | - | - | - | - | 202 | 126 | 232 | 213 |

### 5.2.1 Plant and soil sampling

On July 17 2013, after a fresh yield of 500 g was reached (i.e. the commercial weight), the plants were harvested. A comparison of the production data (crop height and fresh weight) was made among treatments along with an assessment of nutrient concentrations in lettuce. Representative plant material samples for fresh weight (FW) determination were obtained by harvesting twelve random plants per plot, not taking into account the plants that were located in the border line of plots.

Composite soil samples were obtained as a mixture of five random sample points per plot, taken from the 0-30 cm soil layer. Furthermore, each soil sample was divided into two fractions, one of which was immediately stored (-23°C) without drying for $NO_3^-$-N, $NH_4^+$-N and dry matter (DM) analysis. The other fraction was air-dried and sieved to < 2mm for further physicochemical analysis.

### 5.2.2 Physicochemical analysis

***Product analysis***

DM content was determined after drying to a constant weight for 96 h at 105°C. Organic matter (OM) was measured after incineration of the samples during 4 h at 550°C in a muffle furnace, where the loss of mass on ignition was addressed as the OM. EC and pH were determined by using a WTW-LF537 (WTW GmbH, GE) conductivity electrode and an Orion-520A (Orion Research Inc, USA) pH-meter. For liquid samples, EC and pH measurements were performed without prior equilibration and filtration, while for solid samples equilibration in deionized water for 1 h at a 5:1 liquid to dry sample ratio was performed along with subsequent filtration (MN 640 m, Macherey-Nagel, GE). Total N and $NH_4^+$-N were determined as described in section 3.2.2 (Chapter 3). Nitrate-N was determined by flow analysis (CFA and FIA) and spectrometric detection (ISO 13395: 1996) from an 1M KCl extract (BRAN+LUEBBE AA3, GE). After wet digestion (2 ml $HNO_3$ and 1 ml $H_2O_2$), total P, S, K, Cu and Zn were measured as described in section 3.2.2 (Chapter 3). Plant available amounts of P and K were analyzed using the colorimetric Scheel method and using ICP-OES (Varian Vista MPX, USA) after ammonium lactate extraction at pH 3.75 (VITO, 2010) and 0.01 M $CaCl_2$ extraction (Van Ranst *et al.*, 1999), respectively.

***Soil analysis***

The soil moisture content and OM were determined as described in section 3.2.1 (Chapter 3). Soil $EC_{2:1}$ was measured with a WTW-LF537 (GE) electrode after 1h equilibration in deionized water at a 2:1 (v/v) liquid to dry sample ratio (Sonneveld and van den Ende, 1971) and subsequent filtration (MN 640 m, Macherey-Nagel, GE). For pH measurement, potential soil acidity (pH-KCl) was measured as described in section 3.2.1 (Chapter 3). Soil conductivity and pH were monitored on weekly basis. Total N-content in soil was determined using the Kjeldahl destruction method, while nitrate-N (ISO 13395:1996) and ammonium-N (ISO 11732:1997) in soil were analyzed from 1M KCl extract using flow analysis (CFA and FIA) and spectrometric detection (BRAN+LUEBBE AA3, GE). Soil total P, K, S, Cu and Zn were analyzed according to section 4.2.2 (Chapter 4), while plant available amounts of P and K were analyzed as described in previous paragraph (section 'Product analysis').

***Plant analysis***

At harvest, twelve plant samples per plot (collected for FW determination) were cut in half and afterwards oven-dried at 60°C for determination of the DM content. The dry samples were ground and sieved to <1 mm using a Culatti DCFH 48 grinder (GE), and subsequently incinerated for 4 h at 550°C to determine the ash content. After hot plate mineralization digestion (5 ml 6 M $HNO_3$ and 5 ml 3 M $HNO_3$), total P, K, Cu and Zn were analyzed as described in section 4.2.1 (Chapter 4), while total S content was determined as described in section 4.2.3 (Chapter 4). Finally, total N was analyzed using the Kjeldahl method (Van Ranst *et al.*, 1999).

### 5.2.3 Crop quality assessment

As a part of crop quality control, a more extended assessment including tipburn, basal rot, yellow leaves, presence of bremia, volume, uniformity, crop filling, colour and crop closure was conducted. These parameters were determined by a trained observer and evaluated on a scale basis (1-9), not taking into account the plants that were located in the border line of plots. A high score indicates a positive effect while a lower score represents the negative impact of the fertilization treatment on the tested parameter. For some criteria, like diseases and physiological deficiencies, the highest score is given when there is total absence of disease.

### 5.2.4 Economic assessment

A basic economic comparison of conventional synthetic fertilizers and their bio-based alternatives was conducted by summing up the fertilization costs from the view of the arable farmer. The fertilization cost includes the fertilizer retail price, transport and application cost. The retail price of CAN, TSP and PAT includes production, packing and transport cost. It was based on the average market price in 2013 amounting to 28.40 € $100kg^{-1}$ (1.05 € $kg^{-1}$ $N^{-1}$), 45.10 € $100kg^{-1}$ (2.25 € $kg^{-1}$ $P^{-1}$) and 36.85 € $100kg^{-1}$ (1.48 € $kg^{-1}$ $K^{-1}$), respectively (LEI Wageningen, 2014). For bio-based materials, ASW, effluent from CW and LF of digestate, the retail price at this time is zero due to the fact that in a surplus market these materials are exchanged at zero cost. For ASW, the zero cost was based on the fact that Flemish livestock farmers are legally obliged to reduce ammonia emissions coming from animal and digestate facilities (VLM, 2010). The reduction is usually achieved with acid air scrubbers resulting in ASW, a side bio-based material. Next, zero cost for effluent from CW was based on the wetland construction and maintenance costs (3 - 4 € $t^{-1}$ of pre-treated manure coming from biological treatment) which are in the range with the costs (3 - 5 € $t^{-1}$) to spread the effluent from the biological treatment on arable land (Meers *et al.*, 2008). In this case, the investment cost for CW is regained by the free discharge cost. Further, zero cost for LF of digestate was based on the fact that livestock farmers use mechanical separation primary to reduce the water content in manure and digestate, which allows them easier export of P rich materials. Finally, struvite production cost of 50 € $t^{-1}$ (personal communication, NuReSys 2015) was based on the market prices encountered in practice. Next to these costs, it is important to remark that an arable farmer in a surplus market might gain financial benefits for accepting LF of digestate as bio-based fertilizer. These benefits, however, were not included in calculations due to the price uncertainty which might vary from 5 – 10 € $tonnes^{-1}$ $FW^{-1}$ (Vaneeckhaute *et al.*, 2013a; personal communication, VCM 2015). For all used liquid bio-based materials, a specific density of 1000 kg $m^3$ was assumed. For bio-based materials a transport cost of 0.075 € $FW^{-1}$ $tonnes^{-1}$ $km^{-1}$ (Van der Straeten and Buysse, 2013) was assumed, whereas an application cost of 2.5 € $FW^{-1}$ $tonnes^{-1}$ was considered for applying bio-based liquid materials (Van der Straeten and Buysse, 2013; Vaneeckhaute *et al.*, 2013a). The application cost for granular fertilizers (i.e. synthetic fertilizers and struvite) was assumed to be zero because most of the arable farmers have their own equipment to apply conventional fertilizers (INAGRO, personal communication). However, arable farmer has costs in the form of the lost leisure time, fuel cost and depreciation cost of the used machinery which in this assessment are considered to be negligible.

In general, the fertilization cost of the arable farmer can vary depending on the transport distance and whether he owns the application equipment or not. To tackle these effects, a sensitivity analysis was introduced where fertilization cost was calculated for all tested treatments under two different case scenarios (Sc.):

- Sc. 1: transport distance is 5 km and arable farmer owns equipment to apply bio-based liquid materials
- Sc. 2: transport distance is 40 km and arable farmer hires a contractor to apply bio-based liquid materials

These two scenarios represent the most favourable (Sc.1) and the least favourable (Sc. 2) conditions for arable farmer. Finally, potential changes in fertilizer policy might lead to the recognition of N bio-based materials as substitutes for synthetic N fertilizers. To assess these effects, it was assumed that in the case of recognition the retail price for N coming from LF of digestate and ASW might amount to 50%, 75% or 100% of the current market price paid per kg of N. These percentages correspond to the percentages of mineral N (i.e. $NH_4^+$-N/$N_{total}$ ratio) that can be found in common N bio-based materials such as digestate (c. 50-70%), LF of digestate (c. 60-80%), mineral concentrate (c. 90-100%) and air scrubber water (100%). Since the main focus of the dissertation is on N fertilizers, the above described economic assessment was only done for the reference treatment (Ref G1 and Ref G2) and treatments where LF of digestate and ASW were used as N sources. The treatments CAN+STR+PAT and CAN+TSP+CW were not economically assessed since economic effect of using struvite and effluent from CW can be seen also from the treatments where these products were applied in combination with LF of digestate or ASW.

### 5.2.5 Data analysis

The fertilizer use efficiency (FUE) and Fertilizer replacement use efficiency (FRUE %) of each treatment were calculated using Eq. 3 and Eq. 4, respectively (Chapter 1). FRUE was obtained as the ratio of the FUE of the treatments with bio-based material(s) to the FUE of a reference of the respective greenhouse (Ref G1 and Ref G2). For the calculation purposes of comparing efficiency of bio-based materials and synthetic fertilizers, the FRUE of 100% has been assigned to the conventional fertilization practice of using synthetic fertilizers (Ref G1 and Ref G2). The assigned value of 100% corresponds to the $NH_4^+$-N/$N_{total}$ ratio of synthetic N fertilizers.

Statistical analyses were performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL). One way ANOVA was used to determine the effect of the applied fertilizers on soil properties, crop yield and nutrient concentration, based on the obtained physicochemical data. Additional post hoc assessment was performed using Tukey's Test ($p < 0.05$, n=4) when significant differences between means were observed. The condition of normality was checked using the Shapiro-Wilk test.

## 5.3 Results

### 5.3.1 Soil properties and $NO_3^-$-N residue

During the growing period (data not shown) and at harvest time (Table 5.3), no statistical differences with respect to soil $EC_{2:1}$ and potential acidity (pH-KCl) were observed among the eight different fertilization treatments. For the individual treatment substitutions, as hypothesized, results showed no statistical difference ($p > 0.05$) among treatments in greenhouse 1 with respect to total macronutrients (NPKS), plant available nutrients ($NO_3^-$-N, $NH_4^+$-N, P, K) and heavy metals (Cu, Zn). Thus, no significant differences were observed in a soil properties between treatments with bio-based materials and the treatment Ref G1 (Table 5.3).

In contrast, a strong statistical effect ($p = 0.002$) on the $NO_3^-$-N residue in the soil was observed in greenhouse 2 (Table 5.3) where complete substitution of synthetic fertilizers in treatment ASW+STR+CW resulted in a significantly lower $NO_3^-$-N concentration with respect to the treatment Ref G2 ($p = 0.046$) and treatment LFDIG+STR+PAT ($p = 0.001$). Furthermore, the combination treatments significantly ($p = 0.010$) influenced plant available K in greenhouse 2. This observation is a result of differences between treatments ASW+STR+CW, LFDIG+STR+PAT and ASW+STR+PAT in which bio-based materials were applied (Table 5.3). Individually, treatments ASW+STR+CW, LFDIG+STR+PAT and ASW+STR+PAT are not statistically different as compared to the reference, with p-values of 0.805, 0.059 and 0.993, respectively. Finally, treatments in greenhouse 2 did not lead to ($p > 0.05$) a significant difference in soil DM, total macronutrients (NPKS), plant available $NH_4^+$-N and P, and heavy metals (Cu, Zn).

### 5.3.2 Crop production

Determination of the fresh and dry matter yield of lettuce for the eight different fertilization treatments (Table 5.4) over the two greenhouses, showed no differences ($p > 0.05$) in crop yield production. For crop N, P, K, S, Cu and Zn concentration, no significant effects ($p > 0.05$) were observed in treatments with bio-based materials as compared to conventional fertilization (Table 5.4). Additionally, the obtained plant nutrient concentration values were compared with the available reference values from the Hill Laboratories (HL, 2002) which indicate the appropriate range of macro- and micronutrients in lettuce grown within the greenhouse.

***Table 5.3** Soil characterization for the applied fertilization treatments in greenhouse 1 and greenhouse 2 at harvest (17/07/2013). Results are expressed on dry matter (DM) basis as means ± deviation of the four independent samples. Treatments Ref G1 and Ref G2 present conventional fertilization with synthetic fertilizers (CAN+TSP+PAT) as a reference of the respective greenhouse. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO ad 42% $SO_3$); STR: struvite; ASW: air scrubber water; CW: effluent from constructed wetlands; LFDIG: liquid fraction of digestate.*

| Parameter | Treatments in Greenhouse 1 | | | | | Treatments in Greenhouse 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ref G1 | CAN+STR +PAT | ASW+TSP +PAT | CAN+TSP +CW | LFDIG+TSP +PAT | Ref G2 | ASW+STR +CW | LFDIG+STR +PAT | ASW+STR +PAT |
| Moisture (%) | 14 ± 2 | 14 ± 1 | 14 ± 2 | 14 ± 1 | 14 ± 1 | 12 ± 1 | 13 ± 2 | 14 ± 1 | 13 ± 1 |
| Organic matter (%) | 7.0 ± 0.1 | 7.4 ± 0.5 | 7.3 ± 0.6 | 7.1 ± 0.6 | 7.1 ± 0.6 | 6.4 ± 0.2 | 6.4 ± 0.5 | 6.9 ± 0.5 | 6.5 ± 0.4 |
| pH-KCl | 6.1 ± 0.1 | 6.1 ± 0.1 | 6.0 ± 0.1 | 6.2 ± 0.1 | 6.1 ± 0.2 | 6.2 ± 0.1 | 6.3 ± 0.0 | 6.3 ± 0.1 | 6.2 ± 0.1 |
| $EC_{2:1}$ (dS $m^{-1}$) | 0.6 ± 0.2 | 0.7 ± 0.1 | 0.8 ± 0.2 | 0.7 ± 0.1 | 0.8 ± 0.2 | 0.7 ± 0.2 | 0.7 ± 0.1 | 1.0 ± 0.2 | 0.9 ± 0.3 |
| Total N (g $kg^{-1}$) | 2.0 ± 0.1 | 2.2 ± 0.3 | 2.0 ± 0.2 | 2.2 ± 0.4 | 2.2 ± 0.2 | 1.9 ± 0.1 | 1.9 ± 0.1 | 2.0 ± 0.2 | 2.1 ± 0.1 |
| $NH_4^+$-N (mg $kg^{-1}$) | 2.8 ± 0.7 | 3.2 ± 1.4 | 3.3 ± 0.8 | 3.3 ± 2.1 | 6.4 ± 5.6 | 2.8 ± 0.6 | 2.9 ± 0.4 | 4.8 ± 2.7 | 3.5 ± 1.0 |
| $NO_3^-$-N (mg $kg^{-1}$) | 31 ± 11 | 35 ± 4 | 30 ± 11 | 39 ± 29 | 36 ± 28 | 35 ± 14 bc | 12 ± 7 a | 52 ± 12 c | 25 ± 8 ab |
| Total P (mg $kg^{-1}$) | 789 ± 103 | 735 ± 34 | 724 ± 25 | 738 ± 14 | 777 ± 17 | 749 ± 37 | 781 ± 27 | 742 ± 39 | 765 ± 53 |
| Available P (mg $kg^{-1}$) | 0.50 ± 0.05 | 0.46 ± 0.03 | 0.51 ± 0.03 | 0.49 ± 0.04 | 0.50 ± 0.05 | 0.51 ± 0.02 | 0.50 ± 0.03 | 0.53 ± 0.06 | 0.52 ± 0.05 |
| Total K (mg $kg^{-1}$) | 643 ± 19 | 633 ± 64 | 623 ± 26 | 625 ± 33 | 655 ± 33 | 630 ± 56 | 584 ± 44 | 572 ± 41 | 536 ± 38 |
| Available K (mg $kg^{-1}$) | 43 ± 5 | 40 ± 11 | 54 ± 7 | 45 ± 10 | 50 ± 10 | 72 ± 22 abc | 63 ± 8 a | 100 ± 13 c | 66 ± 7 ab |
| Total S (mg $kg^{-1}$) | 329 ± 26 | 360 ± 39 | 403 ± 38 | 345 ± 63 | 353 ± 62 | 318 ± 48 | 317 ± 28 | 371 ± 49 | 365 ± 76 |
| Total Cu (mg $kg^{-1}$) | 7.6 ± 0.6 | 7.6 ± 0.2 | 7.1 ± 0.5 | 7.7 ± 0.2 | 7.3 ± 0.4 | 7.3 ± 0.4 | 7.6 ± 0.9 | 7.1 ± 0.4 | 8.0 ± 1.4 |
| Total Zn (mg $kg^{-1}$) | 28 ± 1 | 28 ± 0 | 27 ± 1 | 28 ± 0 | 27 ± 1 | 27 ± 2 | 28 ± 2 | 27 ± 3 | 27 ± 2 |

*According to ANOVA, mean values observed among the treatments in greenhouse 2 for $NO_3^-$-N and Available K are significant at the probability level p<0.05. However, ANOVA does not tell at which factor levels these effects manifest. For that purpose Tukey's post-hoc test was used in order to denote which means are significantly different and which not. Where ANOVA indicted significant factor effects (p<0.05) different letters indicate significant differences between the means according to Tukey's test at the 5% probability level.*

***Table 5.4*** *Mean values ± deviation of fresh weight (FW), dry matter (DM) and plant nutrient concentration (measured on DM basis) for the eight different fertilization (n=4, size 10m$^2$) treatments in greenhouse 1 and greenhouse 2 at harvest (17/07/2013). Treatments Ref G1 and Ref G2 present conventional fertilization with synthetic fertilizers (CAN+TSP+PAT) as a reference of the respective greenhouse. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO ad 42% $SO_3$); STR: struvite; ASW: air scrubber water; CW: effluent from constructed wetlands; LFDIG: liquid fraction of digestate.*

| Parameter [a] | Treatments in Greenhouse 1 | | | | | Treatments in Greenhouse 2 | | | | Proposed range [b] |
|---|---|---|---|---|---|---|---|---|---|---|
| | Ref G1 | CAN+STR +PAT | ASW+TSP +PAT | CAN+TSP +CW | LFDIG+TSP +PAT | Ref G2 | ASW+STR +CW | LFDIG+STR +PAT | ASW+STR +PAT | |
| FW (kg 40m$^{-2}$) | 246 ± 46 | 245 ± 32 | 254 ± 36 | 244 ± 14 | 250 ± 21 | 222 ± 18 | 236 ± 10 | 229 ± 12 | 224 ± 3 | - |
| DM (%) | 5.5 ± 1.0 | 5.2 ± 0.7 | 5.4 ± 0.8 | 4.9 ± 0.3 | 5.1 ± 0.4 | 5.2 ± 0.4 | 4.8 ± 0.2 | 5.0 ± 0.3 | 5.2 ± 0.1 | - |
| Total N (g kg$^{-1}$) | 45 ± 1 | 43 ± 3 | 43 ± 2 | 43 ± 1 | 41 ± 1 | 45 ± 2 | 41 ± 2 | 44 ± 3 | 44 ± 5 | 31 - 45 |
| Total P (g kg$^{-1}$) | 3.7 ± 0.2 | 4.0 ± 0.2 | 4.1 ± 0.6 | 3.9 ± 0.2 | 4.1 ± 0.5 | 3.5 ± 0.3 | 3.6 ± 0.1 | 3.5 ± 0.2 | 3.6 ± 0.4 | 3.5 - 6.0 |
| Total K (g kg$^{-1}$) | 52 ± 7 | 51 ± 4 | 56 ± 5 | 57 ± 8 | 44 ± 9 | 66 ± 10 | 68 ± 4 | 72 ± 4 | 62 ± 5 | 45 - 80 |
| Total S (g kg$^{-1}$) | 3.1 ± 0.3 | 3.0 ± 0.3 | 3.3 ± 0.3 | 3.2 ± 0.0 | 3.1 ± 0.4 | 3.2 ± 0.3 | 3.3 ± 0.1 | 3.3 ± 0.2 | 3.2 ± 0.2 | 2 - 3 |
| Total Cu (mg kg$^{-1}$) | 7.5 ± 1.6 | 7.6 ± 0.9 | 7.0 ± 0.9 | 8.0 ± 0.9 | 8.3 ± 2.3 | 7.7 ± 0.6 | 7.5 ± 1.8 | 6.8 ± 1.2 | 6.6 ± 1.0 | 7 - 80 |
| Total Zn (mg kg$^{-1}$) | 72 ± 12 | 69 ± 10 | 80 ± 12 | 86 ± 9 | 76 ± 22 | 60 ± 4 | 62 ± 9 | 67 ± 9 | 63 ± 7 | 25 - 250 |

*[a]Analyzed parameters were subjected to ANOVA by comparing treatments with the reference (Ref G1 and Ref G2) of their respective greenhouse. No significant factor effects (p<0.05) were observed at the probability level p<0.05.*

*[b] Reference values from the Hill Laboratories (HL, 2002) which indicate the appropriate range of macro- and micro- nutrients in lettuce grown within the greenhouse.*

### 5.3.3 Lettuce quality control assessment

During the entire growing period, the lettuce uniformity and volume was significantly lower in treatments LFDIG+TSP+PAT and LFDIG+STR+PAT. Those treatments were the only two treatments where LF of digestate was added as a substitute of synthetic N fertilizer. However, at harvest time no difference in crop volume between the eight different fertilization treatments including the scenarios with LF of digestate was noted (Table 5.5). Only lettuce uniformity in treatment LFDIG+TSP+PAT was still significantly lower at harvest time with respect to treatment Ref G1. Furthermore, marketable yield assessment indicated that the crop FW yield and DM (%) were not negatively influenced by the application of bio-based materials. Finally, crop quality assessment (data not shown) for typical lettuce diseases showed that none of the eight different fertilization treatments had a significantly negative or positive effect on basal rot, bremia (*Bremia lactucae L.),* yellow leaves and tipburn.

***Table 5.5*** *Observation score for crop volume (1 = small volume, 9 = voluminous) and uniformity (1 = heterogeneous and 9 = homogenous) during the growing period (21/06/2013 and 04/07/2013) and at harvest (17/07/2013) as a part of crop quality assessment. Treatments Ref G1 and Ref G2 present conventional fertilization with synthetic fertilizers (CAN+TSP+PAT) as a reference of the respective greenhouse. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO ad 42% $SO_3$); STR: struvite; ASW: air scrubber water; CW: effluent from constructed wetlands; LFDIG: liquid fraction of digestate.*

| | Treatment | 21/06/2013 | | 04/07/2013 | | 16/07/2013 | |
|---|---|---|---|---|---|---|---|
| | | Volume | Uniformity | Volume | Uniformity | Volume | Uniformity |
| Greenhouse 1 | Ref G1 | 6.50 cd | 7.25 | 6.75 abc | 6.25 bcd | 8.00 a | 8.00 a |
| | CAN+STR+PAT | 6.75 bcd | 7.00 | 7.25 abc | 6.25 bcd | 7.75 a | 7.50 ab |
| | ASW+TSP+PAT | 7.25 abc | 7.25 | 7.50 ab | 6.75 Bc | 7.75 a | 7.50 ab |
| | CAN+TSP+CW | 7.75 abc | 7.00 | 6.25 bc | 5.25 bcd | 7.00 ab | 7.00 abc |
| | LFDIG+TSP+PAT | 6.00 d | 6.25 | 6.25 bc | 5.00 cd | 7.00 ab | 6.75 bc |
| Greenhouse 2 | Ref G2 | 6.75 bcd | 7.00 | 7.25 abc | 6.50 Bc | 8.00 a | 7.00 abc |
| | ASW+STR+CW | 8.00 a | 7.00 | 7.50 ab | 6.75 Bc | 7.00 ab | 7.50 ab |
| | LFDIG+STR+PAT | 5.75 d | 6.25 | 6.00 c | 4.50 d | 7.50 a | 6.25 c |
| | ASW+STR+PAT | 7.25 abc | 7.25 | 7.25 abc | 7.00 B | 8.00 a | 7.75 ab |

[a] *Mean values denoted by the same letter in a column are not statistically different according to Tukey's test at the 5% probability level.*

### 5.3.4 Fertilizer replacement use efficiency of bio-based materials

A balanced use of NPK nutrition has a remarkable influence on crop growth and yield. Table 5.6 gives more insight into fertilizer replacement use efficiency (FRUE) of each treatment as compared to the reference of their respective greenhouse. According to the Nitrates Directive (91/767/EEC) it has been assumed that mineral fertilization (Ref G1 and Ref G2) is 100% efficient. Hence, results in Table 5.6 indicate that N FRUE of air scrubber water was similar (treatment ASW+TSP+PAT) or slightly higher (treatment ASW+STR+PAT) with respect to CAN, while LF of digestate resulted in moderately lower N FRUE in treatments LFDIG+TSP+PAT and LFDIG+STR+PAT. For struvite, P FRUE was higher than the P FRUE of triple superphosphate, while application of effluent from CW resulted in similar (treatment ASW+STR+CW) or slightly lower (treatment CAN+TSP+CW) K FRUE as compared to potassium sulfate.

***Table 5.6*** *Fertilizer use efficiency (FUE) and its relation to the mineral fertilization reference Ref G1 and G2 as being 100% efficient in their respective greenhouse (i.e. FRUE = fertilizer replacement use efficiency). Treatments Ref G1 and Ref G2 present conventional fertilization with synthetic fertilizers (CAN+TSP+PAT) as a reference of the respective greenhouse. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO ad 42% $SO_3$); STR: struvite; ASW: air scrubber water; CW: effluent from constructed wetlands; LFDIG: liquid fraction of digestate.*

| | Treatments in Greenhouse 1 | | | | | Treatments in Greenhouse 2 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Ref G1 | CAN+STR +PAT | ASW+TSP +PAT | CAN+TSP +CW | LFDIG+TSP +PAT | Ref G2 | ASW+STR +CW | LFDIG+STR +PAT | ASW+STR +PAT |
| N | | | | | | | | | |
| FUE | 0.72 | 0.67 | 0.70 | 0.61 | 0.62 | 0.61 | 0.56 | 0.60 | 0.64 |
| FRUE (%) | 100[a] | 94 | 99 | 86 | 87 | 100[a] | 92 | 97 | 103 |
| P | | | | | | | | | |
| FUE | 0.25 | 0.29 | 0.30 | 0.27 | 0.29 | 0.22 | 0.23 | 0.22 | 0.23 |
| FRUE (%) | 100[a] | 116 | 120 | 108 | 116 | 100[a] | 105 | 100 | 105 |
| K | | | | | | | | | |
| FUE | 0.88 | 0.82 | 0.96 | 0.86 | 0.69 | 0.97 | 0.97 | 1.04 | 0.95 |
| FRUE (%) | 100[a] | 93 | 109 | 98 | 77 | 100[a] | 101 | 107 | 99 |

## 5.4 Discussion

### 5.4.1 Effects on $NO_3^-$-N residue and soil properties

Excessive nutrient inputs could negatively affect the environment through leaching or gaseous losses. Nitrate residues are of interest as they can be indicative for unwanted leaching losses after harvest. This parameter is environmentally relevant in open air cropping, but also in a greenhouse settings nutrient losses by leaching should be minimized. In this study, the $NO_3^-$-N content in the soil was significantly ($p = 0.002$) different only among treatments in greenhouse 2, where treatment ASW+STR+CW with complete synthetic fertilizer replacement (air scrubber water, struvite and effluent from CW as NPK-fertilizer) exhibited a statistically significant effect in comparison to the Ref G2 ($p = 0.046$). This may be partially a consequence of soil saturation and consequent nitrate leaching by applying at once high liquid volume based on nutrient concentration: 157 L of effluent from CW and 2 L of air scrubber water as a K and N-fertilizer on a surface area of 10 $m^2$. To a certain extent, it might have been as well influenced by short-term microbial N transformations in soil, since there were no differences observed with respect to crop N- concentration, total N and $NH_4^+$-N content in soil as compared to Ref G2. Furthermore, if we look at other treatments where ASW and LF of digestate were applied as N-fertilizer, no statistical differences were reported in crop N- concentration and soil total N as compared to reference treatments G1 and G2. Similar results have been obtained in a maize trial by Vaneeckhaute *et al.* (2013b), where ASW and LF of digestate were used as a NS-fertilizer and N-fertilizer, respectively. These results demonstrate that ASW and LF of digestate can be a valuable substitute for mineral N fertilizer.

For soil total and plant available P, as expected, no significant differences were observed among the eight different fertilization treatments at harvest time. Additionally, struvite as a substitute for mineral P (TSP) exhibited similar yields in comparison to mineral fertilization. These findings indicate that struvite can be a valuable substitute for mineral P (Gonzáles-Ponce *et al.*, 2009), which is of great importance in the frame of global P resource depletion over the course of coming centuries.

Furthermore, for soil total and plant available K, significant statistical differences were observed only with respect to plant available K in greenhouse 2. This is a result of a high plant available K in treatment LFDIG+STR+PAT by applying products with higher plant available K content: LF of digestate, struvite and potassium sulfate (Table 5.1). An important nuance however is that no difference in comparison to the Ref G2 was found, only between treatments where bio-based materials were used as a synthetic fertilizer replacement.

In this study, S was supplied through ASW, LF of digestate and potassium sulfate. Since a high dose of sulfate can lead to salt accumulation in soils (Vaneeckhaute *et al.*, 2013b), special attention concerning the soil $EC_{2:1}$ was given to treatments where ASW and LF of digestate were applied. Results of $EC_{2:1}$ weekly assessment and soil analysis indicate higher $EC_{2:1}$ values in treatments with ASW and LF of digestate, along with slightly higher soil and crop S concentrations at harvest. However, these observations were not statistically significant and $EC_{2:1}$ values did not exceed the upper limit value of 1.8 dS $m^{-1}$ set for this crop. Nevertheless, special attention should be given to these materials for potential salt stress during the fertilization (Alburquerque *et al.*, 2012a; Vaneeckhaute *et al.*, 2013a), especially in the cultivation of a salt-sensitive crop as lettuce. In order to avoid any detrimental effects on plant growth such as leaf burning, ASW and LF of digestate should be applied at least one day before planting (Vaneeckhaute *et al.*, 2013b), as it was done in the current study.

Even though Cu and Zn are in fact plant micronutrients, their accumulation in the soil could eventually lead to phytotoxicity which may have negative influence on plant growth (Alburquerque *et al.*, 2012a). In this study, characterization of soil and plant material did not show any significant differences among the eight different fertilization treatments with respect to soil and crop Cu and Zn concentration. This observation is even more important due to fact that lettuce is a plant known for metal accumulation (Peijnenburg *et al.*, 2000). Nevertheless, digestate as a residue of anaerobic digestion may contain significant amounts of Cu and Zn whose concentration is increased remarkably when animal manure is used as a substrate in anaerobic co-digestion (Alburquerque *et al.*, 2012a; Vaneeckhaute *et al.*, 2013b; Saveyn and Eder, 2014). In this study LF of digestate was used, which contains a relatively low amount of Cu and Zn due to the fact that heavy metals are mostly concentrated in the solid fraction (SF). Moreover, LF of digestate complies with environmental quality standards for all heavy metals that are specified in currently proposed End-of-waste criteria (Saveyn and Eder, 2014). However, potential risks of phytotoxicity could be overcome by defining the optimal ratio of input substrates in anaerobic co-digestion which result in lower concentrations of heavy metals, and as such reduce the risk of phytotoxicity toward plants.

### 5.4.2 Effects on crop yield and nutrient concentration

The use of ASW and LF of digestate resulted in a slightly higher mean fresh weight yield compared to the reference and the other treatments (Table 5.4). However, no significant statistical differences ($p > 0.05$) were observed among the eight different fertilization treatments with respect to both DM and FW yield, demonstrating that mineral fertilizers could be safely substituted by bio-based materials without adversely affecting productivity. Similar results have been reported by other authors, where in open field trials lettuce was fertilized with digestate from wine distillery and olive pomace compost (Montemurro *et al.*, 2010) and digestate from fruit and wine distillery wastes (Nicoletto *et al.*, 2014). Vaneeckhaute *et al.* (2013b) also reported small improvement in maize yield where ASW and a mixture of digestate and LF of digestate were applied as N-source in comparison to synthetic N reference.

Furthermore, the nutrient assessment of lettuce showed no significant differences in crop nutrient concentration among the eight different fertilization treatments (Table 5.4). Moreover, crop nutrient concentration values were within the expected range of macro and micronutrients that are present in lettuce grown within the greenhouse (HL, 2002). This indicates that applied bio-based materials can fulfil the total nutrient requirements of the crop.

Nowadays, with an increasing public concern about food quality along with crop nutritional value, it is important to assess the safety aspects with respect to different plant diseases that may affect crop health. In frame of crop quality control, only in treatments LFDIG+TSP+PAT and LFDIG+STR+PAT where LF of digestate was used as N- source, lettuce had a difficult start that was visible in crop volume and uniformity. At harvest time, this negative influence was still notable in lettuce uniformity of the treatment LFDIG+TSP+PAT as compared to the Ref G1 (Table 5.5). This could be result of two crucial variables in LF of digestate application, namely EC and presence of ammonia. The former may be the limiting variable in the case where the acceptable salt tolerance of lettuce for this particular cultivar would be below 1.3 dS $m^{-1}$ since that was the maximum observed value in treatment LFDIG+STR+PAT. On the other hand, in treatment LFDIG+TSP+PAT with LF of digestate, the measured soil $EC_{2:1}$ values were lower as compared to treatments ASW+TSP+PAT and ASW+STR+PAT with air scrubber water. Therefore, we can conclude that the affected lettuce volume and uniformity in treatments with LF of digestate could not be attributed to high salt content since volume and uniformity of crop in treatments with ASW was not affected by the presence of even higher $EC_{2:1}$ values. Until now researchers have reported contradictory results concerning digestate phytotoxicity, implicating $NH_4^+$-N and organic acid concentrations in digestate as limiting factors in plant growth (Fuchs *et al.*, 2008; Möller and Müller, 2012). No data about the expected duration of phytotoxic effects were found. Nevertheless, it is believed that phytotoxicity should decrease within a short time period after field application of digestate (Möller and Müller, 2012). Moreover, Wong *et al.* (1983) reported that plant growth might be inhibited not only due to ammonia but also in a lesser extent by ethylene oxide in animal manure. These findings may give indications about the difficult start of the lettuce that occurred in treatments with LF of digestate. It is however important to remark that in this study no significant differences as compared to a reference were observed at harvest time with respect to crop yield and volume. The negative influence was only visible in lettuce uniformity which was less homogeneous in comparison to the reference. Finally, it should be noted that this negative effect on lettuce uniformity and volume was not observed in previous study (Chapter 4), where LF of digestate was applied as a N source in pot cultivation. This might be a result of lower material dosage that is given on laboratory scale (dosage on weight basis vs. dosage on hectare basis). Conversely, on commercial scale higher dosage of material is required, hence leading to more visible effects on crop production.

### 5.4.3 Economic assessment

Optimizing use of nutrient resources is one of the biggest challenges facing sustainable agriculture. Economic assessment is therefore a vital tool, since it can enumerate the potential costs and value the anticipated benefits of the proposed fertilization treatments. In this study, regardless of the potential acceptance of N bio-based materials as substitutes for synthetic N fertilizer, LF of digestate or ASW as a N source seemed to be more profitable choice for an arable farmer if the transport distance is not larger than 5 km and the farmer owns application equipment (Sc. 1; Figure 5.2). Moreover, under these conditions LF of digestate appeared to be economically better N source than ASW since it contains not only N, but also K and in a lesser extent P. In LFDIG+TSP+PAT treatment, for example, application of LF of digestate resulted not only in absence of CAN application, but also in a reduction of TSP and PAT by 27 kg $P_2O_5$ $ha^{-1}$ and 165 kg $K_2O$ $ha^{-1}$, respectively.

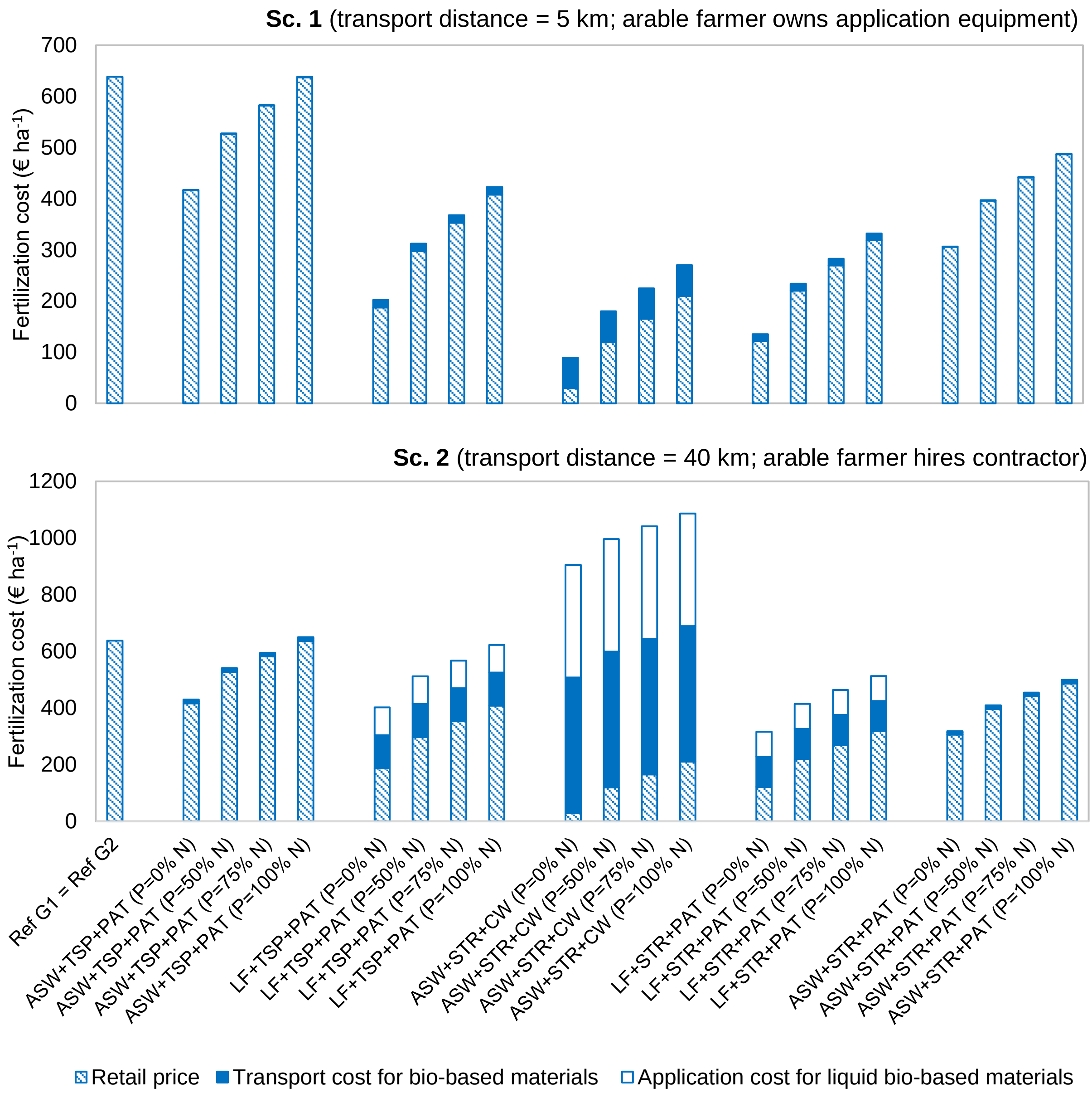


***Figure 5.2*** *Fertilization cost (€ ha$^{-1}$) of the applied treatments under two case scenarios (Sc. 1 and Sc. 2). Treatments Ref G1 = Ref G2 present conventional fertilization with synthetic fertilizers (CAN+TSP+PAT). Treatments with bio-based N materials were additionally assessed with regard to the potential changes of their retail price: zero cost (P=0% N), 50% (P=50% N), 75% (P=75% N) and 100% (P=100% N) of the market price for kg of N. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO and 42% $SO_3$); STR: struvite; ASW: air scrubber water; CW: effluent from constructed wetlands; LFDIG: liquid fraction of digestate.*

As expected, the lowest fertilization cost under Sc.1 conditions was observed with ASW+STR+CW treatment where complete replacement of synthetic fertilizers occurred. Conversely, this treatment under Sc. 2 conditions (transport distance = 40km; arable farmer hires contractor) resulted in the highest fertilization cost regardless of the potential changes in retail price of ASW. This is a consequence of transporting and applying effluent from CW, a material with a low nutrient concentration which led to significantly higher transport and application cost (Sc. 2; Figure 5.2). Therefore, the more bio-based material is up concentrated, the lower fertilization cost will be generated. Finally, despite of observed increases in application and transport cost of LF of digestate under Sc. 2, LFDIG+TSP+PAT treatment still resulted in slightly lower fertilization cost than ASW+TSP+PAT treatment. This indicates that potential economic value of using bio-based materials as substitutes for synthetic fertilizers is quite material specific, depending mostly on the nutrient concentration, presence of economically valuable nutrients and water content in the material.

## 5.5 Conclusion

Air scrubber water and struvite gave the best results with respect to all tested parameters concerning (i) crop nutrient concentration, (ii) crop quality, (iii) marketable crop yield and (iv) soil properties. Nevertheless, additional research is required to assess the role of sulfur in air scrubber water when consistently using this substitute instead of CAN in subsequent crop cycles on the same soil/substrate. Analogously, nutrient use efficiency of phosphorus in struvite should be investigated in consecutive crop cycles to ascertain the P FRUE remains at the initial (high) level as was observed in the current study. Effluent from CW from manure treatment facilities can be considered as a valuable K- source for plant production. However, for economic reasons it is advised to apply the product in more concentrated form (e.g. after membrane filtration) to avoid high transport and fertilization costs. LF of digestate can be considered as a valuable N-source for plant production. However, in horticulture it may have a negative effect on crop uniformity. This issue should be investigated further by conducting biological assays to identify potential phytotoxic effects on plant growth.

**Acknowledgment**

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# **CHAPTER 6:** LIQUID FRACTION OF DIGESTATE AND MINERAL CONCENTRATE AS NITROGEN FERTILIZERS IN A SINGLE-YEAR FIELD SCALE CULTIVATION OF *ZEA MAYS* L.


*Experimental site in Roeselare (Belgium)(Picture: Vervisch, B.)*

***This chapter has been redrafted after:***

Sigurnjak, I., Michels, E., Ryckaert, B., Tack, F.M.G., De Neve, S., Meers, E. Liquid fraction of digestate and mineral concentrate as a N fertilizer in maize cultivation. *In Preparation.*

## Abstract

Liquid fraction of digestate (LFDIG) and mineral concentrate (MC) are bio-based materials with relatively high N/P and $NH_4^+$-N/N ratio, and as such have the potential to be used as N fertilizers. Their fertilizer performance with respect to maize yield and soil properties was evaluated in a single-year field experiment, with reference to calcium ammonium nitrate (CAN). Treatments included the individual material application i.e. LFDIG, MC and CAN, and combination with animal manure (AM), i.e. LFDIG+AM, MC+AM and CAN+AM. No significant differences were observed among individual and combination treatments with respect to maize yield, crop nutrient uptake and soil properties at harvest time, suggesting that LFDIG and MC have a potential to replace synthetic N fertilizers. Fertilizer use efficiency (FUE), apparent N recovery (ANR) and N fertilizer replacement value (NFRV) where lower in combination treatments due to the observed N over-fertilization. The N over-fertilization was caused by the application of AM whose concentration of N varied significantly between two sampling moments of the bio-based materials: i) before fertilization to determine application dosage and ii) during the actual fertilization. Finally, economic assessment indicated that the use of LFDIG and MC as N source can result in economic benefits for an arable farmer.


## 6.1 Introduction

End- and by-materials of animal manure processing currently attract considerable attention in the European Union (EU) due to the ongoing revision of the Fertilizer regulation 2003/2003. It has been estimated that 43.7 million tonnes of liquid fraction (LF) is produced annually from raw animal manure and/or raw digestate in the EU (Flotats *et al.*, 2013). Despite of favorable N/P ratio of the material, LF of digestate is defined currently as a waste (i.e. animal manure). Consequently, farmers in manure surplus areas need to process this bio-based material further. In 2015, 81 out of 118 manure processing installations in Flanders (Belgium) used biological treatment as primary technique, accounting for the loss of around 12.7 million kg N in the form of $N_2$ (VCM, 2016). In the emerging bio-based economy, the loss of N from biological treatment should be prevented by utilizing LFs as N fertilizers or by upgrading them via reverse osmosis to a mineral concentrate solution where most of the water and organic material is removed (Klop *et al.*, 2012; Schröder *et al.*, 2014).

Mineral concentrate may exhibit similar N dynamics as synthetic N fertilizer (Sigurnjak *et al.*, 2017a). For LF of digestate, a higher N release was observed as compare to animal manure, but it was still lower than the one observed for mineral concentrate and calcium ammonium nitrate (CAN). The pattern of N release depends on the $NH_4^+$-N/$N_{total}$ ratio of the material (Sigurnjak *et al.*, 2017a; Sigurnjak *et al.*, 2017b; Tambone and Adani, 2017). However, laboratory incubations are conducted in the absence of plants whose presence might additionally affect N dynamics in the soil. Importantly, in contrast to incubation, greenhouse or pot-trials, field trials are conducted under uncontrolled conditions where weather is seen as a key factor in determining the agricultural productivity. Therefore, before recommending these materials as an equivalent to synthetic N fertilizer, their efficacy in open field cultivation should be tested by determining N fertilizer replacement value (NFRV) of these bio-based materials as compare to synthetic N fertilizer. The aim of this chapter was to evaluate the impact of using LF of digestate and mineral concentrate as replacements for synthetic N fertilizer in fodder maize field cultivation. Fodder maize is one of the most important crops grown in Belgium. It has a dual purpose, as a silage for animals or as an input stream for anaerobic digestion (i.e. as an energy maize). Currently, around 5000 ha of land in Flanders is used for energy maize production (De Vliegher *et al.*, 2012; EC, 2014a). The performance of LF of digestate and mineral concentrate was evaluated with reference to CAN via application of the individual materials and via combination treatments involving application of LF of digestate and mineral concentrate on top of animal manure. It is hypothesized that the use of these bio-based materials in individual or combination treatments will not cause significant differences in maize yield, nitrate leaching and soil properties as compared to CAN and conventional practice of using animal manure supplemented with synthetic fertilizers.

## 6.2 Materials and methods

### 6.2.1 Experimental set-up

The field experiment was performed on a sandy-loam soil in Roeselare (50° 54′ 53″N, 3° 6′ 41″E), Belgium. The soil characteristics of the 0-30 cm soil layer prior to the experiment were organic carbon (OC) = 1.2 % on dry matter (DM); $NO_3^-$ -N = 19 kg $ha^{-1}$; $NH_4^+$-N = 21 kg $ha^{-1}$; ammonium lactate extractable P (P-AL) = 420 mg $kg^{-1}$ DM and ammonium lactate extractable K (K-AL) = 204 mg $kg^{-1}$ DM. The $NO_3^-$-N amount in soil prior to the experiment was 7 kg $ha^{-1}$ per each soil layer (i.e. 30-60 and 60-90 cm), whereas for $NH_4^+$-N 11 kg $ha^{-1}$ was measured in 30-60 cm and <4 kg $ha^{-1}$ in 60-90 cm soil layer. Note that soil from this field was also used for incubation experiment in Chapter 3. As a test crop, fodder maize (*Zea mays L.*) cv. Atletico KWS (FAO Ripeness Index: 280), was grown in 2014. The preceding crop in 2013 and 2012 was chicory (i.e. Belgian endive) and fodder maize, respectively. The monthly rainfall and average soil temperature during the experimental period were collected at the meteorological station within 16 km from the site, and is presented in Table 6.1.

Experimental treatments were tested in a randomized block design with quadruplicate plots of 7 m x 7 m (n = 4) spread across the field to minimize potential influence of variable soil conditions on the results (Figure 6.1). Since Flemish soils are rich in P and located in the NVZ, the $P_2O_5$ and effective N application rates are limited by legislation. In this study according to the Flemish manure regulation for the cultivation of maize on non-sandy soils (FMD, 2011), the application dosage was set at 150 kg effective N, 80 kg $P_2O_5$, 220 kg $K_2O$ and 60 kg MgO $ha^{-1}$. The amount of effective N in animal manure was accepted to be 60 % of the total N content, according to the Flemish manure regulation. For LF of digestate and mineral concentrates it was hypothesized (as in Chapter 3) that 100 % of total N present in these bio-based materials would be available during the experiment. This is similar to what is expected from the application of synthetic N fertilizer.

***Table 6.1*** *Average rainfall (mm) and soil temperature (°C) measured at Zarren station (16 km from the field location) during the experimental year (Source: Waterinfo.be).*

| Year 2014 | Rainfall (mm) | Temperature (°C) |
|---|---|---|
| January | 68 | 5.8 |
| February | 90 | 5.8 |
| March | 29 | 7.8 |
| April | 25 | 11.9 |
| May | 74 | 14.1 |
| June | 60 | 18.5 |
| July | 117 | 19.4 |
| August | 107 | 17.7 |
| September | 5 | 16.4 |
| October | 49 | 14.2 |
| November | 50 | 10.1 |
| December | 59 | 6.2 |

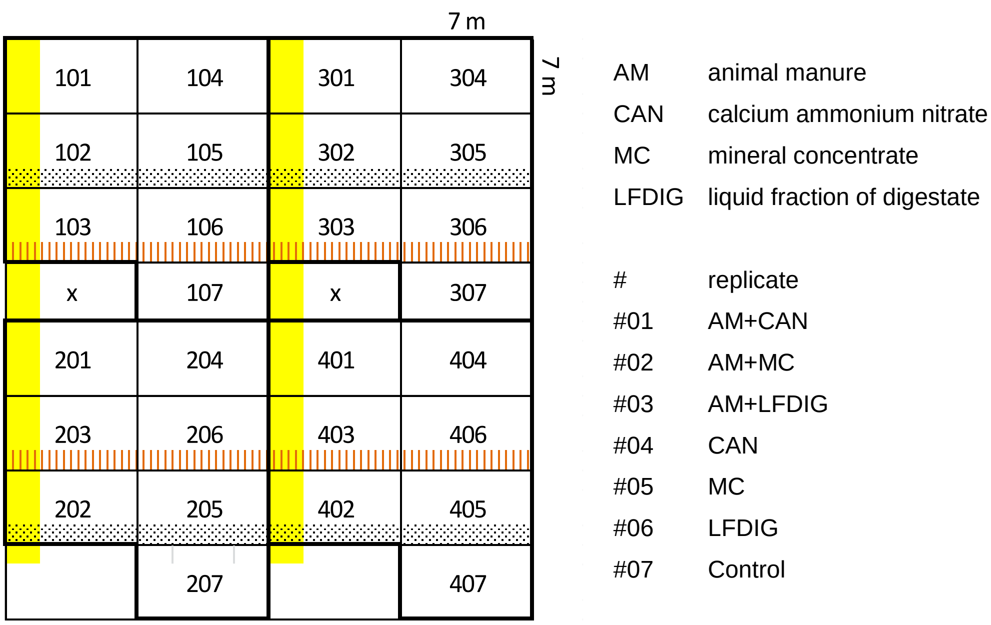


***Figure 6.1*** *Overview of plots on the experimental site. The different colours and patterns indicate where animal manure (yellow pattern), liquid fraction of digestate (red striped pattern) and mineral concentrate (grey shaded area) were applied.*

As it can be seen from Table 6.2, the $N_{total}$ and $P_{total}$ concentration of animal manure (AM) was, respectively, 31% and 75% higher during the actual fertilization as compare to analysis obtained before fertilization. As a result, N and $P_2O_5$ over-fertilization occurred in [AM+CAN], [AM+MC] and [AM+LFDIG] treatments. The nutrient composition of LF of digestate (LFDIG) and mineral concentrate (MC) was far more stable. Finally, in order to make N as the only responsive nutrient, synthetic P and K fertilizers were added in control treatment in the form of triple superphosphate (TSP; 46 % $P_2O_5$) and potassium sulfate (PAT; 30 % $K_2O$, 10 % MgO and 42 % $SO_3$), respectively. The aim was for all treatments, including the control, to receive at least 65 kg $P_2O_5$ $ha^{-1}$ and 210 kg $K_2O$ $ha^{-1}$ regardless of the nutrient source. Nutrient application rates for seven different treatments are summarized in Table 6.3.

AM, LFDIG and MC were applied on May 2 2014, by use of PC controlled injection (Bocotrans, NL). The PC controlled injection allows the driver to control the amount of slurry that is injected per ha from the tractor, whereas in the conventional system the driver needs to perform a certain balance between speed and pumping dosage. The synthetic N and K fertilizers were applied on May 12, while synthetic P fertilizer was applied on May 19. All synthetic fertilizers were applied to the plots by hand-application. Fodder maize was sown on May 6 2014 at a seed density of 100 000 $ha^{-1}$.

***Table 6.2*** *Characterization of bio-based materials before (14/04/2014) and during the field application (02/05/2014) based on the fresh weight. Value between brackets indicate the content of bio-based materials measured during the fertilization.*

| Parameters | Animal manure | Liquid fraction of digestate | Mineral concentrate |
|---|---|---|---|
| Dry matter (g $kg^{-1}$) | 34 (121) | 28 (34) | 32 (49) |
| Organic matter (g $kg^{-1}$) | 20 (78) | 17 (21) | 14 (31) |
| Organic carbon (g $kg^{-1}$) | 11 (43) | 9 (12) | 8 (17) |
| $N_{total}$ (g $kg^{-1}$) | 4.2 (6.1) | 4.2 (4.9) | 4.0 (3.9) |
| $NH_4^+$-N (g $kg^{-1}$) | 2.9 (3.7) | 2.9 (3.8) | 3.8 (3.5) |
| $N_{organic}$ (g $kg^{-1}$) | 1.3 (2.4) | 1.3 (1.1) | 0.2 (0.4) |
| $P_{total}$ (g $kg^{-1}$) | 0.63 (2.6) | 0.26 (0.41) | 0.03 |
| $K_{total}$ (g $kg^{-1}$) | 2.9 (3.0) | 2.9 | 3.3 (3.2) |
| $Ca_{total}$ (g $kg^{-1}$) | 1.57 (8.8) | 0.25 (0.54) | 0.11 (0.10) |
| $Mg_{total}$ (g $kg^{-1}$) | 0.40 (2.1) | 0.06 (0.09) | 0.19 (0.17) |
| $Na_{total}$ (g $kg^{-1}$) | 1.0 (0.96) | 1.6 (1.8) | 2.8 |
| $Cu_{total}$ (mg $kg^{-1}$) | 15 (68) | 1.4 (1.6) | < 1.6 |
| $Zn_{total}$ (mg $kg^{-1}$) | 29 (156) | 4.9 (8.1) | < 0.79 |
| $C/N_{total}$ | 2.6 (7.1) | 2.2 (2.4) | 2.0 (4.4) |
| $C/N_{organic}$ | 8.5 (18) | 6.9 (11) | 40 (43) |
| N/P | 6.6 (1.4) | 16 (9.3) | 126 (120) |
| $NH_4^+$-N/$N_{total}$ | 0.69 (0.61) | 0.69 (0.78) | 0.95 (0.90) |
| $N_{organic}$ /$N_{total}$ | 0.31 (0.39) | 0.31 (0.22) | 0.05 (0.10) |

***Table 6.3*** *Dosage of total nitrogen (kg ha$^{-1}$) applied for the seven (n = 4) different fertilization treatments; $K_2O$ and $P_2O_5$ contribution (kg ha$^{-1}$) brought to the field via applied fertilization regime of total nitrogen; additional application of synthetic $K_2O$ and $P_2O_5$ (kg ha$^{-1}$) in order to obtain similar application rate in all treatments. Values present the intended dosage and values between brackets present the actual dosage, indicating the difference caused in variability between sampling at farm/digester and sampling during the actual fertilization. The contribution of effective N (kg ha$^{-1}$) from animal manure amounts to 60% of the total N applied, while for mineral concentrate and liquid fraction of digestate amounts to 100%.*

| Treatment | Animal manure N | Synthetic N | Mineral concentrate N | Liquid fraction of digestate N | $K_2O$ contribution | $P_2O_5$ contribution | Synthetic $K_2O$ addition | Synthetic $P_2O_5$ addition | Total N | Effective N | Total $P_2O_5$ | Total $K_2O$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AM + CAN | 170 (246) | 48 | - | - | 141 (145) | 59 (242) | 65 | 0 | 218 (295) | 196 | 65 (242) | 210 |
| AM + MC | 170 (246) | - | 48 (47) | - | 189 (192) | 59 (243) | 18 | 0 | 218 (293) | 195 | 65 (243) | 210 |
| AM + LFDIG | 170 (246) | - | - | 48 (56) | 181 (185) | 65 (253) | 25 | 0 | 218 (302) | 204 | 65 (253) | 210 |
| CAN | - | 150 | - | - | - | - | 210 | 65 | 150 | 150 | 65 | 210 |
| MC | - | - | 150 (146) | - | 150 (146) | 2.7 (2.5) | 64 | 63 | 150 (146) | 146 | 65 | 210 |
| LFDIG | - | - | - | 150 (175) | 125 | 21 (34) | 85 | 32 | 150 (175) | 175 | 65 | 210 |
| Control | - | - | - | - | - | - | 210 | 65 | - | - | 65 | 210 |

*AM: animal manure; CAN: calcium ammonium nitrate; MC: mineral concentrate; LFDIG: liquid fraction of digestate; Control: application of synthetic $P_2O_5$ and $K_2O$.*

### 6.2.2 Bio-based material sampling and analysis

AM used in the trial was collected at a local pig farm in Beitem, Belgium. LFDIG was sampled from an anaerobic co-digestion plant at the site of Bioelectric (Beernem, Belgium; denoted as LFDIG_AM in Chapter 3), whereas MC was sampled at the site of Ampower (Pittem, Belgium; denoted as MC_LDIG in Chapter 3). These products were also used in Chapter 3 where their N dynamics were evaluated on soil that was sampled from the field on which the current experiment occurred. For more information on the description of biogas installations, please see Chapter 3 section 3.2.2. All products were collected in polyethylene sampling bottles (2 L), stored (4 °C) and characterized (Table 6.2) to determine the required application dosage. Product characterization was done as described in section 3.2.2 (Chapter 3). Product Cu and Zn content was determined and analyzed in the same manner as K, Mg, Ca and Na (section 3.2.2, Chapter 3).

### 6.2.3 Soil sampling and analysis

Soil samples were taken during (September 24 2014) and after the harvest (October 1, October 22 and November 14 2014). At each soil sampling moment, homogenized soil subsamples were taken per plot at three depths (0-30 cm, 30-60 cm, 60-90 cm) using an auger. In order to obtain a representative soil sample in each plot, samples were collected from five sampling points (the center and the 4 corners) of 7.5m$^2$ area which was located in the middle of the plot (49 m$^2$ area) and corresponds to the area that was harvested for determination of maize FW yield. The samples were collected in polyethylene sampling bags and transported from the test site to the laboratory where each sample was divided into two fractions. A fresh fraction was sealed in polyethylene cups and stored in the freezer (-18°C) for the mineral N and DM determination, while the second fraction of the soil sample was air-dried at room temperature (25°C).Soil moisture content, OC, pH, total N and mineral N were determined as described in section 3.2.1 (Chapter 3), whereas soil conductivity and total P, K, Ca, Mg, Na, S, Cu and Zn were analyzed as described in section 4.2.2 (Chapter 4).

### 6.2.4 Plant sampling and analysis

Maize was harvested on September 24 2014. The 7.5m$^2$ area in the middle of each plot was measured, and the maize within that surface area was harvested manually by use of trimming scissors. The FW biomass yield of the maize within 7.5 m$^2$ area was determined at the field. Ten maize plants (stem with cob and leaves) per plot were taken to the lab, chopped and homogenously mixed. From this mixture 200 – 350 g of sample was oven-dried at 55 °C for determination of the DM (%) content. The dry samples were grinded to pass a 1 mm sieve (Retsch SM-2000, Germany) and incinerated at 550 °C during 4 h in order to determine the OC content. Plant nutrient concentrations were determined and analyzed as described in section 4.2.3 (Chapter 4).

### 6.2.5 Economic assessment

Fertilization cost was determined according to the methodology described in Chapter 5 (section 5.2.4). As compared to the greenhouse trial (Chapter 5), larger quantities of bio-based materials were applied in this study due to the larger experimental area. This requirement led to the visible differences in nutrient variability between intended and actual applied dosages. The economic assessment was based on the intended dosage since in practice the arable farmer would base his acceptance/purchase decision on the results of the most recent laboratory report, and that is before fertilization.

### 6.2.6 Data analysis

Based on the physicochemical data, the apparent N recovery (ANR) and N fertilizer replacement value (NFRV) were determined according to Eq. 1 and Eq. 2 (Chapter 1). NFRV of individual treatments was compared to CAN treatment, whereas for combination treatments comparison was made with AM+CAN treatment. In order to compare different experimental designs (with and without unfertilized treatment) N fertilizer use efficiency (FUE) was determined according to Eq. 3 (Chapter 1).

An additional way to assess the fertilizer efficiency is to measure post harvest nitrate residue, which corresponds to residual nitrate that is left in 0-90 cm soil layer, between October 1 and November 15. The measured nitrate residue gives an estimation of the nitrate amount that can potentially leach to ground and surface water. This instrument is used in Flanders (Belgium) since 2004, and in Bretagne (France) since 2014 (Buysse, 2015).

Statistical analyses were performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL). One way ANOVA was used to determine the effect of the applied fertilizers on soil properties along with the effect on crop yield and nutrient uptake, based on the obtained physicochemical data. When significant differences between means were observed, additional post hoc assessment was performed using Tukey's Test ($p < 0.05$, $n=3$). The condition of normality was checked using the Shapiro-Wilk test, whereas the homogeneity was tested with the Levene Test. Significant parameter correlations were determined using the Pearson correlation coefficient (r).

Note that during the fertilization a technical error occurred when two replicates (plot 207 and 407) of control treatment, located at the edge of the experimental area (Figure 6.1), received N fertilizer. This error was confirmed by observed differences between maize yield and nitrate residue among 4 replicates of the control treatment. Therefore, the mean and standard deviation for control treatment is based only on two replicates. As a result, the control treatment was not subjected to ANOVA analysis.

## 6.3 Results

### 6.3.1 Maize yield and nutrient uptake

The lowest crop FW and DM yield was observed in the control treatment (Table 6.4). When comparing proposed fertilization treatments, no significant differences with respect to FW and DM yield were observed among the combination treatments (reference AM+CAN) nor among individual treatments (reference CAN). The lowest crop nutrient uptake was observed on average in control treatment (Table 6.4). No significant differences were recorded with respect to crop nutrient uptake among individual and combination treatments.

***Table 6.4*** *Mean ± standard deviation of maize fresh weight, dry weight, nutrient uptake, N fertilizer use efficiency (N FUE), Apparent N recovery (ANR) and N fertilizer replacement value (NFRV) for the seven different fertilization treatments (n = 4; for control n=2) at harvest time.*

| Parameter | AM + CAN | AM + MC | AM + LFDIG | CAN | MC | LFDIG | Control |
|---|---|---|---|---|---|---|---|
| Fresh weight (tonnes $ha^{-1}$) | 83 ± 3 | 76 ± 8 | 81 ± 5 | 78 ± 3 | 77 ± 3 | 80 ± 2 | 59 ± 2 |
| Dry weight (tonnes $ha^{-1}$) | 24 ± 1 | 24 ± 3 | 23 ± 1 | 23 ± 1 | 23 ± 1 | 23 ± 0 | 19 ± 1 |
| Total N (kg $ha^{-1}$) | 282 ± 17 | 274 ± 33 | 262 ± 20 | 256 ± 18 | 261 ± 19 | 264 ± 26 | 216 ± 17 |
| Total P (kg $ha^{-1}$) | 42 ± 3 | 41 ± 7 | 36 ± 7 | 39 ± 3 | 42 ± 5 | 39 ± 4 | 32 ± 1 |
| Total K (kg $ha^{-1}$) | 309 ± 19 | 298 ± 29 | 315 ± 49 | 320 ± 55 | 333 ± 59 | 287 ± 50 | 237 ± 4 |
| Total S (kg $ha^{-1}$) | 25 ± 3 | 24 ± 2 | 20 ± 7 | 22 ± 1 | 22 ± 3 | 23 ± 2 | 19 ± 1 |
| Total Ca (kg $ha^{-1}$) | 37 ± 6 | 31 ± 3 | 38 ± 9 | 39 ± 7 | 40 ± 13 | 32 ± 9 | 27 ± 4 |
| Total Mg (kg $ha^{-1}$) | 29 ± 1 | 24 ± 3 | 26 ± 1 | 26 ± 2 | 28 ± 6 | 24 ± 6 | 21 ± 1 |
| Total Na (kg $ha^{-1}$) | 5.0 ± 1.0 | 4.0 ± 0.8 | 5.4 ± 1.1 | 5.3 ± 0.8 | 6.6 ± 2.3 | 4.0 ± 1.2 | 4.1 ± 0.2 |
| N FUE (%) | 96 ± 6 | 94 ± 11 | 87 ± 7 | 171 ± 12 | 179 ± 13 | 151 ± 15 | - |
| ANR | 0.22 ± 0.06 | 0.20 ± 0.11 | 0.15 ± 0.07 | 0.27 ± 0.12 | 0.31 ± 0.13 | 0.28 ± 0.15 | - |
| NFRV (%) | 100 | 89 | 69 | 100 | 115 | 104 | - |

*Analyzed parameters were subjected to a one-way ANOVA by comparing separately individual (i.e. MC and LFDIG vs. CAN) and combination (i.e. AM+M and, AM+LFDIG vs. AM+CAN) treatments. No significant different means between fertilizer treatments (Tukey's Test ($p$ <0.05)) were detected. Treatments AM+CAN and CAN, as references, were considered to be 100% efficient (i.e. NFRV = 100). AM: animal manure; CAN: calcium ammonium nitrate; MC: mineral concentrate; LFDIG: liquid fraction of digestate; Control: application of synthetic $P_2O_5$ and $K_2O$.*

Even though there were no differences observed with respect to crop N uptake, the calculated N FUE indicates that the relative export of N with harvest was higher in CAN, MC and LFDIG treatments than in combination treatments (Table 6.4). As a result, higher ANR was observed on average in treatments with individual product application. Nevertheless, due to the high standard deviations ANR of individual treatments and ANR of combination treatments was not statistically ($p>0.05$) different. On average, AM+MC and AM+LFDIG treatments resulted in lower NFRVs as compared to AM+CAN, while individual application of MC and LFDIG has led to higher NFRV as compared to CAN.

### 6.3.2 Soil properties and $NO_3^-$-N residue

The post-harvest $NO_3^-$-N residue was measured on three moments between October 1 and November 15, in order to determine the potential risk for $NO_3^-$ leaching. On all three occasions, the measured $NO_3^-$-N residue was below the legally stipulated limit of 80 kg $NO_3^-$-N $ha^{-1}$ for all treatments (Figure 6.2). On average higher $NO_3^-$-N residue was observed in treatments where LFDIG was applied.

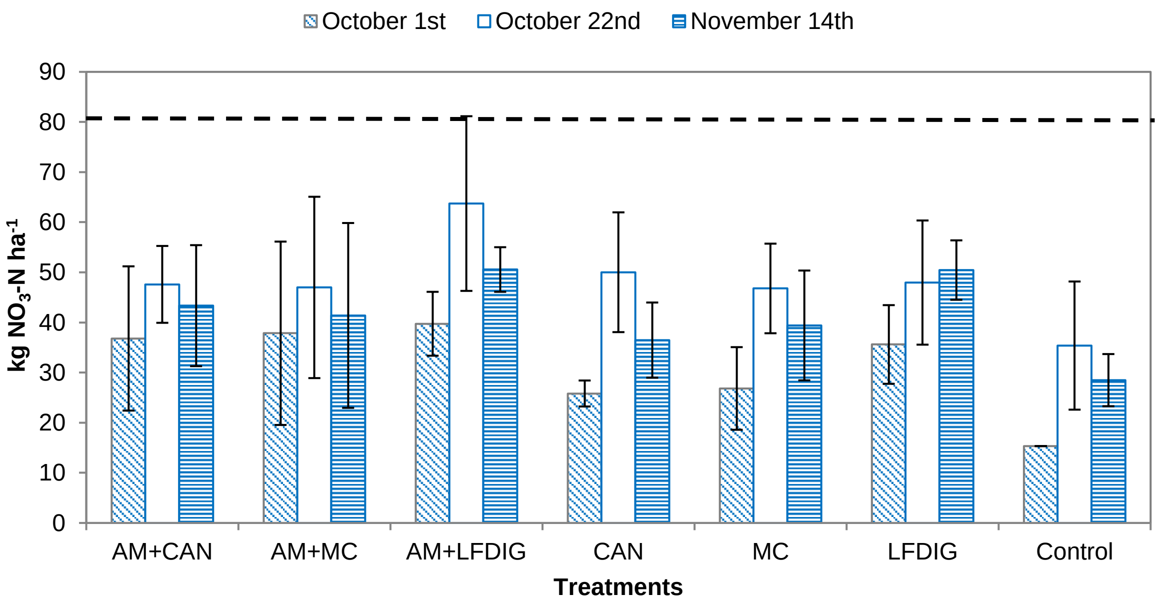


***Figure 6.2*** *$NO_3^-$-N residue (kg $ha^{-1}$) in soil layer 0-90 cm for the seven (AM: animal manure; CAN: calcium ammonium nitrate; MC: mineral concentrate; LFDIG: liquid fraction of digestate; Control: application of synthetic $P_2O_5$ and $K_2O$) different fertilization treatments after the harvest. The dash line indicates the maximum allowable level of nitrate residue in soil (80 kg $NO_3^-$-N $ha^{-1}$ for sandy-loam soil in 2014) between October 1st and November 15th according to Flemish environmental standard. Error bars indicate standard deviations (n=4; for control n=2).*

At harvest time, no significant effects of fertilizer treatment were observed with respect to soil pH, $EC_{5:1}$ and soil total N, P, K, S, Ca, Mg, Cu and Zn (Table 6.5). The effect of the fertilizer treatment was notable only in individual fertilized treatments, where a significantly higher soil total Na amount was measured at harvest time in MC treatment as compared to CAN reference.

***Table 6.5*** *Total mean ± standard deviation of soil moisture content (%; on soil fresh weight basis), pH-$H_2O$, $EC_{5:1}$ and nutrient amounts (0-30 cm; on soil dry weight basis) at harvest time for the seven different fertilization treatments (n=4; for control n=2).*

| Parameters | AM + CAN | AM + MC | AM + LFDIG | CAN | MC | LFDIG | Control |
|---|---|---|---|---|---|---|---|
| Moisture (%) | 14 ± 1 | 14 ± 0 | 14 ± 0 | 15 ± 1 | 14 ± 0 | 14 ± 0 | 14 ± 1 |
| Total N (g $kg^{-1}$) | 1.09 ± 0.10 | 1.04 ± 0.06 | 1.03 ± 0.12 | 1.00 ± 0.04 | 1.03 ± 0.05 | 0.99 ± 0.02 | 0.99 ± 0.02 |
| Total P (mg $kg^{-1}$) | 766 ± 75 | 807 ± 76 | 814 ± 80 | 855 ± 64 | 766± 41 | 734 ± 17 | 840 ± 76 |
| Total K (g $kg^{-1}$) | 2.00 ± 0.07 | 2.02 ± 0.21 | 1.96 ± 0.05 | 2.00 ± 0.14 | 2.06 ± 0.12 | 2.07 ± 0.13 | 1.97 ± 0.16 |
| Total S (mg $kg^{-1}$) | 443 ± 53 | 449± 55 | 417 ± 46 | 435 ± 50 | 441± 77 | 423 ± 58 | 423 ± 46 |
| Total Ca (g $kg^{-1}$) | 2.38 ± 0.15 | 2.33 ± 0.14 | 2.23 ± 0.16 | 2.41 ± 0.22 | 2.25 ± 0.22 | 2.20 ± 0.15 | 2.11 ± 0.19 |
| Total Mg (g $kg^{-1}$) | 1.64 ± 0.09 | 1.60 ± 0.08 | 1.54 ± 0.06 | 1.65 ± 0.17 | 1.55 ± 0.10 | 1.54 ± 0.07 | 1.52 ± 0.11 |
| Total Na (mg $kg^{-1}$) | 173 ± 12 | 188 ± 10 | 197 ± 40 | 171 ± 14a | 195 ± 9b | 197 ± 29ab | 154 ± 4 |
| Total Cu (mg $kg^{-1}$) | 17 ± 1 | 17 ± 2 | 16 ± 1 | 16 ± 1 | 16 ± 1 | 16 ± 0 | 16 ± 0 |
| Total Zn (mg $kg^{-1}$) | 44 ± 2 | 44 ± 3 | 42 ± 2 | 42 ± 1 | 42 ± 1 | 41 ± 1 | 41 ± 0 |
| pH-$H_2O$ | 6.99 ± 0.07 | 6.92 ± 0.11 | 7.03 ± 0.06 | 6.97 ± 0.06 | 6.87 ± 0.05 | 6.96 ± 0.06 | 6.87 ± 0.15 |
| $EC_{5:1}$ (µS $cm^{-1}$) | 114 ± 26 | 106 ± 35 | 91 ± 10 | 110 ± 24 | 116 ± 10 | 107 ± 32 | 88 ± 7 |

*Analyzed parameters were subjected to a one-way ANOVA by comparing separately individual (i.e. MC and LFDIG vs. CAN) and combination (i.e. AM+M and AM+LFDIG vs. AM+CAN) treatments. Lower-case letters (a and b) in a single row indicate significant different means between fertilizer treatments - Tukey's Test ($p < 0.05$). AM: animal manure; CAN: calcium ammonium nitrate; MC: mineral concentrate; LFDIG: liquid fraction of digestate; Control: application of synthetic $P_2O_5$ and $K_2O$.*

## 6.4 Discussion

### 6.4.1 Nutrient variability of bio-based materials and sampling

Bio-based materials are known for a high variability in nutrient composition (Galvez *et al.*, 2012; EC, 2014a) as compared to manufactured synthetic fertilizers. Materials used in the current study exhibited important differences in their nutrient composition between sampling prior to the fertilization and the sampling on the day of the fertilization itself. The highest variability in nutrient composition was observed with AM which during the field application contained four times more P than the sample of AM that was taken from the same storage three weeks earlier to determine the application dosage of the material (Table 6.2). In general, nutrient variability can occur due to the variation in diet, use of cleaning water, ammonia losses from storage and sampling strategies. The variation in diet can be detected from manure solids concentration which are related to nutrient concentration: the higher solids concentration is, the higher nutrient concentration will be (Lorimor et al., 2004). This was noted also with AM which during the fertilization, next to the four times more P, contained also almost four times higher DM content than the analyzed AM before fertilization. The DM content is dependent on the animal feed and water intake. If feed contains less salt or crude protein pigs will consume less water, resulting in manure with higher DM content (Kendall et al., 2000). In this study, however, no significant changes in feed strategy were expected during the three week period between sampling moments. Effect of cleaning water and ammonia losses would lead respectively to dilution of nutrient concentrations and reduction of $NH_4^+$-N, which did not occur in this study (Table 6.2). Therefore, the most likely cause for observed nutrient variability lies in sampling strategies. The issue is that most of the manure storages in practice are not mixed, which leads to settling and consequently makes it challenging to obtain homogeneous sample. Variability in nutrient composition was also observed for LFDIG and MC, however, to a lesser extent. For these bio-based materials the variability is mostly attributed to non-stable feeding patterns of co-digesters, which occurs due to the dependence on feedstock market availability. In general, these observations were briefly noted in other studies (Schröder *et al.*, 2013; Schröder *et al.*, 2014; Cavalli *et al.*, 2016), but their relevance and impact on the conducted field experiments was not discussed since only application rates during the actual fertilization were reported. In this study, the observed variability with AM led to N and P over-fertilization in all combination treatments, where on average 27% more N was added as compared to what was stipulated (Table 6.3). Consequently, the exceedance of the maximum stipulated limit for P application rate occurred by applying four times more P than it is legally allowed. The variability in nutrient composition of the bio-based materials needs to be tackled in the future to prevent the potentially negative effects of over-fertilization on the environment. Possible solutions would be to keep the time between the first sampling and the actual fertilization the shorter as possible (<3 weeks) and consider the use of Near Infra-Red (NIR) devices which would allow to adapt the application dosage during fertilization.

### 6.4.2 Effects on maize yield and nutrient uptake

As hypothesized, the use of LFDIG and MC as an individual N source or in combination with AM did not cause any differences in maize yield nor maize nutrient uptake as compared to CAN and AM+CAN treatment, respectively. Nevertheless, despite the similar N uptake of maize, the observed N FUE (%) differed significantly between the individual and combination treatments, with individual treatments exhibiting on average 45% higher N FUE (Table 6.4). This can be attributed to the N over-fertilization which occurred in combination treatments and the fact that N FUE largely accounts for differences in N application rate (Klop *et al.*, 2012). The observed negative correlation ($r=-0.995$, $p<0.05$) between applied rate of total N and N FUE (%) was also reflected on ANR and NFRV values which tended to be higher for individual treatments. However, due to the high standard deviations ANR and NFRV values did not differ significantly among fertilized treatments (Table 6.4), suggesting that LFDIG and MC have a fertilizer value similar to that of synthetic N fertilizer. The NFRVs of MC reported in this chapter are in agreement with the ones reported by Dutch researchers (Klop *et al.*, 2012; Schröder *et al.*, 2013; Schröder *et al.*, 2014) whose NFRV value of soil injected mineral concentrate ranged from 72 – 96% as compared to CAN being 100% efficient. For LF of digestate, Cavalli *et al.* (2016) reported that NFRVs of tested LF of digestate varied on average across the three year field trial from 20-75% as compared to ammonium sulfate as a synthetic N source. In general, studies on NFRVs tend to show a notable variation across different field experiments. This variation stems from the effects of variable weather conditions on the performance of both bio-based materials and the used references (Schröder *et al.*, 2013).

Despite the lack of N fertilization, control treatment resulted in 19 tonnes DM $ha^{-1}$ and N export of 216 kg N $ha^{-1}$. It is quite known that unfertilized control can benefit from previous fertilizer application (Brentrup and Palliere, 2010; Riva *et al.*, 2016), especially in regions with historical manure utilization such as Flanders. According to Flemish demonstration project 'Nitrogen monitoring network' that was on-going for 4 years, the following assumptions for the N mineralization from SOM were made (VLM, 2014b): i) a standard mineralization rate of 0.8 kg N $ha^{-1}$ $day^{-1}$ can be considered, ii) on poor land where only occasionally animal manure was used, an average mineralization rate of 0.5 kg N $ha^{-1}$ $day^{-1}$ seems to be more appropriate, iii) on plots where in past a lot of animal manure was applied, an average mineralization rate of 1 kg N $ha^{-1}$ $day^{-1}$ is more appropriate. The latter is in agreement with our prediction which was based on incubation of bare soil in Chapter 3. Incubation results showed that 93 kg N $ha^{-1}$ would be mineralized during the growing period of maize (141 days) on top of 69 kg mineral N $ha^{-1}$ that was present in soil prior to the experiment (section 6.3.1). This prediction of 162 kg N $ha^{-1}$ is in agreement with calculations of N mineralization at harvest time as follows (D' Haene *et al.*, 2014): N uptake + mineral N at harvest – mineral N at the start – N deposition = 216 + 17 – 69 – 9 = 155 kg N $ha^{-1}$. The N deposition was 23.8 kg N $y^{-1}$ in 2014 (MIRA, 2017). As shown, N mineralization in nutrient surplus regions can be considerable. In this study, the potential effect was taken into consideration by accounting for maize N uptake in control treatment when determining ANR and NFRV. In the absence of control treatment, as it occurs in practice, it is assumed that field is homogenous and the effect of N mineralization will be similar on all tested treatments.

### 6.4.3 Effects on $NO_3^-$-N residue and soil properties

N which is not taken up by the plant is prone to leaching thus causing environmental concern. The determination of the $NO_3^-$-N residue in soil is therefore seen as an important parameter to assess the nitrogen load in the environment. The post-harvest $NO_3^-$-N residue was measured on three moments between October 1 and November 15, and on all three occasions was below the legally stipulated limit of 80 kg $NO_3^-$-N $ha^{-1}$ for all treatments (Figure 6.2). This indicates that utilization of LF of digestate and mineral concentrate as N fertilizers should not additionally increase the risk of nitrate leaching compared to synthetic N fertilizer during the winter period, used individually or in combination with animal manure.

As hypothesized, the use of LFDIG and MC as an individual N source or in combination with AM did not cause differences in soil properties as compared to the respective references (Table 6.5). However, treatments with LFDIG and MC at harvest time tended to have on average higher amount of total Na in soil. In MC treatment the measured amount of Na in soil was significantly ($p<0.05$) higher than the one in CAN treatment. In general, LF of digestate and mineral concentrate contain considerable amounts of Na, which in this study were two to three times higher than the ones detected in AM (Table 6.2). Thus, when using these bio-based materials a significant amount of Na is applied. Some arable crops such as sugar beet respond positively to applied Na (Velthof, 2015), while some such as grass might exhibit scorching effect if salt concentrations of applied materials are too high and too close to the grass roots (Klop *et al.*, 2012). Moreover, if Na in soil dominates significantly over Ca and Mg, soil pores might clog resulting in limited water infiltration (Horneck *et al.*, 2007). This problem, however, is not expected in regions with temperate climate (e.g. Flanders) where precipitation exceeds evaporation, but might be an issue in arid regions where evaporation is often greater than precipitation.

### 6.4.4 Economic assessment

The use of bio-based materials as substitutes for their mineral counterparts can result in significant economic benefits for the farmer (Vaneeckhaute *et al.*, 2013a; Dahlin *et al.*, 2015). In this study, under the most favorable scenario (Sc. 1: transport distance = 5 km; arable farmer owns application equipment) for an arable farmer, the highest fertilization cost was observed in the conventional fertilization regime that uses manure and synthetic fertilizers (AM+CAN and CAN treatments) as a nutrient source for plants (Figure 6.3). This was mostly due to high purchase costs of synthetic fertilizers. Under the least favorable scenario (Sc. 2: transport distance = 40 km; arable farmer hires contractor) treatments with MC and LFDIG are still economically better option if, in the case of market acceptance, their retail price does not exceed 75% of the current market price paid for 1 kg of N. If the retail price of N from MC and LFDIG is equal to the one currently paid for N from CAN, bio-based treatments become 2-5% more expensive for the arable farmer under conditions of Sc. 2. In this case, arable farmer might still decide to use MC or LFDIG if there is an option to buy them from the closer location (< 40 km) or with higher N up-concentration (i.e. higher $NH_4^+$-N/$N_{total}$ ratio). This reduction in transport costs would compensate for 2-5% higher fertilization costs. Also, if the retail price for bio-based materials is positive, livestock farmers/biogas plant owners (i.e. producers of bio-based materials) might aim to produce bio-based fertilizers with higher $NH_4^+$-N/$N_{total}$ ratio in order to remain competitive on the fertilizer market.

Finally, nutrient variability might be better controlled because arable farmer would want to know the exact amount of N that he is receiving for his payment.

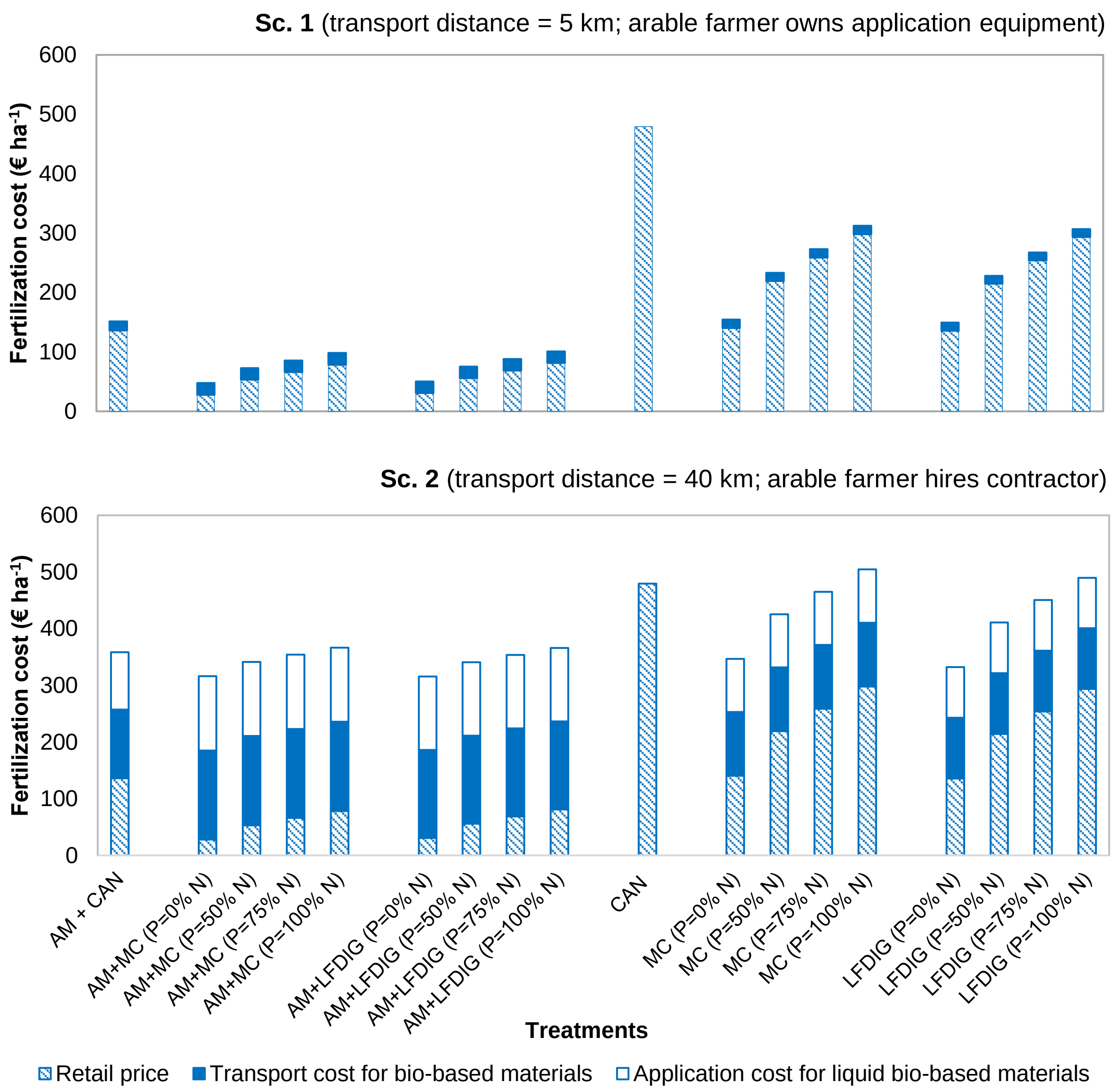


***Figure 6.3*** *Fertilization cost (€ $ha^{-1}$) of the applied treatments under two case scenarios (Sc. 1 and Sc. 2). Treatments AM+CAN and CAN present conventional fertilization with synthetic fertilizers. Treatments with bio-based N materials were additionally assessed with regard to the potential changes of their retail price: zero cost (P=0% N), 50% (P=50% N), 75% (P=75% N) and 100% (P=100% N) of the market price for kg of N. CAN: calcium ammonium nitrate (27% N); TSP: triple superphosphate (46% $P_2O_5$); PAT: potassium sulfate (30% $K_2O$, 10% MgO and 42% $SO_3$); AM: animal manure; MC: mineral concentrate; LFDIG: liquid fraction of digestate.*

## 6.5 Conclusion

In a single-year field maize cultivation, mineral concentrate and LF of digestate proved to be effective substitutes for conventional fertilizers applied in arable crops. Moreover, economic assessment indicated that their use can lead to economic benefits for an arable farmer, even in the case of their acceptance on the fertilizer market. Future perspectives indicate that point of attention for using the liquid fraction of digestate and mineral concentrate as substitutes for synthetic N fertilizer should at least focus on variability of their nutrient composition. By reducing the variability, potential N over-application and N loss during the crop growth could be reduced as well.

# **CHAPTER 7:** LIQUID FRACTION OF DIGESTATE AS A N FERTILIZER IN A THREE-YEAR CONSECUTIVE *ZEA MAYS* L. ROTATION

*Product sampling, fertilization, soil sampling and determination of the maize fresh weight yield at the experimental site (Pictures: Vaneeckhaute, C.)*

***This chapter has been redrafted after:***

Sigurnjak, I., Vaneeckhaute, C., Michels, E., Ryckaert, B., Ghekiere, G., Tack, F.M.G., Meers, E. 2017. Fertilizer performance of liquid fraction of digestate as synthetic nitrogen substitute in silage maize cultivation for three consecutive years. Science of the Total Environment, 599-600, 1885-1894.

## Abstract

Following changes over recent years in fertilizer legislative framework throughout Europe, phosphorus (P) is taking over the role of being the limiting factor in fertilizer application rate of animal manure. This results in less placement area for spreading animal manure. As a consequence, more expensive and energy demanding synthetic fertilizers are required to meet crop nitrogen (N) requirements despite existing manure surpluses. Anaerobic digestion followed by mechanical separation of raw digestate, results in liquid fraction (LF) of digestate, a bio-based material poor in P but rich in N and potassium (K). A 3-year field experiment was conducted to evaluate the impact of using the LF of digestate as a (partial) substitute for synthetic N fertilizer. Two different fertilization strategies, the LF of digestate in combination with respectively animal manure and digestate, were compared to the conventional fertilization regime of raw animal manure with synthetic NK-fertilizers. Results from the 3-year trial indicate that the LF of digestate may substitute synthetic N fertilizers without crop yield losses. Through fertilizer use efficiency assessment it was observed that under-fertilization of soils with a high P status might reduce P availability and consequently the potential for P leaching. Under conditions of lower K application, more sodium was taken up by the crop. In arid regions, this effect might reduce the potential risk of salt accumulation that is associated with organic fertilizer application. Finally, it seems that the nutrient variability in bio-based fertilizers will be one of the greatest challenges to address in the future utilization of these materials.

## 7.1 Introduction

In 2014, the average synthetic fertilizer use in Europe (EU-27) reached 10.7 million tonnes of nitrogen (N), 2.5 million tonnes of phosphate ($P_2O_5$) and 2.7 million tonnes of potash ($K_2O$) (FE, 2015). Contrary to this, the European (EU-27) agri-food sector is yearly generating 12 million tonnes of N and 2.5 million tonnes of phosphorus (P) via production of animal manure, sewage waste and food chain waste (Buckwell and Nadeu, 2016). At the moment, small part of nutrients in these waste streams is recovered and reused. In the case of animal manure, only 7.8% of all livestock manure in Europe is currently processed (Flotats *et al.*, 2013). This is often done via anaerobic co-digestion process, resulting in valuable biogas and nutrient rich digestate. The latter has the potential to be used as a fertilizer, however, the current European legislation limits its use by identifying it as a waste rather than a product (Article 2(g) of the Nitrates Directive 91/676/EC; EC, 1991). This implies that digestate from co-digestion with animal manure retains the status of animal manure (Schröder *et al.*, 2013; Vaneeckhaute *et al.*, 2013a; EC, 2014a) and thus directly competes with animal manure for possible disposal on arable land. This disposal is limited in nitrate vulnerable zones by the maximum allowable N application rate of 170 kg N $ha^{-1}$ $y^{-1}$ (Nitrates Directive 91/676/EC; EC, 1991), and as such constitutes a serious obstacle for profitable development of the biogas sector (Galvez *et al.*, 2012; Lebuf *et al.*, 2012; EC, 2013).

In order to enhance both the production of renewable energy and the sustainability of the agro-food value chain, it has become an important challenge to adopt biorefinery concepts in which nutrients present in animal manure and digestate are maximally recovered (Alburquerque *et al.*, 2012a; Galvez *et al.*, 2012) by generating various end- and by-materials that can be used as substitutes for synthetic fertilizers. As part of the Circular Economy Package, the European Union is revising its regulatory framework (Fertilizer Regulation 2003/2003) around mineral fertilizers with the intention to include bio-based fertilizers into the market. However, insufficient knowledge about the properties and the impact of these materials on soil characteristics and crop yield limit their use. This study aimed to evaluate the fertilizer potential of liquid fraction (LF) of digestate, a by-material of anaerobic digestion, and its impact on soil and crop production during three consecutive years of fodder maize cultivation.

Until now, many studies have investigated the fertilizer potential of digestate (Garfí *et al.*, 2011; Alburquerque *et al.*, 2012b; Herrmann *et al.*, 2013; Svoboda *et al.*, 2013; Bachmann *et al.*, 2014; Vanden Nest *et al.*, 2015). However, little is known, about the fertilizer potential of the LF of digestate (Riva *et al.*, 2016). In 2011, Vaneeckhaute *et al.* (2013b) conducted a field trial aimed at evaluating the fertilizer performance of LF of digestate as an alternative to synthetic N fertilizer. In this study, results on the leaching potential were however inconclusive due to unfavorable weather conditions which led to an exceedance of the Flemish legally allowed limit for $NO_3^-$-N $ha^{-1}$ residue in 0-90 cm soil layer for all tested treatments.

Field trial assessment is indispensable to determine the impact of newly proposed fertilizers on crop yield and soil characteristics. Moreover, in order to stimulate the legislative changes related to agronomic use of the LF of digestate as a potential replacement of synthetic N fertilizer, multi-year field assessments (> 1 year) are of utmost importance. The current study reports the results from three consecutive growing seasons (2011 – 2013) and aims to compare on the longer term the impact of LF of digestate application on soil properties and maize production with conventional treatment of raw animal manure supplemented with synthetic fertilizers. It is hypothesized that the three year use of LF of digestate in combination with animal manure or digestate will not cause significant differences in maize yield, nitrate leaching and soil properties as compared to conventional fertilization practice of using animal manure supplemented with synthetic fertilizers.

## 7.2 Materials and methods

### 7.2.1 Experimental set-up

The field experiment was performed on a sandy soil in Wingene (51° 3′ 0″N, 3° 16′ 0″E), Belgium for three consecutive years (2011, 2012 and 2013). The soil characteristics of the 0-30 cm soil layer prior to the experiment were organic carbon (OC) = 1.9 % on dry matter (DM); ammonium lactate extractable P (P-AL) = 820 mg $kg^{-1}$ DM and ammonium lactate extractable K (K-AL) = 120 mg $kg^{-1}$ DM. The $NO_3^-$-N amount in 0-30, 30-60 and 60-90 cm of soil layer was 25, 10 and 5 kg $ha^{-1}$, respectively. The $NH_4^+$-N amount was 4, 6 and 5 kg $ha^{-1}$ for 0-30, 30-60 and 60-90 cm, respectively. As test crops, fodder maize

(*Zea mays L.*) cv. Atletico KWS (FAO Ripeness Index: 280), Fernandez (FAO Ripeness Index: 260) and Millesim (FAO Ripeness Index: 240) were grown in 2011, 2012 and 2013, respectively. During the experimental period, Italian rye grass was mechanically sown as a catch crop at a seeding rate of 40 kg $ha^{-1}$, every year (October 22 2011, November 13 2012, October 8 2013) after the harvest of maize. The use of catch crops is a common practice in Western Europe where farmers use them with the aim to reduce the potential risk of nitrate leaching during the winter period. In this study, Italian rye grass was incorporated back to the field before fertilization in April. The monthly rainfall and average air temperature during the experimental period are presented in Table 7.1. The temperate marine climate of the region, with an average annual precipitation of 800 mm and an average annual air temperature of 10 °C (RMI), is favorable for high crop yields but entails conditions favorable for N leaching.

***Table 7.1*** *Weather conditions in Flanders in the period 2011 – 2013 and degree of abnormality by means of the statistical characteristics (SC) based on the reference period: 1833 – 2010 for 2011, 1833 – 2011 for 2012 and 1833 – 2012 for 2013.*

| | Average temperature (°C) | | | | | | Total rainfall (mm) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | SC | 2012 | SC | 2013 | SC | 2011 | SC | 2012 | SC | 2013 | SC |
| January | 4.0 | n | 5.1 | n | 2.1 | n | 90.5 | n | 86.4 | n | 53.6 | n |
| February | 5.4 | n | 0.7 | va | 1.4 | a | 44.0 | n | 30.0 | n | 55.3 | n |
| March | 7.7 | n | 8.9 | va | 3.0 | ve | 22.4 | e | 32.9 | n | 64.2 | n |
| April | 14.1 | ve | 8.4 | va | 9.0 | n | 25.8 | n | 104.1 | va | 25.8 | n |
| May | 14.8 | n | 14.3 | n | 11.1 | a | 22.5 | ve | 63.4 | n | 132.5 | ve |
| June | 16.8 | n | 15.4 | n | 15.8 | n | 72.3 | n | 133.1 | ve | 55.3 | n |
| July | 16 | e | 17.3 | n | 20.2 | a | 55.6 | n | 115.7 | a | 65.6 | n |
| August | 17.3 | n | 19.2 | n | 18.6 | n | 189.3 | ve | 22.5 | va | 48.3 | n |
| September | 16.5 | a | 14.5 | n | 14.8 | n | 83.1 | n | 51.6 | n | 58.1 | n |
| October | 12.1 | n | 11.1 | n | 12.8 | a | 48.8 | n | 119.4 | va | 77.5 | n |
| November | 8.6 | a | 7.1 | n | 6.4 | n | 8.5 | ve | 44.7 | a | 102.6 | a |
| December | 6.1 | e | 5.1 | n | 6.1 | e | 152.1 | a | 172.7 | ve | 77.1 | e |
| Mean/Sum | 11.6 | | 10.6 | | 10.1 | | 814.9 | | 976.5 | | 815.9 | |

*SC = statistical characteristic: n = normal, a = abnormal (averages one time in 6 years), va = very abnormal (averages one time in 10 years), e = exceptional (averages one time in 30 years), ve = very exceptional (averages one time in 100 years). All data is determined and available by RMI (Royal Meteorological Institute of Belgium).*

As previously mentioned in Chapter 6, bio-based fertilizers are known for their high variability in nutrient composition (Galvez *et al.*, 2012; EC, 2014b). Accordingly, product sampling and characterization were done at two moments. Before fertilization, all materials were collected to determine the required application rate for the test crops, while respecting the legal limits imposed by the Flemish Manure Decree (FMD, 2011). During the actual fertilization, bio-based materials were again sampled and analyzed (Table 7.2) to determine the nutrient content applied to the field (Table 7.3). Digestate and LF of digestate were sampled at Sap Eneco Energy (Merkem, Belgium; for more information on description of the biogas installation, please see Chapter 5, section 5.2.1). The digestate (DIG) and consequently the LF of digestate (LFDIG) underwent a hygenization step (1 h at 70 °C) and received a quality certification according to the quality standards of the Flemish compost organization (VLACO). The LF of digestate was obtained via a sieve band press separator. Both materials were collected from mixed storage tanks. Animal manure (AM) used in the field trial was collected at a local pig farm (Huisman; Aalter, Belgium), from non-mixed storage. The collection of bio-based materials from storage tanks was done according to the official sampling procedure from the Flemish institute for technological research (VITO, 2014), where it is advised to take several samples from different sampling points (i.e. bottom, middle and top of the storage tank) and mix them to obtain a homogenous sample. All materials were collected in polyethylene sampling bottles (5 L) and stored at 4 °C. As a reference treatment, calcium ammonium nitrate (CAN; 27 % N) and potassium sulfate (PAT; 30 % $K_2O$, 10 % MgO and 42 % $SO_3$), which in Flanders are commonly used synthetic N and K fertilizers, were applied in combination with animal manure.

***Table 7.2*** *Characteristics of bio-based materials (n=2) applied as fertilizer in fodder maize cultivation. Data is presented on fresh weight (FW) basis within a range of all three experimental years.*

| Parameter | Animal manure | Raw digestate/LF of digestate mixture[a] | LF of digestate |
|---|---|---|---|
| DM (%) | 4.3 - 11.0 | 6.2 - 7.1 | 2.5 - 4.3 |
| EC (dS $m^{-1}$) | 31 - 35 | 29 – 35 | 33 – 34 |
| pH | 7.7 - 7.8 | 8.0 - 8.2 | 7.4 - 7.8 |
| Total N (g $kg^{-1}$ FW) | 5.3 - 8.3 | 4.7 - 7.2 | 3.6 - 7.2 |
| $NH_4^+$-N (g $kg^{-1}$ FW) | 3.2 - 5.6 | 3.1 - 4.3 | 2.8 - 6.2 |
| P (g $kg^{-1}$ FW) | 1.0 - 2.4 | 0.87 - 1.57 | 0.27 - 0.74 |
| K (g $kg^{-1}$ FW) | 2.4 - 5.6 | 2.2 - 3.8 | 2.5 - 3.7 |
| Ca (g $kg^{-1}$ FW) | 1.9 - 4.1 | 1.3 - 2.1 | 0.11 - 0.64 |
| Mg (g $kg^{-1}$ FW) | 1.1 - 1.4 | 0.34 - 0.86 | 0.02 - 0.30 |
| Na (g $kg^{-1}$ FW) | 1.3 - 2.2 | 2.0 - 3.4 | 2.7 - 3.1 |
| S (g $kg^{-1}$ FW) | 0.42 - 0.80 | 0.39 - 0.84 | 0.11 - 0.27 |
| N/P | 3.5 - 5.0 | 4.6 - 5.4 | 10 – 13 |

*LF: liquid fraction; DM: dry matter; EC: electrical conductivity.*

[a] *50 vol % digestate and 50 vol % liquid fraction of digestate in 2011, 40 vol % digestate and 60 vol % liquid fraction of digestate in 2012 and 2013*.

### 7.2.2 Fertilization strategies and experimental design

Based on the soil characteristics and crop demand, fertilizer application dosage was advised by the Provincial Advice Centre for Agriculture and Horticulture (INAGRO, Beitem, Belgium) at 150 kg effective N $ha^{-1}$, 180 kg $K_2O$ $ha^{-1}$ and 30 kg MgO $ha^{-1}$ in 2011, and 135 kg effective N $ha^{-1}$, 250 kg $K_2O$ $ha^{-1}$ and 60 kg MgO $ha^{-1}$ in 2012 and 2013. The effective N is the amount of N from the applied product that is expected to be available for crop uptake in the season of application (Webb *et al.*, 2010). In accordance with Flemish legislation, the effective N from organic fertilizers (pig manure, digestate and LF of digestate) was set at 60 % of the total N content. The maximum allowable rate of 80 kg $P_2O_5$ $ha^{-1}$ for maize cultivation was respected (FMD, 2011).

Experimental treatments were tested in a fully randomized block design with three replicates of 9 m x 7.5 m each, spread across the field to minimize potential influence of variable soil conditions on the results. The same treatments were tested on the same test plots in the consecutive years. Nutrient application rates for the different treatments over three years are summarized in Table 7.3. Treatment [SF+AM]$_{REF}$ represents the reference treatment where only pig manure and synthetic fertilizers (CAN, PAT) were used. In treatment [LFDIG+DIG], mixtures (see below for detail) of digestate and LF of digestate obtained from anaerobic co-digestion of animal manure, energy crops and organic waste were spread on the field, with (2011 and 2013) or without (2012) supplemented addition of synthetic fertilizers (CAN, PAT) depending on crop requirements. In the reference treatment, $P_2O_5$ was the limiting nutrient for manure application, while in the treatment [LFDIG+DIG] N became the limiting nutrient. Based on the product characterizations an optimal combination of raw digestate and its LF after mechanical separation was determined to create a concentrated mixture with high effective N content but reduced $P_2O_5$ content, thereby reducing synthetic N requirements (2011: 50/50 digestate/LF of digestate; 2012-2013: 40/60 digestate/LF of digestate). Finally, in the treatment [LFDIG+AM], LF of digestate was applied as P-poor fertilizer in combination with pig manure, and with the additional supply of synthetic starter N during the maize sowing. Synthetic starter N is usually applied in practice to correct for the lower content of available N in organic fertilizers at the start of the growing period, which might otherwise cause a delay in maize development.

Fertilizers were applied to the soil over a period of three days (April 28 - 30 2011, May 28 - 30 2012 and April 26 - 28 2013) due to logistic reasons. On the first day of fertilization, LF of digestate was applied manually to ensure high precision for the targeted application on the test plots. The adequate amount was applied using watering cans, and within 2 hours incorporation was done with standard farming equipment. The mixture of digestate and LF of digestate, as well as pig manure were applied to the field by use of PC controlled injection (Bocotrans, NL) during the second day of the fertilization. Finally, on the third day synthetic fertilizers were applied to the plots by hand-application. All fertilizers were incorporated or injected at 5-10 cm depth, which is considered as an adequate measure to reduce the extent of ammonia volatilization (Webb *et al.*, 2010).

In 2012, the fertilization was conducted at a later date than usual due to very exceptional wet weather conditions in spring of that year (Table 7.1). Fodder maize was sown on May 5 2011, June 2 2012 and May 3 2013 at a seed density of 102 000 $ha^{-1}$, 100 000 $ha^{-1}$ and 100 000 $ha^{-1}$, respectively. The synthetic start N fertilizer was applied together with maize seeds to boost the development of plant at the beginning of the growing period. Crops were harvested on October 7 2011, November 9 2012 and October 4 2013.

***Table 7.3*** *Dosage of total nitrogen (kg ha$^{-1}$) applied for the three (n = 3) different fertilization treatments (TRT); $K_2O$, MgO and $P_2O_5$ amount (kg ha$^{-1}$) brought to the field via applied fertilization regime of total nitrogen; additional application of synthetic $K_2O$ (kg ha$^{-1}$) in order to obtain similar application rate in all treatments; the total and effective amount of applied N for each TRT. Values present the intended dosage and values between brackets present the actual dosage, indicating the difference caused in variability between sampling at farm/digester and sampling during the actual fertilization.*

| TRT | Year | Synthetic start N | Synthetic N | Animal manure | Digestate/LF of digestate mixture | LF of digestate | $K_2O$[a] | MgO[a] | $P_2O_5$[a] | Synthetic $K_2O$ | Total N applied | Effective N applied |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| [SF+AM]REF | 2011 | 25 | 29 | 160 *(163)* | | | 95 | 69 | 80 *(108)*[d] | 78 | 217 | 151 |
| | 2012 | 30 | 30 | 125 *(97)* | | | 75 | 30 | 80 *(45)* | 161 | 157 | 118 |
| | 2013 | 30 | 30 | 125 *(152)* | | | 122 | 50 | 80 *(96)*[d] | 128 | 212 | 151 |
| [LFDIG+DIG] | 2011 | 25 | 18 | | 178[b] *(175)* | | 139 | 86 | 81 *(75)* | 29 | 218 | 148 |
| | 2012 | | | | 223[c] *(232)* | | 231 | 60 | 80 *(101)*[d] | 0 | 232 | 139 |
| | 2013 | | | | 225[c] *(305)* | | 193 | 72 | 80 *(150)*[d] | 57 | 305 | 183 |
| [LFDIG+AM] | 2011 | 25 | | 140 *(143)* | | 58 *(60)* | 142 | 85 | 77 *(105)*[d] | 33 | 228 | 147 |
| | 2012 | 33 | | 112 *(87)* | | 58 *(65)* | 127 | 76 | 76 *(49)* | 121 | 185 | 124 |
| | 2013 | 29 | | 118 *(143)* | | 58 (110) | 184 | 95 | 80 *(117)*[d] | 66 | 282 | 181 |

[SF+AM]REF*: synthetic fertilizer + animal manure = reference;* [LFDIG+DIG]*: liquid fraction of digestate + digestate;* [LFDIG+AM]*: liquid fraction of digestate + animal manure.*

[a] *No synthetic $K_2O$, MgO and $P_2O_5$ was added;*

[b] *Mixture of 50 vol % raw digestate and 50 vol % LFDIG;*

[c] *Mixture of 60 vol % LFDIG and 40 vol % raw digestate;*

[d] *Maximum allowable fertilization level of 80 kg ha$^{-1}$ was exceeded.*

### 7.2.3 Plant and soil sampling

Soil samples were taken at harvest time (October 2011, November 2012 and October 2013) and in November 2011 and 2013. At each sampling time, homogenized soil subsamples were taken per plot at three depths (0-30 cm, 30-60 cm, 60-90 cm) using an auger. In 2011 one soil sample from the middle of the plot was taken, whereas in 2012 four random sampling points were chosen (Vaneeckhaute *et al.*, 2013b; Vaneeckhaute *et al.*, 2014). In 2013, a representative soil sample in each plot was obtained from five sampling points (the center and the 4 corners) of 7.5m$^2$ area which was located in the middle of the plot and corresponds to the area that was harvested for determination of maize fresh weight (FW) yield. The maize was harvested with a maize chopper. The crop FW yield (i.e. above ground plant) was determined at the field by hand harvesting 6.5 m$^2$, 10 m$^2$ and 7.5 m$^2$ per plot in 2011, 2012 and 2013, respectively. The samples were collected in polyethylene sampling bags and transported from the test site to the laboratory. In the laboratory, the replicate samples were stored at 1 °C to 5 °C until further analysis.

### 7.2.4 Physicochemical analysis

***Bio-based material analysis.*** Product DM, OM, total N and $NH_4^+$-N content were determined as described in section 3.2.2 (Chapter 3). Conductivity and pH were determined potentiometrically using a WTW-LF537 (Germany) conductivity electrode and an Orion-520A pH-meter (USA), respectively, without prior product equilibration and filtration. Nitrate-N was determined by flow analysis (continuous flow analysis (CFA) and flow injection analysis (FIA)) and spectrometric detection (BRAN+LUEBBE AA3, Germany) from a 1M KCl extract (ISO 13395: 1996). After wet digestion (2 ml $HNO_3$ and 1 ml $H_2O_2$), total P was analyzed using the colorimetric Scheel method (Van Ranst *et al.*, 1999), while total S, K, Ca, Mg and Na were analyzed using Inductively coupled plasma optical emission spectrometry (ICP-OES) (Varian Vista MPX, USA).

***Soil analysis.*** Soil moisture, OM, OC, pH-KCl, EC and total N content were determined as described in section 3.2.1 (Chapter 3), whereas soil nitrate-N content was analyzed as described in the previous paragraph ('Bio-based product analysis'). After aqua regia digestion (1 g sample + 7.5 ml HCl, 2.5 ml $HNO_3$ and 2.5 ml demineralized water), total P, K, S, Cu and Zn were analyzed as described in section 4.2.2 (Chapter 4). Finally, plant available amounts of P, K and Na were analyzed after filtering (MN 640 m, Macherey–Nagel, Germany) a suspension of 2.5 g sample and 50 ml ammonium lactate (AL) at pH 3.75 (VITO, 2010a) that was previously shaken for 4 h. The Sodium Adsorption Ratio (SAR) was determined from a water extract of a saturated soil paste (Van Ranst *et al.*, 1999) in terms of meq l$^{-1}$ and reported according to Horneck *et al.* (2007):

$$SAR = \frac{[Na^+]}{\sqrt{0.5([Ca^{2+}] + [Mg^{2+}])}}$$

SAR is used as a parameter to assess salt-affected soil. When Na dominates over Ca and Mg (high SAR), soil pores clog and water infiltration is limited (Horneck *et al.*, 2007).

***Plant analysis.*** Plant samples (i.e. above ground plant) collected in the field were weighed for determination of the FW biomass yield and oven-dried at 55 °C for determination of the DM content. The dry samples were ground to pass a 1 mm sieve (Retsch SM-2000, Germany) and used for further analysis. Total N was determined using the Kjeldahl method. Total P was determined using the method of Scheel (Van Ranst *et al.*, 1999) after incineration of the dry samples during 4 h at 550 °C and digestion of the residual ash (1 g ash + 5 ml 3 mol $HNO_3$ $L^{-1}$ + 5 ml 6 mol $HNO_3$ $L^{-1}$). Na, K and metals in the digested samples were determined using ICP-OES.

### 7.2.5 Data analysis

Based on the physicochemical data, the fertilizer use efficiency (FUE) of each treatment was determined using the Eq. 3 (Chapter 1). Fertilizer use efficiencies were evaluated throughout time for N, P and K. A value above 1 implies a deficit of the relevant nutrient while a value below 1 indicates a surplus on the soil balance. Furthermore, the fertilizer replacement use efficiency (FRUE) was obtained as the ratio of the FUE of the treatments with bio-based materials to the FUE of conventional fertilization with mineral fertilizers and animal manure (Eq. 4; Chapter 1). As previously (Chapter 4, 5 and 6), it was assumed that the conventional fertilization practice is 100% efficient.

Statistical analyses were performed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL). The data from the plant and soil analysis corresponding to the 3 replicates were first subjected to one-way ANOVA for each year separately to measure the effect of treatment on yearly basis (due to the observed variability in nutrient application between treatments and the experimental years). When significant differences between means were observed, additional post hoc assessment was performed using Tukey's Test ($p < 0.05$, n=3). These differences are indicated by the different lower case letters. In order to examine the effect of the treatments and the experimental year over the 3-year field study, all data was subjected to a two-way ANOVA, considering year and replication as random and fertilizer treatments as fixed factors. Compound symmetry was used as a covariance structure. When a significant factor effect was observed, additional post hoc assessment was performed using Tukey's Test ($p < 0.05$, n=3). These differences are indicated by the different upper case letters. The condition of normality was checked using the Shapiro-Wilk test, whereas the homogeneity was tested with the Levene Test. Significant parameter correlations were determined using the Pearson correlation coefficient (r).

## 7.3 Results

### 7.3.1 Maize yield and nutrient uptake

A treatment effect on the fresh weight (FW) based yield was only observed in 2011, when the treatment [LFDIG+AM] resulted in a higher FW yield as compared to the conventional fertilization regime (i.e. $[SF+AM]_{REF}$; Table 7.4). This effect did not recur in the following years. Overall, no significant effects of the fertilizer treatments were observed over the 3-year field experiment with respect to crop FW and dry matter (DM) based yield (Table 7.4). The experimental years however significantly ($p < 0.05$) influenced the maize yield, indicating the effect of environmental conditions on treatment performance and maize development. This is a result of the unfavorable weather conditions in 2012, characterized by a wet spring, which delayed the planting date (Table 7.1) and led to the lowest crop yields over the 3-year period. Nevertheless, no significant differences ($p > 0.05$) were observed for DM content (%) over the 3-year period. The average DM content at harvest was 28 ± 1 % in 2011, 29 ± 0 % in 2012 and 28 ± 1% in 2013.

Modest effects of the treatments on a yearly basis were observed with respect to crop P, K and Na uptake (Table 7.4). However, these effects did not persist throughout the 3-year field experiment. As a result, no significant effects of the fertilizer treatments were measured with respect to crop nutrient uptake over the 3-year field study. In line with the observation for maize yield, weather conditions in 2012 significantly ($p < 0.05$) influenced the crop nutrient uptake as compare to 2011 and 2013 (Table 7.4). There were no systematic differences in trace element uptake.

### 7.3.2 $NO_3^-$-N residue and soil properties

Significant differences ($p < 0.05$) in the soil $NO_3^-$-N residue among treatments were only observed in 2013, when the treatment [LFDIG+DIG] resulted in a higher $NO_3^-$-N residue as compared to the reference (i.e. $[SF+AM]_{REF}$; Figure 7.1). However, $NO_3^-$-N levels were still below the legislative maximum allowable level of 80 kg $NO_3^-$-N $ha^{-1}$ (0-90 cm soil layer) for 2012 (VLM, 2013) and 2013 (VLM, 2014). In 2011, $NO_3$-N levels exceeded the limit for all treatments, including the reference.

***Table 7.4*** *Effects of fertilizer treatment and year on maize fresh weight (FW) yield, dry matter (DM) yield and crop nutrient uptake in the 3 year field experiment. Results of two-way mixed effect ANOVA and post hoc comparison of means (mean ± standard deviation; n=3).*

| | FW (t ha$^{-1}$) | | | DM (t ha$^{-1}$) | | | N (kg ha$^{-1}$) | | | P (kg ha$^{-1}$) | | | K (kg ha$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 |
| *Treatment (TRT)* | | | | | | | | | | | | | | | |
| [SF+AM]$_{REF}$ | B81±2a | A60±4 | B83±4 | B22±2 | A18±2 | B24±1 | B293±40 | A140±23 | B322±11 | AB56±8 | A42±14 | B58±2b | A280±28a | A225±76 | A278±5a |
| [LFDIG+DIG] | B84±1a | A58±6 | B79±7 | B23±1 | A17±2 | B22±1 | B316±25 | A146±9 | B298±31 | B60±5 | A46±7 | A48±6a | B353±6b | A236±93 | A290±28ab |
| [LFDIG+AM] | B86±1b | A56±2 | B84±10 | B25±1 | A16±2 | B23±3 | B344±51 | A106±40 | B323±45 | A66±10 | A51±6 | A60±4b | B306±17a | A211±73 | B323±16b |
| *Significance of the p-values* | | | | | | | | | | | | | | | |
| TRT | | 0.892 | | | 0.713 | | | 0.958 | | | 0.126 | | | 0.399 | |
| Year | | 0.001 | | | 0.003 | | | 0.001 | | | 0.025 | | | 0.027 | |
| TRT x Year | | 0.423 | | | 0.211 | | | 0.185 | | | 0.567 | | | 0.530 | |

| | Na (kg ha$^{-1}$) | | | Cu (g ha$^{-1}$) | | Zn (g ha$^{-1}$) | |
|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | 2011 | 2013 | 2011 | 2013 |
| *Treatment (TRT)* | | | | | | | |
| [SF+AM]$_{REF}$ | B6.2±1.9ab | A1.6±0.4 | B4.3±0.7 | 81±14 | 140±22 | 646±153 | 904±47 |
| [LFDIG+DIG] | C5.7±0.7a | A1.3±0.2 | B3.5±0.8 | 96±3 | 100±31 | 601±16 | 694±166 |
| [LFDIG+AM] | C7.4±0.3b | A1.3±0.4 | B4.1±1.3 | 77±8 | 118±24 | 716±100 | 908±23 |
| *Significance of the p-values* | | | | | | | |
| TRT | | 0.256 | | 0.790 | | 0.187 | |
| Year | | 0.001 | | 0.164 | | 0.063 | |
| TRT x Year | | 0.475 | | 0.086 | | 0.408 | |

*Lower-case letters (a and b) in a single column indicate significant different means between fertilizer treatments within single year; upper-case letters (A, B and C) indicate significant different means between years; Tukey's Test (p <0.05; n=3); [SF+AM]$_{REF}$: synthetic fertilizer + animal manure = reference; [LFDIG+DIG]: liquid fraction of digestate + digestate; [LFDIG+AM]: liquid fraction of digestate + animal manure.*

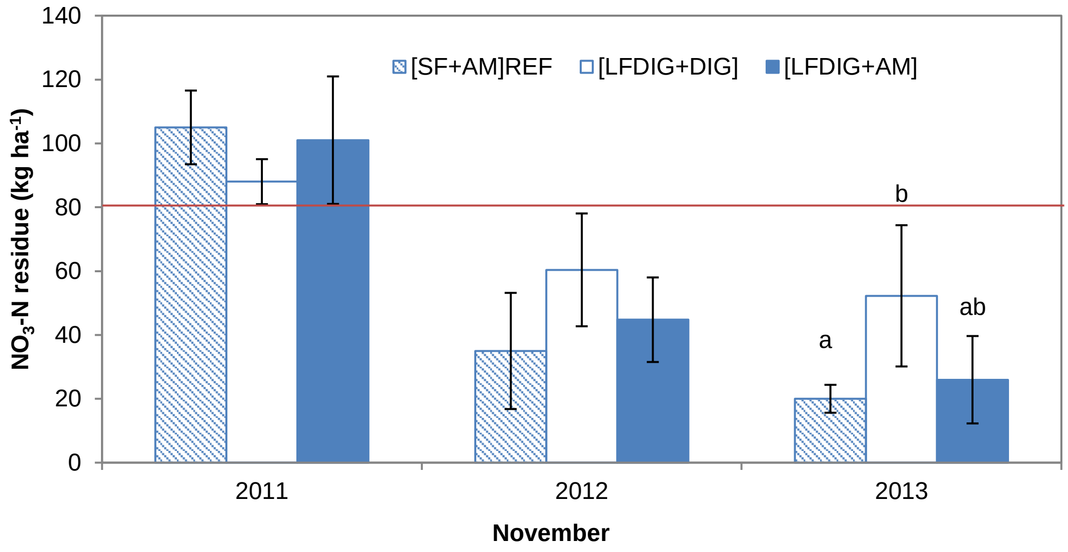


***Figure 7.1*** *$NO_3^-$-N residue (kg ha$^{-1}$) in soil layer 0-90 cm for the three ([SF+AM]$_{REF}$: synthetic fertilizer + animal manure = reference; [LFDIG+DIG]: liquid fraction of digestate + digestate; [LFDIG+AM]: liquid fraction of digestate + animal manure) different fertilization treatments in November for each experimental year. The line indicates the maximum allowable level of nitrate residue in soil (80 kg $NO_3^-$-N ha$^{-1}$ for sandy soil in 2012 and 2013) between October 1st and November 15th according to Flemish environmental standard. In 2011 the maximum allowable level of nitrate residue in soil was 88 kg $NO_3^-$-N ha$^{-1}$. Error bars indicate standard deviations (n=3), and lower-case letters (a and b) indicate significant different means between fertilizer treatments within single year.*

Fertilizer treatments did not cause significant differences in the soil available nutrient pool (Table 7.6), the soil total nutrient pool, pH-KCl or $EC_{5:1}$ (Table 7.5) at harvest time. However, a significant effect of the experimental year on soil available P-AL and total K was observed in 2012. A year effect was also measured in 2013 when a significant decrease in K-AL and a significant increase of the total Na in the soil was detected. Consequently, a negative correlation was found between soil K-AL and total Na amount (r = - 0.643, p = 0.00). Furthermore, the potential three year impact of the proposed fertilization strategies on Na build-up was examined through SAR analysis. Results showed that in July 2013, the [LFDIG+DIG] treatment resulted in a significantly ($p < 0.05$) higher SAR ratio of 1.22 ± 0.28 as compared to the conventional fertilization regime [SF+AM]$_{REF}$ exhibiting a SAR ratio of 0.75 ± 0.12. The SAR ratio of treatment [LFDIG+AM] was 1.03 ± 0.31.

***Table 7.5*** *Effects of fertilizer treatment and year on soil pH-KCl, $EC_{5:1}$, and soil total N, P and K amount (0-30 cm; expressed on dry matter basis) at harvest time in the 3 year field experiment. Results of two-way mixed effect ANOVA and post hoc comparison of means (mean ± standard deviation; n=3).*

| | pH - KCl | | | $EC_{5:1}$ (µS $cm^{-1}$) | | | N (g $kg^{-1}$) | | | P (g $kg^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 |
| *Treatment (TRT)* | | | | | | | | | | | | |
| $[SF+AM]_{REF}$ | 5.4±0.1 | 5.7±0.6 | 5.1±0.2 | 104±12 | 75±7 | 74±23 | 2.2±0.0 | 2.1±0.5 | 2.1±0.2 | 1.6±0.1 | 1.0±0.7 | 1.6±0.3 |
| [LFDIG+DIG] | 5.4±0.1 | 5.5±0.3 | 5.3±0.3 | 106±15 | 85±13 | 71±18 | 2.2±0.0 | 2.7±0.6 | 2.1±0.2 | 1.6±0.1 | 1.4±0.1 | 1.5±0.1 |
| [LFDIG+AM] | 5.3±0.1 | 5.8±0.3 | 5.5±0.1 | 106±4 | 83±11 | 113±49 | 2.1±0.2 | 2.9±0.1 | 2.2±0.0 | 1.6±0.1 | 1.5±0.1 | 1.7±0.1 |
| *Significance of the p-values* | | | | | | | | | | | | |
| TRT | | 0.402 | | | 0.387 | | | 0.488 | | | 0.474 | |
| Year | | 0.086 | | | 0.182 | | | 0.163 | | | 0.173 | |
| TRT x Year | | 0.524 | | | 0.329 | | | 0.164 | | | 0.311 | |

| | K (mg $kg^{-1}$) | | | Na (mg $kg^{-1}$) | | | Cu (mg $kg^{-1}$) | | | Zn (mg $kg^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 |
| *Treatment (TRT)* | | | | | | | | | | | | |
| $[SF+AM]_{REF}$ | B497±68 | A315±52 | B515±74 | B80±7 | A52±8a | C101±11a | 34±2 | NA | 31±1 | 52±5 | NA | 50±4 |
| [LFDIG+DIG] | B524±67 | A361±47 | B573±45 | B86±7 | A74±2b | C121±8b | 34±4 | NA | 30±1 | 51±6 | NA | 47±5 |
| [LFDIG+AM] | B549±49 | A372±60 | B579±19 | AB78±4 | B76±15ab | B113±23ab | 34±2 | NA | 32±1 | 45±2 | NA | 52±2 |
| *Significance of the p-values* | | | | | | | | | | | | |
| TRT | | 0.142 | | | 0.093 | | | 0.629 | | | 0.813 | |
| Year | | 0.001 | | | 0.003 | | | 0.072 | | | 0.867 | |
| TRT x Year | | 0.992 | | | 0.404 | | | 0.561 | | | 0.089 | |

*Lower-case letters (a and b) in a single column indicate significant different means between fertilizer treatments within single year; upper-case letters (A, B and C) indicate significant different means between years; Tukey's Test ($p < 0.05$; n=3); $[SF+AM]_{REF}$: synthetic fertilizer + animal manure = reference; [LFDIG+DIG]: liquid fraction of digestate + digestate; [LFDIG+AM]: liquid fraction of digestate + animal manure.*

***Table 7.6*** *Effects of fertilizer treatment and year on available soil P, K and Na amount extracted through ammonium lactate (AL) at harvest time (0-30 cm) in the 3 year field experiment. Results of two-way mixed effect ANOVA and post hoc comparison of means (mean ± standard deviation; n=3).*

| | P-AL (mg kg$^{-1}$) | | | K-AL (mg kg$^{-1}$) | | | Na-AL (mg kg$^{-1}$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 | 2011 | 2012 | 2013 |
| *Treatment (TRT)* | | | | | | | | | |
| [SF+AM]$_{REF}$ | B915±44 | A405±270 | B941±92 | B143±33 | B146±18 | A93±13 | 43±2 | 37±8 | 57±44 |
| [LFDIG+DIG] | B908±138 | A544±104 | B959±134 | B155±55 | B141±39 | A114±25 | 60±20 | 52±12 | 55±10 |
| [LFDIG+AM] | B903±68 | A600±24 | B1054±70 | B193±7 | B165±2 | A93±6 | 49±7 | 45±4 | 90±29 |
| *Significance of the p-values* | | | | | | | | | |
| TRT | | 0.222 | | | 0.347 | | | 0.396 | |
| Year | | 0.001 | | | 0.022 | | | 0.193 | |
| TRT x Year | | 0.623 | | | 0.373 | | | 0.328 | |

*Upper-case letters (A, B and C) indicate significant different means between years; Tukey's Test ($p < 0.05$; n=3); [SF+AM]$_{REF}$: synthetic fertilizer + animal manure = reference; [LFDIG+DIG]: liquid fraction of digestate + digestate; [LFDIG+AM]: liquid fraction of digestate + animal manure.*

### 7.3.3 Fertilizer replacement use efficiency

The fertilizer use efficiency (FUE) and fertilizer replacement use efficiency (FRUE) of each treatment as compared to the conventional fertilization regime $[SF+AM]_{REF}$ are presented in Table 7.7. FUE values above 1 indicate that during the first growing season more nutrients were taken up by the crop than were added to the field. During 2012 and 2013, treatments [LFDIG+DIG] and [LFDIG+AM] resulted in moderate N FRUE ratios as compared to the reference (i.e. $[SF+AM]_{REF}$). During the same period, treatment [LFDIG+DIG] resulted on average in 50% lower P FRUE ratio, which is probably the result of higher P application rates in this treatment and the observed negative correlation between the applied P dosage and P FUE of the tested treatments ($r = -0.950$, $p = 0.00$).

***Table 7.7*** *Fertilizer use efficiency (FUE; as a ratio) and its relation to the conventional fertilization treatment (i.e. $[SF+AM]_{REF}$) as a reference (FRUE; as a %) assuming that the latter is 100% efficient (FRUE $[SF+AM]_{REF}$ =100%).*

| | 2011 | | | 2012 | | | 2013 | | |
|---|---|---|---|---|---|---|---|---|---|
| | [SF+AM] REF | [LFDIG +DIG] | [LFDIG +AM] | [SF+AM] REF | [LFDIG +DIG] | [LFDIG +AM] | [SF+AM] REF | [LFDIG +DIG] | [LFDIG +AM] |
| N | | | | | | | | | |
| FUE | 1.35 | 1.43 | 1.51 | 0.89 | 0.63 | 0.57 | 1.52 | 0.98 | 1.14 |
| FRUE | 100[a] | 106 | 112 | 100[a] | 71 | 64 | 100[a] | 64 | 75 |
| P | | | | | | | | | |
| FUE | 1.19 | 1.83 | 1.42 | 2.09 | 1.05 | 2.39 | 1.38 | 0.73 | 1.17 |
| FRUE | 100[a] | 153 | 119 | 100[a] | 50 | 114 | 100[a] | 53 | 85 |
| K | | | | | | | | | |
| FUE | 1.95 | 2.53 | 2.10 | 1.15 | 1.23 | 1.02 | 1.34 | 1.40 | 1.56 |
| FRUE | 100[a] | 130 | 108 | 100[a] | 107 | 89 | 100[a] | 104 | 116 |

*ND: not determined; $[SF+AM]_{REF}$: synthetic fertilizer + animal manure = reference; [LFDIG+DIG]: liquid fraction of digestate + digestate; [LFDIG+AM]: liquid fraction of digestate + animal manure.*
*[a] Assumption that conventional fertilization treatment is 100% efficient.*

## 7.4 Discussion

### 7.4.1 Fertilization regime and applied nutrient dosage

An important prerequisite for efficient organic fertilizer use is accurate knowledge of nutrient concentration, especially with respect to N and P. Over the course of this 3-year experiment, it was observed that the actual N doses varied in some instances from the intended N doses (Table 7.3). This was caused by differences in organic fertilizer composition between sampling at the farm/digester versus sampling during the actual fertilization. As a limiting factor in N fertilization, the applied dosages of P were positively correlated ($r = 0.907$, $p = 0.00$) with applied N, which led to exceedances of the maximum legal level of 80 kg $P_2O_5$ $ha^{-1}$ (FMD, 2011) in 2011 and 2013 for all treatments, except for [LFDIG+DIG] in 2011. In 2012, the actual applied dosages of P were below the maximum legal level for all treatments, except for treatment [LFDIG+DIG]. These observations indicate that nutrient variability in bio-based fertilizers will be one of the greatest challenges to address in the future utilization of these materials.

### 7.4.2 Effect of fertilization strategies on biomass yield and nutrient uptake

As hypothesized, the three year use of LF of digestate in combination with animal manure or digestate did not cause significant differences in maize FW or DM yield as compared to conventional fertilization. However, there was a significant effect ($p < 0.05$) of the experimental year. The lowest FW and DM maize yields were observed in 2012 as compared to the other experimental years as a result of wet weather conditions (Table 7.1; Boerenbond, 2012) and delayed planting which may lead to delayed leaf area index (LAI) development. Interestingly, there were no differences noted in the DM (%) content, although each year a different cultivar (Atletico KWS in 2011, Fernandez in 2012 and Millesim in 2013) was grown. As a plant that uses C4 carbon fixation, maize is most sensitive to drought at the time of silk emergence, when the flowers are ready for pollination (Wang *et al.*, 2010). Due to unfavorable weather conditions in the spring of 2012, maize was sown relatively late (June 2 2012). The first two months of its growing period were characterized by exceptionally heavy rainfall (249 mm) and the pollination stage occurred in August when the lowest rainfall (22.5 mm) during the maize growing period 2011 – 2013 was recorded (Table 7.1). It is likely that the heavy rainfall at the beginning of the maize cropping period has influenced the crop uptake by leaching N and other essential nutrients out of the crop root zone and making them less available, leading to lower fresh yields. However, the characteristics of the C4 pathway suggest that maize with limited water supply during the period of active growth might exhibit a high DM (%) content, while at the same time lower fresh yields are obtained. This is a result of the high water use efficiency in C4 plants, which allows them to produce biomass and to set seed on a limited amount of water (Sage, 2005). When comparing season 2011 and 2013, treatments [LFDIG+DIG] and [LFDIG+AM] in 2013 on average received 20% more effective N (Table 7.3). The increase in effective N rate, however, did not lead to higher maize yield. This can probably be explained by a yield dose response curve and the fact that increase in effective N dose will not simultaneously lead to a yield increase if the maximum yield has already been reached. This was also observed in a study on maize response curve by D' Haene *et al.* (2014) where application of effective N above 150 kg effective N $ha^{-1}$ did not lead to a visible yield response.

Similar to the maize yield, no effect of fertilization treatment was observed with respect to crop nutrient uptake. The effect of unfavorable weather conditions in 2012 was however reflected in a reduced nutrient uptake for all treatments, including the reference. In 2013, maize nutrient concentrations again reached similar levels as these of 2011 (Table 7.4), further supporting that the observed decrease of nutrient uptake was a consequence of exceptional weather conditions and not an effect of the applied fertilization.

### 7.4.3 Nitrogen use efficiency

In general, organic fertilizers such as animal manure, digestate and LF of digestate are more susceptible to nitrate leaching due to the asynchrony between crop demand versus the slow release of organically bound N (Schröder *et al.*, 2013; Svoboda *et al.*, 2013). N that is not taken up by the plant is prone to leaching and therefore is of environmental concern. In order to assure efficient N use, N losses should not exceed those of synthetic fertilization if we aim to (partially) substitute them in the future. In Flanders, the level of $NO_3^-$-N residue (kg $ha^{-1}$) in the soil profile (0 – 90 cm) in the post-harvest period between October 1st and November 15th is used as an indicator for quantifying unwanted leaching to surface and ground water. The legal maximum allowable $NO_3^-$-N level has been reduced from 88 in 2011 (VLM, 2012) to 80 kg $NO_3^-$-N $ha^{-1}$ in 2012 (VLM, 2013), and 75 kg $NO_3^-$-N $ha^{-1}$ in 2014 (VLM, 2015). The results of this study indicate that the effect of the experimental year was stronger ($p < 0.05$) than the effect of the treatment. In the first year of this study, the maximum allowable $NO_3^-$-N level was exceeded for all treatments, including the reference scenario. This was attributed to unfavorable weather conditions, an exceptionally dry spring followed by a moist summer, which has led to the exceedance of maximum allowable $NO_3^-$-N level in 40% of all taken measurements in West Flanders (Vaneeckhaute *et al.*, 2013b). In the following years, the $NO_3^-$-N residue for all treatments was below the legal stipulated limit. Catch crops are sown with the aim to reduce potential $NO_3^-$-N leaching during the winter period. An effect of Italian rye grass on the $NO_3^-$-N residue dynamics was not observed over the 3-year field experiment because the date of sowing catch crop was too close to the legally stipulated date of measuring the post harvest $NO_3^-$-N residue (i.e. November 15th). The impact of Italian rye grass on $NO_3^-$-N residue dynamics after November 15th was not the focus of this chapter. In general, we do not expect for the practice of growing catch crops to have an impact on the agronomic nor environmental performance of the maize under the tested fertilization scenarios. The effect of fertilizer type on the $NO_3^-$-N residue was only significant in November 2013 ($p < 0.05$) as a result of 93 kg N $ha^{-1}$ over-fertilization in treatment [LFDIG+DIG] above that of the conventional fertilization where N import was 212 kg N $ha^{-1}$ (Table 7.3). The over-fertilization was caused by the above mentioned nutrient variability in bio-based fertilizers. Regardless of the over-fertilization in 2013, results of the $NO_3^-$-N residue indicate that derivatives of digestate processing have similar nitrate leaching potential as conventional fertilization treatment (Figure 7.1), which is in accordance with the findings of Svoboda *et al.* (2013).

Next, since all treatments had a similar crop N uptake, the over-fertilization in 2013 ([LFDIG+DIG] > [LFDIG+AM] > $[SF+AM]_{REF}$) reflected the N FRUE, which was 36 % and 25 % lower for [LFDIG+DIG] and [LFDIG+AM] treatments, respectively, as compared to the reference scenario (assumption FRUE = 100%; Table 7.7). A similar decrease in N FRUE was observed in 2012 as a result of exceptional weather conditions, which led to a lower crop N uptake and subsequently to low N FRUE levels. In this respect, the high N FRUE for bio-based fertilization in 2011 was not validated in the following two years. This indicates that the FRUE observed for bio-based fertilizers depends on many factors, such as soil texture (appears to be low in sand sites), weather conditions, as well as the variability in nutrient composition. It seems that in the open field experiments FRUE might not be the best efficiency indicator and other ways of assessing FRUE are needed. Nevertheless, fertilization strategies where the LF of digestate was used as a bio-based N fertilizer have resulted in similar agronomic and environmental values as compared to conventional fertilization of using synthetic N and animal (pig) manure.

### 7.4.4 Phosphorus use efficiency

In general Flemish soils are quite rich in P due to the long-term application of animal manure and inorganic fertilizers. Over the course of this 3-year experiment, a negative correlation was found between the P application and the P FUE of the treatments (r = - 0.950, p = 0.00). This indicates that the less P applied to the soil (< 80 kg $P_2O_5$ $ha^{-1}$ via [LFDIG+DIG] in 2011 and [LFDIG+AM] in 2012), the higher P FUE will be. This opportunity to reduce P leaching in soils with high P status was also reported by Vanden Nest *et al.* (2015) who stated that introducing zero P-fertilizer application on soils with high P status can reduce P availability and consequently lower the potential for P leaching in an arable crop rotation without decreasing crop yields. However, in this 3-year experiment, no significant differences in soil ammonium lactate extractable P (P-AL) available pool were observed in time, except in 2012, when a decrease in P-AL was detected, probably due to lower P application rates. This is in good agreement with the study of Vanden Nest *et al.* (2015) who observed no effects of P fertilizer rate and organic amendments on the P-AL level during a 4-year experimental trial on Flemish soil.

### 7.4.5 Potassium and sodium use efficiency

Application of LF of digestate in [LFDIG+DIG] and [LFDIG+AM] treatments led to significantly lower or no use of synthetic K as compared to the reference scenario. This can result in significant economic and ecological benefits (Miyamoto *et al.*, 2012; Vaneeckhaute *et al.*, 2013b). Moreover, after three years of fertilization the K supply was negatively correlated with the crop Na uptake (r = - 0.708, p = 0.00) and positively with the soil total Na (r = 0.516, p = 0.001) at harvest time. This effect was clearly visible in 2013 when at increased K dosage, less Na was taken up by the crop and soil total Na amounts were the highest over time for all treatments. Simultaneously, the mean values of K-AL have decreased while Na-AL increased, but, due to high variability, the Na-AL levels were not significantly different from these in 2011 and 2012. This is in accordance with Alam (1999) who observed that addition of K suppressed the rice and tomato Na uptake. As such, the observation is of practical importance for bio-based materials whose utilization might be restricted by their salinity (Alburquerque *et al.*, 2012b), particularly in arid regions. In the case of LF of digestate, it would mean that under lower K conditions less Na is applied while crop Na uptake is higher, subsequently reducing the potential risk of salt build up in the soil. This relationship between K and Na is based on the fact that in their ionic form both are similar in charge and as such might compete for crop uptake since many $K^+$ transporters do not discriminate sufficiently between these two cations (Pardo and Quintero, 2002).

In order to assess salt-affected soils, SAR and $EC_{5:1}$ are used as parameters to indicate potential problems related to soil dispersion (Horneck *et al.*, 2007). Although the total Na in the soil increased significantly in 2013, no significant effect of the fertilizer type was observed on total and plant available Ca and Mg (exception 2012 due to exceptional weather conditions; data not shown). As such, SAR remained far below the critical threshold value of 5 (Horneck *et al.*, 2007). Salt accumulation in soils can also occur by a high dose of sulfate (Vaneeckhaute *et al.*, 2013b; Sigurnjak *et al.*, 2016). After three years of fertilization, no significant effect of the fertilizer type was observed with respect to the soil total and plant available S (data not shown). During the entire field experiment, soil $EC_{5:1}$ values (Table 7.5) were significantly below the critical threshold value of 4 dS $m^{-1}$ (Horneck *et al.*, 2007). These measurements indicate that the tested substitutes for synthetic fertilizer did not pose a risk for degradation of soil properties and fertility after three years of consecutive fertilization. This low risk is largely related to the temperate marine climate, which tends to leach salts from the soil and prevent their accumulation.

### 7.4.6 Fate of trace metals

Along with the potential salinity problem, the use of bio-based materials might also require close assessment of the micronutrient and heavy metal amount, in particular Cu and Zn (Alburquerque *et al.*, 2012b; Sigurnjak *et al.*, 2016) when animal manure is used as a substrate in anaerobic co-digestion. The presence of Cu and Zn in co-digested animal manure originates from their addition to livestock feed as metabolic enhancers. Although Cu and Zn are essential plant micronutrients, their accumulation in the soil could eventually lead to phytotoxicity. Flemish soil environmental quality standards for Cu (17 mg Cu $kg^{-1}$ DM; FSD, 2006) and Zn (62 mg Zn $kg^{-1}$ DM; FSD, 2006) have been imposed, and in this study they were exceeded for Cu in all scenarios, including the reference. Even though no significant effect of fertilizer type was observed when comparing soil Cu and Zn concentrations between seasons 2011 and 2013, it is interesting that in treatment [LFDIG+DIG] where the highest dose of the LF of digestate was applied (50 % in 2011 and 60 % in 2012 and 2013) the lowest mean values of Cu and Zn soil levels were observed (Table 7.5). This is associated to the fact that most of Cu and Zn ends up in solid fraction and thus their addition in relation to the NPK added is lower in LF of digestate as compared to digestate and animal manure. As such no adverse effects on Cu and Zn soil levels are expected when applying LF of digestate as compared to animal manure (whose historical application has contributed to exceedance for Cu in Flemish soil). Moreover, the LF of digestate complies with the European environmental quality standards (100-200 mg Cu $kg^{-1}$ DM and 400-600 mg Zn $kg^{-1}$ DM) for all heavy metals that are specified in the currently proposed End-of-waste criteria (Saveyn and Eder, 2014).

## 7.5 Conclusion

In this 3-year field trial, LF of digestate as a NK- source in treatments with animal manure or digestate had similar effects on biomass yields and soil properties as the classical fertilization regime which uses animal manure and synthetic NK- fertilizers. Nevertheless, experiments on longer term (> 3 years) may be required to fully evaluate the effects of continuous application of LF of digestate on crop growth and soil fertility. Next, due to the high nutrient variability of all bio-based fertilizers (animal manure, digestate and LF of digestate), product sampling should be taken on two occasions, before and during fertilization. In order to move towards replacing mineral fertilizers, a higher composition reliability will be required for bio-based materials. FRUE assessment for open field conditions appeared not to be the best indicator to assess environmental efficiency, since it was highly influenced by weather conditions. Finally, current findings may support recognition of the LF of digestate as a valuable N fertilizer.

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# **CHAPTER 8:** GENERAL CONCLUSIONS AND FUTURE RESEARCH PERSPECTIVES

## 8.1 Introduction

Bio-based materials from nutrient recovery processes have the potential to be used as substitutes for synthetic nitrogen (N) fertilizers. However, their fertilizer performance with regards to crop yield and soil properties remains unclear. As a result, their use is currently hampered by legal restrictions which categorize these materials as waste (i.e. animal manure). The aim of this dissertation was to investigate both short-term and multi-year effects of using, in particular, liquid fraction (LF) of digestate, air scrubber water and mineral concentrate as N fertilizers in horticulture (test crop: *Lactuca sativa L.*) and agriculture (test crop: *Zea mays L.*). This evaluation was conducted through a series of experiments (laboratory, greenhouse and field scale) and compared to utilization of calcium ammonium nitrate (CAN) as one of the most widely used types of synthetic N fertilizers. The main hypothesis of the current dissertation is that LF of digestate, air scrubber water (ASW) and mineral concentrate will not cause significant differences in crop yield, nutrient uptake, soil N dynamics and soil properties as compared to the use of CAN. In this concluding chapter, the main findings of the dissertation are synthesized, recommendations are given and answers are provided for the four research questions:

*i.* Do bio-based materials behave similarly to animal manure or similarly to synthetic N fertilizer with respect to N dynamics? (section 8.2)

*ii.* Does acidification increase N mineralization and N fertilizer replacement value of bio-based materials? (section 8.3)

*iii.* Can bio-based materials be used as synthetic N substitutes in commercial greenhouse production of vegetables? (section 8.4)

*iv.* What are single-year and multi-year effects of using bio-based materials on an open field scale production? (section 8.5)

Suggestions for future research are given in section 8.6.

## 8.2 Do bio-based materials behave similarly to animal manure or similarly to synthetic N fertilizer with respect to N dynamics?

The Nitrates Directive (91/676/EC) poses one of the main obstacles for recognition of bio-based materials as valuable products as it categorizes their fertilizer performance equal to animal manure. Consequently, the utilization of LF of digestate and mineral concentrate (derived from animal manure) as potential substitutes for synthetic N fertilizers is currently hampered.

Through an incubation experiment (Chapter 3) it was shown that animal manure processing increases the N availability of LF of digestate and mineral concentrates as compared to unprocessed raw manure. N release from mineral concentrates exhibited patterns similar to CAN, suggesting that this type of bio-based material could provide plant available N in a similar fashion as synthetic N fertilizers. The N release from LF of digestate (derived from animal manure origin) was higher as compared to raw animal manure, but it followed to a lesser extent (19% lower N release) the pattern of CAN. In general, the observed N release was positively correlated with the amount of $NH_4^+$-N (r=0.898, p=0.015) and $N_{total}$ (r=0.929, p=0.007) that was added through the bio-based materials. Although no significant correlation (r=0.693, p>0.05) was observed between N release and $NH_4^+$-N/$N_{total}$ ratio, it still appears by taking into consideration the observed significant correlations with applied $NH_4^+$-N and $N_{total}$ that the higher $NH_4^+$-N/$N_{total}$ ratio is, the higher N release will be. This was confirmed by the second incubation experiment (Chapter 4) where LF of digestate with higher $NH_4^+$-N/$N_{total}$ ratio ($NH_4^+$-N/$N_{total}$ = 0.82), as compared to tested LF of digestate in Chapter 3 ($NH_4^+$-N/$N_{total}$ = 0.76), exhibited a similar N release pattern as CAN. Increasing the $NH_4^+$-N/$N_{total}$ ratio in LFs of digestate can be achieved through different practices, such as increasing the proportion of animal manure and food waste in anaerobic co-digestion and/or performing mechanical separation of digestate at higher separation efficiencies (e.g. combination of low efficient separation with subsequent centrifuge). With regard to the N mineralization potential, a negative correlation was observed with the C/$N_{organic}$ ratio (r = -0.847, p = 0.033) of the applied bio-based materials. As a result, net N mineralization was not observed for mineral concentrate that contained only 5% of organic N, whereas it was observed for mineral concentrate with 20% of organic N. In general, the contribution from mineralization to total mineral N availability was very limited (6-14%) compared to the mineral N initially present in the bio-based materials. Overall, Chapter 3 indicates that bio-based materials with relatively low C/$N_{organic}$ and high $NH_4^+$-N/$N_{total}$ ratio exhibit similar N dynamics in soil as synthetic N fertilizers. Air scrubber water was not included in the incubation experiment as its N is entirely in mineral form, suggesting that from a N dynamics perspective it will behave similarly as synthetic N fertilizer.

***Recommendation.*** Due to a high variability in nutrient composition of bio-based materials, most of the Member states throughout the EU have introduced in their national legislation a so called N working coefficient, in previous chapters referred to as effective N. In Flanders, a N working coefficient of 60% is currently set for LF of digestate (Chapter 7). Results from this dissertation (Chapter 3 and 4) indicate that in practice the N release of LF of digestate can be higher than 60%. This could potentially lead to unwanted over-application of effective N, suggesting that revision of N working coefficients is required. Results of this dissertation indicate that effective N should be based on the actual mineral N content (i.e. $NH_4^+$-N/$N_{total}$ ratio) of the used bio-based materials rather than a fixed percentage. Moreover, results from the single-year field trial (Chapter 6) indicate that LF of digestate, with effective N experimentally set at 100%, provides similar amounts of N to the crop as CAN. The potential acceptance of LF of digestate as synthetic N substitute on a European level will in future definitely require setting criteria stating when this bio-based material should be considered as synthetic N replacement and when not. These criteria will probably take into consideration dry weight, organic carbon, total N and $NH_4^+$-N/$N_{total}$ ratio as the main parameters. The results from this dissertation indicate that the $NH_4^+$-N/$N_{total}$ ratio of LF of digestate should be above 0.80, if we want this product to exhibit similar N release as synthetic N fertilizer.

Mineral concentrate, as stated earlier (Chapter 2), is of interest in the Netherlands due to the on-going project that aims to examine the performance of mineral concentrate as synthetic N substitute. The assessment has started in 2009 (Velthof, 2015) and currently the decision of the EU is expected to see if the project on mineral concentrate will continue or terminate. The first indications suggest that the EU will impose an obligation on the sum of total N and K concentration in mineral concentrate, which should be c.5-7% (WUR, personal communication). At the moment, mineral concentrate contains 1-2% of total N and 1-2% of total K (Chapter 3 and 6). As such, it seems an impossible mission to reach the limit of 5-7% that probably will be stipulated by the EU. The potential decision of the EU is already acknowledged by some Dutch processing installations, whose focus is shifting from NK mineral concentrate to K mineral concentrate (Nijhuis Industries, personal communication). This will be achieved by integration of the stripper/scrubber unit before membrane filtration to recover N as ASW, and integrating the evaporation unit after the membrane filtration to up-concentrate K. This approach of up concentrating N and K at high cost (i.e. stripping/scrubbing + membrane filtration + evaporator) is of interest in the case if transportation over long distances is required. Otherwise for direct land use, up-concentration of N at lower cost via efficient separation (i.e. $NH_4^+$-N//$N_{total}$ ratio of LF of digestate > 80%) or stripping/scrubbing unit ($NH_4^+$-N//$N_{total}$ ratio of ASW = 100%) would be sufficient.

Finally, it should be noted that the Nitrates Directive was implemented in 1991 with the aim to protect an environment and not to stimulate a fertilizer market. Nevertheless, the Directive should acknowledge that in last 16 years quite some progress has been made in the field of manure processing and that nowadays certain processed manure (i.e. liquid fraction of digestate, mineral concentrate and air scrubber water) can behave similarly as synthetic N fertilizers. The 'need for change' has been recognized by the DG Environment, which has recently initiated a 2 year study (2018-2020) with the Joint Research Centre (JRC) to: i) assess the performance of processed manure on the basis of the available scientific literature, and ii) determine if certain modifications in the Article 2(g) of the Nitrates Directive can be made.

**General conclusions of Research question No. 1:**

- N dynamics of LF of digestate and mineral concentrate tend to better follow the pattern of synthetic N fertilizer than animal manure
- N release from bio-based materials over the course of the growing season is highly dependent on the initial $NH_4^+$-N/$N_{total}$ ratio
- $NH_4^+$-N/$N_{total}$ ratio seems to be a better indicator of effective N than the currently imposed N working coefficient of 60% for LF of digestate

## 8.3 Does acidification increase N mineralization and N fertilizer replacement value of bio-based materials?

The N mineralization potential from mineral concentrate, LF of digestate, digestate and animal manure is quite limited (6-14%) as compared to the mineral N initially present in these bio-based materials (Chapter 3). Regardless of the low N mineralization, mineral concentrate still exhibits similar N dynamics as CAN due to the high initial $NH_4^+$-N/$N_{total}$ ratio, whereas other bio-based materials tend to exhibit a lower N release. Several studies indicated that acidification of bio-based materials stimulate N mineralization, decrease potential N immobilization and delay or inhibit nitrification (Fangueiro *et al.*, 2009; Fangueiro *et al.*, 2010; Fangueiro *et al.*, 2013). Chapter 4 investigated if acidification of animal manure, LF of animal manure, digestate and LF of digestate increases N mineralization and the N fertilizer replacement value (NFRV) of these bio-based materials.

Incubation experiment (Chapter 4) showed that acidification of bio-based materials actually led to a delay in N release, resulting in lower N uptake and a lower fresh yield of *Lactuca sativa* L (pot experiment). Consequently, lower NFRV values were observed in acidified treatments as compared to non-acidified materials and CAN. A recent study by Fangueiro *et al*. (2016) reported that the application of acidified slurry can lead to a more significant immobilization than application of non-acidified slurry and as such reduce the potential N mineralization, especially in soils with a high buffering capacity. The study also mentions the possibility that acidification reduces the activity of nitrifies since the levels of $CO_2$, as their sole source of carbon, are decreased as a result of $CO_2$ emissions that occur during acidification process. In the case of reduced nitrification we should have seen higher presence of $NH_4^+$-N in the soil (Figure 4.1), except if $NH_4^+$-N as an energetically favourable mineral N source has been quickly immobilized. Moreover, it cannot be excluded that acidification triggers the release of substances that inhibit the activity of microorganisms or their enzymes, negatively affecting N mineralization as observed in acidified treatments. This type of negative priming effect was reported by Kuzyakov *et al.* (2000) and was attributed to the release of substances that are toxic to the microbial community. Potential explanation might be that addition of $H_2SO_4$ stimulated lignin degradation and resulted in release of polyphenols. Polyphenols are known to reduce the microbial activity (toxic to a number of bacteria) and form complexes with amino compounds which are very stable and hence not bio-available. This toxic effect has been reported more with regard to N release and N mineralization of crop residues (Agneessens *et al.*, 2014), and at the moment there is no indication if this might be the case with acidified manure. Another toxic effect might have resulted from addition of sulphur and potential increase of aluminium concentration due to pH reduction. In poultry industry, often aluminum sulfate [alum; $Al_2(SO_4)_3$] is added to bedding material to reduce environmental pollution from poultry production. Gandhapudi *et al.* (2006) observed in N incubation experiment that i) alum retarded the nitrification by lowering pH of soil and reducing the potential enzyme activity and ii) acidification of slurry environment could briefly inhibit mineralization by suppressing population of soil organisms. In our study, there was no difference in soil pH between acidified and non-acidified treatments at t=20 and t=120 of the incubation experiment. As such, we can exclude pH effect of acidified materials on microbial community and conclude that observed immobilization was more likely result of material modification in terms of potential $CO_2$ reduction, release of polyphenols or aluminum and sulfur interaction. Finally, the initial mineral N present in acidified bio-based materials at t=0 was reached from day 60 for acidified materials with lower $NH_4^+$-N/$N_{total}$ ratio (< 0.65). The exception was acidified LF of digestate that was characterized by an $NH_4^+$-N/$N_{total}$ ratio of 0.80 where strong N immobilization was observed throughout the entire incubation experiment. The higher the $NH_4^+$-N/$N_{total}$ ratio of an acidified bio-based materials is, the higher N immobilization will occur ($r = - 0.989$, $p < 0.05$).

***Recommendation.*** In this experiment, acidification did not stimulate N mineralization and thus did not increase NFRV of bio-based materials. Rather, acidification of materials rich in mineral N might inhibit or immobilize the mineral N. The immobilization of mineral N could be of interest considering the potential risk of $NO_3^-$ leaching that may occur by applying bio-based materials in an open field cultivation. This is especially relevant at the beginning of the growing season when plants are too small to take up large amounts of N and excess mineral N might leach in case of excess precipitation. However, N leaching losses may be increased if N release is postponed too long after the peak in crop N uptake.

**General conclusions of Research question No. 2:**

- Acidification does not increase the N mineralization potential and NFRV of bio-based materials
- Acidification tends to reduce N release in acidified materials that are rich in mineral N
- The reduction of N release could be of interest with regard to $NO_3^-$ leaching that may occur by applying bio-based materials in an open field cultivation

## 8.4 Can bio-based materials be used as synthetic N substitutes in commercial greenhouse production of vegetables?

The laboratory incubations (Chapter 3 and 4) were conducted in the absence of plants to avoid their potential effects on N dynamics in the soil. However, for bio-based materials to be considered as potential N fertilizers, comparison with synthetic N fertilizer should be done on commercial production scale where crop yield and quality are assessed. Chapter 5 evaluated if the use of LF of digestate and ASW results in similar yield, crop quality and nutrient uptake of *Lactuca sativa L*. as compared to CAN utilization.

Chapter 5 demonstrated that utilization of LF of digestate and ASW in commercial production of lettuce does not cause significant impact on crop yield, crop nutrient concentrations and soil properties at harvest time as compared to CAN. Lettuce in treatments where LF of digestate was used as N source had a difficult start, visible in a smaller crop volume and heterogeneous uniformity. At harvest time, this negative influence was still notable in lettuce uniformity as compared to the CAN treatment. This could be attributed to two crucial variables in LF of digestate, namely electrical conductivity (EC) and presence of ammonia. The former was excluded as a potential cause because lettuce volume and uniformity was not affected in treatments with ASW where even higher soil EC values were measured. Until now contradictory results have been reported concerning digestate phytotoxicity, with $NH_4^+$-N and organic acids in the digestate as factors negatively affecting plant growth (Fuchs *et al.*, 2008; Möller and Müller, 2012). No data about the expected duration of phytotoxic effects were found. Nevertheless, it is believed that phytotoxicity should decrease within a short time period after field application of the digestate (Möller and Müller, 2012). These findings may explain the difficult start of lettuce that occurred in treatments with LF of digestate. Finally, it should be noted that this negative effect on lettuce uniformity and volume was not observed in the pot experiment (Chapter 4), where LF of digestate was applied as a N source in lettuce cultivation. This might be the result of lower material dosage that was given on laboratory scale (application dosage on weight basis). While, on a commercial scale (application dosage on hectare basis) higher dosage of material is required, leading to more visible effects on crop production. No effects on maize development were observed when LF of digestate was used as a N source in an open field cultivation (Chapter 6 and 7).

***Recommendation.*** Regardless of the fact that both LF of digestate and ASW have similar effects on crop yield and soil properties at harvest time as CAN, LF of digestate appeared to be less appropriate for greenhouse cultivation. In utilization of this type of materials, along with their performance also the material handling and perception of horticulturists and the end-consumer is highly important (Case *et al.*, 2017). The unpleasant odor of LF of digestate upon the application and incorporation generated a negative perception among practicing horticulturists (i.e. PCG; Vegetable Research Centre). At the same time, despite the fact that LF of digestate is subjected to a hygenization process (1h and 70°C), there is a risk that consumers have a negative perception of raw eaten vegetable fertilized with processed animal manure such as LF of digestate instead of CAN.

ASW on the other hand was perceived positively by practicing horticulturists as a N source in greenhouse cultivation. In this dissertation, the product was obtained from an acid air washer connected on animal stables. As such, the product is accepted within the Flemish legislation as a substitute for synthetic N fertilizer. This derogation on national level was introduced because N in the product is completely present in mineral form. Conversely, for ASW that is obtained from stripping/scrubbing of animal manure or digestate, it is not yet clear if this can also be accepted as synthetic N fertilizer. Although both products have the same product characteristics, the Flemish legislation currently does not accept this product as synthetic N fertilizer since there is no indication if EU would allow its acceptance. Again a formal derogation needs to be asked to the European Commission (VCM, personal communication).

**General conclusions of Research question No. 3:**

- Air scrubber water and LF of digestate have similar effects on crop yield and soil properties as CAN
- LF of digestate caused a difficult start in lettuce development (reduced uniformity and volume)
- LF of digestate is currently perceived as not suitable for the use in greenhouse cultivation due to its unpleasant odor and potential negative perception of the end-consumer

## 8.5 What are single-year and multi-year effects of using bio-based materials on an open field scale production?

In contrast to laboratory (incubation and pot-trials) and greenhouse experiments, field trials are conducted under uncontrolled conditions in which weather is seen as a key factor in determining the agricultural productivity. To recommend bio-based materials as an equivalent to synthetic N fertilizer, it is therefore crucial to test their efficacy on an open field cultivation. Single-year effects (Chapter 6) of utilizing LF of digestate and mineral concentrate were assessed in *Zea mays L*. cultivation, whereas multi-year effects (Chapter 7) were examined only with regard to the use of LF of digestate as a N source.

In a single-year field maize cultivation, mineral concentrate and LF of digestate proved to be effective substitutes for CAN, resulting in similar crop yields and risk for $NO_3^-$ leaching. In the multi-year trial (3 years), the effect of weather conditions was stronger ($p < 0.05$) than the effect of tested treatments ($p > 0.05$). As such, there were no significant differences between conventional fertilization practice (animal manure + CAN) and the use of LF of digestate, as a (partial) substitute of CAN, in combination with digestate or animal manure. In both trials, application of bio-based materials tended to increase the soil Na concentration since these materials contain considerable amounts of Na. Some arable crops respond positively to applied Na, whereas for other crops (e.g. grass) application too close to the root of the plant should be avoided (Klop *et al.*, 2012; Velthof, 2015). An important observation was that the actual applied N doses differed considerably from the intended N doses. This was caused by differences in bio-based material composition between sampling at the farm/digester versus sampling during the actual fertilization. The highest variability was observed in animal manure which is usually attributed to variation in storage and sampling strategies. Variability in nutrient composition of LF of digestate and mineral concentrate is mostly attributed to non-stable feeding patterns of co-digester as a result of dependence on feedstock market availability. In general, variability may result in significant over- or under- fertilization with N and P (Chapter 7).

***Recommendation.*** In practice a time gap of a few weeks to one month can be observed between the first sampling in storage and actual fertilization (INAGRO, personal communication). One of the measures to reduce variability in batches of bio-based materials between product characterization and actual application would be to reduce the time gap up to maximum 1 week. However, time needed for sampling and analysis may be prohibitive in this respect because farmers are dependent on external laboratory analysis. Additional storage could be introduced where additional flow of bio-based material is not allowed between the first sampling moment (to determine material application dosage) and the moment of fertilization. As a result, the composition of bio-based material in the storage will not change due to the variation in feeding patterns of digesters. Finally, for on-site measurements there might be potential to use Near Infra-Red (NIR) sensors to know the exact composition of the used bio-based material and adjust the previously set application dosage during the moment of fertilization.

Another aspect of nutrient variability is that it might lead to N over-fertilization. In Chapter 6, treatments with animal manure received double dosage of total N (c. 300 kg N $ha^{-1}$) as compared to CAN, LFDIG and MC treatment (c. 150 kg N $ha^{-1}$). Regardless of the difference in N application rate, the post-harvest $NO_3^-$-N residue was below the legal stipulated limit and no significant differences were observed among tested treatments. This does not mean that the Flemish legislation is too severe, but rather that weather is unpredictable and N is quickly responsive to change. In this specific experiment, month July and August (Table 6.1) were characterized by high temperature and precipitation. Under these conditions some N was probably lost via denitrification. Moreover, animal manure contains organic N which by the time of the $NO_3^-$-N residue determination will not be completely mineralized. Nevertheless, it should be acknowledged that determination of the $NO_3^-$-N residue is highly influenced by the weather and it might not always indicate the potential N over-fertilization. From scientific point of view, it would be interesting to combine the $NO_3^-$-N residue with methods that can determine N status in plants. For example, content of chlorophyll and polyphenols has been used in practice as an indicator of the plant N status. These parameters can be measured in the field by portable and affordable tools, however, the readings of chlorophyll and polyphenols can be affected by growth stage, cultivars, soil water and deficiency of nutrients other than N (Muñoz-Huerta *et al.*, 2013).

**General conclusions of Research question No. 4:**

- Under open field conditions the performance of LF of digestate and mineral concentrate was similar to synthetic N fertilizer
- Both LF of digestate and mineral concentrate contain considerable amount of Na. This is an attention point, especially in arid regions and in cultivation of Na sensitive crops
- The nutrient variability in bio-based materials between the moment of sampling at the storage and the actual fertilization needs to be reduced in order to avoid risk of over-fertilization

## 8.6 Future research perspectives

In this section, recommendations for potential future research are presented based on the experience and insights gained during this research.

***Effect of acidification on the microbial community.*** The observed decrease of net N release (Chapter 4) appears to be due to the release of toxic substances that inhibit the activity of microorganisms or their enzymes, negatively affecting N mineralization in acidified treatments. This type of negative priming effect was also reported by Kuzyakov *et al.* (2000). Additional research is needed to confirm this hypothesis. In general, there is a significant lack of knowledge regarding the effect of acidification on the microbial community (Fangueiro *et al.*, 2015).

***Effect of liquid fraction of digestate on crop quality in greenhouse production.*** Until now contradictory results concerning digestate phytotoxicity were reported (Fuchs *et al.*, 2008; Möller and

Müller, 2012), implicating $NH_4^+$-N and organic acid concentrations in digestate as limiting factors in plant growth. Moreover, Wong *et al.* (1983) reported that plant growth might be inhibited not only due to ammonia but also by presence of ethylene oxide in animal manure. These hypotheses were used as possible explanation for the observed delay in lettuce uniformity and volume when LF of digestate was used as a N source. Additional research is required to determine what caused these observations and if a similar effect would be observed on other horticultural crops grown within the greenhouse. This could be assessed via germination bioassays and pot experiments where material application occurs on hectare basis, involving pots with higher volume (> 5L) and thus higher application dosage.

***Legally allowable fertilization rates versus the optimal fertilization.*** Flemish farmers currently apply animal manure and bio-based fertilizers according to the legally allowable fertilization rates which are not always considered to be optimal. For example, even though soil properties prior to the fertilization and crop P requirement indicate that 40 kg $P_2O_5$ $ha^{-1}$ is required, arable farmer will more likely follow the legal maximum limit that allows him application of 80 kg $P_2O_5$ $ha^{-1}$. The farmer will opt for the higher $P_2O_5$ application rate because it will simultaneously allow him to apply more N coming from animal manure. From scientific point of view, it would be interesting to assess the optimal fertilization through incremental rates and compare it to the currently used legally allowable fertilization rates. This can also lead to the determination of economic optimum which is of interest for farmers.

***Variability in nutrient composition of bio-based materials***. Bio-based materials are known for their high variability in nutrient composition (Galvez *et al.*, 2012; EC, 2014a) as compared to manufactured synthetic fertilizers. In this dissertation, the largest variability was observed in animal manure, whereas lower variability was observed in LF of digestate and mineral concentrate (Chapter 6 and 7). As application of animal manure is highly popular by farmers throughout Europe, it is important to determine critical points of variability (i.e. storage, digester outlet, separation outlet, transport tank and fertilizing tank) within the management chain of animal manure and processed materials such as LF of digestate. To study the impact of the management chain on the composition of bio-based materials, special focus should be given to the storage, digester and fertilizing tank that is used during the actual application. Sampling at these points of interest might result in correlations between the sampling point and the nutrient value that we might expect on the moment of the actual fertilization. Alternatively, there is always an option of introducing an additional storage (section 8.5) where addition of bio-based material will not be allowed between the moment of first sampling and the moment of fertilization. Another approach might be the use of Near Infra-Red (NIR) sensors in determining the nutrient content of bio-based materials at the moment of actual fertilization. Current research indicates the potential of NIR (Millmier *et al.*, 2000; Saeys *et al.*, 2005), however further investigation of the applicability of the NIR procedure to all forms of manure, regardless of moisture content is needed. Especially it seems difficult to predict P (Millmier *et al.*, 2000; Saeys *et al.*, 2005), which is next to N the most important parameter in application of bio-based materials.

Finally, it would be interesting to test the use of LF of digestate as the only N source in cultivation of arable crops, without additional supply of animal manure. It appears that higher nutrient variability tends to occur with animal manure, leading to N and consequently also P over-fertilization. LF of digestate is a material that has a high $NH_4^+$-N/$N_{total}$ and N/P ratio. Therefore, it would be interesting to observe the long term effects of applying LF of digestate as the only N source in cultivation of arable crops. Flemish soils are known to be rich in P, and it is believed that crop cultivation could be successful in the first years without additional application of P. Of course, this might lead to a situation where LF of digestate is seen not only as competitor for synthetic N fertilizer, but also for animal manure. On the other hand, this might stimulate farmers to transport their manure to the AD plant where LF of digestate would be produced as a N fertilizer. In this case, question might arise if Flemish biogas sector is ready for higher processing activities and if the transition to centralized biogas sector should occur. Finally, some limitations should be imposed on the minimum $NH_4^+$-N/$N_{total}$ ratio and N/P ratio that are desirable in bio-based materials. Tuning of these parameters also requires future research with regard to performance of mechanical separators. In general, the biggest issue of reducing the direct use of animal manure is simultaneous reduction of organic carbon that is usually supplied via animal manure.

This dissertation clearly shows the potential of bio-based materials derived from animal manure to offset energy intensive and import dependent synthetic N fertilizers. The creation of a playing field between synthetic fertilizers and the manure based alternatives is of utmost importance, however, it presents certain challenges. The nutrient variability will be one of the greatest challenges to address in the future utilization of these materials. Nevertheless, valuable bio-based materials are available, challenges have been identified and now actions are required.

# Summary

Bio-based materials from animal manure and digestate processing contain a significant amount of nitrogen (N) that could potentially be re-used in agriculture as a substitute for synthetic N fertilizers. Legally, however, these materials are still perceived as animal manure. The aim of this dissertation was to investigate the impact of using liquid fraction (LF) of digestate, air scrubber water (ASW) and mineral concentrate as substitutes for synthetic N fertilizer (i.e. calcium ammonium nitrate (CAN)) with regard to crop yield, crop quality, nutrient uptake and soil properties. More specific, four research questions were addressed: i) Do bio-based materials behave similarly to animal manure or similarly to synthetic N fertilizer with respect to N dynamics?; ii) Does acidification increase N mineralization and N fertilizer replacement value (NFRV) of bio-based materials?; iii) Can bio-based materials be used as synthetic N substitutes in commercial greenhouse production of vegetables? and iv) What are single-year and multi-year effects of using bio-based materials in an open field scale production?

In a first study, N release and mineralization potential of several bio-based materials, including LF of digestate and mineral concentrate, were assessed via N incubation experiment and compared with N availability from CAN. The N release appeared to be highly dependent on the $NH_4^+$-N/$N_{total}$ ratio of the material, whereas N mineralization contributed only to a limited extent (6-14%) on top of mineral N initially present in the bio-based materials. In general, our results indicate that further processing of animal manure and digestate can increase the N value of processed bio-based materials. In this study, only the N release from mineral concentrates exhibited similar patterns to CAN, suggesting that this material will provide plant available N in a similar fashion as synthetic N fertilizers. The N release from LF of digestate was higher in comparison to animal manure, but it followed to a lesser extent the pattern of CAN.

From a first incubation experiment it was clear that N mineralization from bio-based materials is quite limited. In a second experiment, we hypothesized that acidification of bio-based materials might increase N mineralization and thus indirectly lead to higher NFRV of animal manure, LF of animal manure, digestate and LF of digestate. The performance of acidified bio-based materials was compared to non-acidified counterparts and CAN with regard to (i) crop development via a pot experiment with lettuce (*Lactuca sativa* L.) and (ii) soil N dynamics via a soil incubation experiment. Findings suggest that acidification does not result in an increased use efficiency of applied N as the NFRVs of acidified materials were 6-13% and 11-18% lower compared to non-acidified materials and the CAN treatment, respectively. This might be explained by an inhibitory delay in the net N release which in our experimental design proved to be negative for crops with short production cycles, as lettuce. This pattern was revealed in the incubation experiments in which net N release in acidified materials remained below that of non-acidified, in this study tentatively attributed to immobilization of mineral N

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In the next phase the agronomic performance (i.e. crop yield, crop quality, fertilizer use efficiency (FUE) and soil properties) of bio-based materials was assessed through a commercial greenhouse production experiment in which LF of digestate and ASW were used as N fertilizers for lettuce production. No significant differences in crop yield and soil properties at harvest time between conventional fossil-based mineral fertilizers and selected bio-based mineral alternatives were observed. However, LF of digestate fertilization resulted in a difficult start with regard to the crop uniformity and volume. The mechanism behind this observation was tentatively attributed to the ammonia presence. This effect diminished towards the end of the experiment, and only the effects on lettuce uniformity remained visible.

Finally, the single-year and multi-year effects of applying bio-based materials in an open field cultivation of maize (*Zea mays* L.) were assessed. In a single-year trial, the use of LF of digestate and mineral concentrate as N fertilizers led to similar crop yield, crop nutrient uptake and soil properties at harvest time as in CAN treatments, suggesting that these materials have a potential to replace synthetic N fertilizers. Moreover, no significant differences were observed with regard to N FUE, apparent N recovery (ANR) and NFRV between treatments with bio-based materials and CAN. The multi-year effects focused solely on the use of LF of digestate. The performance of LF of digestate in combination with animal manure or digestate was assessed and compared to conventional fertilization. During this 3-year study, the effect of weather conditions was stronger than the effect of the applied treatments, suggesting that LF of digestate has potential to be used as a (partial) substitute for synthetic N fertilizers. Importantly, through both field experiments a high variability in nutrient composition of bio-based materials was observed, especially in the case of animal manure.

This dissertation clearly shows that processed bio-based materials, such as LF of digestate, ASW and mineral concentrate, tend to behave more as synthetic N fertilizer than animal manure. With regard to their $NH_4^+$-N/$N_{total}$ ratio, additional N mineralization should not be expected in a significant extent. Hence, producers of these materials, especially in the case of LF of digestate, should tend to increase the efficiency of mechanical separators to obtain materials with even higher $NH_4^+$-N/$N_{total}$ and N/P ratio. Overall, these materials should be considered as products and as such be integrated in the European fertilizer market. Some of their characteristics (form, nutrient concentration, stability) differ from the characteristics of synthetic N fertilizers, however their N performance indicates the potential of labelling LF of digestate, ASW and mineral concentrate as processed mineral fertilizers.

Future research perspectives should address the issue of variability in nutrient composition of bio-based materials, examine mechanisms behind the observed N immobilization in acidified bio-based materials and determine if there is a toxicity effect of LF of digestate with regard to greenhouse crop cultivation.

# Samenvatting

Bio-gebaseerde materialen uit dierlijke mest en digestaat bevatten significante hoeveelheden stikstof (N). Deze zouden potentieel hergebruikt kunnen worden in de landbouw ter vervanging van synthetische stikstofmeststoffen. Wettelijk gezien worden de bemestingseigenschappen van deze materialen echter gelijkgesteld aan die van dierlijke mest. Het doel van dit doctoraat was om de impact te onderzoeken van de vloeibare fractie (LF) van digestaat, effluent van een luchtwasser (ASW) en mineraal concentraat (MC) ter vervanging van synthetische stikstofmeststoffen. De impact werd geëvalueerd op basis van de bodemeigenschappen, nutriëntopname van de gewassen en hun opbrengst. Vier onderzoeksvragen werden vooropgesteld: i) Gedraagt N uit bio-gebaseerde materialen zich gelijkaardig aan N uit dierlijke mest of eerder zoals synthetische stikstofmeststoffen? ii) Verhoogt verzuring de N-mineralisatie en de waarde als stikstofmeststof (NFRV) van de bio-gebaseerde materialen? iii) Kunnen bio-gebaseerde materialen gebruikt worden ter vervanging van synthetische N-meststoffen voor commerciële serreteelten? en iv) Wat zijn de eenjarige en meerjarige effecten van het gebruik van deze materialen in een veldproef?

In een eerste studie, een N-incubatie experiment, werd de N-vrijstelling en -mineralisatie van enkele bio-gebaseerde materialen, waaronder LF van digestaat en MC onderzocht en vergeleken met kalkammonsalpeter (CAN). De N-vrijstelling bleek vooral afhankelijk van de $NH_4^+$-N/$N_{total}$ ratio, terwijl N-mineralisatie maar voor een klein deel (6-14%) bijdroeg bovenop de minerale N initieel aanwezig in de bio-gebaseerde materialen. Onze resultaten tonen wel aan dat behandeling van dierlijke mest en digestaat de N-meststofwaarde kan verhogen. In deze studie vertoonde enkel de vrijstelling uit MC een gelijkaardig patroon aan CAN. Dit wijst erop dat MC op een gelijkaardige manier N zal vrijstellen voor de plant als synthetische meststoffen. De N-vrijstelling uit LF van digestaat was hoger in vergelijking met dierlijke mest, maar volgde in mindere mate het N vrijstellingspatroon van CAN.

Uit het eerste incubatie-experiment kwam duidelijk naar voor dat N-mineralisatie uit bio-gebaseerde materialen gelimiteerd is. Daarom werd in het tweede experiment onderzocht of een verzurende behandeling de N-mineralisatie en NFRV kan verhogen. De waarden werden vergeleken met hun niet-verzuurde tegenpolen en CAN op vlak van (i) de ontwikkeling van sla (*Lactuca sativa* L.) in een potexperiment en (ii) het gedrag van N in de bodem in een incubatie experiment. De resultaten tonen aan dat verzuring niet leidde tot een verhoogde efficiëntie van de toegediende N. De NFRVs van de verzuurde materialen waren respectievelijk 6-13% en 11-18% lager in vergelijking met de niet-verzuurde materialen en CAN. Dit kan mogelijks toegeschreven worden aan een inhibitorische vertraging in de netto N-vrijstelling hetgeen in onze experimenten nefast bleek te zijn voor gewassen met een korte productiecyclus zoals sla. Dit patroon kon ook vastgesteld worden in de incubatie-experimenten waarbij de netto N-vrijstelling uit verzuurde materialen lager bleef dan die uit niet-verzuurde materialen, hetgeen in deze studie werd toegeschreven aan immobilisatie van minerale N.

In een volgende stap werd de agronomische performantie van bio-gebaseerde materialen onderzocht bij commerciële serreteelt van sla met gebruik van LF van digestaat en ASW als N-meststof. Dit werd geëvalueerd op basis van de gewas opbrengst, de kwaliteit van de sla, de efficiëntie van de meststof (FUE) en de bodemeigenschappen in vergelijking met CAN gebruik. Er werden geen significante verschillen in productie en bodemeigenschappen vastgesteld op moment van de oogst tussen de conventionele fossiel-gebaseerde meststoffen en de geselecteerde bio-gebaseerde materialen. Sla die bemest was met LF kende echter een moeilijke start op vlak van gelijkheid en volume. Als reden hiervoor werd de aanwezigheid van ammoniak naar voor geschoven. Naar het einde van het experiment toe bleek enkel uniformiteit nog een aandachtspunt te zijn.

Finaal werd het 1- en meerjarig effect onderzocht van gebruik van bio-gebaseerde materialen aan de hand van een veldproef met mais (*Zea mays* L.). In een 1-jarig experiment werd het gebruik van LF van digestaat en MC als N-meststof onderzocht. In vergelijking met de met CAN behandelde planten werd eenzelfde productie, opname van nutriënten en bodemeigenschappen vastgesteld. Dit suggereert het duidelijke potentieel van biogabeseerde materialen ter vervanging van synthetische fossiele N-meststoffen. Verder werden er geen significante verschillen op vlak van FUE, N-benuttingspercentage en NFRV tussen de planten behandeld met CAN of met bio-gebaseerde materialen geobserveerd. De meerjarige effecten van het gebruik van LF van digestaat in combinatie met dierlijke mest of digestaat werden bestudeerd en vergeleken met conventionele bemesting. Tijdens het 3-jarige experiment bleek het effect van de weeromstandigheden groter dan het effect van de behandelingen. Dit toont aan dat LF van digestaat het potentieel heeft om (deels) synthetische N-meststoffen te vervangen. Een belangrijke vaststelling in beide veldexperimenten was de hoge variabiliteit in nutriënt samenstelling van de bio-gebaseerde materialen, vooral in dierlijke mest.

Deze doctoraatsthesis toont duidelijk aan dat bio-gebaseerde materialen die een verwerkingsstap hebben ondergaan zoals LF van digestaat, ASW en MC een gelijkaardiger gedrag vertonen met synthetische N-meststoffen dan N uit dierlijke mest. Op basis van hun $NH_4^+$-N/$N_{totaal}$ ratio wordt echter geen significante N-mineralisatie na toediening verwacht. Daarom is het aangeraden dat de producenten van deze materialen (vooral van LF van digestaat) pogingen ondernemen om de efficiëntie van hun mechanische scheidingsstap te verbeteren om zo hogere $NH_4^+$-N/$N_{totaal}$ ratio en N/P ratio's te genereren. In het algemeen zouden deze materialen erkend moeten worden als producten en aldus geïntegreerd worden op de Europese meststoffenmarkt. Enkele van hun eigenschappen (vorm, nutriëntconcentratie, stabiliteit) verschillen wel van die van synthetische N-meststoffen maar de N performantie LF van digestaat, ASW en MC suggereert dat ze potentieel gelabeld kunnen worden als verwerkte minerale meststoffen.

Verder onderzoek zou zich moeten toespitsen op de variabiliteit in de nutriëntconcentraties van bio-gebaseerde materialen. Voorts dienen de mechanismen achter de N-immobilisatie in verzuurde bio-gebaseerde materialen bepaald te worden en dient de mogelijkse toxiciteit van LF van digestaat op serreteelten verder onderzocht te worden.

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WRAP, 2016. Digestate and compost use in agriculture. Banbury, UK, pp. 1-32.

# Curriculum Vitae


**PERSONALIA**

| | |
|---|---|
| | Ivona Sigurnjak |
| Address: | Dorenhofweg 19<br>9230 Wetteren, Belgium |
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| E-mail: | ivona.sigurnjak@yahoo.com |
| Date of birth: | 02/01/1988 |
| Place of birth: | Sisak, Croatia |
| Nationality: | Croatian |


**EDUCATION**

| | | |
|---|---|---|
| *Doctoral training – Specialist Courses (30 ECTS)* | *2013 - 2014* | ***Ghent University, Belgium***<br><br>Master courses: Nutrient management, Environmental impact assessment: integrated project, Environmental soil science, Membrane processes in environmental technology, Biosolids and solid waste treatment. |
| *Master of Finance* | *2010 - 2011* | ***University of Zagreb, Croatia***<br><br>Summa Cum Laude – GPA 4.9/5.0 · Faculty of Economics and Business<br><br>Thesis title: Development of population policy through Financial Support in the Republic of Croatia<br><br>Supervisor: Prof. Dr. Anđelko Akrap<br><br>Description: The degree focused on Fiscal policy, Monetary policy of the European Union and Liquidity management |
| *Bachelor of Finance* | *2006 - 2010* | ***University of Zagreb, Croatia***<br><br>Summa Cum Laude – GPA 4.6/5.0 · Faculty of Economics and Business<br><br>Description: The degree focused on Marketing, Public Finance, Banking, Project Organization, Insurance and Risks |
| *High school* | *2002 - 2006* | ***High school of Economics, Sisak, Croatia***<br><br>Description: The degree focused on Marketing, Accounting and Entrepreneurship |

**WORK EXPERIENCE**

| | | |
|---|---|---|
| *Ghent University (Ghent, Belgium)* | *2013 - Present* | ***Doctoral Researcher***<br>- Conducted lab and field scale assessments on utilization of manure and digestate derivatives in the field of horticulture (*Lactuca sativa L.*) and agriculture (*Zea Mays L.*)<br>- Tutored 7 master students during their thesis work |
| *Province of East Flanders (Lam Dong, Vietnam)* | *Oct – Nov 2016* | ***Consultant***<br>- Conducted a market potential analysis for the introduction of new technologies in horticulture in the province of Lam Dong, Vietnam<br>- Developed questionnaires, conducted a survey among Vietnamese farmers, processed data obtained from questionnaires and reported results |
| *Sisak-Moslavina County (Sisak, Croatia)* | *Nov 2012 – Jan 2013* | **Project Administrator**<br>- Worked on execution of FP7 European project 'Improved Nutrient and Energy Management through Anaerobic digestion' (INEMAD)<br>- Developed questionnaires, conducted a survey among Croatian farmers, processed data obtained from questionnaires and reported results |
| *DLV (Zolder, Belgium)* | *Jun – Oct 2012* | **Trainee in agricultural consultancy**<br>- Monitored biogas installations and wetlands in Flanders by examining if their performance is within legally stipulated environmental standards<br>- Supported development of various European projects |

## PUBLICATIONS

*Peer reviewed (A1)*

1. **Sigurnjak, I.**, Michels, E., Crappé, S., Buysens, S., Tack, F.M.G., Meers, E. 2016. Utilization of derivatives from nutrient recovery processes as alternatives for fossil-based mineral fertilizers in commercial greenhouse production of *Lactuca sativa* L. Scientia Horticulturae 198, 267-276. (IF = 1.624)
2. **Sigurnjak, I.**, Vaneeckhaute, C., Michels, E., Ryckaert, B., Ghekiere, G., Tack, F.M.G., Meers, E. 2017. Fertilizer performance of liquid fraction of digestate as synthetic nitrogen substitute in silage maize cultivation for three consecutive years. Science of the Total Environment 599-600, 1885-1894. (IF= 4.900)
3. **Sigurnjak, I.**, De Waele, J., Michels, E., Tack, F.M.G., Meers, E., De Neve, S. 2017. Nitrogen release and mineralization potential of derivatives from nutrient recovery processes as substitutes for fossil fuel based nitrogen fertilizers. Soil Use and Management 33, 437-446. (IF=2.117)
4. **Sigurnjak, I.**, Michels, E., Crappé, S., Buysens, S., Biswas, J.K., Tack, F.M.G., De Neve, S., Meers, E. 2017. Does acidification increase the nitrogen fertilizer replacement value of bio-based fertilizers in *Lactuca sativa* L. cultivation? Journal of Plant Nutrition and Soil Science 180, 800-810. (IF=2.102)
5. **Sigurnjak, I**., Michels, E., Ryckaert, B., Tack, F.M.G., De Neve, S., Meers, E. Liquid fraction of digestate and mineral concentrate as a N fertilizer in maize cultivation. *In Preparation.*

*Book chapters*

1. **Sigurnjak, I.**, Vaneeckhaute, C., Michels, E., Meers, E. xxxx. Manure as a resource for energy and nutrients. In Eds. Meers, E. and Velthof, G. Nutrient recovery book. Wiley Press. *Major Revision*
2. **Sigurnjak, I.**, Michels, E., Meers, E. xxxx. Liquid fraction of digestate and air scrubber water as sources for mineral N. In Eds. Meers, E. and Velthof, G. Nutrient recovery book. Wiley Press. *Major Revision*

*National publications (A4)*

1. **Sigurnjak, I.**, Michels, E., Tack, F.M.G., Meers, E. 2017. The fate of heavy metals in anaerobic digestion process. Ghent University. Comm. Appl. Biol. Sci., 82(4):99-101.
2. **Sigurnjak, I.**, Vaneeckhaute, C., Michels, Ryckaert, B., Ghekiere, G., Tack, F.M.G., Meers, E. 2017. Liquid fraction of digestate as good as chemical fertilizer? – Observations from a three-year field experiment. Ghent University. Comm. Appl. Biol. Sci., 82(4):118-121.
3. Ghyselbrecht, K., Monballiu, A., Somers, M.H., **Sigurnjak, I.**, Meers, E., Apples, L., Meesschaert, B.D. 2017. The issues of nitrogenous components during anaerobic digestion. Ghent University. Comm. Appl. Biol. Sci., 82(4):122-126.
4. Van de Sande, T., Ryckaert, B., De Dobbelaere, A., Annicaert, B., Meers, E., **Sigurnjak, I.** 2015. Bemestingwaarde van spuiwater en dunne fractie van digestaat in bloemkool. Proeftuinnieuws 25(5):22-25.
5. Schollier, C., Michels, E., De Wachter, H., Ryckaert, B., **Sigurnjak, I.**, Meers, E. 2014. Spuiwater in de praktijk. Varkensbedrijf 25(1):22-23.
6. Meers, E., Michels, E., De Wachter, H., Crappé, S., Schollier, C., Ryckaert, B., **Sigurnjak, I.** 2013. Spuiwater van luchtwasser als groene minerale meststof. Varkensbedrijf 24(12):22-23.

*Published reports*

1. De Clercq, L., Michels, E., Meers, E., **Sigurnjak, I.**, Vaneeckhaute, C., Annicaert, B., Cougnon, M., Reheul, D., Vanden Nest, T., Willaert, L., De Dobbelaere, A., Ryckaert, B., Van de Sande, T., Vandaele, E., Lebuf, V.,

Crappé, S., Vercammen, J. 2015. Veldproeven met biogebaseerde meststoffen. Ghent University, pp. 1-50.

2. Lebuf, V., Snauwaert, E., Michels, E., Meers, E., **Sigurnjak, I.**, De Clercq, L., De Dobbelaere, A., Ryckaert, B. 2015. Nutrient recovery from digestate: case study report.

*Proceedings in international conferences*

1. **Sigurnjak, I.,** Michels, E., Crappé, S., Buysens, S., Tack, F.M.G, De Neve, S., Meers, M. Can acidification increase nitrogen fertilizer value of bio-based fertilizers? *Proceedings of the 3rd International conference on manure management and valorization (ManuResource), 27-28-29 November, 2017, Eindhoven, the Netherlands, pp. 58-59.*

2. **Sigurnjak, I.**, Michels, E., Tack, F.M.G., Meers, E. 2016. The fate of heavy metals in anaerobic digestion process*. Proceedings of the 18th International Conference on Heavy Metals in the Environment, 12 to 15 September 2016, Ghent, Belgium.* Electronic version available at: http://ojs.ugent.be/ichmet/article/view/4044/4027

3. **Sigurnjak, I.**, Michels, E., Crappé, S., Buysens, S., Tack, F.M.G., Meers, E. 2016. Can acidification increase nitrogen fertilizer value of bio-based fertilizers? *Proceedings of the 12th International Conference on Renewable Resources & Biorefineries (RRB-12), 30-31 May and 1 June, 2016, Ghent, Belgium, pp. 104-105.*

4. **Sigurnjak, I**., De Mey, J., Michels, E., Crappé, S., Buysens, S., Tack, F.M.G., Meers, E. 2016. Can digestate derivatives be used to substitute fossil based mineral fertilizers? *Proceedings of the 12th International Conference on Renewable Resources & Biorefineries (RRB-12), 30-31 May and 1 June, 2016, Ghent, Belgium, pp. 49.*

5. **Sigurnjak I**., Michels E., Tack F.M.G., Meers E. 2015. Heavy metal concentration in manure and digestate: a simulation model. *Proceedings of the 2nd International conference on manure management and valorization (ManuResource), 2-3-4 December, 2015, Ghent, Belgium, pp. 58.*

6. **Sigurnjak I.**, Michels E., De Neve, S., Meers E. 2015. Nitrogen mineralization potential of bio-based fertilizers. *Proceedings of the 11th International Conference on Renewable Resources & Biorefineries (RRB-11), 3-4- 5 June, 2015, York, UK.*

7. **Sigurnjak I.**, Vaneeckhaute C., Michels E., Ryckaert, B., Vandenbulcke, J., Ghekiere G., Tack F.M.G., Meers E. 2014. Nutrient use efficiency of the liquid fraction of digestate: a three-year field trial. *Proceedings of the 10th International Conference on Renewable Resources & Biorefineries (RRB-10), 4-5-6 June, 2014, Valladolid, Spain.*

8. **Sigurnjak I**., Michels E., Crappé, S., Dolmans, E., Tack F.M.G., Meers E. 2014. Bio-based fertilizer for greenhouse cropping: a lab and field scale assessment. *Proceedings of the 10th International Conference on Renewable Resources & Biorefineries (RRB-10), 4-5-6 June, 2014, Valladolid, Spain.*

9. **Sigurnjak I**., Dolmans, E., Michels E., Ryckaert, B., Lebuf, V., Tack F.M.G., Meers E. 2013. Optimization of nutrient fluxes in European agriculture by using bio-based mineral fertilizer substitutes: a field experiment. *Proceedings of the 1st International conference on manure management and valorization (ManuResource), 5-6 December, 2013, Bruges, Belgium, pp. 155.*

10. **Sigurnjak I**., Michels E., Crappé, S., Dolmans, E., Tack F.M.G., Vergote, N., Meers E. 2013. Bio-based fertilizer for greenhouse cropping: a lab and field scale assessment. *Proceedings of the 1st International conference on manure management and valorization (ManuResource), 5-6 December, 2013, Bruges, Belgium, pp. 18.*

11. **Sigurnjak I.**, Vaneeckhaute C., Michels E., Ghekiere G., Lebuf V., Accoe F., Tack F.M.G., Meers E. 2013. Field and lab scale assessment of biobased mineral fertilizer substitutes. *Proceedings of the 9$^{th}$ International Conference on Renewable Resources & Biorefineries, 5 – 6 – 7 June, 2013, Antwerp, Belgium, pp. 81.*

## TUTORSHIP

1. Maxime Dedecker: '*Replacement of fossil-based fertilizer by bio-based substitutes: a greenhouse trial on Lactuca Sativa L.*', Master thesis, Ghent University.
2. Albana Luta: '*The fate of trace metals in anaerobic digestion of animal manure'*, Master Thesis, Ghent University.
3. An Chen: '*Optimizing nutrient use efficiency in agriculture by utilizing digestate derivatives as biobased fertilizer'*, Master Thesis, Ghent University.
4. Cindy Geraldine Irusta Torrez: '*Liquid fraction of digestate and mineral concentrate as sources for fossil-based N fertilizer'*, Master Thesis, Ghent University.
5. Aruk Ojong Bawak: '*Field scale assessment of bio-based mineral fertilizer substitutes',* Master Thesis, Ghent University.
6. Simon Mosaso Egbe: '*The use of acidification to increase nitrogen fertilizer value of bio-based fertilizers'*, Master Thesis, Ghent University.
7. Talina Zeidan: '*Reducing ammonia inhibition in anaerobic digestion by stripping/scrubbing process'*, Master Thesis, Ghent University.

## TRIVIA – additional participation

1. Transbio summer school 'Advances in biogas technology: from a sustainable input, over conversion process to energy and end-product valorization', September 26-30, Ghent (Belgium) – award for the best poster contribution.
2. Summer school 'Novel approaches towards a sustainable agro-industry', March 21-22, 2016, Milano (Italy) – oral contribution.
3. Summer school 'Environmental technology for treatment and management of the bio-waste manure', August 24-29, 2015, Aarhus University (Denmark)
4. FIRe: Joint scientific workshop: "Innovative strategies to improve the recycling of energy, nutrients and organic matter from waste materials", 26th May 2015, Erfurt (Germany) – oral contribution.
5. FLAMES Summer School In Methodology And Statistics, Flanders Training Network for Methodology and Statistics (FLAMES), Leuven, September 16 - 20, 2013.