Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

Nutrient dynamics in pak choi cultivation fertilized with biogas digestate

– effects of non-nutrient digestate elements and amendment with mineral nutrients

Kristina Weimers

Degree Project • 30 hec
Hortonomprogrammet/Horticultural Science
Alnarp 2020

Nutrient dynamics in pak choi cultivation fertilized with biogas digestate
– effects of non-nutrient digestate elements and amendment with mineral nutrients

Växtnäringsdynamik i en pak choi-kultur gödslad med biogasrötrester

Kristina Weimers

Supervisor: Håkan Asp, SLU, Department of Biosystems and Technology.
Examiner: Malin Hultberg, SLU, Department of Biosystems and Technology.

Credits: 30 hec
Project level: A2E, Master's thesis
Course Title: Independent Project in Biology, A2E
Course Code: EX0856
Subject: Biology

Programme: Hortonomprogrammet/Horticultural Science

Place of Publication: Alnarp
Year of Publication: 2020
Cover Art: Kristina Weimers
Online Publication: http://stud.epsilon.slu.se

Keywords: Biogas digestate, pak choi, *Brassica rapa*, ssp. *chinensis*, 'Joy Choi', greenhouse, soilless production, peat-based growing media, biostimulants, nitrification, nutrient recovery efficiency, phosphorus, sulphur.

SLU, Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Biosystems and technology

Preface

This Master's thesis is the result of a degree project at level A2E at the Master of Science programme in Horticulture, performed at the Department of Biosystems and Technology at the Swedish University of Agricultural Sciences, Alnarp. I would like to take the opportunity to extend my sincere thanks to SLU Partnerskap Alnarp for funding the trials and C4 Energi in Kristianstad for their gift of digestate. I would also like to express my profound gratitude to Håkan Asp for supervision and guidance during the project, Olle Pelayo Lind for advice and generous lending of equipment, Anita Gunnarsson at HIR Skåne for sharing her expertise, and Jean WH Yong for taking the trouble to help me with measurements during early mornings in the greenhouse. Last but not least, I would like to thank my sister Johanna Weimers for invaluable help with irrigation during some critical summer weeks and Tomellen Boije for love and support throughout this journey.

Abstract

Anaerobic digestion in biogas plants produces renewable energy and a residue which is rich in plant nutrients. This residue is called digestate. Today, these digestates are mostly spread directly onto fields as manure. However, due to their high content of plant-available macro- and micronutrients, digestates also have the potential to replace synthetic fertilizers in protected horticulture in soilless systems, contributing to the completion of global energy and nutrient cycles. However, this places more demands on their nutrient composition because, unlike fertilizers in soil systems, fertilizers in soilless systems have to provide the crop with all essential macro- and micronutrients at sufficient levels during the whole cropping cycle. Most reports from trials in soilless systems emphasize that the high ammonium nitrogen (NH_4-N) to nitrate nitrogen (NO_3-N) ratio in digestates constitutes a problem. Furthermore, low concentrations and recovery efficiency of phosphorus (P) and sulphur (S) have been highlighted as limiting factors for growth. However, as all digestates differ in composition, other nutrients might also be present at insufficient levels. Accordingly, it has been recommended that a share of the NH_4-N in the digestate is converted to NO_3-N before application, and that the digestate is supplemented with the missing macro- and micronutrients. However, to date, trials with digestate fertilizers in protected horticulture are limited, and the results are conflicting.

In addition to plant nutrients, digestates contain a complex mixture of partially degraded organic matter and inorganic compounds, including substances that, when derived from other organic source materials, have been reported to have biostimulatory properties. Digestates derived from protein-rich feedstocks have been reported to contain the auxin indole-3-acetic acid (IAA), as well as other plant hormones, at concentrations sufficient to regulate plant development. This has been related to improved growth and nutrient stress tolerance in digestate growth trials.

As part of this thesis, a greenhouse pot trial with pak choi (*Brassica rapa*, ssp. *chinensis*, ‘Joy Choi’) grown in peat was set up to evaluate the plant-nutrient dynamics and biostimulatory effects of a digestate collected at the municipal Karpalund biogas plant in Kristianstad, southern Sweden. The digestate was nitrified in a moving bed biofilm reactor prior to the experiment in order to lower the NH_4-N:NO_3-N ratio. The study was designed with three objectives: (i) to assess the plant availability of macro- and micronutrients in the digestate with particular focus on P and S recovery; (ii) to assess the plant availability and effect of added mineral P, S, magnesium (Mg), manganese (Mn), boron (B), and molybdenum (Mo) to the slightly alkaline digestate; and (iii) to assess the possible biostimulatory properties of the Karpalund digestate (i.e., the effects unrelated to the nutrient content) on plant yield and stress tolerance.

The result showed that the recovery of P and S was significantly lower in the digestate treatment than the mineral control with the same total P and S content (65% for P and 67% for

S was recovered in the above-ground parts of the plant in the digestate treatment compared to 83% for P and 95% for S in the mineral control). The shoot tissue concentrations of S (1.6 g kg^{-1}) and B (10 mg kg^{-1}) in the digestate treatment were below the threshold recommended for optimal growth. The value for P (2.8 g $kg^{-}1$) was within the recommended limits but on the verge of a possible shortage of P. Supplementing the digestate with mineral P, S, Mg, Mn, Mo, and B resulted in sufficient plant tissue concentrations of all nutrients with the exception of S, and in higher fresh matter yields. The supplemented digestate performed as well as the synthetic control with respect to fresh matter yield, and outperformed it with respect to dry matter yield. It might be speculated that the higher dry matter yield was a result of biostimulatory compounds contained in the digestate. However, it cannot be excluded that it was caused by higher concentrations of potassium (K) and chlorine (Cl). Finally, the digestate was not found to alleviate plant response to nutrient stress.

To summarize, the results are promising and show that, after some modifications, the Karpalund digestate can be used successfully as a fertilizer in soilless production of leafy vegetables.

List of content

Abbreviations

DM	Dry matter
EC	Electrical conductivity
FAO	Food and Agriculture Organization of the United Nations
FM	Fresh matter
GHG	Greenhouse gases
IAA	Indole-3-acetic acid
MBBR	Moving bed biofilm reactor
N_{min}	Mineral nitrogen
N_{org}	Organic nitrogen
N_{tot}	Total nitrogen
NH_4-N	Ammonium nitrogen
NO_3-N	Nitrate nitrogen
OM	Organic matter

Introduction

Climate crisis and food crisis

The world's population is expected to reach almost 10 billion by 2050 and there is a need to take action to avoid catastrophic and irreversible climate change (IPCC, 2018; UN, 2019). Thus, agriculture is facing its greatest ever challenge to date (FAO, 2017). Through the adoption of the Paris Agreement on climate change and the 2030 Agenda for Sustainable Development in 2015, most countries of the world agreed to the ambition to limit the global temperature increase to 1.5°C compared to pre-industrial levels, and committed themselves to securing a stable climate and a healthy planet for present and future generations. In addition, among 17 sustainability goals, they also agreed to end extreme poverty and ensure food security (UN, 2015; UNFCCC, 2015). To achieve this, greenhouse gas (GHG) emissions need to be net zero by the middle of the twenty-first century at the latest (IPCC, 2018). Sweden's Climate Act, which was adopted by a broad majority of the Swedish Parliament on the 1st of January 2018 is even more ambitious. It sets out that Sweden's long-term target is to reach net zero GHG emissions by 2045 at the latest, followed by negative emissions (IPCC, 2018; Swedish Climate Policy Council, 2019).

At the same time as GHG emissions are to be drastically reduced, in order to combat hunger and feed a growing world population agricultural production needs to increase globally by almost 50% until 2050, as compared to 2012 levels (FAO, 2017). Big increases in agricultural production have been achieved before in comparable time frames. Between 1961 and 2011, for example, global agricultural production more than tripled (FAO, 2012). However, this historical jump in production was achieved by the introduction of high-yielding cultivars, dependent on large inputs of synthetic fertilizers and irrigation, and on the expansion of cultivated land, which has resulted in negative effects on the natural resource base of agriculture, such as soil degradation and salinization of irrigated areas. It has also resulted in negative effects on the wider environment, including deforestation, nitrate pollution of water bodies, and the emission of GHGs (FAO, 2017). As a result, land and water resources, as well as climate systems, are significantly more stressed today than in the past. Achieving the required intensification of food production under the current conditions, without further compromising the ability of future generations to meet their basic needs, represents one of the greatest challenges ever for agricultural science.

Greenhouse gas emissions from agriculture

Together with forestry and other land use, agriculture accounted for around 23% of global net anthropogenic GHG emissions between 2007–2016 (13% of carbon dioxide [CO_2], 44% of methane [CH_4], and 82% of nitrous oxide [N_2O] emissions) (IPCC, 2019c). The major emission sources are land-use changes (CO_2 from deforestation), cattle farming (CH_4 from enteric fermentation), and crop production (N_2O emissions from organic and synthetic N inputs) (IPCC, 2019c). The agricultural sector is the largest anthropogenic source of N_2O

emissions. This is mainly due to the application of N to soils, where there is a lack of synchronization between the soil's N supply and the crop's N demand; approximately 50% of the N applied is not being taken up by the crop (IPCC, 2019c). However, the IPCC method does not allocate GHG emissions from the production and transport of agricultural inputs to agriculture but to industrial and transportation sectors. If all emissions associated with pre- and post-production in the global food system are taken into account, the emissions from the sector are estimated to be 21–37% of total emissions from human activities globally (FAO, 2019). According to the Swedish national inventory report of 2019, GHG emissions from Swedish agriculture amounted to 7.2 million tons of CO_2 equivalents (Mton CO_2eq) in 2017, corresponding to 14% of national GHG emissions (Swedish Environmental Protection Agency, 2019). However, the figure almost doubles to 13.5 Mton CO_2eq if the GHG emissions from machinery, premises, and production of agricultural inputs, such as synthetic fertilizers and lime, are included (Swedish Board of Agriculture, 2018).

Production of synthetic N fertilizers

Since 1961, the use of synthetic N fertilizers has increased by almost nine times (IPCC, 2019b). Today, fertilizer production accounts for roughly 1.2% of global primary energy demand and is responsible for about the same share of global GHG emissions (Kongshaug, 1998; Swaminathan and Sukalac, 2004). Production of N fertilizers makes up approximately 90% of this energy use (Swaminathan and Sukalac, 2004). Greenhouse gas emissions from the production of N fertilizer are mainly from two sources: CO_2 from the use of fossil hydrocarbons as raw material and fuel in ammonia synthesis, and the N_2O emitted from the production of nitric acid (Brentrup and Palliere, 2008). The production of ammonia—the raw material for most nitrogen containing fertilizers—is largely based on modifications of the Haber-Bosch process and demands significant energy use, with the average energy use being 37 GJ ton^{-1} NH_3 (Williams and Al-Ansari, 2007). When ammonia is oxidized to nitric acid, N_2O is formed as a by-product. This gas has a global warming potential of 265, and its leakage into the atmosphere accounts for the largest share of GHG emissions from mineral fertilizer production (Brentrup and Palliere, 2008; Myhre et al., 2013). However, the implementation of catalytic N_2O cleaning equipment reduces the extent of N_2O leakage (IPCC, 2007). The emissions from European ammonium nitrate production averaged at approximately 6 t CO_2eq ton^{-1} N in 2006 (Brentrup and Palliere, 2008). Yara, which accounts for 60% of the nitrogen fertilizer market in Sweden, reports emissions of approximately 2.9 t CO_2eq ton^{-1} N (Markensten et al., 2018). Despite the relatively low figure, the manufacturing of synthetic N fertilizers is one of the largest fossil energy inputs in Swedish agriculture (Ahlgren et al., 2011). In a study on conventional grain cropping systems in Maryland, United States, Hoffman et al. (2018) found that the production of N fertilizers accounted for 45–46% of total energy use.

Challenges ahead

According to the Food and Agriculture Organization of the United Nations (FAO), the consensus view is that current systems are capable of producing enough food, but to do so in a sustainable manner will require major transformations and new technologies (FAO, 2017). A report from the Swedish Board of Agriculture (2018) states that there are no existing measures that could be applied presently to attain the necessary emission reductions, and that significant leaps in technology are required (Markensten et al., 2018). What is clear is that plant nutrient management will play a central role in the agricultural intensification that lies ahead (IPCC, 2019b). A critical issue will be to develop alternative fertilizer production systems, as 30–50% of current yields are suggested as being attributable to synthetic fertilizers, directly linking food production to the combustion of fossil fuels (Stewart et al., 2005).

Biogas digestate

Circular bioeconomy approach: the potential of biogas and biogas digestates

An environmental challenge, caused by growing populations and increased consumption of natural resources, is the management of waste. When the organic fractions of agricultural and municipal solid wastes are disposed of in landfill sites (which is still the dominant method of handling waste internationally), anaerobic decomposition releases large amounts of methane to the atmosphere (Psomopoulos et al., 2009). Biological treatment of organic residues in biogas plants is an efficient method for reducing the amount of waste and mitigating the methane emissions. The process produces renewable energy and a nutrient-rich digestion residue, which is known as digestate. The digestate, which contains all the nutrients of the treated organic waste, can be used as a fertilizer directly or after further processing, contributing to the completion of global energy and nutrient cycles (Alburquerque et al., 2012; Möller and Müller, 2012). When evaluating the effects of a large number of organic waste products, Tambone et al. (2010) found that digestates had very good fertilizing properties due to a high content of mineralized plant nutrients, including nitrogen (N), potassium (K), and phosphorous (P).

According to calculations made by the World Biogas Association based on data from IEA/OECD (2018), if all currently available and sustainably grown/recovered major feedstocks (food waste, livestock manure, waste from the food and drink industry, crop residues, energy crops, and sewage) in the world were utilized, anaerobic digestion in biogas plants would have the potential to meet 6–8% of the world's primary energy consumption (IEA, 2018; Jain et al., 2019). However, the current estimate of 87 TWh of electricity generation constitutes only about 2% of the industry's potential. Given this, the potential for the growth of the biogas industry is considerable (IEA, 2018; Jain et al., 2019). In Sweden, biogas production accounted for about 2 TWh in 2018 (Klackenberg, 2019). A national biogas strategy has suggested a national biogas use of 15 TWh per year by 2030 (Swedish Energy Gas, 2018). In Sweden, 2.8 million tons of digestate were produced in 2018, and 86% of this was used as fertilizer in agriculture (Klackenberg, 2019). According to Jain et al. (2019), digestate can replace 5–7% of inorganic fertilizer currently in use globally if the potential of the biogas industry is fully utilized.

As the production of biogas and digestates increases, finding sustainable, economical and safe applications of them becomes a matter of urgency. Currently, digestates are mostly spread directly onto fields as manure (Odhner et al., 2015). However, due to their high content of plant-available macro- and micronutrients, digestates also have the potential to replace synthetic fertilizers in protected horticulture in soilless systems. Furthermore, digestates have the potential to improve crop nutrition management in organic horticultural production, where difficulty in matching crop need with nutrient supply has been pointed out as a major limitation (Gunnarsson and Norup, 2018). When used as liquid fertilizers in these systems, digestates can, among other things, meet the demand for an organic quick-release N fertilizer.

However, more knowledge on the dynamics of plant nutrients in digestates is needed, as well as development and optimization of technology for separating the digestate into solids and liquids, and for its application. This thesis will focus on the dynamics of plant nutrients in digestates, as well as their possible biostimulatory effects.

Anaerobic digestate as fertilizer

During anaerobic digestion in the biogas reactor, biogas—which is primarily composed of methane (CH_4) and CO_2—is produced through bacterial degradation of organic matter (Möller, 2015). The resulting digestate is a complex matrix of partially degraded organic matter, inorganic compounds, and microbial biomass (Möller, 2015). Its content depends on the compositions of the biomass feedstock and the process parameters (e.g., operating temperature and hydraulic retention time) (Alburquerque et al., 2012; Risberg, 2015). As reviewed by Möller and Müller (2012), most plant nutrients in the raw substrate are retained during the digestion process, and digestates normally contain all essential macro-and micronutrients in varying proportions, reflecting the composition of the ingoing substrate. Furthermore, due to the mineralization and carbon removal taking place during the microbial anaerobic digestion, digestates are characterized by high ammonium nitrogen (NH_4-N) to total nitrogen (N_{tot}) ratios, an alkaline pH (7.5–8.5), and increased solubilization of essential plant nutrients (Möller and Müller, 2012; Svehla et al., 2020). However, due to the complex matrices and slightly alkaline pH of digestates, the bioavailability of P, S, magnesium (Mg), and iron (Fe) might be decreased due to the formation of solid phase complexes and precipitates such as carbonates, struvite, and phosphates (Möller and Müller, 2012). Moreover, the soil amending properties are influenced by the anaerobic digestion, due to changes in the chemical, organic and biological composition. Thus, the variability in biochemical properties of anaerobic digestates is considerable (e.g., Abubaker et al., 2012; Kirchmann and Witter, 1992; Sogn et al., 2018; Tampio et al., 2016). The variation in nutrient composition and biochemical properties across digestates has been put forward as a mean for a customized and optimized fertilization of crops with different nutrient demands (Risberg, 2015).

Plant nutrients

Nitrogen

Multiple studies on livestock manures have found increased availability of N and N use efficiency after anaerobic digestion compared to the undigested raw material (e.g., Gunnarsson et al., 2010; Möller et al., 2008). In the digester, organic N-compounds are mineralized to NH_4-N. Although a fraction of the mineralized N is assimilated by the digester micro-organisms, precipitated as struvite and ammonium carbonate and volatilized in the biogas stream (Möller and Müller, 2012), the losses are marginal and N_{tot} in the biomass input and the digestate has been reported to remain the same (Risberg, 2015). Consequently, the digestates' NH_4-N content has been shown to be directly related to N_{tot} in the biomass feedstock (Webb and Hawkes, 1985). N-rich feedstocks (i.e., biomasses rich in proteins) will therefore result in digestates high in N. Protein-rich feedstocks include food waste, cereal grain, clover and grass silage, slaughterhouse waste, and manure from livestock which have been fed protein-rich diets (such as poultry and pigs) (Möller and Müller, 2012). Feedstock rich in N and low in C will result in digestates with a high NH_4-N:N_{tot} ratio, as their low C:N ratio renders them a high degradability. Accordingly, feedstock low in N and high in C, such as fibrous feedstock or cattle manure, leads to a low NH_4 -N:N_{tot} ratio (Möller and Müller, 2012). The total N content has been reported to vary from 3.1–14.0% of dry matter (DM) and the fraction NH_4-N of N_{tot} has been reported to vary between 44–81% (Möller and Müller, 2012). During storage, the high pH might lead to a shift in equilibrium from NH_4 to ammonia (NH_3), prevalent as a dissolved gas, which is more toxic to plants than NH_4 and which favors N-losses by NH_3 emissions (Möller and Müller, 2012).

Phosphorus

The phosphorus (P) content of the feedstock has been reported to decrease moderately (< 10%) during anaerobic digestion in biogas plants (Möller and Müller, 2012; Schievano et al., 2011).The slightly alkaline pH in digestates (pH of 7.5–8.5) might cause precipitation of P into poorly soluble compounds such as calcium or magnesium phosphates (e.g., $Ca_3(PO_4)_2$), struvite ($MgNH_4PO_4 * 6 H_2O$), and hydroxylapatite ($Ca_5(PO_4)_2OH$), which decrease the plant-available P fraction, and of which some might be retained inside the reactor (Banks et al., 2011; Güngör et al., 2007; Field et al., 1984; Möller and Müller, 2012). A recovery rate of P as low as 32.8% was reported after digestion of domestic food waste (Banks et al., 2011). The authors speculated that the low recovery was due to precipitation of, for example, struvite ($NH_4MgPO_4 * 6H_2O$) within the digester. However, several studies have found similar crop uptake of P and readily available P pools in soil after application of raw and digested animal slurries, and a P availability comparable to high soluble P fertilizers like TripleSuper-P has been reported (Bachmann et al., 2016, 2011; Loria and Sawyer, 2005; Möller and Stinner, 2010). Zirkler et al. (2014) reported that Mg losses in the fermenter due to struvite precipitation did not correspond with P losses, and concluded that P losses due to struvite formation are negligible when P concentrations are relatively high. However, several growth trials have highlighted low levels of P as a limiting factor for growth when digestate alone is used as fertilizer (Abubaker et al., 2012; Liu et al., 2011; Lošák et al., 2016; Pokhrel et al., 2018; Stoknes et al., 2018; Svensson et al., 2004).

Potassium, calcium, and magnesium

The content of potassium (K) has not been reported to change during anaerobic fermentation, nor has the plant availability of K (Field et al., 1984; Massé et al., 2007; Möller and Müller, 2012). The content and plant availability of calcium (Ca) and Mg, on the other hand, have been reported to decrease (Marcato et al., 2008; Zirkler et al., 2014), partially due to formation of phosphates and carbonates (Möller and Müller, 2012). If struvite is formed, corresponding Mg losses will occur (Zirkler et al., 2014). Since K is the most abundant cation in plant cells, and is not built into organic complexes but is retained in cationic form

(Marschner, 2012), plant-derived digestates and digestates derived from animal slurry, including straw from bedding material, are typically high in directly plant-available K (Table 1) (Gunnarsson et al., 2010b; Liedl et al., 2006; Möller et al., 2008b; Pelayo Lind et al., 2020; Pokhrel et al., 2018; Sogn et al., 2018). A high content of K in digestates might be problematic because it might depress the uptake of Ca, Mg, and micronutrients such as zinc (Zn) and because the amount of K relative to N and P is important for optimized fertilization (Marschner, 2012).

The uptake of Mg is highly influenced by the K:Mg ratio (Marschner, 2012). Therefore, high levels of K become particularly problematic when Mg levels are low due to precipitation during the digestion and low levels of Mg in plant-based feedstocks if plants were grown on soils low in Mg due to unbalanced NPK fertilization (Zalewska et al., 2017). The optimal K:Mg ratio in soil for plant uptake depends on the crop and soil type; however, recommendations in literature suggest that it should not be higher than 3–4:1, but that the optimal ratio is lower (Li et al., 2018; Loide, 2004). As shown in Table 1, plant-based feedstocks resulted in K:Mg ratios above 10:1, and 28:1 when the distiller's waste from ethanol production and cereals was used as feedstock (Abubaker et al., 2012). In addition, digested poultry litter resulted in high K:Mg ratios, probably due to bedding material being rich in K. Nevertheless, many crops have successfully been cultivated in these digestates, probably due to sufficient availability of Mg in soils. However, in soilless cultivation, the balance of the nutrient solution is more critical, and Liedl et al. (2004b) reported Mg deficiency in tomatoes hydroponically grown in a poultry-litter digestate with a K:Mg ratio of 16:1. On the other hand, Stoknes et al. (2018) reported satisfactory yields of tomatoes which had been grown in a nutrient solution with a 10:1 K:Mg ratio; however, extra Mg was provided by the digestate solids (K:Mg ratio of 2:1) which were used as the substrate. Similar to competition with K^+, Ca^{2+} levels which are too high inhibit the uptake of Mg (Marschner, 2012). Furthermore, high applications of NH_4-N fertilizers have been shown to enhance the risk of Mg deficiency due to cation competition, which might pose a problem when using NH_4-N-rich digestates as fertilizers (Lasa et al., 2000; Mulder, 1956).

Table 1*: N:P:K ratio and K:Mg ratio as influenced by digester feedstock.*

Anaerobic digester feedstock	N:P:K*	K:Mg	Scale	Temp.	Reference
Food waste					
Source-separated household waste	10:1:5	8:1	F		Haraldsen et al. (2011)
Food waste	14:1:6		F	42°C	Banks et al. (2011)
Food waste	18:1:10		L	M	Tampio et al. (2016
Food waste, autoclaved at 160°C	16:1:13		L	M	Tampio et al. (2016)
Food waste	40:1:17		L/F	M	Tampio et al. (2016)
Organic household waste	4:1:5		F		Sogn et al. (2018)
Kitchen waste	21:1:9		F		Odlare et al. (2011).
Food and vegetable waste	19:1:20		L	M	Krishnasamy et al. (2012)
Standardized organic household waste	2:1:2a		L	M	Båth and Rämert (1999)
Food waste co-digested					
Slaughterhouse waste and kitchen waste	9:1:2/6:1:2a	8:1	F	M	Abubaker et al. (2012)
Slaughterhouse waste and kitchen waste	13:1:6/10:1:6a	11:1	F	T	Abubaker et al. (2012)
Silage from ley and kitchen waste	13:1:9/8:1:9a	12:1	F	M	Abubaker et al. (2012)
Kitchen waste and animal manure	6:1:5a	10:1	F	N/A	Stoknes et al. (2018)
Animal slurries/manure					
Cattle slurry	5:1:1	2:1	L		Kirchmann and Witter (1992)
Pig slurry	2:1:1	1.5:1	L		Kirchmann and Witter (1992)
Poultry slurry	3:1:1	4:1	L		Kirchmann and Witter (1992)
Cattle slurry	6:1:7	2.5:1	L		Möller et al. (2008)
Manure	4:2:16		L		Sogn et al. (2018)
Poultry litter	5:1:8a	16:1	L	T	Liedl et al. (2006)
Pig manure and maize silage	8:1:4	10:1	N/A	N/A	Lošák et al. (2011)
Plant-based feedstocks					
Distiller's waste from ethanol production and cereals	8:1:4/5:1:4 a	28:1	F	T	Abubaker et al. (2012)
Plant material	9:1:11/5:1:11a	12:1	N/A	N/A	Gunnarsson et al. (2010)
Ensiled red clover and white mustard	12:1:22a	13:1	L	T	Pokhrel et al. (2018)
Crop residues, plant-based residues from food industry	6:1:6a	10:1	F	M	Pelayo Lind et al. (2020)
Crop residues, plant-based residues from food industry	4:1:6ab	12:1	F	M	Pelayo Lind et al. (2020)
Others					
Whey permeate and fish silage co-digested with manure	4:4:24		L		Sogn et al. (2018)
Whey permeate co-digested with manure	4:1:10		L		Sogn et al. (2018)

* N = total N unless otherwise stated; a = mineral N; b = N:P:K ratio after controlled nitrification treatment
L = Laboratory or pilot scale fermenter; F = Full-scale biogas plant
M = mesophilic; T = thermophilic
N/A = Data not available

Sulphur

Sulphur (S), which is assimilated by plants as sulphate (SO_4^{2-}), is introduced into the biogas reactor mainly as a constituent of proteins (Scherer, 2001; Straka et al., 2007). Anaerobic digestion has been shown to clearly decrease the S content (as well as the SO_4-S:S ratio) of the ingoing feedstock by emissions of hydrogen sulphide (H_2S) and other volatile S-containing compounds (Fontaine et al., 2020; Massé et al., 2007; Peu et al., 2011; Wahid et al., 2018). Recently, S losses of up to 30% during anaerobic digestion of cover crops, straw, and cattle manure were reported by Fontaine et al. (2020). Likewise, Wahid et al. (2018) reported S losses of up to 39% in a biogas reactor digesting a mixture of lucerne and forbs, while Massé et al. (2007) observed that more than 50% of S contained in swine manure was lost during digestion. The H_2S formation in the digester has been related to three dominant genera of sulphate-reducing bacteria—*Desulfomicrobium*, *Desulfobulbus* and *Desulfovibrio*—of which the latter is the most ubiquitous in soils (Kushkevych et al., 2017; Saha et al., 2018). The gas has corrosive properties which cause damage to equipment. It is therefore removed from the biogas stream via a number of techniques (Moestedt et al., 2013). In Sweden, the removal of the gas at large-scale plants is achieved through the addition of iron salts to the digester, resulting in the precipitation of dissolved sulphides with ferric or ferrous iron which limits the formation of H_2S (Moestedt et al., 2013). Accordingly, the S speciation in eight full-scale biogas reactors in Sweden was reported to be dominated by iron sulphide precipitates (between 27–62% of the total S), including Fe-monosulphide (FeS), greigite (Fe_3S_4), and pyrite (FeS_2) (Yekta, 2014). The process resembles the process in waterlogged soils, where insoluble sulphides—usually iron sulphides—tend to accumulate (Freney, 1967). When such soils are drained, the iron sulphides will slowly be oxidized to plant-available sulphate by soil micro-organisms (e.g., *Thiobacilli*), followed by an increase in acidity (Freney, 1967). A similar process could be expected upon the application of digestates containing iron sulphides to soils, increasing plant-available Fe and S over time. The rate of oxidation is dependent on the iron sulphide species because iron polysulphides, for example pyrite (FeS_2), are more resistant to oxidation than Fe-monosulphides (Freney, 1967).

There are conflicting reports on the plant availability of S in digestates supplemented with Fe-salts, possibly related to different proportions of Fe and S in the biogas reactor. Low S uptake, similar to an unfertilized control, was reported by Assefa (2013), while Pelayo Lind et al. (2020) reported a high uptake of S, exceeding an inorganic control.

However, low uptake of S cannot be attributed to iron sulphide precipitation alone given that a lower availability of S than expected was also reported where Fe-salts were not added to the digester (Fontaine et al., 2020). The findings have been attributed to high SO_4^{2-} immobilization after digestate application (Fontaine et al., 2020). A net mineralization of S from organic material is generally believed to take place when the C:S ratio is less than 200, although mineralization might occur up to a C:S ratio of 400 (Barrow, 1960; Eriksen, 2005; Eriksen et al., 2004). However, this relationship does not seem to apply to digestates; Fontaine et al. (2020) reported a net immobilization of SO_4^{2-} after application of digestates with C:S ratios as low as 67 (in a cattle-manure digestate), in both the short term and the longer term (180 days). Furthermore, the immobilization of SO_4^{2-} was stronger in the digestate treatment than the undigested raw materials, despite the lower content of easily degradable C and mineral S (Fontaine et al., 2020). In contrast, Elfstrand et al. (2007) reported similar S uptake in leeks with anaerobically digested, composted, mulched, and directly incorporated red clover; however, the uptake was significantly lower in comparison to an inorganic reference and was similar to an unfertilized control. Assefa (2013) analyzed biogas digestates from eight biogas plants in Germany with a broad spectrum of feedstocks, where iron chloride and/or injections of air were used to purify the biogas stream. For six of the digestates, Assefa reported C:S ratios in the liquid fractions ranging between 50 and 76, and for two of the digestates, ratios of 1.4 and 14.8. The solid fraction had a ratio that was approximately twice as high as the liquid fraction for most digestates. Only the digestate with the C:S ratio of 1.4 (a digestate from poultry manure, maize silage, grass silage, grass meal and whole plant silage of rye, barley and sorghum) resulted in S uptake by pak choi plants grown in soil, similar to the inorganic reference; all digestates with a C:S ratio above 30, on the other hand, resulted in S uptake by the pak choi plants at similar rates to the negative control where no S had been added (Assefa, 2013).

In soil, S cycling through immobilization of inorganic S and mobilization of organically bound S is thought to be microbially mediated (Kertesz, 2004; Saha et al., 2018). Microbes contain about 40% C and 1% S, and it has been shown that the addition of cellulose to soils reduced the S content in plants as well as in crop yields (Kertesz, 2004). However, due to degradation of the labile organic fraction during biogas production, digestates contain less and more recalcitrant C compared to the raw materials and typically have narrower C:S ratios; nevertheless, this does not result in higher uptake of S by plants fertilized with the digested material compared to plants fertilized with the raw material (Elfstrand et al., 2007; Fontaine et al., 2020; Molinuevo-Salces et al., 2013). Consequently, the explanation for the observed high SO_4^{2-} immobilization after the application of digestate remains unknown as there is currently limited knowledge on the dynamics of S in soils that have been amended with digestate (Fontaine et al., 2020; Möller and Müller, 2012). Even less is known about the fate of S in digestates when used as fertilizers in soilless systems.

Micronutrients and heavy metals

The anaerobic digestion process has been reported to decrease the bioavailability of several micronutrients, and significant losses during fermentation have been reported for manganese (Mn), zink (Zn), cupper (Cu), and cadmium (Cd) (Bloomfield and McGrath, 1982; Lavado et al., 2005; Marcato et al., 2009b; Massé et al., 2007; Zirkler et al., 2014). In addition, Fe might be precipitated as iron sulphide and iron carbonate (Möller and Müller, 2012). Accumulations of the micronutrients Fe, nickel (Ni) and Mn, and the heavy metal chromium (Cr) during digestion have also been reported, probably due to the attrition of stirrers and pump apparatus in the digester during digestion (Trzcinski and Stuckey, 2011; Zirkler et al., 2014).

Furthermore, the use of trace metals such as Fe-salts as process additives influences the micronutrient balance.

High concentrations of Cu and Zn found in some digestates from pig and cattle slurry (José Antonio Alburquerque et al., 2012; Zirkler et al., 2014) have been thought to originate from metal additives to commercial animal feeds (Demirel et al., 2013). Zirkler et al. (2014) found that Cu and Zn levels in a pig-slurry digestate were above the threshold values of the German decree for bio waste. In contrast, when reviewing eight studies on digestates derived from food waste and agricultural feedstock, Sheets et al. (2015) found that the concentrations of heavy metals in all studies were all well below the threshold levels set by the United States Environmental Protection Agency (Sheets et al., 2015). When monthly samples from three Norwegian biogas plants processing source-separated organic wastes and industrial food waste were analyzed over a twelve-month period, the concentrations of Ni, Cr, lead (Pb), Cd, mercury (Hg), and Cu were found to be low enough to meet the quality criteria for organic farming in Norway, while the Zn concentrations were found to exceed the threshold values for fertilizers in organic farming (Govasmark et al., 2011). A survey of eight commercial anaerobic digestion plants (not including wastewater treatment plants) in Switzerland revealed that heavy metal concentrations found in digestates were mostly below the Swiss threshold values with the exception of Cu, Ni, and Pb, which were occasionally much higher (Kupper et al., 2014). However, the authors did not find any correlation between the contamination level and the composition and origin of feedstock materials, the treatment process, the season of feedstock materials collection, the particle size, or the amount of impurities. They suggest that the random occurrence of high heavy metal content was probably caused by metal-enriched individual lots within the feedstock (Kupper et al., 2014). However, not much is known about the plant bioavailability of heavy metals in digestates, nor their effect on the soil environment, and it is not certain that a heavy metal input from digestate which exceeds threshold levels correlates with negative effects on plants and soil (Kupper et al., 2014).

Organic matter

During the anaerobic digestion, depending on the feedstock's type and recalcitrance, between 20–95% of the C in the feedstock is lost due to the degradation of organic matter into gaseous C compounds, primarily CH_4 and CO_2 (Möller and Müller, 2012). However, although the C content is decreased, the remaining organic fraction is much more recalcitrant compared to the undigested feedstock (Tambone et al., 2019). This might explain why several studies report similar long-term reproduction of soil organic matter after the application of digestate and the undigested or composted feedstock (reviewed by Möller, 2015).

The evolution of the organic matter in the anaerobic digester has been reported to be almost identical to the first, thermophilic phase in a composting process, but not to a humification process (Marcato et al., 2009a). Volatile fatty acids, raw proteins, and hemicellulose are degraded to a high degree while degradation of cellulose is at a rate of approximately 50% and lignin is degraded to a lesser extent still (Molinuevo-Salces et al., 2013).

As a consequence of the degradation of the labile organic fraction, the remaining organic N in the digestate is relatively stable, with a reported net mineralization rate of 8% and 12% of organic N during the first three and six months respectively after application in soil (Moorhead et al., 1987; Gunnarsson et al., 2010). Since the total N content is relatively stable during the anaerobic digestion process, the change in $C:N_{tot}$ ratio is dependent on the degradability of the feedstocks' C and the degree of degradation obtained via the anaerobic digestion (reviewed by Nkoa, 2014).

The dry matter (DM) content is clearly impacted by the feedstock and digester operational parameters, but is typically between 2% and 9% in digestates from large-scale biogas plants fermenting animal manure and food waste (Abubaker et al., 2012; Alburquerque et al., 2012; Fouda et al., 2013). The total carbon content varies between 28% and 47% of the dry matter (Möller, 2015).

Previous trials with biogas digestate as a fertilizer in soil and soilless systems: the effect on plant growth

Scientific trials on the effect of digestates on crop yield and quality have been conducted in a wide range of cropping systems over the last few decades, including field and pot experiments in various soils and trials in soilless systems. Most experiments in soils have been conducted on agronomic crops (e.g., Abubaker et al., 2012; Bougnom et al., 2012; Chantigny et al., 2008, 2007; Gunnarsson et al., 2010; Loria et al., 2007; Möller et al., 2008; Odlare et al., 2011; Šimon et al., 2016; Svensson et al., 2004). However, some field studies have investigated the effect of digestates on horticultural crops including tomatoes, cauliflower, sweet potatoes, lettuce, spinach, Japanese mustard spinach, broccoli, and watermelon (Alburquerque et al., 2012; Barzee et al., 2019; Furukawa and Hasegawa, 2006; Liedl et al., 2006; Nicoletto et al., 2013, 2014, 2017; Yu et al., 2010). Pot and bed experiments in soil on horticultural crops have included leeks, cucumber, and kohlrabi (Båth and Elfstrand, 2008; Båth and Rämert, 1999; Duan et al., 2011; Lošák et al., 2016, 2011).

The soilless systems investigated include pot experiments on pak choi (xiao bai cai, *Brassica rapa* var. *chinensis*) and Indian mustard (*Brassica juncea*) in unfertilized peat-based growing media (Cheong et al., 2020; Pitts, 2019). Also, soilless pot experiments have been performed on tomatoes, lettuce, parsley, basil, and peppermint with nutrient-rich organic substrates such as digestate solids, compost, or peat mixed with organic fertilizers (Pokhrel et al., 2018; Ronga et al., 2019, 2018; Stoknes et al., 2018). Studies on hydroponic systems include lettuce, silver beet, tomatoes, Japanese mustard spinach, and pak choi (Kamthunzi, 2015; Krishnasamy et al., 2012; Liedl et al., 2004b, 2004a; Liu et al., 2009a, 2011; Neal and Wilkie, 2014; Pelayo Lind et al., 2020; Uchimura et al., 2014).

Furthermore, several experiments with digestate as a fertilizer of vegetable crops have been conducted in China, and the results are only published in Chinese. According to a review of Chinese papers by Liu et al. (2009b), studies on digestates as fertilizers of vegetable crops grown in soil have been conducted on celery, Chinese cabbage, pak choi, lettuce, green pepper, mustard, and water spinach (*Ipmoea aquatica*) with good results. Furthermore, trials in soilless systems with modified digestates have resulted in good yields of lettuce, Okinawan spinach (*Gynura bicolor*), the medicinal herb *Gynura divaricata*, cucumber, peppers, and tomatoes (Liu et al., 2009b).

In the above studies, digestates were derived from anaerobic biogas reactors of all scales, from large-scale municipal and industrial biogas plants to small laboratory bioreactors, with different management practices with regards to retention times and operating temperatures. The feedstocks for the fermentation process were manures from stables, crop residues, municipal wastes, wastes from the food industry, and dedicated energy crops, fermented separately or in mixtures. In addition, the digestates were either spread directly as manures, or treated by solid/liquid separation and/or dilution before being fed to plants. As a result, the nutrient composition and organic matrix of the digestate fertilizers differed greatly, making the results difficult to compare. Additionally, when an inorganic fertilizer control was used, it varied in composition between studies, as it was usually designed to resemble common practice in the area (N alone, NPK, NPS, or NPK with the addition of other macro- and micronutrients), further complicating the comparison of results between studies.

Trials in soil

Fertilizing trials involving digestates in soils have shown conflicting results on crop yields and N uptake. However, it could generally be said that if the application of digestates and inorganic NPK fertilizers was based on the same mineral N (N_{min}) content, or the digestates' organic N (N_{org}) fraction was small when applications were based on the total N (N_{tot}) content, the digestate performed as well as, or better than, inorganic fertilizers, provided that: (1) the digestate contained (or was supplemented with) sufficient levels of other essential plant nutrients, especially P, in relation to the nutrient status of the soil; (2) the digestate was properly incorporated into the soil in order to avoid loss of N; (3) applications of the digestate were timed according to crop need, especially in long-cycle crops.

For organic manures, it is generally assumed that the amount of N available to the plant during the first year is closely related to the manure's NH_4^+-N content, and this has been shown to apply to digestates as well (Fouda et al., 2013; Furukawa and Hasegawa, 2006). The N_{org} fraction in digestates is largely unavailable: net mineralization of 8% and 12% has been reported during the first three and six months after application, respectively (Moorhead et al., 1987; Gunnarsson et al., 2010). However, due to differences in chemical composition and the wide variation in C:N ratios between different digestates which highly influences the short-term availability of N, it is difficult to make general statements about N mineralization rates in digestates (Fouda et al., 2013). In addition, N losses due to NH_3 volatilization after application can be considerable (Chantigny et al., 2007; Möller and Stinner, 2009).

Odlare et al. (2011) compared the fertilizer value of a kitchen waste digestate (N:P:K:S ratio of 21:1:9:1) with an inorganic NPS fertilizer (N:P:K:S ratio of 7:1:0:1) and reported lower barley yields with the digestate. The fertilizers were applied at the same N_{tot} rate and the authors attributed the lower yields to the lower content of N_{min} in the digestate. It was also concluded that kitchen-waste digestates generally need P supplementation in order to maintain the same oat and barley grain quality and yields as produced by a traditional inorganic fertilizer (Svensson et al., 2004).

Despite the reported low N_{org} mineralization rates of digestates, several trials in soils have reported similar yields to those of inorganic controls, when digestate and controls were applied at the same N_{tot} rate, supporting the notion that some digestates have a better biodegradability—and thus a higher N recovery rate—than others. However, the high N_{tot} recovery might also be a result of a larger N_{min} fraction; the NH_4^+ share on N_{tot} has been reported to vary between 44–99% across different digestates (Furukawa and Hasegawa, 2006; Möller and Müller, 2012). For example, corn grain yields, as well as the corn's uptake of N and P, were reported to be similar with a pig-slurry digestate and an inorganic NPK fertilizer applied at the same N_{tot} rate (77–85% of N_{tot} was NH_4-N) to corn grown in clay and loam (Chantigny et al., 2008). In addition, a pig-slurry digestate and a NPK (1:1:1) fertilizer applied at the same N_{tot} rate (NH_4-N content not specified) were found to result in the same watermelon yields in a two-year trial on sandy loam in Valencia, Spain (Alburquerque et al., 2012). In both trials, the digestates were incorporated into the soil immediately after application to avoid loss of N (Alburquerque et al., 2012; Chantigny et al., 2008). Moreover, Nicoletto et al. (2014, 2013) reported similar cauliflower and lettuce yields when a digestate from anaerobic fermentation of by-products from fruit distillation (supplemented with P and K to the same levels as the inorganic mineral control) was compared to a NPK fertilizer applied on the same N_{tot} -basis (soil type not specified). However, in the abovementioned studies, the high yields in some of the digestate treatments were contrasted by lower yields in others, which was attributed to inaccurate timing of digestate application, to leaching of N caused by rain, and to low temperatures, slowing microbial mediated nitrification (Alburquerque et al., 2012; Nicoletto et al., 2014).

When digestate and an inorganic fertilizer were applied at the same N_{tot} basis, similar kohlrabi (*Brassica oleracea*) yields were reported by Lošák et al. (2011). The trial was set up as a one-year pot experiment with a medium heavy soil characterized as fluvial soil. The digestate was collected from a digester fed with pig manure and maize silage (fresh weight ratio of 10:16) and had a N_{tot}:P:K:Mg ratio of 8:1:4:0.4. The inorganic control was composed to contain the same levels of N_{tot}, P, K, and Mg as the digestate. In a subsequent two-year pot trial in the same soil, which was found to be low in P, the effect of adding P to a similar digestate was evaluated in terms of yield and quality of the kohlrabi compared to the unamended digestate (digestate: N:P:K:Mg ratio of 6:1:6:0.6; supplemented digestate: N:P:K:Mg of 3:1:3:0.3) (Lošák et al., 2016). Adding P was found to significantly increase kohlrabi bulb yield.

When applied on the same N_{min} basis and thus eliminating the risk that the beneficial effects of the digestate are held back by a shortage of N, higher yields with digestate fertilizers than with synthetic fertilizers, have been reported (Abubaker et al., 2012; Barzee et al., 2019; Duan et al., 2011; Gunnarsson et al., 2010). In a Swedish six-month pot experiment on Italian ryegrass (*Lolium multiflorum*), grown in soil classified as Arenosol, the total biomass was significantly higher in a digestate treatment than in an inorganic control (Gunnarsson et al., 2010). The digestate in the trial was derived from an experimental biogas reactor which was run under mesophilic conditions and fed with grass, red and white clover and sugar beet leaves. The inorganic control was composed of N, P, K, sodium (Na), and chlorine (Cl) in the same proportions as those of the digestate (Gunnarsson et al., 2010). In a recent Californian field trial, the biomass production of tomatoes was reported to be higher after digestate fertilization than when a mineral N control was used (UAN32) (Barzee et al., 2019). The trial was performed in a Rincon silty clay loam which had received a basal N-P-K-Zn fertilizing prior to transplanting. The digestate was an ultra-filtered dairy manure digestate (N:P:K 256:1:336, NH_4-N 90% of total N) which was fertigated to the tomatoes using a drip irrigation system (Barzee et al., 2019). In the same trial, a concentrated mixed food-waste digestate

(N:P:K 6:1:2, NH_4-N 50% of total N) resulted in similar, but not higher, tomato fruit yields as those produced with the inorganic N control (Barzee et al., 2019).

When the fertilizing performance of four digestates of three different origins (slaughterhouse waste and source-separated organic household waste; distiller's waste from ethanol production and cereals; and silage from ley and source-separated organic household waste) derived from different biogas plant reactors operating at different temperature and retention times was evaluated, all digestates were found to performe as well as, or better than, an inorganic control (Abubaker et al., 2012). The trial was set up as a pot experiment where spring wheat was grown in sandy soil, all treatments were applied at fixed N_{min} levels, and the control was a NPK (30:11:24) fertilizer which also contained Mg and Ca (Abubaker et al., 2012). However, although the digestate-treated plants had higher yields than the inorganic control, they had lower yields than a pig slurry control which contained higher levels of P and K (Abubaker et al., 2012). The authors concluded that the lower yield in the inorganic control was most likely a result of the nutrient-poor sandy soil used in the experiment, which had a limited ability to provide the wheat with essential nutrients not contained within the fertilizer. The authors speculated that the relatively low content of P, K, and Zn in the digestates compared to the pig slurry treatment most likely limited the yields in the digestate treatments. Finally, they recommended that when digestates are used as fertilizers on nutrient-poor soils, they should be supplemented with macro- and micronutrients (Abubaker et al., 2012).

In a series of trials over six years in West Virginia, United States, Liedl et al. (2006) studied the effect of poultry-litter digestate, collected from a thermophilic biogas reactor (56.6°C), on crop yield. The digestate was separated into a solid fraction and a liquid fraction in order to evaluate the fractions separately. Field application of the solids resulted in low yields of vegetables and blueberries, while application of the liquids (N_{min}:P:K 5:1:8) to plots containing potatoes, broccoli, tomatoes, or grass resulted in similar or superior yields compared to plots treated with commercial N fertilizers. The application of liquid digestate was determined by equalizing the N_{min} contained within the commercial fertilizer in one treatment, and doubling it in a second treatment. Application based on equal content of N_{min} yielded similar results as with the commercial fertilizer. When the digestate was applied at the double dose, it resulted in significantly higher tomato yields as well as potato tuber yields (Liedl et al., 2006). On turf plots, the liquid digestate performed significantly better in one of the years compared to a commercial mineral fertilizer (Liedl et al., 2006).

Digestate compared to raw or composted substrate

Several trials have compared the fertilizing performance of digestates to the raw or composted substrate. Möller et al. (2008) showed that digestion of liquid cattle slurry has the potential to increase crop yields compared to raw slurry, but only if the digestate was incorporated into the soil shortly after field spreading. In a three-year field study, Loria et al. (2007) found that digested and raw swine manure resulted in similar corn grain yields and plant uptake of N. Båth and Rämert (1999) compared composted and anaerobically digested organic household wastes as N sources for leeks (*Allium porrum* L.) grown in outdoor frames and found that the digestate resulted in a higher fresh weight yield of leeks than the compost or chicken manure (the amount of mineral N in the digestate treatment was 84 kg ha^{-1}, compared to 2 and 11 kg ha^{-1} in the compost and chicken manure, respectively). In a study where the amount of available N applied was equalized, no differences in the yield of leeks were observed between anaerobically digested grass/clover ley, composted grass/clover ley, or inorganic fertilizer (Båth and Elfstrand, 2008).

Trials in soilless systems

The difficulties associated with using digestate as fertilizer to plants grown in soils are emphasized in soilless systems and some new problems arise as well. First, when digestates are used as fertilizers in soils, they are mainly considered as N fertilizers, or N and P fertilizers, with little emphasis on the other macro- and micronutrient content. In soilless systems, on the other hand, the fertilizer has to provide the crop with all essential macro- and micronutrients, which places higher demands on its nutrient composition. Second, the negative effects of an unbalanced nutrient composition and high pH are more prominent in soilless substrates without the nutrient and pH buffering capacity of soils, which increases the risk for salt toxicity and pH-related nutrient deficiencies. Third, the high NH_4-N:NO_3-N-ratio in digestates is a problem for many crops in the absence of nitrifying bacteria (Stoknes et al., 2018). In addition, soilless systems are input-intensive, which makes the systems sensitive to unbalances.

Trials in soilless systems can be divided into pot experiments conducted in active substrates, often peat-based media, and hydroponic trials with digestate solids as substrates or inert/no substrates. In general, peat-based growing media with a high cation exchange capacity (CEC) should, to some extent, be able to buffer against toxicity caused by cations which are too highly concentrated (for example, NH_4^+, K^+, Na^+, Mg^+), while the hydroponic systems do not have this capacity, making them even more sensitive to salinity stress.

Pot experiments in peat-based growing media

Parsley grown with digestate from ensiled red clover and white mustard

Pokhrel et al. (2018) reported low yields of parsley fertilized with the liquid fraction of a digestate derived from thermophilic (50°C) fermentation of ensiled red clover and white mustard (N_{min}:P:K ratio of 12:1:22). The parsley plants were grown in pots filled with a peat-based growing medium enriched with lime, clay, and composted chicken manure. The yield was compared to an inorganic control containing all essential macro- and micro nutrients (N:P:K ratio of 6:1:7) applied at similar N_{min} rates as the digestate, and to a chicken manure extract (N:P:K ratio of 4:1:16) applied at about half the N_{min}-dose to avoid too high an application of K. The digestate treatment resulted in a lower yield than the mineral control but a similar yield to that produced by the chicken manure extract treatment. Pokhrel et al. (2018) hypothesized that the result might be explained by pH-related N-losses in the digestate (pH of 8.0), a theory supported by low mineral N rates in the digestate substrate at harvest compared to the mineral control. However, N did not seem to have been the limiting factor given that the chicken manure extract treatment, which was applied at about half the N-rate, produced a similar yield to the mineral control. In addition, the chicken manure extract contained about twice as much Cl and Na as the digestate, and about the same amount of K, so salinity stress induced by these cations cannot explain the lower yield produced by the digestate treatment. Further, the NH_4 to NO_3 ratio and the NH_4 concentration were higher in the growing medium solution in the chicken manure extract treatment than in the digestate treatment at harvest. Consequently, NH_4 toxicity alone cannot explain the lower yields, even though it might have reduced the yields in both the organic treatments. A factor that differed greatly between the treatments was the P content, which was 50% higher in the chicken manure extract compared to the digestate, and 80% higher in the inorganic control (Pokhrel et al.,2018).

Pak choi grown with food-waste digestate

In Singapore, a greenhouse pot experiment was recently conducted on xiao bai caim, a small variety of pak choi (*Brassica rap* var. *chinensis*) which was fertilized with digestate from a pilot-scale anaerobic digester treating food waste from a university canteen, at different dilution levels (Cheong et al., 2020). The pots were filled with a substrate consisting of 60% coco peat and 40% biochar and were fertigated with the nutrient solutions. A commercial NPK fertilizer with trace elements dissolved in water was used as a mineral control. At harvest after 20 days of cultivation, Cheong et al. (2020) reported that, overall, fresh and dry yields produced by the digestate treatment and the control treament were similar. However, the rates at which the treatments were applied are unclear.

Hydroponics with digestate solids as growing media

Tomatoes grown with digestate from mixed food waste and animal manure, nitrification biofilter included.

Stoknes et al. (2018) reported good yields of tomatoes grown with a digestate derived from source-separated food waste and animal manure (80:20 on dry matter basis) in a cropping system in which the solid parts of the digestate (NH_4-N:P:K ratio of 1.4:1:1.1) were vermicomposted and used as the growing media together with green waste compost. The whole digestate (NH_4-N:P:K ratio of 6:1:5) was used as a fertilizer in a recirculating system with an integrated nitrification biofilter (Stoknes et al., 2018). The unseparated digestate was used because it was shown that using only the liquid phase resulted in tomato plants with a deficiency of P and Cl levels that were too high. When a synthetic mineral control was included, consisting of equal parts Kristalon Brown and YaraLivaTM Calcinit and a peat-based substrate, similar yields to those produced by the digestate treatment resulted. However, the authors highlighted that even though the yield was similar, or higher (not significantly so) in the digestate treatment, and although the plants did not show clear signs of P shortage, P might still have been a limiting factor as the recirculating nutrient solution was very low in P in the digestate treatment.

Basil and peppermint grown with digestate from mixed crop residues and cow slurry

A similar approach to that used by Stoknes et al. (2018), but without the nitrification step, was used by Ronga et al. (2018, 2019), who observed good yields of basil (*Ocimum basilicum* L.), peppermint (*Mentha x piperita* L.), and lettuce (*Lactuca sativa* L.'Chiara'), grown with the solid and liquid fractions of a separated digestate derived from crop residues and cow slurry (70:30 on fresh weight basis). The solids were utilized as growing media and were compared to an agriperlite substrate, and the liquids were utilized as a recirculating nutrient solution and were compared to a commercial hydroponic nutrient solution (Ronga et al., 2018, 2019). In the first trial, the performance of basil (*Ocimum basilicum* L.) and peppermint (*Mentha x piperita* L.) grown in the system was evaluated (Ronga et al., 2018). The results showed that using the solid digestate as the growing medium increased most agronomical traits compared to the control, and that liquid digestate performed in a similar way to the commercial control (Ronga et al., 2018). However, as pointed out by the authors, the surplus of nutrients contained in the solid digestate was not included when treatments were calculated and equalized, and obviously allowed a better crop growth in the digestate treatment. In a subsequent trial on baby leaf lettuce (*Lactuca sativa* L.'Chiara'), the highest shoot dry yields were achieved with agriperlite + liquid digestate and with solid digestate + commercial nutrient solution (Ronga et al., 2019). The combination of solid digestate and liquid digestate performed lower than the other combinations, a result Ronga et al. (2019) attributed to a high NH_4 to NO_3 ratio and high pH in the digestate.

Hydroponics with inert substrates or no substrates

Lettuce grown with digestate supplemented with P and Fe

Higher lettuce yield, a larger number of expanded leaves, and lower nitrate concentrations were found in lettuce plants which were grown in sand and fertilized with digestate (unspecified origin, N:P:K ratio of 26:1:26) compared to a commercial inorganic nutrient solution where all N was present as NO_3-N (Liu et al., 2009). In a subsequent trial, Liu et al. (2011) showed that supplementing the above-described digestate, which apart from being low in P also had relatively low levels of Fe, with K_2HPO_4 and EDTA-Fe significantly increased lettuce shoot biomass and decreased the root to shoot ratio of lettuce compared to the original digestate (Liu et al., 2011).

Lettuce, cucumber, and tomatoes grown with poultry-litter digestate

In experiments involving liquid poultry-litter digestate in hydroponics, Liedl et al. (2006, 2004a, 2004b) found contradicting results for lettuce yields and lower cucumber yields, but, after modification of the digestate, satisfying yields of tomatoes. The trials were part of a larger series of experiments investigating the fertilizing potential of poultry-litter digestate (see "Trials in soil", page 21). The liquid digestate was derived from thermophilic (56.6°C) digestion of poultry litter, and the fertilizing properties were compared with a commercial nutrient solution. In one experiment consisting of three subsequent trials, lettuce (*Lactuca sativa* L. 'Rex' or 'Vegas') was grown using a hydroponic nutrient film technique (NFT) system and fertilized with digestate at different dilution levels. In two of the three trials, the digestate diluted to 100 mg NH_4-N L^{-1} resulted in similar fresh shoot weight and a similar number of leaves as produced by the inorganic control (in which all N was present as NO_3-N). However, in a following trial with digestate diluted to 50, 100, and 150 mg NH_4-N L^{-1}, the digestate treatments resulted in lower yields. The N:P:K ratio was 1:9:5 in the 100 mg N L^{-1} dilution (Liedl et al., 2004b). In hydroponic trials on tomato plants (*Lycopersicon esculentum* Mill. 'Trust'), the poultry-litter digestate was found to result in slow growth and small fruit yields, which was attributed to high NH_4-N and low Mg concentrations (Liedl et al., 2004a). By removing approximately 75% of the ammonia through heating and air sparging, replacing N with $Ca(NO_3)_2$, and by adding $MgSO_4$, toxicity and deficiency symptoms disappeared and satisfying fruit yields were obtained, although the yields were not as high as those produced by the inorganic control (Liedl et al., 2004a). In trials involving hydroponic cucumber (*Cucumis sativus* L., 'Manar') production, the average fruit weight decreased from 84 g to 75 g when plants were grown with the poultry-litter digestate at the same N_{min} concentration as a commercial hydroponic fertilizer; however, the percentage of fruits classified as grade 1 increased (Liedl et al., 2006). It should be noted that the pH in the above experiments using poultry-litter digestate was adjusted with phosphoric acid to 5.6–6.0, which led to high P-levels (Liedl et al., 2006, 2004a, 2004b).

Tomatoes grown with food-waste digestate

Difficulties with growing tomatoes hydroponically in digestate were also reported by Neal and Wilkie (2014). They reported that only one of four tomato plants survived when grown hydroponically in aerated containers and fed an unnitrified kitchen-waste digestate. Interestingly, a share of the NH_4-N in the digestate nutrient solution in the container of the surviving tomato plant had converted to NO_3, indicating that nitrification may occur by simple aeration within hydroponic reservoirs. This was confirmed in a subsequent laboratory nitrification test where the NO_3-concentration in an aerated digestate increased from 9.13 ppm to 17.25 ppm in 48 hours (Neal and Wilkie, 2014).

Chard in food and vegetable-waste digestate

Krishnasamy et al. (2012) found that the growth of chard (*Beta vulgaris* subsp. *vulgaris*), cultivated in a hydroponic setup with unaerated containers filled with nutrient solutions, was significantly lower when using diluted food and vegetable-waste digestate (N:P:K ratio of 19:1:20) than when a commercial hydroponic solution (N:P:K ratio of 4:1:6) was used. At harvest after 50 days, the highest yielding digestate-fed plants weighed 38 g compared to 462 g in the control (Krishnasamy et al., 2012). The authors suggested that low dissolved oxygen levels and NH_4-concentrations that were too high (24–47 mg^{-1} in the diluted digestate solutions) might be responsible for the low yields (Krishnasamy et al., 2012). Low P-levels might also have contributed to the low yields.

Nitrification of digestate in hydroponic cultivation

To overcome the problems in soilless systems with NH_4-toxicity related to high NH_4-N content in digestates, some researchers included a nitrifying step in their experimental set-up. As described above (see “Hydroponics with digestate solids as growing media”, page 24), Stoknes et al. (2018) included a nitrification biofilter in their hydroponic system with good results on fruit yields of tomatoes. Other experiments where nitrification was included are presented below.

Lettuce grown with a nitrified dairy-manure digestate

Kamthunzi (2015) found that nitrification of dairy-manure digestate resulted in significantly higher yields of lettuce in a hydroponic hybrid NFT-ebb-and-flow system than the raw digestate (Kamthunzi, 2015). The low growth in the raw digestate treatment was concluded by the author to be due to ammonia toxicity (tested solutions contained 70 mg L^{-1} and 223 mg L^{-1} NH_4-N), resulting in inhibition of root growth (Kamthunzi, 2015). Although the nitrified digestate contained very low levels of P (2 mg L^{-1} PO_4^{3-}) which was caused by losses during the nitrification treatment, this resulted in vigorous plants whose shoot fresh yield was 70–75% of the commercial control (Kamthunzi, 2015).

Japanese mustard spinach in sewage-sludge digestate

Positive effects of digestate nitrification were also reported by Uchimura et al. (2014), who compared the effect of a nitrified sewage-sludge digestate with raw digestate and a commercial control with respect to crop growth and nitrogen use efficiency of Japanese mustard spinach (*Brassica rapa* var. *perviridis)* grown in a NFT-system (Uchimura et al., 2014). The digestate nutrient solutions were supplemented with mineral P, Ca, Mg, and SO_4 to the same concentrations as the commercial control (Uchimura et al., 2014). At harvest three weeks after transplanting, the plants that had been fed nitrified digestate showed the same shoot fresh weight and nitrogen uptake as the commercial control, while the raw digestate resulted in significantly smaller plants (Uchimura et al., 2014). (The concentrations of N in the nutrient solutions were not specified.)

Pak choi in plant-based digestate

In a hydroponic trial involving pak choi (*Brassica rapa* ssp. *Chinensis cv.* 'Joy Choi'), Pelayo Lind et al. (2020) compared the effects of different nitrification and digestate input strategies on pH-dynamics in a NFT-system, and how these in turn, as well as the NH_4-N concentration in the system, affected plant growth. The digestate used in the experiment was collected from a Swedish biogas plant treating crop residues and plant-based residues from the food industry with the addition of iron chloride, which was operated under mesophilic conditions with a retention time of 80 days. Prior to the experiment, solids were removed from the digestate by filtration. The N_{min}:P:K ratio in the resulting liquid fraction was 5.5:1:6.3, and nitrification of the digestate in an external moving bed biofilm reactor resulted in a ratio of about 4.4:1:6.1. A commercial control, composed of Kristalon TM Indigo and Calcinit TM, Yara, was diluted to the same EC (2.0 mS cm^{-1}) as the digestate nutrient solution. The use of both external and integrated nitrification bioreactors was evaluated, as well as different pH-managements regimes (no synthetic pH-regulation was used). The highest shoot dry weight among the digestate treatments was observed in the system with the lowest pH oscillations— i.e., the pH was regulated continuously by adding 0.02 liters of unnitrified digestate when the pH in the recirculating nutrient solution dropped below 5.8, in a system where the nitrification bioreactor was integrated. This system resulted in plants with shoot dry weights that were not significantly different from the inorganic control. However, cultivation time of an additional week (= 20%) was needed in order for the digestate-grown plants to catch up with the inorganically grown plants. The authors hypothesized that the need for a longer cultivation time might be related to a need for acclimatization when seedlings were moved from the pure inorganic synthetic solution in which they were sown to the digestate nutrient solution (Pelayo Lind et al., 2020).

In summary

High NH_4-N:NO_3-N ratio

Trials in soil have shown that the N fertilizer value of digestates is closely related to the NH_4-N content (Fouda et al., 2013; Furukawa and Hasegawa, 2006; Gunnarsson et al., 2010a). When applied to soils, the NH_4-N is converted to NO_3 by micro-organisms; however, if the soil has a low microbial activity, nitrification might be slow, which can result in, for example, reduced growth at low temperatures due to a direct impact of the NH_4-N:NO_3-N ratio on growth rate and C and N partitioning, or an indirect effect due to the slower transport of NH_4-N in the plant than NO_3-N (reviewed by Gunnarsson et al., 2010). Despite this, successful cultivation of short-cycle leafy *Brassica* crops have been reported in limed peat, with a supposed low initial microbial activity, without any prior nitrification of the digestate (Cheong et al., 2020). There are also a few reports on successful hydroponic cultivation of lettuce in unnitrified digestate (Liedl et al., 2004a; Liu et al., 2009). However, most reports from trials in soilless systems emphasize that the high NH_4-N:NO_3-N ratio in digestates constitutes a problem, with negative impacts on growth and biomass production (Liedl et al., 2004a; Neal and Wilkie, 2014; Pokhrel et al., 2018; Ronga et al., 2019). Accordingly, nitrification of digestates prior to application, or in integrated biofilters in the system, has been reported to be successful and has resulted in, for example, tomato, lettuce, and pak choi yields similar to those of commercial fertilizers (Pelayo Lind et al., 2020; Stoknes et al., 2018).

Low concentrations of P, K, S, Mg, Fe, and Zn

Several authors have highlighted low P levels as a limiting factor for growth when digestate alone is used as fertilizer (Abubaker et al., 2012; Liu et al., 2011; Lošák et al., 2016; Pokhrel et al., 2018; Stoknes et al., 2018; Svensson et al., 2004). Even after maximizing the levels of P by using the digestate solids as the substrate (N_{min}:P:K ratio of 1.4:1:1.1) and the whole digestate instead of only the liquid fraction as the nutrient solution (N_{min} -N:P:K ratio of 6:1:5), the risk of P-limitation was reported in tomatoes (Stoknes et al., 2018). Accordingly, the supplementation of P in digestate nutrient solutions has been reported to increase yields of lettuce and kohlrabi (Liu et al., 2011; Lošák et al., 2016). Yields of lettuce and tomatoes have also been found to increase after additions of Fe and Mg, respectively, to digestate nutrient solutions low in these nutrients (Liedl et al., 2004b; Liu et al., 2011). Furthermore, a low S uptake by plants has been reported in digestate fertilized crops (Assefa, 2013; Elfstrand et al., 2007; Fontaine et al., 2020) and levels of K and Zn in digestates which are too low have also been reported (Abubaker et al., 2012). Consequently, it has been recommended that digestates might need supplementation of macro- and micronutrients before being utilized as fertilizers.

Higher yields with digestate

In the above-reviewed trials, the effect of digestate application to cropping systems has been related to the ability of the digestates to provide the crops with essential macro- and micronutrients. Lower yields in digestate treatments have successfully been addressed by converting NH_4-N to NO_3-N and by supplementing missing nutrients. Higher yields from digestate treatments than from commercial synthetic fertilizers have been related to the insufficient supply of nutrients by the latter, which has been the case when, for instance, the commercial fertilizer was composed of only macronutrients and the soil/substrate had a limited ability to provide the plant with the missing nutrients (Abubaker et al., 2012; Barzee et al., 2019; Gunnarsson et al., 2010). However, as will be reviewed in the next section, there are several reports of beneficial effects of digestates on plant growth that are not related to their content of plant nutrients.

Benefits of digestates beyond their nutrient content

Most research into the effects of digestates on plant growth and quality has evaluated their fertilizer value, and plant yields have been related to their content of NH_4-N and other nutrients. However, there are indications that positive effects of digestates on plant growth may go beyond their nutrient content (Kostenberg et al., 1995; Li et al., 2016; Scaglia et al., 2017, 2015). As a result of the anaerobic digestion of the feedstock, digestates contain a complex mixture of partially degraded organic matter and inorganic compounds, including monosaccharides, free amino acids, fatty acids, polypeptides, nucleic acids, vitamins, phytohormones, etc., as well as compounds of higher molecular weight (Möller and Müller, 2012; Scaglia et al., 2017). The same substances, when derived from other organic source materials, have been reported to act as biostimulants (Calvo et al., 2014; du Jardin, 2015). A plant biostimulant has been defined as any micro-organism or substance that, regardless of nutrient content, is applied with the aim of enhancing abiotic stress tolerance, nutrition efficiency, and/or crop quality traits (du Jardin, 2015). However, other definitions have also been proposed (Calvo et al., 2014).

A problem when trying to predict, or draw any conclusions about, the biostimulant properties of complex mixtures of components such as digestates is that they contain compounds with known modes of action, and compounds of unknown function, making it difficult, or even impossible, to identify with certainty the components responsible for the biological activity (Yakhin et al., 2017). Digestates have been reported to contain several plant hormones, such as gibberellic acid and abscisic acid (ABA) (Feng et al., 2011; Li et al., 2016; Shen, 2001). However, the vast majority of studies on biostimulant properties of anaerobic digestates have attributed the observed biostimulatory effects to the presence of plant-active levels of the phytohormone auxin and auxin-like compounds (Kostenberg et al., 1995; Li et al., 2016; X. Li et al., 2018; Lu et al., 2019; Scaglia et al., 2017, 2015).

Auxins are a class of phytohormones which are fundamental in regulating most aspects of plant growth and development and which are also involved in the response to biotic and abiotic stress (Taiz et al., 2015). Among the auxins, indole-3-acetic acid (IAA) is the most abundant naturally occurring auxin in higher plants (Taiz et al., 2015). Auxin signaling and transport has been reported to play important roles in plant responses to nutrient deficiency stress by inducing a rapid alternation in root architecture in response to low soil nutrient concentrations (reviewed by Pandey et al., 2019). However, it is unclear whether, and in what doses, exogenous auxin can facilitate this stress response. It is known that exogenous auxin alters elongation of the primary root and stimulates the formation of lateral roots and root hairs, a morphological change which is also induced by the plant in response to low levels of immobile nutrients such as P, in order to assist the exploration of a larger soil volume and increase the absorbing root surface area (Casimiro et al., 2001; Torrey, 1950). The P-starvation induced change in root system architecture has been observed in *Arabidopsis* to be caused by changes in auxin transport and increased sensitivity to auxin rather than to increased auxin synthesis (Hammond et al., 2003; López-Bucio et al., 2002; Nacry et al., 2005). Plant responses to N, S, and Fe starvation, on the contrary, involve the upregulation of genes involved in auxin biosynthesis, increased levels of auxin in roots/root tips, and increased root growth which allows the root system to penetrate a larger volume of soil (reviewed by Pandey et al., 2019). With severe K deficiency, reduced root growth was found to be related to lower auxin concentrations and transport due to the crucial role of K-transporters in auxin transport in roots (reviewed by Pandey et al., 2019). Accordingly, exogenous auxin application has been reported to alleviate K deficiency in tobacco plants through increased lateral root formation and elongation (Song et al., 2015).

Auxin and auxin-like compounds in digestates

Auxin-like activity was found in the water-soluble organic fraction of pig-slurry digestate—i.e., the fraction readily available to micro-organisms and plants (Scaglia et al., 2015). The findings were confirmed in a subsequent study, where auxin-like properties, similar to those reported for recognized biostimulants, were found in the water-soluble fraction of five digestates from large-scale biogas plants in Italy (Scaglia et al., 2017). The authors identified the major factors responsible for the observed auxin-like effect to the presence of two auxin active forms (IAA and hydroxyphenylacetic acids) and auxin-like molecules typically produced during organic matter anaerobic degradation (fatty acids, linear carboxylic acids, aromatic carboxylic acids, and amino acids). They concluded that anaerobic fermentation is a useful way to produce biostimulants (Scaglia et al., 2017). Increased IAA concentration during anaerobic fermentation was also reported by Kostenberg et al. (1995), Li et al. (2016), and Sensel and Wragge (2008). Kostenberg et al. (1995) reported almost ten times the concentration of IAA, present in its free form, in digested coffee waste than in undigested waste (approximately 23.5–33.0 nmol/g dry weight), and that the total amount of IAA was almost twice that of raw coffee beans (Kostenberg et al., 1995). Li et al. (2016) reported higher content of plant hormones, including IAA, in animal manure after anaerobic digestion: both IAA and ABA were found in concentrations sufficient to regulate plant development (13–23 mg/L and 13–36 mg/L respectively). However, the authors also reported significant losses of plant hormones during storage of digestate, with higher losses at higher temperatures. For example, the IAA concentration decreased by 26.2%, 48.1%, and 70.5% at 4, 20, and 37°C, respectively, after 88 days of storage (Li et al., 2016).

IAA is a common product from the degradation of the aromatic amino acid L-tryptophan via several pathways, present in, for example, soil bacteria and fungi (Frankenberger and Arshad; 1995; Gruen, 1959; Lynch, 1985), microorganisms found in animal intestines or faeces (Yokoyama and Carlson, 1979), and anaerobic ruminal microbes (e.g., Mohammed et al., 2003). Although there are large environmental and microbial differences in, for example, animal intestines/rumens/faeces and anaerobic biogas reactors, and it has been shown that environmental factors like temperature, pH, N- and C source, as well as the strains of the micro-organism, largely influence the types and concentration of microbial metabolites produced from L-tryptophan (reviewed by Li et al., 2018), it was recently shown that IAA is also synthesized from L-tryptophan in biogas reactors. In a batch reactor experiment, the levels of tryptophan and its metabolites were monitored for 30 days during anaerobic digestion of dairy, chicken, and pig manure under thermophilic conditions (55 ± 1°C) (Li et al., 2018). During the first 15 days, the levels of L-tryptophan rapidly increased as a result of the hydrolysis of large amounts of proteins present in the animal manure (10%–25%), followed by decreased concentrations as L-tryptophan was transformed into other products or was mineralized to CH and CO (Li et al., 2018). At the end of the experiments, the only indolic derivates detected in the liquid digestate were L-tryptophan, IAA, skatole, and indole. IAA concentrations had increased by between 3–5 times compared to the initial values, and were about 133 µmol L^{-1} (23.2 mg L^{-1}) in the dairy and pig-manure digestate, and about 76 µmol L^{-1} (13.3 mg L^{-1}) in the chicken-manure digestate. Skatole was maintained at low levels in the digester and indoles were almost completely absent after 30 days. In subsequent experiments using single indolic components, two pathways for L-tryptophan metabolism under alkalescent anaerobic conditions were reported: (i) L-tryptophan was converted to skatole via IAA but the conversion from IAA to skatole was completely inhibited by soluble carbon, which is available in increasing amounts during animal-manure hydrolysis; (ii) L-tryptophan was directly converted to indole, which was then degraded in a process enhanced by other amino acids produced from protein hydrolysis in the digestate. The authors concluded that the high levels of IAA and low levels of indole found in the animal-manure digestates were probably a result of the abundant mono saccharides and amino acids produced from hydrolysis and conversion of carbohydrates and proteins, which influenced the metabolism of tryptophan into IAA, and that, theoretically, the concentration of IAA in the digestate might be increased by the addition of an exogenous carbon source (Li et al., 2018).

In what concentrations are IAA active?

When IAA was applied as soil drench to established corn seedlings, it was reported that at concentrations between $2.2 * 10^{-5}$ and $2.2 * 10^{-2}$ mg kg^{-1}, soil had a positive effect on plant growth, especially on root growth, while 22 mg kg^{-1} soil had a significant negative effect (Sarwar and Frankenberger, 1994). Ahmad et al. (2008) reported that L-tryptophan-enriched compost, resulting in an IAA concentration of 3.34 mg kg^{-1} in the compost, had a positive effect on maize growth when applied at a rate corresponding to 1032 mg IAA ha^{-1} (Ahmad et al., 2008).

Biostimulatory effect of digestate alkaline extracts

In accordance with how humic substances have traditionally been studied (and defined) through alkaline extractions, the same method of extraction has been applied to the study of bioactive compounds in digestates. Although the alkaline soluble hydrolysates from digestates are sometimes also referred to as “humic substances” or “humic-like substances” (Ertani et al., 2013a; Scaglia et al., 2017), these must be considered as strictly operational uses of the term (= the alkaline soluble fraction), as the stabilization of the organic matter in the digester is reported to be due not to a “humification” process (i.e., it has no resemblance to the process in the later phase of composting), but to the accumulation of stable compounds in the dry matter, such as hemicellulose-like and lignin-like molecules, as described by Marcato et al. (2009a), for example. Therefore, the broader term “complex organic material” proposed by

Jardin (2012) seems more appropriate when denoting the alkaline soluble compounds in digestates. It has to be kept in mind, though, that research on this fraction does not necessarily add to the understanding of properties of the whole digestate, as the alkaline treatment might ionize compounds that would never dissociate within the pH range of the digestate itself, nor the substrates, soils, or hydroponic systems where they are applied (Lehmann and Kleber, 2015; Lynch, 1985). Furthermore, alkaline extraction only extracts between 30–50% of the organic carbon, leaving the potential biostimulatory properties of the rest unexplored (Lehmann and Kleber, 2015). Finally, extracts from digestates contain plant nutrients, which have to be accounted for when interpreting the results. Despite the difficulties, the results from these trials might provide some information on the potential biostimulatory effects of digestates.

The extracts comprise a mixture of molecules with a molecular weight ranging from 5 to several hundred kDa, including a variety of aliphatic and aromatic C atoms bonding to different basic and acid functional groups (Montoneri et al., 2011). The first reported trial involving alkaline extracts of digestates was performed with an extract from livestock-manure digestate by Ertani et al. (2013), who reported the prescense of IAA, phenolic acids, and flavanoids at concentrations which, according to the authors, can be physiologically active when applied to plants; the IAA concentration was reported to be 32.63 nmol L^{-1} ($5.71*10^{-3}$ mg L^{-1}). The extract was found to have auxin-like effects when assessed in a watercress root growth bioassay and, further, to improve growth of hydroponically grown maize plants (Ertani et al., 2013a). (The addition of the extract caused only a low increase in N and K content, which is why the authors state that it is unlikely that the observed effect on the plants was due to the presence of these ions.)

Sortino et al. (2014) reported a significant increase in the height of tomato plants when an alkaline extract of a digestate from the humid organic fraction of municipal solid waste was applied at 500 kg ha^{-1}, but no increase in fruit yield. However, the extract contained approximately 8% N and 9% K (on dry matter basis), as well as all other plant nutrients (Sortino et al., 2014). The nutrient content was not equalized between treatments and control, and this fact may have influenced the result. The same extract was investigated for biostimulant properties by Fascella et al. (2018, 2015) on the growth and quality of the ornamental hybrid *Euphorbia x lomi* and two *Lantana* species. Positive results were reported. However, the alkaline extract provided the plants with an extra 0.21 g of N per plant, as well as other plant nutrients, which might have influenced the result (Fascella et al., 2018, 2015).

In a recent trial, Guilayn et al. (2020) studied the effect of alkaline extracts from digestate from two different feedstocks (sewage sludge and manure), obtained from full-scale anaerobic digester plants, on the growth of lettuce in a hydroponic system. Both digestate treatments resulted in significantly higher (20–60%) biomass yields than those of a negative control, while a commercial leonardite extract product did not increase yields significantly. However, although the experiment was performed under no nutrient limitation, the result cannot be dissociated from the supplementary amounts of nutrients applied by the digestate extracts (the

N, P, and K content in the highest performing digestate extract treatment was about 40, 32, and 190% higher, respectively, compared to the negative control) (Guilayn et al., 2020).

In yet another experiment with alkaline hydrolysates of a digestate derived from the organic humid fraction of municipal solid waste, Massa et al. (2018) reported biostimulant properties in hibiscus grown under nutritional stress in an experiment where the treatments and control were arranged to receive comparable amounts of N, P, and K. The authors reported that the nutrient-stressed hibiscus plants treated with alkaline digestate hydrolysates performed significantly better than the control with regards to fresh and dry weight as well as other plant growth parameters. Interestingly, in relation to N and micronutrients, the mineral leaf content (g kg^{-1}) was the same in the digestate treatments and control, while P, K, and S levels were higher in the digestate treatment while Ca and Mg levels were lower. The authors conclude that the alkaline hydrolysates of the digestate improved the capability of the hibiscus plants to face nutrient stress, probably by enhancing the photosynthetic capacity (Massa et al., 2018).

Comparing the biostimulatory effect of digestate and digestate alkaline extracts: growth trials

In the study of Massa et al. (2016), the effects of a digestate and its soluble alkaline hydrolysate were compared for their capacity to boost the production and quality of *Hibiscus* plants which were grown under optimal growing conditions in a peat and pumic substrate. The digestate was obtained from the organic humid fraction of urban wastes, and was applied at low doses (4.2 g digestate pot^{-1} on dry matter basis, corresponding to 0.17 g N pot^{-1}) while the hydrolysate provided the plants with 0.257 g N pot^{-1}. Overall, the hydrolysate performed better than the whole digestate, but the digestate performed better than the negative control. The effect of the hydrolysate was comparable to a commercial biostimulant. Both the digestate and its hydrolysate resulted in a similar photosynthetic rate (20.7–21.1 mol m^{-2} s^{-1}), which was higher than that of the control (17.5 mol m^{-2} $s^{-1)}$, and in similar leaf dry matter.

In summary

When reviewing the literature on biostimulatory effects of digestates, the majority of studies have attributed observed biostimulatory effects to the presence of plant-active levels of IAA present in the digestate as a result of catabolism from L-tryptophan in the digester (Kostenberg et al., 1995; Li et al., 2016; X. Li et al., 2018; Lu et al., 2019; Scaglia et al., 2017, 2015). However, the IAA is unstable and has been reported to degrade during storage (Li et al., 2016). Evaluation of the plant-growth promoting properties of digestates and their extracts beyond their nutrient content is complicated by their high content of macro- and micronutrients, which were only equalized between treatments in one of the reviewed studies (Massa et al., 2018). Some authors have argued that the surplus of nutrients added by the digestate or its extract was negligible but, when calculated, was considerable. Others have argued that surplus nutrients applied to plants at optimal nutrient levels would not result in an extra nutrient effect on growth; however, taking into account the whole range of macro- and micro nutrients supplied with the digestate or its extracts, this assumption seems precarious. Thus, the observed positive effects on plant growth after digestate/digestate extract application in the reviewed studies cannot be distinguished from plant nutrient effects in all but the trial of Massa et al. (2018).

Aims and objectives

In this study, a trial was set up to assess the plant availability of macro- and micronutrients and the possible biostimulatory effects of a digestate collected at the municipal Karpalund biogas plant in Kristianstad, southern Sweden, which was nitrified in a moving bed biofilm reactor prior to the experiment. The treatments were evaluated with respect to their effect on shoot mineral content and the growth of pak choi grown in a peat substrate. The study was designed with three objectives:

i. To assess the plant availability of macro- and micronutrients in the digestate, with particular focus on P and S. As a result of the slightly alkaline pH during the digestion (pH of 7.5–8.5) and the reduced conditions, the plant availability of P and S have been reported to decrease due to the formation of poorly soluble compounds such as calcium- or magnesium phosphates (e.g., $Ca_3(PO_4)_2$, struvite ($MgNH_4PO_4 * 6\ H_2O$), hydroxylapatite ($Ca_5(PO_4)_2OH$) and iron sulphides. Several authors have highlighted P as a limiting factor for growth when digestate alone is used as fertilizer (Abubaker et al., 2012; Liu et al., 2011; Lošák et al., 2016; Pokhrel et al., 2018; Stoknes et al., 2018; Svensson et al., 2004). Moreover, losses of P have been reported after nitrification (Kamthunzi; 2015). A very low plant-uptake of S (similar to that seen in unfertilized controls) in digestate-grown crops has been reported in trials in soil (Assefa, 2013; Elfstrand et al., 2007; Fontaine et al., 2020); in contrast, a high uptake of S (similar to an inorganic reference) was reported in a hydroponic trial (Pelayo Lind et al., 2020). In addition to decreased plant availability due to iron sulphide precipitation during digestion, there are reports of high SO_4^{2-} immobilization after digestate application (Fontaine et al., 2020). In order to assess the relation of plant-available nutrients to the total nutrient content, the digestate was compared to a mineral nutrient solution composed to resemble the total amounts of macro- and micronutrients in the digestate. The hypothesis tested was that S and P uptake would be lower in the digestate treatments compared to the mineral control.

ii. To assess the plant availability of added mineral P, S, Mg, Mn, B, and Mo to the digestate. When the total nutrient content of the digestate was compared to a nutrient solution which had been formulated to optimize the growth of Asiatic vegetables including pak choi (Bergstrand and Hultin, 2014), the digestate was found to be low in P, S, Mg, Mn, B, and Mo. Mineral amendments have been added successfully to various digestates in previous studies. For example, Liu et al. (2011) added K_2HPO_4 and EDTA-Fe directly into a diluted digestate and Liedl et al. (2004) mixed a pig-slurry digestate with $MgSO_4$ and H_3PO_4, both with positive effects on yields. The hypothesis tested was that the addition of the above-mentioned plant nutrients would increase their availability to the plant in the digestate and would thus improve yields.

iii. To assess the possible biostimulatory properties of the Karpalund digestate (i.e., the effects of the digestate beyond its nutrient content) on plant yield, quality, and stress tolerance. Digestates derived from protein-rich feedstocks have been reported to contain IAA, as well as other plant hormones, at concentrations sufficient to regulate plant development (Li et al., 2016; X. Li et al., 2018) which have been related to improved growth and nutrient-stress tolerance in digestate growth trials (Massa et al., 2018). The hypotheses tested were:

   a) Plants fertilized with digestate will perform better in terms of yield and/or quality at optimal N-levels than plants fertilized with a mineral nutrient solution with the same plant nutrient content. A plain digestate and a digestate with mineral amendments were assessed in order to exclude possible negative effects caused by unbalances in the digestate nutrient composition.

   b) Plants fertilized with digestate will cope better with nutrient stress than plants fertilized with a mineral nutrient solution with the same plant nutrient content. In order to rule out effects caused by unbalances in the nutrient composition, the effect of digestate with mineral amendments, at 50% of optimal N-dose, was assessed.

Materials and methods

Plant materials and growing conditions

A greenhouse study was conducted between May and June 2019 at the Department of Biosystems and Technology, Swedish University of Agricultural Science, Alnarp, Sweden. A growing medium of peat moss (0–25 mm, H2–4, H5–7; SW Horto AB, Sweden), with 5.5 kg m^{-3} dolomite lime ($CaMg(CO_3)_2$, Björka mineral AB, Sweden) and a pH of 6.1, was used for all treatments. Pak choi (*Brassica rapa,* ssp. *chinensis*, 'Joy Choi', Olsson Seed, Sweden), was used as an experimental crop. On the 30th of April, seeds were sown in a plug tray, one seed per plug. The plantlets were subirrigated, and nine days after sowing, the plug tray was soaked in a standard nutrient solution (0.5 + 0.5 g L^{-1}, respectively, of CalcinitTM and KristalonTM Indigo; Yara, Oslo, Norway). On the 14th of May, the plantlets were transferred to 2-liter pots. The plug tray and the pots were kept on a table in a 100 m^2 greenhouse compartment where the temperature was set to 18°C and the roof ventilation was opened at 20°C. The greenhouse screen was closed when the outdoor light intensity was above 1200 µmol m^{-2} s^{-1}. The plants were harvested on the 19th of June, 51 days after sowing.

Beginning three days after planting, the plants were fed nutrient solution every second or third day, a total of 13 times. The nutrient dose was increased incrementally during the cultivation period with a starting dose that was half the final dose. Plants were irrigated according to need, which was every seventh day in the beginning of the experiment and once a day at the end of the experiment. Water was poured by hand into each pot until the tray was covered with 5 mm water. In total, approximately 3.75 liters of tap water were fed to the plants in the full nutrient dose treatments. Tap water (pH 8.2) was used as irrigation water, containing the following compounds at detectable levels (mg L^{-1}): Ca 20, Mg 1.5, Na 8.3, Zn 0.025, Cl 26, Fe 0.064, and SO_4 2.6 (analysis performed by Eurofins Agro Testing Sweden).

Nutrient requirements

The N requirement for *Brassica rapa* 'Joi Choi' was calculated using 250 grams per plant as an estimated shoot fresh matter yield, 30% weight addition for root fresh matter, 95% water content, and 3.5% N content in dry matter, resulting in an estimated N assimilation of 570 mg $plant^{-1}$. 15% of the added N was estimated to stay unavailable to the plant in the substrate, resulting in an estimated N requirement of 650 mg plant $^{-1}$. The plant-available N in the peat substrate was assumed to be negligible (in accordance with the manufacturer's information).

A slightly modified Sonneveld & Straver lettuce nutrient solution, formulated to optimize growth of Asiatic vegetables including pak choi in hydroponic sytems, was used as a positive control (Bergstrand & Hultin, 2014; Sonneveld & Straver, 1994). The solution contained (parts by weight): NO_3-N 0.93, NH_4-N 0.07, P 0.2, K 1.37, Ca 0.64, Mg 0.15, S 0.18, Fe 0.012, Mn 0.003, Zn 0.0017, B 0.00014, Cu 0.0002, Mo 0.00024, (Bergstrand & Hultin, 2014; Sonneveld & Straver, 1994).

The nutrient solutions were formulated to give the same N_{min} in all full-dose treatments.

Digestate nutrient composition

Biogas digestate was collected at Karpalund biogas plant, operated by Kristianstad's Biogas AB, southern Sweden, in February 2019. The organic substrate going into to the plant consisted of: 37% organic household waste, 29% manure (of which 2/3 were pig manure and 1/3 cattle manure), 21% slaughter waste, 5% fat from grease separators, 8% other food waste, and iron chloride and iron sludge as processing aids (< 0.3%) (c4energi, 2019; personal communication Bengt Stuhre, Kristianstad Biogas AB, 2019-11-06). The average temperature during the anaerobic digestion was 44°C, and the retention time in the reactors was 50 days.

After being sieved through a 0.8 mm mesh, the digestate's nutrient content was analyzed by an accredited laboratory (Eurofins Environment Testing, Sweden AB, Lidköping) using the following methods: Kjeldahl's and Devarda's for total-N, Kjeldahl's for NH_4-N (Standard Methods 4500-N mod.) (APHA, 1998), silver nitrate titration for Cl, and the remaining substances by extraction with aqua regia (HNO_3 + 3 HCl) and determination of elements by inductively coupled plasma atomic emission spectroscopy (ICP-AES), in accordance with ISO 11466. The content per kg^{-1} (fresh weight) was as follows: total-N 5.3 g, NH_4-N 3.7 g, P 0.25 g, K 1.5 g, Ca 0.7 g, Mg 0.045 g, S 0.28 g, Na 0.8 g, Cl 1,8 g, Fe 325 mg, Zn 9.25 mg, Mn 5.25 mg, Cu 3.25 mg, B 0.6 mg, Mo 0.1225 mg, Co 0.05 mg. The total solids were 2.5% and the pH was 8.1.

A second analysis, performed by Eurofins Steins Laboratory, Denmark, resulted in 5.06 g kg^{-1} total-N (Kjeldahl's and Devarda's) and 4.0 g kg^{-1} NH_4-N (Kjeldahl according to Commission Regulation EC 152/2009) (EC, 2009). An average of the two results (5.18 g kg^{-1} tot-N and 3.85 g kg^{-1} NH_4-N) was used for calculating the N levels when setting up the experiment. For all other nutrients, the results from Eurofins Environment Testing Sweden AB, Lidköping, were used for calculations, as Mo and Cl were not included in the second analysis, and the accuracy of the measurements was higher for several nutrients in the former lab's analysis report.

Nitrification of digestate

Setting up the moving bed biofilm reactor

A small-scale moving bed biofilm reactor (MBBR) was set up to convert a share of the NH_4-N in the digestate to NO_3-N. During the first attempt to set up and run the reactor, no chemical pH control was used, as this is not allowed in organic production in Sweden. The attempt failed as most N accumulated as nitrite. The procedure described below is from a second attempt, when a pH control was in place and the NH_4-N loading rate was proportioned to keep NH_4-N concentration in the reactor below 2 mg L^{-1} during the start-up. Three liters of digestate were processed during the 51 days the reactor was running. (The initial goal was to process four liters, but at about 2.5 liters, no more K_2CO_3, which was used to raise the pH, could be added as it would have caused the levels of K to be too high in the final nutrient solution, and the reactor had to be shut down before the goal was achieved. The remaining digestate was added to the digestate nutrient solution unnitrified.)

The equipment used was a 120-liter plastic barrel, 18 liters biofilm carriers (K3 from AnoxKaldnes; protected surface area 500 m^2 m^{-3}), an air pump and four air stones, PVC hoses (ø 5 mm for air and pH regulation, ø 13 mm for pumping digestate), an aquarium pump, a peristaltic dosing pump (Luxorparts), a timer, a pH controller (MC122 from Milwaukee), 0.1 M K_2CO_3 for pH regulation, and a 10-liter polypropylene bucket with a lid to hold the digestate prior to pumping it into the reactor. A Hach DR1900 spectrophotometer was used to measure the concentrations of NH_4^+, NO_2^- and NO_3^{2+} in the reactor (Hach lange tests LCK 303 for [NH_4^+], LCK 342 for [NO_2^-], and LCK 340 for [NO_3^{2+}]) (Hach, Loveland, USA).

The biofilm carriers were taken from a previous MBBR digestate nitrification experiment at the department, where they had been kept in an aerated batch of fully nitrified digestate (running on digestate from the biogas plant Gasum Jordberga AB), with a pH of 3.9, for five months, before the start of the experiment.

To start the MBBR, the 120-liter barrel was filled with 70 liters of distilled water, 18 liters of biofilm carriers (26% filling degree; maximum filling degree for K3 is 70%), and three deciliters of fully nitrified digestate (as extra bacterial inoculum) from the same batch of nitrified digestate as the biofilm carriers (AnoxKaldnes, 2019). For appropriate dosing, the raw digestate was diluted three times to 12 liters (1727 mg L^{-1} total-N, 1283 mg L^{-1} NH_4-N). To start up the reactor, a digestate volume containing 100 mg NH_4-N was added (78 mL of the diluted digestate), resulting in an initial NH_4^+ concentration of 1.43 mg L^{-1} in the MBBR. The pH controller was set to keep the pH above 6.6.

Running the MBBR

The reactor was operated with incremental increases in the NH_4-N loading rate. During the first 10 days, digestate was added manually using pH, NH_4^+, and NO_2^- levels in the reactor as guidelines for appropriate timing of digestate injections. Ultimately, more digestate was not added until the NH_4^+ and NO_2^- levels in the reactor were close to zero in order to avoid accumulation; levels that are too high result in the inhibiting of ammonia-oxidizing and nitrite-oxidizing bacteria. When proportioning the digestate doses, consideration also had to be given to keep pH oscillations as low as possible in the reactor in order to optimize the conditions for the bacteria. (Since the reactor had a pH of 6.7, the digestate had a pH of 8.1, and the first step in the nitrification reaction has an acidifying effect, every digestate load caused a pH oscillation). The automatic digestate injection system was enabled on day 10, and the dose and injection frequency were incremental increased to reach 60 mL with an interval of 2 hours 40 minutes as a maximum loading rate (Table 2).

On day 11, a large volume of digestate was accidently pumped into the reactor at once. The pH, NH_4^+, and NO_2^- levels rose to suboptimal levels (Table 2). However, after five days, the conditions in the reactor had returned to favorable levels.

On day 22, the pH set point was lowered to 6.0 in an attempt to use less K_2CO_3 and to avoid high levels of K in the final digestate nutrient solution. On day 27, the set point was lowered to 5.6. On day 30, the pH regulation was turned off. Nitrification worked well at a pH of both 6.0 and 5.6, and as the amount of digestate added to the reactor was raised, the nitrification rate reached its maximum of around 10 g NH_4-N m^{-3} day^{-1} on days 28–29. However, when the pH regulation was turned off, and the pH dropped below 5.0, NH_4-N accumulated and nitrification was slowed down. Digestate injections were stopped, and during the following days, the pH dropped to 3.0 and nitrification stopped completely. On day 48, pH regulation was turned on again for 24 hours (pH set to 5.5) in an attempt to start the nitrification process again. It worked, but as the reactor had to be shut down in two days and there was still around 1.5 liters of digestate with a pH of 8.1 to be added to the solution in the reactor (in order to reach the nutrient concentrations necessary for the treatments), the pH regulation was turned off again. Thus, the pH could drop again to levels low enough, when mixed with the unnitrified digestate, to create a nutrient solution with an acceptable pH.

At shut down, the volume in the reactor had decreased from the initial 70 liters to 49 liters. In order to keep concentrations of N high enough in the final nutrient solutions, the vaporized 29 liters of water were not replaced during the MBBR's running time. The larger volume in the beginning made it possible for the reactor to process larger volumes of digestate while keeping the initial NH_4 concentration well below 2 mg L^{-1}.

To create enough nutrient solution to serve all digestate treatments, the volume was increased again with some water, and raw digestate was added to reach 250 mg N_{min} L^{-1}, resulting in 35% NH_4-N and 65% NO_3-N in the final digestate nutrient solutions. The final pH was 7.7.

***Table 2**: Digestate input, pH, NH_4^+, NO_2^-, and NO_3^{2+} concentrations and calculated nitrification rate in the MBBR running for 51 days at 18–20°C.*

Day	Digestate added (mL/day)	NH_4-N added (mg/day)	Contribution of added NH_4-N to $[NH_4^+]$ in reactor (mg/L)	pH (a) Max	pH (a) Min	NH_4^+ mg/L (b)	NO_2^- mg/L (b,c)	NO_3^{2-} mg/L (b)	Nitrification rate $NH_4 \rightarrow NO_3$ g(N)/m^3/day (d)
1	26	100	1.4		6.7				0.29
6-7	8	32	0.5	7.0	6.7	< 2.0	< 0.6		0.47
8	17	64	1.0	6.9	6.7		< 0.6		0.95
9	8	32	0.5	7.0	6.8		< 0.6		0.48
10	8	32	0.5	6.9	6.7		< 0.6	18	0.48
11	216	834	12.6	7.8	7.8				0.53
12		-		7.8	7.8				0.53
13		-		7.8	7.8	11.1	4.1	20.3	0.53
14		-		6.7	6.7	3.9	5.5	32	5.53
15		-		6.7	6.7	< 2.0	2.8	> 35	5.53
16	17	64	1.0	6.9	6.7		< 0.6		1
17	27	103	1.6	6.9	6.7	< 2.0	< 0.6		1.61
18-21	50	192	3.0	6.9	6.7				3.06
22-24	75	289	4.7	6.4	6.0	< 2.0	< 0.6		4.71
25-26	135	520	8.6	6.4	6.0			92.2	8.59
27	135	520	8.7	6.3	5.6	2.3	< 0.6		8.7
28	180	693	11.7	6.4	5.8	< 2.0	< 0.6		11.68
29	180	693	11.8	6.4	5.6	2.1	< 0.6		9.62
30	180	693	11.8		4.9	6.0	< 0.6		8.02
31-34	180	693	11.9		3.6				9
35		-		3.6	3.6	20.6		186.6	9
38		-		3.5	3.5	19.5			0.01
41		-		3.0	3.0	15.3			0.03
42		-		3.0	3.0	15.6			-0.01
44		-		3.0	3.0	15.4		183.3	0.002
48	67	257	5.0	6.1	5.5	16.6		223.9	3.76
49	53	205	4.0	7.1	6.9	20.5	1.3	209.7	0.1
50				5.1	5.1				
51	454	1747	34.81	4.4	3.9	18.2	< 0.6	202.6	
51				*Reactor shut down.*					
51	1000 (e)	3849		7.3	7.3	71.1	< 0.6	202.1	

(a) A pH controller set to keep the pH above 6.6 (using 0.1 M K_2CO_3). On day 22, the setting point was changed to a pH of 6.0, and on day 27 to a pH of 5.6. On day 30, the pH controller was turned off in order to avoid levels of K that were too high in the final nutrient solution. It was turned on again on day 48 for 24 hours in an attempt to start the nitrification process again.

(b) Measurements taken just before the new digestate was added. Values are affected by the decreasing volume in the reactor (volume drops from 70 liters to 49 liters during the running time).

(c) No access to nitrite analysis during days 31–48.

(d) Nitrification rate calculated as the difference between the calculated $[NH_4^+]$ in the reactor after digestate injection and the measured $[NH_4^+]$ in the reactor.

(e) Added 4 hours after shut down, when the pH had dropped to 3.4.

Treatments

The seven treatments were as follows: (1) nitrified digestate (D1); (2) nitrified digestate with added minerals to resemble the nutrient levels in the standard mineral nutrient solution used in the experiment (D2); (3) D2 in half dose (D3); (4) mineral nutrient solution designed to mimic the nutrient levels in the nitrified digestate (M1); (5) standard mineral nutrient solution (M2); (6) M2 in half dose (M3); and (7) water as a negative control (W). The treatments and the variables tested are listed in Table 3.

Table 3: *The treatments and the variables tested.*

Treatment			Variable tested
Digestate treatments	D1	Nitrified digestate	
	D2	Nitrified digestate + P, Mg, S, Mn, B, and Mo, to resemble the nutrient composition of M2.	Compared to D1: To assess the plant availability of added minerals and their effect on plant growth.
	D3	D2 in half dose.	
Mineral treatments	M1	Mineral nutrient solution designed to imitate the total nutrient composition of D1.	Compared to D1: (1) The plant availability of nutrients in the digestate. (2) The effect of digestate components other than nutrients at plain digestate nutrient levels.
	M2	Standard mineral nutrient solution, designed for optimal growth.	Compared to D2: The effect of digestate components other than nutrients at optimal nutrient levels.
	M3	M2 in half dose.	Compared to D3: The effect of digestate components other than nutrients at nutrient stress.
Negative control	W	Water	Negative control

Preparation of nutrient solutions

The mineral nutrient solutions were mixed separately for each treatment and diluted to 250 mg N L^{-1} and 125 mg N L^{-1} for the full- and half-dose treatments respectively. Sodium hydroxide (NaOH) was used to adjust the pH in M2 and M3. Table 4 shows the composition of mineral salts in each nutrient solution.

The nitrified digestate from the MBBR was diluted with distilled water to the volume needed for the treatments. It was then mixed with concentrated, not nitrified, digestate to reach a N concentration (NO_3-N + NH_4-N) of 250 mg L^{-1}. In the resulting solution, 35% of the N was NH_4-N and 65% NO_3-N. The D2 nutrient solution was created by mixing mineral salts directly into a separated volume of the digestate in order to avoid diluting the digestate (and keep the same NO_3/NH_4 ratio in both digestate treatments). The D3 nutrient solution was created by diluting D2 to 125 mg N L^{-1}.

Table 4: *Recipes of the nutrient solutions. Amounts give a total of 1000 mg N.*

M1		M2		Amendments to D2	
	mmol		mmol		mmol
macronutrients		*macronutrients*		*macronutrients*	
NH_4NO_3	9.75	$Ca(NO_3)_2 * 4H_2O$	15.88	$MgSO_4 * 7H_2O$	1.96
KNO_3	37.84	KNO_3	31.52	$CaSO_4 * 2H_2O$	0.98
NH_4Cl	4.22	$(NH_4)_2SO_4$	2.41	H_2PO_4	1.51
K_2SO_4	1.86	$MgSO_4 * 7H_2O$	2.78		
$MgSO_4 * 7H_2O$	0.65	KH_2PO_4	3.41		
KCl	5.1	$NaNO_3$	3.39		
$(NH_4)_2HPO_4$	4.82	H_3PO_4	2.94		
$CaCl_2 * 2H_2O$	1.37				
micronutrients		*micronutrients*		*micronutrients*	
$C_{10}H_{12}FeN_2NaO_8$	1.6302	$C_{10}H_{12}FeN_2NaO_8$	0.2222	$MnSO_4 * H_2O$	0.0367
$MnSO_4 * H_2O$	0.0269	$MnSO_4 * H_2O$	0.0636	$Na_2MoO_4 * 2H_2O$	0.0022
$MnSO_4 * H_2O$	0.0396	$ZnSO_4 * 7H_2O$	0.0255	H_3BO_3	0.1114
$CuSO_4$	0.0143	$CuSO_4$	0.0032		
$Na_2MoO_4 * 2H_2O$	0.0004	$Na_2MoO_4 * 2H_2O$	0.0024		
H_3BO_3	0.0155	H_3BO_3	0.1269		

Table 5: *Total amounts of nutrients (in mg) supplied to each plant in the different treatments (in total, 2.6 liters of nutrient solution per plant) and the pH of the nutrient solutions and substrate. The total amount of Ca and Mg in the substrate of each pot, provided by the dolomite lime, is also displayed. The numbers highlighted in red show which minerals were added to the digestate in D2.*

	Treatment				Substrate
	Plain digestate	Amended digestate	Mineral as digestate	Standard mineral solution	$CaMg(CO_3)_2$
	D1	D2	M1	M2	
NH_4-N	230	230	214	43	
NO_3-N	420	420	432	605	
K	1241	1241	1243	885	
P	97	128	101	128	
Ca	144	170	36	413	2391
Mg	10	41	10	100	1450
S	54	115	54	116	
Cl	327	327	331	0	
Na	145	145	24	101	
Fe	59.1	59.1	59.1	8.05	
Mn	0.95	2.26	0.96	2.26	
Zn	1.68	1.68	1.68	1.08	
B	0.11	0.89	0.11	0.89	
Cu	0.59	0.59	0.59	0.13	
Mo	0.02	0.16	0.02	0.16	
Ni	0.02	0.02	0	0	
pH	7.7	7.6	7.6	5.9	6.1

Experimental design

The experiment was set up as a completely randomized design consisting of seven treatments (three digestate treatments, three mineral treatments, and one negative control) with eight replicate pots per treatment. In total, 56 pak choi seedlings were planted. The pots were randomly placed on a greenhouse table and moved twice during the experiment

Data collection and measurements

The following data were collected on growing days 45 and 46 from the youngest mature leaf of each plant: (1) chlorophyll content, using a MC-100 Chlorophyll Meter from Apogee Instruments and (2) chlorophyll fluorescence, measured with a Pocket PEA Chlorophyll Fluorimeter from Hansatech Instruments.

The following data were collected after harvest from each plant: (1) fresh and dry weight of shoots, (2) number of leaves over 10 mm in length, (3) total leaf area of leaves over 10 mm in length, measured with a LI-3100 Area Meter from LI-COR, (4) EC, pH, and concentrations of minerals and sugars in plant sap, (5) total mineral content in shoot dry matter, and (6) mineral content, EC, and pH in the substrate. The measurements of (4)–(6) were performed by an accredited laboratory (LMI in Helsingborg, Sweden) using inductively coupled plasma optical emission spectrometry (ICP-OES) for determination of mineral concentrations. The mineral content of the substrate was measured in a Spurway extract (a weak acetic acid extraction of the substrate) (Spurway, 1949). The fresh weight of shoots was measured directly after harvest, and the dry weight after drying at 60°C for three days.

Phosphorus and sulphur recovery efficiency

The fertilizer's P and S recovery efficiency was calculated using the partial balance method—i.e., as the ratio of nutrients removed by crop harvest (nutrient content in shoots) to fertilizer nutrients applied (Fixen et al., 2015).

Statistics

One-way analysis of variance (ANOVA) together with Tukey's HSD test for differences of means, with a confidence interval set to 95%, was used for statistical analysis of the data from the experiment. The software used was Minitab Express version 1.5.1.

Results

Visual observations

At harvest, the plants in the full nutrient dose treatments D1, D2, M1, and M2 had reached a height of about 30 cm and showed no signs of nutrient deficiency (Figure 1). There were no obvious visible differences between the plants in these treatments. The plants in the half nutrient dose treatments D3 and M3 were 5–10 cm shorter and the older leaves displayed a light green color indicating a deficiency of N (Figures 1 and 2). The plants in both D3 and M3 displayed similar phenotypes. The plants in the negative control had grown to about 10 cm in height and showed severe symptoms of nutrient deficiency (Figure 2).

Figure 1. One representative plant from each treatment at harvest. D1 = digestate; D2 = digestate with amendments; D3 = D2 in half dose; M1 = mineral nutrient solution designed to have the same nutrient composition as D1; M2 = mineral nutrient solution designed for optimal growth; M3 = M2 in half dose.

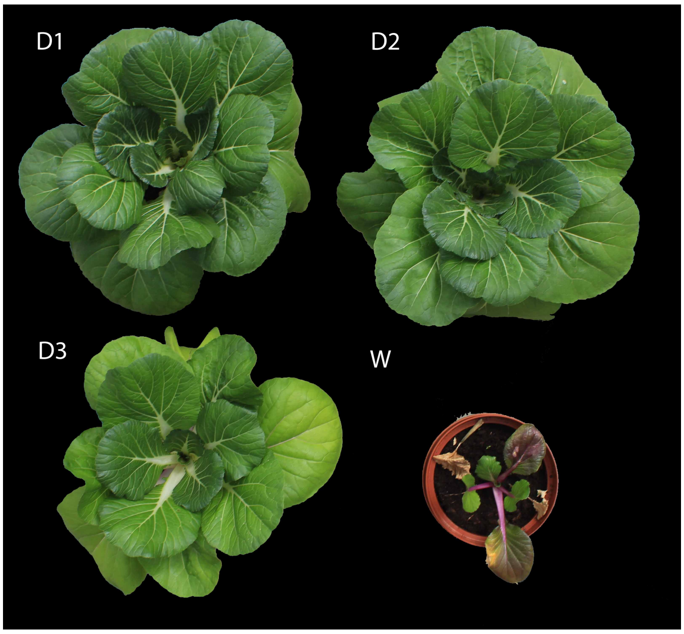

Figure 2: One representative plant from each digestate treatment and a negative control plant. D1 = plain digestate; D2 = digestate with amendments; D3 = D2 in half dose; C1 = negative control.

The root systems showed distinct characteristics at harvest (Figure 3). The root systems of the plants in D1 and M1 had similar characteristics with short and crispy roots while the plants in D2, D3, M2 and M3 exhibited longer, softer roots.

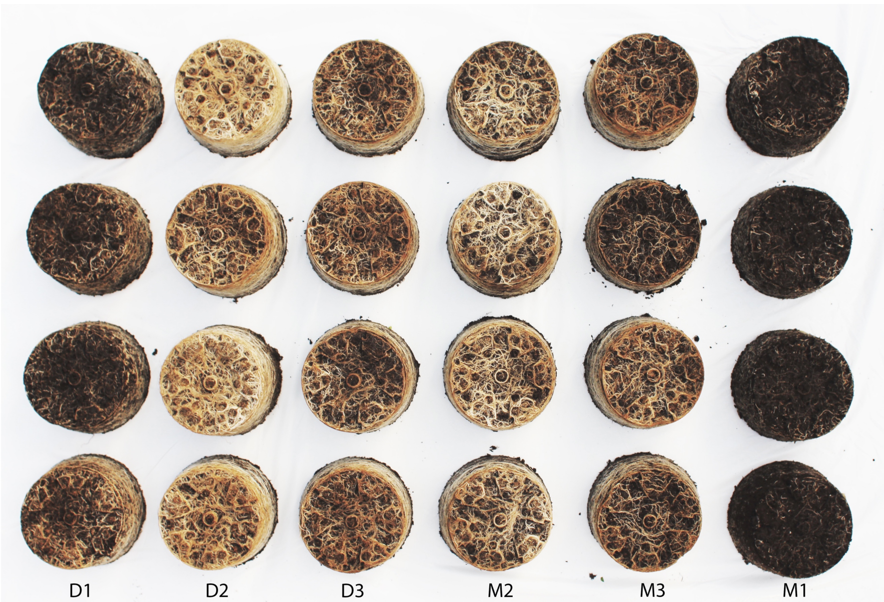

Figure 3: Root systems in substrate turned upside down at harvest. The vertical rows display the six treatments: D1 = plain digestate; D2 = digestate with amendments; D3 = D2 in half dose; M1 = mineral nutrient solution designed to have the same nutrient composition as D1; M2 = mineral nutrient solution designed for optimal growth; M3 = M2 in half dose.

Quality parameters at harvest

Shoot dry and fresh matter yield

As displayed in Table 6, D2 resulted in the highest mean for shoot DM, which was significantly higher compared to M2 (17% higher). However, it did not differ significantly from the two other full nutrient dose treatments. The nutrient-stressed plants in D3 and M3 had significantly lower DM compared to the plants in the full nutrient dose treatments, with the exception of the plants in M2. The shoot fresh matter (FM) also differed between the full-dose treatments (Table 6). The D2 treatment, with the highest DM mean, also resulted in the highest FM mean. The FM yield in D2 was 10% higher than in D1. The M2 treatment, which resulted in the lowest DM among the full nutrient dose treatments, resulted in a FM yield which was not significantly lower than D2. The lowest FM mean was found in D1 and was significantly lower than in D2 and M2, but not M1.

There were no significant differences in water content between the full nutrient dose treatments; they all resulted in plants with approximately 94% water content (Table 6). The half nutrient dose treatments resulted in plants with 92% water content, which was significantly lower. The plants in the negative control had a water content of 89%.

Table 6. *Growth and quality parameters at harvest. Means within each column that do not share a letter are statistically different (P < 0.05).*

Treatment	Shoot fresh weight (g) *(n=8)*	Shoot dry weight (g) *(n=4)*	Chlorophyll content (CCI) *(n=8)*	Leaf number *(n=4)*	Leaf area (dm^2) *(n=4)*	Chlorophyll fluorescence (Fv/Fm) *(n=8)*	Water content (%) *(n=4)*
D1	368 b	22.1 ab	29.8 a	19.5 ab	30.6 a	0.81 a	93.7 ab
D2	402 a	24.4 a	24.2 b	17.8 abc	30.5 a	0.81 a	94.1 a
D3	228 c	19.0 c	21.3 b	15.3 d	22.5 b	0.80 a	91.8 c
M1	385 ab	23.1 ab	31.0 a	19.6 a	29.6 a	0.80 a	94.0 a
M2	393 a	20.8 bc	22.3 b	17.5 bc	28.3 a	0.81 a	94.4 a
M3	232 c	18.8 c	16.0 c	16.5 cd	22.0 b	0.80 a	92.0 bc
W	10 d	1.3 d	24.1 b	6.3 e	1.8 c	0.72 b	89.4 d

Chlorophyll fluorescence and chlorophyll content

The chlorophyll fluorescence (calculated as Fv/Fm) did not differ between the nutrient treatments at harvest (Table 6), unlike the chlorophyll content which did. The plants in D1 and M1 had significantly higher chlorophyll content compared to the other treatments. The nutrient-stressed plants in M3 had significantly lower chlorophyll content compared to all other treatments. D3, however, did not result in lower chlorophyll content compared to D2 and M2.

Leaf area and number of leaves

There was no difference in the total leaf area between the full nutrient dose treatments (Table 6). The means varied between 30.6 dm^2 (D1) and 28.3 dm^2 (M2). The leaf area in the nutrient-stressed treatments was significantly lower: 22.5 dm^2 (D3) and 22.0 dm^2 (M3).

The number of leaves at harvest varied between the full nutrient dose treatments. The M1 treatment resulted in a mean of 19.6 leaves per plant, and the M2 treatment in 17.5 leaves per plant, which was significantly lower. The nutrient-stressed plants in D3 had a mean of 15.3 leaves per plant and the negative control resulted in 6.3 leaves per plant.

Nutrients in shoots and substrate

Nitrogen

The nitrogen (N) concentrations in shoot DM in M2 and M1 were significantly higher those of the D1 treatment, but not the D2 treatment (Table 7). The total N uptake was significantly lower in D1 compared to the other full-dose treatments (but the DM was not). The concentration of NH_4 and NO_3 in the plant sap at harvest varied widely within each treatment, so although the mean values differed greatly, the treatments did not differ statistically (Table 9). The levels of plant-available N in the substrate at harvest also differed between treatments: the substrate in the M2 treatment had significantly lower N levels compared to the substrate in the D2 and M1 treatments, but not compared to the substrate in the D1 treatment (Table 10). The total N uptake, N concentration in shoots, N concentration in plant sap, and N levels in substrate were all found to have a significant positive correlation with DM yield (Pearson correlation; P-value = < 0.0001, 0.0007, 0.0069, 0.022, respectively).

Table 7. *Concentration of nutrients in shoot dry matter at harvest (n=4). Means within each column that do not share a letter are statistically different (P < 0.05).*

	g/kg						
Treatment	N	P	K	S	Ca	Mg	Na
D1	22.4 b	2.80 de	42.7 ab	1.62 e	15.2 abc	7.31 abc	6.61 b
D2	23.0 ab	3.92 b	37.2 bc	3.45 b	14.3 bc	6.66 c	6.94 b
D3	15.2 c	2.63 e	26.9 d	2.24 d	15.6 abc	7.82 abc	5.59 b
M1	26.9 a	3.65 bc	46.6 a	2.24 d	13.5 c	7.15 bc	3.85 c
M2	27.0 a	4.89 a	35.7 c	4.26 a	16.5 ab	7.59 abc	7.02 a
M3	17.1 c	3.22 cd	26.2 d	2.79 c	17.2 a	8.55 a	5.82 b
W	14.4 c	0.69 f	9.43 e	2.21 d	16.2 ab	8.14 ab	9.00 a

	mg/kg					
Treatment	Mn	Fe	Zn	B	Cu	Mo
D1	86.3 bc	49.3 ab	41.8 ab	10.1 c	4.1 ab	1.0 d
D2	115.5 a	51.5 ab	41.5 ab	33.0 a	4.4 ab	3.7 b
D3	102.0 ab	40.3 b	35 bc	22.3 b	3.5 b	2.6 c
M1	46.4 d	53.3 a	31.8 c	8.6 c	5.6 a	1.5 d
M2	94.5 abc	54.8 a	46 a	34.8 a	3.6 b	3.4 bc
M3	70.4 cd	46.5 ab	42.8a	24.5 b	3.5 b	2.7 c
W	70.6 cd	44.3 ab	43 a	8.9 c	3.4 b	6.2 a

Potassium

The plants in the M1 treatment had the highest mean for potassium (K) in shoot plant tissue (Table 7). The concentration was significantly higher compared to D2 and M2, but was not higher than in the D1 treatment. The M2 plants had the lowest levels of K in the plant sap at harvest among the full nutrient dose treatments. Furthermore, the plant-available K in the substrate in the M2 treatment was the lowest among the full nutrient dose treatments (Table 10). A significant correlation between the concentration of K in dry matter and dry weight was found, as well as between the total K uptake and dry weight (Pearson correlation, P-value = < 0.0001).

Table 8. *Total uptake of nutrients (n=4). Means within each column that do not share a letter are statistically different (P < 0.05).*

	mg/plant						
Treatment	N	P	K	S	Ca	Mg	Na
D1	502 c	63 c	958 b	36 c	340 ab	164 ab	148 b
D2	560 b	95 a	906 b	84 a	349 a	162 ab	169 a
D3	289 d	50 d	510 d	43 c	296 c	148 b	106 c
M1	615 a	84 b	1066 a	51 b	311 bc	164 a	88 d
M2	564 ab	102 a	747 c	89 a	345 a	159 ab	147 b
M3	321 d	60 c	490 d	52 b	323 abc	161 ab	109 c
W	19 e	1 e	12 e	3 d	22 d	11 c	12 e

	mg/plant					
Treatment	Mn	Fe	Zn	B	Cu	Mo
D1	1.94 b	1.10 a	0.94 ab	0.23 d	0.093 bc	0.023 d
D2	2.81 a	1.25 a	1.01 a	0.80 a	0.106 b	0.090 a
D3	1.94 b	0.77 b	0.66 c	0.42 c	0.067 d	0.050 c
M1	1.07 c	1.22 a	0.73 c	0.20 d	0.127 a	0.034 d
M2	1.97 b	1.15 a	0.96 a	0.73 b	0.076 cd	0.072 b
M3	1.32 c	0.88 b	0.80 bc	0.46 c	0.065 d	0.051 c
W	0.10 d	0.06 c	0.06 d	0.01 e	0.004 e	0.008 e

Table 9. *Concentration of macronutrients, pH, EC, and sugar content in plant sap at harvest (n=4). Means within each column that do not share a letter are statistically different (P < 0.05).*

	mg/L								
Treatment	NH4-N	NO3-N	P	K	S	Ca	Mg	Na	Cl
D1	6.3	69	86 c	2800 a	77 d	1103	560	290 a	2200 a
D2	5.8	88	185 a	2800 a	318 a	1133	603	338 a	2150 a
D3	4.3	2	133 bc	2175 bc	205 c	1218	698	343 a	2075 a
M1	6.34	56	120 bc	2625 ab	115 d	923	523	170 b	1750 a
M2	5.4	37	183 a	1725 c	283 ab	1123	613	303 a	945 b
M3	5.7	24	150 ab	1650 c	240 bc	995	565	265 a	808 b
	n.s.	*n.s.*				*n.s.*	*n.s.*		

	mg/L					
Treatment	Mn	Fe	Zn	B	Cu	Mo
D1	6.8 ab	0.93	3.1	0.09 c	0.20	0.16 d
D2	10.4 a	1.17	3.2	2.38 a	0.24	0.41 a
D3	10.0 a	1.06	2.5	1.04 b	0.18	0.36 ab
M1	3.3 b	0.96	3.2	0.18 c	0.20	0.20 d
M2	9.1 a	1.72	3.3	1.88 a	0.30	0.32bc
M3	5.1 b	1.01	2.7	1.32 b	0.14	0.29 c
		n.s.	*n.s.*			*n.s.*

Treatment	pH	EC (mS/cm)	Sugar (°Bx)
D1	6.1 ab	10.5 a	5.5
D2	6.2 ab	11.0 a	5.3
D3	6.1 ab	9.1 ab	5
M1	6.3 a	9.4 ab	4.8
M2	6.1 b	7.7 bc	5.3
M3	6.1 ab	6.9 c	5
			n.s.

n.s. = no significant differences

Table 10. *Plant-available nutrients (Spurway, 1949), pH, and EC in substrate at harvest (n=4). Means within each column that do not share a letter are statistically different (P < 0.05).*

	mg/L								
Treatment	N-Kjeldal	NH_4-N	P	K	S	Ca	Mg	Na	Cl
D1	2.13 ab	2.0	2.5 ab	20.8 ab	3.0	345 abc	243 d	51.3 a	< 6
D2	2.45 a	2.3	3.3 a	22.5 a	4.8	335 bc	248 d	38.3 b	< 6
D3	1.64 ab	1.8	2.0 ab	17.8 ab	4.8	343 bc	280 bc	37.3 b	N/A
M1	2.40 a	2.3	1.5 b	18.0 ab	3.5	315 c	265 cd	26.5 b	< 6
M2	1.16 b	1.0	1.8 b	15.8 b	4.8	358 ab	278 bc	26.0 b	< 6
M3	1.80 ab	2.0	2 ab	15.5 b	4.5	378 a	300 ab	30.0 b	N/A
W	1.1 ab	1.0	1.0 b	12.0 b	3.0	360 abc	300 a	36.5 b	16
		n.s.			*n.s.*				

	mg/L				
Treatment	Mn	Fe	B	pH	EC (mS/cm)
D1	0.31 ab	0.45 ab	0.13 a	6.7 a	0.25
D2	0.41 a	0.41 b	0.13 a	6.5 ab	0.25
D3	0.31 abc	0.54 ab	0.13 a	6.7 a	0.25
M1	0.21 bc	0.95 a	0.12 ab	6.3 b	0.25
M2	0.29 abc	0.49 ab	0.13 a	6.6 ab	0.25
M3	0.19 c	0.30 b	0.13 a	6.6 a	0.25
W	0.20 bc	0.42 ab	0.09 b	6.7 ab	0.4
					n.s.

n.s. = no significant differences
N/A = data not available

Phosphorus

The shoot phosphorus (P) content differed between the treatments (Table 7). The nutrient solutions with the highest total P content—D2 and M2—resulted in the highest P values for shoots, plant sap, and substrate (Tables 7, 9, and 10). However, although the total P uptake was similar in D2 and M2, the higher dry weight of the D2 plants resulted in a lower shoot P concentration in this treatment (Table 7 and 8). The P recovery efficiency (calculated as P-uptake by shoots/P added with nutrient solution) in the plain digestate (D1) was significantly lower than M1: only 65% of the applied P was found in shoots at harvest compared to 83% in M1. The low recovery rate in D1 resulted in lower shoot P concentrations than M1, but not lower DM yield.

The addition of H_3PO_4 to the digestate nutrient solution significantly increased the shoot P concentration from 2.8 g kg^{-1} (D1) to 3.9 g kg^{-1} (D2) (Table 7). It also resulted in a P recovery efficiency similar to the mineral treatments M1 and M2 (Table 11).

The P content in the nutrient solutions did not reflect the P content in the substrates at harvest (Table 10). The M2 treatment resulted in significantly lower plant-available P levels in the substrate than D2 (1.75 mg L^{-1} compared to 3.25 mg L^{-1}).

Table 11. *Nutrient recovery efficiency of P and S, calculated as the ratio of nutrients removed by crop harvest (nutrient content in shoots) to fertilizer nutrients applied (n=4). Means within each column that do not share a letter are statistically different (P < 0.05).*

Treatment	Total amount applied (mg/pot)	Total shoot uptake (mg/plant)	Nutrient recovery efficiency (shoot uptake/nutrient added)	Concentration in shoots at harvest (g/kg)
		Phosphorus		
D1	97	63 c	65% c	2.80 c
M1	101	84 b	83% a	3.65 b
D2	128	95 a	75% b	3.92 b
M2	128	102 a	80% ab	4.89 a
		Sulphur		
D1	54	36 c	67% c	1.62 d
M1	54	51 b	95% a	2.24 c
D2	115	84 a	73% bc	3.45 b
M2	115	89 a	77% b	4.26 a

Sulphur

The nutrient solutions with the highest sulphur (S) levels—D2 and M2—resulted in the highest plant-tissue and plant-sap S concentrations (Tables 7 and 9). However, M2 resulted in a higher S uptake than D2, and M1 resulted in a higher S uptake than D1. The substrates did not differ significantly in S content at harvest (Table 10).

The addition of $CaSO_4$ and $MgSO_4$ to the digestate nutrient solution significantly increased the shoot S concentration from 1.62 g kg^{-1} (in D1) to 3.45 g kg^{-1} (in D2) (Table 7). It also resulted in a S recovery efficiency similar to that of M2 (Table 11).

Sodium

The M1 treatment, with the lowest levels of sodium (Na) in the nutrient solution, had significantly lower Na concentrations in both plant tissue and plant sap at harvest (3.85 g kg^{-1} and 170 mg L^{-1}, respectively) than the other full nutrient dose treatments (Tables 7 and 9). The negative control had the highest Na plant-tissue content (9.00 g kg^{-1}), followed by M2 (7.02 g kg^{-1}). Among the full nutrient dose treatments, D2 had the highest mean for Na in plant sap (337.5 mg L^{-1}) (Table 9). The D1 substrates had the highest Na levels at harvest (51.25 mg L^{-1}) and M2 had the lowest (26.00 mg L^{-1}).

Micronutrients

In general, the differences in shoot micronutrient content reflected the differences in nutrient content in the nutrient solutions (Table 5). The plants in D2, which was the digestate treatment with the added micronutrients Mn, B and Mo, had higher Mn, B, and Mo shoot concentrations than the plants in the plain digestate treatment D1 and had the same Mn, B, and Mo content as M2 (Table 7). However, when M1 and D1, with the same lower levels of micronutrients in the nutrient solutions, were compared, M1 had significantly lower shoot tissue concentrations of Zn and Mn (Table 7).

Nutrient-stressed treatments

The only differences found between the two nutrient-stressed treatments was the chlorophyll content and the electrical conductivity (EC) of the plant sap: the plants in the M3 treatment had significantly lower values for chlorophyll content and plant-sap EC compared to the plants in the D3 treatment (Tables 6 and 9). The chlorophyll levels in the half-dose digestate treatment did not differ from the levels in the full-dose digestate treatment. The shoot DM of the nutrient-stressed plants was about 80–85% of the non-stressed plants in treatment D1, D2, and M1, and about 90% of the M2 plants (Table 6). The fresh weights were about 60% of the non-stressed plants and the total leaf area was about 75% of the non-stressed plants. The water content was about 2% lower in the nutrient-stressed treatments.

The concentrations of nutrients in shoots in the nutrient-stressed plants were lower for most nutrients compared to the full-dose treatments (Table 7). The concentrations of N, K, and P were about 70% of the full-dose treatments (N = 65%, K = 73%, P = 66%.) However, the Ca and Mg content was similar to the full-dose treatments.

pH in plant sap and substrate

The plant sap pH at harvest varied between 6.1 (M2) and 6.3 (M1). The substrate pH at harvest varied between 6.3 (M1) and 6.7 (D3). The substrate pH in M1 was significantly lower than in D1 and both of the half-dose treatments.

Electrical conductivity in plant sap and substrate

The electrical conductivity (EC) means in the substrate varied between 0.2 (M2 and M3) and 0.25 (D1 and D2), but there were no significant differences (Table 10). However, the EC in the plant sap differed significanly at harvest: the M2 treatment in both the full and half dose (M3) resulted in significantly lower EC than the other treatments. The EC was 6.9 in M3 and 11.0 in D2.

Shoot sugar content

The sugar content (measured in °Bx) in the shoots did not differ at harvest.

In summary

The plain digestate (D1), and its mineral equivalent (M1), resulted in the same dry matter yield, fresh matter yield, and chlorophyll content. However, the recovery of P and S was significantly lower in D1 than in M1. The addition of P, S, Mg, Ca, Mn, B, and Mo mineral salts to the digestate was found to significantly increase the shoot tissue concentrations of P, S, Mn and B. It was also found to increase the fresh weight but not the dry weight, and to decrease the chlorophyll content. The supplemented digestate (D2) performed as well as the standard mineral nutrient solution (M2) with respect to fresh matter yield, and outperformed it with respect to dry matter yield.

Discussion

The plant availability of S and P in the digestate

Sulphur

The content of S in the Karpalund digestate was relatively low, probably as a result of S losses in the biogas reactor (Fontaine et al., 2020; Massé et al., 2007; Peu et al., 2011; Wahid et al., 2018). The total content of S in the D1 treatment and its mineral equivalent M1 was the same according to the analysis of the digestate performed after filtration but before the nitrification (54 mg per plant). As could be expected, the S concentration in plant tissues at harvest differed between the two treatments: the digestate-fed plants had significantly ($P < 0.0001$) lower values for S. According to current research, the plant-available S can be very low even in digestates with a relatively high S content and narrow C:S ratios (Assefa, 2013; Fontaine et al., 2020).

The result in this trial was probably, at least partly, due to a low SO_4^{2-}:S ratio in the digestate. According to Yekta (2014), when Fe-salts are used as process additives, the S speciation in digestates is dominated by insoluble iron sulphides (27–62%) and the second most abundant S group is reduced organic S (22–46%). The plant-available SO_4^{2-} is only reported to make up 3–8% of the total S in digestates (Fontaine et al., 2020). In cases when Fe-salts are not added, the SO_4^{2-} concentration might rise after application to soil/substrates, as sulphides in the solution are expected to be readily oxidized to SO_4^{2-} under oxic conditions (Eriksen et al., 1995). The high amount of total Fe in the digestate in this trial (59 mg Fe and 54 mg of S per pot, corresponding to 1.1 mmol Fe and 1.7 mmol S) indicates that a large share of the sulphide-S was probably precipitated with Fe. Considering the short cultivation period and the absence of a soil microflora in this trial, oxidation of iron sulphides to SO_4^{2-} at any relevant rate could not be expected. The organic-bound S, on the other hand, is more readily available for microbial degradation (Kertesz, 2004). However, the low plant-uptake of S in D1 indicates that the microflora in the limed peat substrate, originating from the nitrified digestate, was not capable of mineralizing the organic-bound S to SO_4^{2-}, or, alternatively, was outcompeted by microbial SO_4^{2-} immobilization, as has been reported by Fontaine et al. (2020). Additionally, any microbial activity in the peat substrate was probably limited by low C-bioavailability.

It might also be speculated that the S content was lowered by volatilization during storage and handling as the digestate might have contained potentially volatile S compounds not precipitated with the added Fe (Möller and Müller, 2012).

Contrary to the findings in this trial, Pelayo Lind et al. (2020) reported high uptake of S by pak choi plants grown with nitrified digestate as fertilizer in a hydroponic setup. Similar to the digestate in this trial, the digestate was collected at a large-scale biogas plant using 2% FeCl as process additive. However, the feedstock was plant based, whereas the feedstock in the Karpalund biogas plant consisted of a large share of animal manure (29%) and slaughterhouse waste (21%), resulting in different (approximate) N:P:K:S:Fe ratios of 7:2:9:1:0.4 for Pelayo

Lind et al. (2020) and 12:2:23:1:1 for this trial. The higher amount of S in relation to N in the former (resulting in a higher total S application), as well as the higher S:Fe ratio, provides a good explanation for at least part of the higher plant-uptake of S. However, in the trial of Pelayo Lind et al. (2020), the digestate-fertilized plants outperformed the inorganic control in plant uptake of S, despite the S content in the latter being almost twice as high. This is remarkable, considering the literature discussed above. The explanation might be found in the hydroponic setup. However, this has to be further investigated.

The low levels of plant-available S in digestates pose a problem when considering using digestates as sole fertilizers, as S deficiency has been recognized as a constraint in crop production all over the world in the two last decades (Eriksen et al., 2004; Scherer, 2001). *Brassica* crops are considered extra sensitive to S deficiency, and low levels of S have been found to result in lower yields as well as lower contents of valuable S-containing metabolites such as glucosinolates (Scherer, 2001). According to Hawkesford et al. (2012), the S requirement varies between 0.1 and 0.5% of the dry weight of plants. For oilseed rape, *Brassica napus,* the critical concentration for visible deficiency symptoms has been reported to be 0.3–0.35% S, and up to 0.65% S for deficiency without visible symptoms (Schnug and Haneklaus, 1998). For optimal growth of the *Brassica oleracea* crops broccoli, cabbage, and cauliflower, a S content between 0.4–1.3% in plant tissues is recommended (Magnusson et al., 2006). Accordingly, the 0.16% S content in the D1 plants has to be considered very low and in the deficiency range, with potential negative effects on yield and quality. The values were only about 10–15% of the values reported by Pelayo Lind et al. (2020), who reported 1.1–1.4% S in plant tissues.

A lower S availability in the digestate treatments might also provide an explanation of the lower N concentrations observed in the plants in these treatments, as S interacts closely with N uptake in plants (Eriksen et al., 2001). However, the lower S and N concentrations in plant dry matter did not have any negative effects on biomass yield.

As a consequence of iron sulphide precipitation, the plant-available Fe was also probably much lower in D1 than M1. However, this was not visible in differences in plant uptake, probably because Fe was present in excess amounts in all treatments, shown by the similar Fe uptake in all full-dose treatments, including M2 with an Fe content that was 87% lower than M1. The high Fe concentration in M1, similar to D1, did not result in immobilization of S (at least not to the same degree as in D1), showing that high Fe-levels alone do not explain a lower uptake of S. This is explained by the fact that precipitation of FeS requires reducing conditions (i.e., the presence of Fe^{2+} and S^{2-}), and that sulphate reduction is a microbially mediated process (Rickard and Luther, 2007).

Phosphorus

The total P content in the Karpalund digestate was relatively high (N:P ratio of 6.7:1). As a comparison, the commonly used Hoagland lettuce solution for hydroponics has a N:P ratio of 7:1 (Smith et al., 1983) and the synthetic reference solution in this trial, M2, had a N:P ratio of 5:1. However, a considerable fraction of the total P was not plant available: only 65% of P was recovered in shoots, compared to 83% in the digestate synthetic equivalent, M1. The lower uptake of P resulted in a P tissue concentration of 0.28%, which is just within the range recommended for optimal growth of the *Brassica oleracea* crops broccoli, cabbage, and cauliflower (0.3–0.5%) (Magnusson et al., 2006). Accordingly, P was not found to limit growth in the digestate treatment. However, as the value was just on the verge of possible P shortage, the risk of P deficiency when using digestates with a similar or higher N:P ratio to the digestate in this trial (see Table 1) has to be considered. As reviewed in the background section, Stoknes et al. (2018) reported a risk of P limitation in tomatoes even after maximizing the P levels by using the digestate solids as the substrate (N:P ratio of 1.4:1) and the whole digestate instead of the liquid fraction as the nutrient solution (N:P ratio of 6:1). Lošák et al. (2016) also reported P limitations on growth when a digestate with a 6:1 N:P ratio was used as fertilizer. However, this trial was performed in a soil low in P, where P fixation could be expected (Menezes-Blackburn et al., 2016). To summarize, when digestates are used as fertilizers, a P recovery rate that is lower than that experienced when using synthetic fertilizers has to be accounted for.

Another explanation for the lower recovery rate of the P in the digestate treatment D1 might be P losses through precipitation during the nitrification pretreatment, as was reported by Kamthunzi (2015). However, Pelayo Lind et al. (2020) reported a slight decrease in the N_{min}:P ratio during nitrification in a MBBR similar to the one used in this trial (5.5:1 and 4.4:1, before and after nitrification, respectively). The fate of P during nitrification, and the factors influencing its speciation, must be further investigated.

The effect of added mineral P, S, Mg, Mn, B, and Mo

The addition of P to the digestate nutrient solution significantly increased the shoot P-concentration ($P < 0.0001$) and P-recovery efficiency ($P = 0.0139$). The results confirm those of Liedl et al. (2004), who reported positive effects of adding H_3PO_4 to a pig-slurry digestate. The direct addition of K_2HPO_4 into a diluted digestate has also had good results (Liu et al., 2011). The pH of the nutrient solution determines the speciation of P through the dissociation of H_3PO_4 to either H_2PO^{4-} (dominating P species between pH 2.1–7.2), or HPO_4^{2-} (pH > 7.2) (Lindsay, 1979; Marschner, 1995). Both $H_2PO_4^-$ and HPO_4^{2-} have a strong tendency to form ion-ion pairs, complexes, or precipitates with several metal ions such as Fe, Al, Ca, or Mg, influencing the plant availability of P (Hinsinger, 2001). The solubility and concentration of these cations are determined by pH, and in alkaline conditions, Ca and Mg are the dominating cations (Hinsinger, 2001). The slightly alkaline pH of the digestate nutrient solution (7.6) therefore posed a risk for precipitation of the added P to poorly soluble compounds such as calcium- or magnesium phosphates, struvite, and hydroxylapatite (Hinsinger, 2001; Möller and Müller, 2012). However, the recovery efficiency of the added P was > 100% (adding 31 mg of extra P per pot resulted in an average increase in P uptake by shoots of 32 mg), showing that the (bio)chemical properties of the digestate did not negatively influence the plant availability of the added P. An important factor for the high P recovery was probably the decrease in digestate pH after application to the pots. In D2, the substrate pH was 6.5 at harvest. Around this pH, the solubility of P is at its maximum, as the concentration of Al and Fe ions on the one hand, and Ca ions on the other, is minimized (Sims and Sharpley, 2007). There might also have been synergistic effects of P and the other added nutrients. For example, Mo fertilizer has been reported to increase P accumulation in shoots in *Brassica napus* (Liu et al., 2010).

Doubling the total S content in the digestate by adding $MgSO_4$ and $CaSO_4$ to D2 (Table 2) significantly ($P < 0.0001$) increased shoot-tissue S concentration levels from 0.16% to 0.36%, which is close to the minimum level recommended for optimal growth of *Brassica* crops (0.4–1.3%) (Magnusson et al., 2006). The shoot recovery efficiency of the added S was 79 % (adding 61 mg extra S per pot resulted in an average increase in S uptake by shoots of 48 mg). Considering this, a higher S addition (e.g., tripling the total S content in the digestate) would have been more optimal. No correlation was found between the Mg content in nutrient solutions and Mg plant uptake, which was probably a result of the high Mg levels in the substrate due its dolomite content.

The addition of the micronutrients B, Mn, and Mo to the digestate resulted in significant increases in shoot mineral content of the added nutrients. Boron concentration increased from 10 to 33 mg kg^{-1} in supplemented plants. For most dicotyledonous species, the critical deficiency range for B is 20–70 mg kg^{-1} (Broadley et al., 2012). For the *Brassica oleracea* species broccoli and cauliflower, 30–100 mg kg^{-1} B in shoots (the whole plant) has been recommended for optimal growth (Magnusson et al., 2006). Considering these numbers, the D1 plants suffered from B deficiency, and the supplemented D2 plants were just within the range recommended for optimal growth. One of the most rapid responses to B deficiency is inhibition of root elongation which results in stubby and bushy roots (Broadley et al., 2012). The very low tissue concentrations of B in D1 and M1 (10.1 and 8.6 mg kg^{-1}, respectively), might therefore provide an explanation of the distinctly shorter (but not bushy) roots observed in these treatments. However, no aboveground symptoms of B deficiency were detected. Further, there were no differences in DM yields between D1 and D2. This seems strange as inhibited shoot growth is a typical early symptom of B deficiency (Broadley et al., 2012). However, the fresh matter yield was significantly higher in D2, which might be explained by an increase in root volume when B was supplied at sufficient levels, allowing for a higher water uptake. The tissue concentrations of Mn and Mo were above the threshold level for deficiency (10–20 mg kg^{-1} for Mn and 0.1–1.0 mg kg^{-1} for Mo) in both D1 and D2 (Broadley et al., 2012), showing that these nutrients were present in sufficient levels in the digestate.

To summarize, adding mineral nutrients to the nitrified Karpalund digestate increased the fresh matter yield, or, in other words, the marketable yield, which is a parameter of economic importance. In fact, the D2 treatment had the highest mean for fresh weight among all the treatments. The increase might have been a result of S and/or B addition, as the shoot tissue concentration of these nutrients increased from deficient levels to sufficient levels after supplementation.

The influence of digestate beyond its nutrient value

Biostimulatory effects of digestates have been reported in bioassays and growth-trials (Ertani et al., 2013a; Fascella et al., 2015, 2018; Guilayn et al., 2020; Massa et al., 2018; Sortino et al., 2014). The observed effects on growth and stress tolerance have been attributed to high concentrations of auxin and auxin-like compounds in the digestate (Kostenberg et al., 1995; Li et al., 2016; X. Li et al., 2018; Lu et al., 2019; Scaglia et al., 2017, 2015). High concentrations of the auxin IAA in digestates have been related to protein-rich feedstocks, and high levels of the IAA persecutor L-tryptophan after protein hydrolysis (Kostenberg et al., 1995; Li et al., 2016; X. Li et al., 2018). The feedstock of the digestate in this trial consisted of 21% protein-rich slaughterhouse waste as well as relatively protein-rich manure and organic household waste. Therefore, IAA, or other auxin-like compounds derived from protein hydrolysis, could be expected to be contained within the digestate (Nardi et al., 2016; Scaglia et al., 2017). The metabolism of L-tryptophan is dependent on environmental factors and strains of micro-organisms (reviewed by Li et al., 2018), which varies between reactors and feedstocks, and therefore make results from previous studies difficult to apply when discussing the possible content of the digestate in this trial. However, the environmental parameters in the above-mentioned reports varied greatly with regard to process parameters such as the scale of the reactor, temperature, feedstock, and retention time, making it possible to regard IAA, or auxin-like compounds, as a common product of alkaline anaerobic fermentation of protein-rich feedstocks. Other compounds with reported growth-promoting effects could also be expected to be contained within the digestate, such as protein hydrolysates and large molecular weight compounds. In many cases, especially in complex organic matrices, the compound responsible for an observed biostimulatory effect is not known (Yakhin et al., 2017).

Biostimulatory effects at full-nutrient dose

The full-nutrient dose digestate treatments (D1 and D2) and mineral treatments (M1 and M2) resulted in similar yields and quality values in all but one parameter; the amended digestate, D2, resulted in higher shoot DM yield compared to M2, which might indicate a biostimulatory effect. Since the digestate was low in some nutrients, nutrient limitations might have camouflaged these biostimulatory effects in D1. Increased biomass yields with digestates compared to synthetic fertilizers have been reported in previous growth trials (Abubaker et al., 2012; Barzee et al., 2019; Gunnarsson et al., 2010). Furthermore, digestates and their alkaline extracts have been reported to have biostimulatory properties (Ertani et al., 2013; Fascella et al., 2018; Guilayn et al., 2020; Massa et al., 2016; Scaglia et al., 2017; Sortino et al., 2014). However, in the mentioned trials, it cannot be excluded that the positive plant-growth response might be related to the addition of extra plant nutrients with the digestate or digestate extracts compared to the controls. This might also be the case in this trial, as D2 contained more K, Na, Cl, Fe, Zn, and Cu than M2. In addition, the standard nutrient solution (M2) might not have been optimal for pak choi growth even though it was formulated to optimize growth of Asiatic vegetables including pak choi in hydroponic sytems (Bergstrand and Hultin, 2014). If M2 was too low in any of the nutrients supplied at sufficient levels with D2, this would provide a straightforward explanation of the higher DM yield in D2. However, the higher content of K, Na, Fe, Zn, and Cu in D2 did not result in higher DM concentrations of these nutrients compared to M2 (Table 6). On the contrary, the levels of N, K, Na, Fe, and Zn were higher in M2 than D2 at harvest (no values available for Cl), indicating that these nutrients were not limiting growth in M2. However, the plant sap concentration of K was significantly higher in D2 compared to M2, indicating differences in K dynamics with a potential effect on growth, as K can accelerate photosynthesis and also allow for a higher N-uptake, and thus a higher biomass (Mengel, 1987). However, the total N-uptake in M2 was greater than in D2, showing that higher N-assimilation was not the cause behind the higher DM in D2. Another explanation for the lower biomass yield in M2 might be to do with Mg limitations on growth caused by a high K:Mg ratio, which was 3:1 and 9:1 in the D2 and M2 nutrient solutions respectively. However, taking into account the relatively large amounts of Mg applied with the dolomite to the substrate, the ratios in the substrate solution were probably much smaller and more similar. Accordingly, no statistical differences in Mg uptake between the treatments were observed (Tables 6 and 7). Considering the nutrient composition of the M2 nutrient solution, the absence of chloride (Cl) in the solution might provide an explanation of the lower DM yield, although some was applied with tap water (~100 mg per pot in total). Chloride is essential for photosynthesis as well as stomatal regualtion, osmotic adjustment, vacuolar transport, and activation of certain enzymes, and is required in micronutrient concentrations by higher plants (Broyer et al., 1954; Heckman, 2007). Moreover, the addition of Cl to growth mediums above the micromolar levels needed for, for example, photosynthesis, has been shown to significantly increase growth and biomass in plants (reviewed by Wege et al., 2017). The D2 treatment contained about four times more Cl than the M2 treatment, including the Cl provided by the tap water. However, considering the total amount of Cl applied, both treatments contained Cl well above micronutrient concentration levels. The supply, on the other hand, was unequally distributed over the cultivation period in the M2 treatment, as a consequence of the limited need for irrigation during the first weeks, when most water was supplied via the nutrient solutions. Therefore, the M2 plants might have suffered from low Cl levels in the beginning of the experiment. In summary, that there were positive growth effects of having more K and Cl in D2 compared to M2 cannot be ruled out. Another trial, with an additional mineral control attempting to mimic the nutrient content of D2, would be needed to be able to draw any conclusions on possible growth-enhancing biostimulatory properties in the Karpalund digestate.

Biostimulatory effects at half-nutrient dose

It has been reported that many biostimulants only induce genes and enhance plant growth when the plant is challenged by biotic or abiotic stress (reviewed by Yakhin et al., 2017). To assess any such properties of compounds in the digestate, the digestate with amendments (D2) was applied at half dose (D3) in order to induce nutrient stress. The result was compared to the mineral equivalent at the same low dose (M3).

In a previous study, Massa et al. (2018) reported significantly increased biomass production after application of extracts from a muncipal solid waste digestate to nutrient-stressed Hibiscus plants, grown in a mixture of peat and pumice. Even though alkaline digestate extracts do not necessarily reflect compounds present in the whole digestate, and the concentrations of certain compounds are expected to be higher, the total applied dose of active substances might not be higher with extracts compared to digestate used as fertilizers when considering the larger volumes applied. Alkaline extractions are estimated to extract 30–50% of the organic carbon (Lehmann and Kleber, 2015), and even though the numbers are rough estimates, it can be concluded that the amount of organic material supplied with the digestate in this trial was in the same range as in the trial by Massa et al. (2018). Therefore, their results might also have relevance for understanding the properties of digestate fertilizers.

However, contrary to the results by Massa et al. (2018), the nutrient-stressed plants in the digestate treatment in this trial did not have a higher yield than the plants in the mineral treatment. The only differences found between D3 and M3 was the chlorophyll content and the plant sap EC, which were significantly ($P = 0.0476$ and 0.0322, respectively) higher in the digestate treatment (Table 5). Increased chlorophyll content has been reported as a common mechanism of action of animal- and organic waste-derived biostimulants (reviewed by Yakhin et al., 2017). However, it cannot be excluded that the result in this trial was a consequence of a slightly higher—though not significant—water content in the mineral treatment, causing a dilution of the pigments and salts. This is supported by the fact that the lower chlorophyll content in M3 did not result in lower biomass yield, indicating that the total photosynthetic capacity was not lower in this treatment. The higher EC in the digestate treatment was probably a result of its higher content of K, Na, and Cl.

Absence of biostimulatory effects

The absence of visible hormone-like effects on the tolerance of nutrient stress might be explained by the fact that any IAA present in the digestate may have been degraded prior to fertilization. Although IAA has been found in concentrations sufficient to regulate plant development in some digestates (Li et al., 2016; X. Li et al., 2018), it is known to be an unstable compound which is rapidly broken down into inactive products by light and micro-organisms (Lee, 2003). Consequently, IAA has been found to degrade in digestates during storage (Li et al., 2016). If nitrification of the digestate prior to application is needed, which was the case in this trial, the process is likely to decrease the IAA content, as it exposes the digestate to oxygen and temperatures of around 20°C for an extended period of time.

Additionally, application of exogenous IAA does not per se entail positive effects on plant growth or response to nutrient stress. As an example, the deficiency of N in *Arabidopsis* was not reported to be alleviated by exogenous application of auxin even though auxin has been shown to mediate plant response to N deficiency (Zhang et al., 2007). This indicates that auxin is not the only factor regulating the response and that the lack of interacting factors can cause the response to fail.

Finally, although digestates were not found to alleviate the plants' response to nutrient stress in this trial, it would be interesting to further investigate its effect on other stress responses, such as the response to draught.

In summary

The result showed that the recovery of P and S was significantly lower in the digestate treatment compared to the mineral control with the same total P and S content (65% for P and 67% for S was recovered in the above-ground parts of the plant in the digestate treatment in contrast to 83% for P and 95% for S in the mineral control). The shoot tissue concentrations of S (1.6 g kg^{-1}) and B (10 mg kg^{-1}) in the digestate treatment were below the threshold recommended for optimal growth. The value for P (2.8 g kg^{-}1) was within the recommended limits, but on the verge of a possible shortage of P. Supplementing the digestate with mineral P, S, Mg, Mn, Mo, and B resulted in sufficient plant tissue concentrations of all nutrients with the exception of S, and in higher fresh matter yields. The supplemented digestate performed as well as the synthetic control with respect to fresh matter yield, and outperformed it with respect to dry matter yield. It might be speculated that the higher dry matter yield was a result of biostimulatory compounds contained in the digestate; however, it cannot be excluded that it was caused by higher concentrations of K and Cl. Finally, the digestate was not found to alleviate plant response to nutrient stress.

Conclusion

In conclusion, the results were promising and showed that, after some modifications, the Karpalund digestate can be used successfully as a fertilizer in the production of leafy vegetables in peat-based substrates. From a climate change mitigation perspective, this is encouraging because the replacement of inorganic fertilizers with digestate fertilizers in protected horticulture reduces the sector's dependence on fossil fuels. This is crucial in the agricultural intensification that lies ahead if the targets of the Paris Agreement are to be reached. What is needed now is development and optimization of technology for separating the digestate into solids and liquids, and for its application. In addition, the use of digestate fertilizers in more nutrient-demanding, long-cycle crops, with varying nutrient needs during plant development needs to be further investigated.

The results showed that if a digestate is low in plant-available P and S, which is often the case, the fertilizer value can be improved by adding these nutrients in mineral form to the digestate. When calculating the amount of nutrients to add, the lower nutrient recovery rate of P and S in digestates compared to synthetic nutrient solutions must be taken into account. This is of additional importance when Fe-salts are used as process additives in the biogas reactor. However, more knowledge is needed to understand the factors influencing the recovery efficiency of S in digestate fertilizers as previous trials have reported conflicting results.

When digestates are used as fertilizers to crops grown in soil or compost, as is the case for organic producers in Sweden, the need for nutrient supplementation might be different, taking into account the specific soil's/compost's capacity to provide the crop with macro- and micronutrients. However, according to the literature reviewed and discussed in this thesis, the immobilization of S might be greater in soils than in soilless systems, and greater with digestates than with other organic fertilizers. Supplementing the digestate or the soil with, for example, potassium- or magnesium sulphate (depending on the K and Mg content in the digestate) which are water soluble and allowed in organic production, could therefore probably be generally recommended. However, this has to be further investigated.

References

Abubaker, J., Risberg, K., Pell, M., 2012. *Biogas residues as fertilisers – Effects on wheat growth and soil microbial activities.* Applied Energy 99, 126–134. https://doi.org/10.1016/j.apenergy.2012.04.050

Ahlgren, S., Hansson, P.A., Kimning, M., Aronsson, P., Lundkvist, H., 2009. *Greenhouse Gas Emissions from Cultivation of Agricultural Crops for Biofuels and Production of Biogas from Manure***.** Swedish University of Agricultural Sciences, Uppsala, Sweden.

Ahmad, R., Khalid, A., Arshad, M., Zahir, Z.A., Mahmood, T., 2008. *Effect of compost enriched with N and L-tryptophan on soil and maize.* Agron. Sustain. Dev. 28, 299–305. https://doi.org/10.1051/agro:2007058

AnoxKaldnes, 2019: Homepage (online): https://www.veoliawatertechnologies.com/en/products/anoxkaldnes-mbbr?bu=doc

Alburquerque, J.A., de la Fuente, C., Campoy, M., Carrasco, L., Nájera, I., Baixauli, C., Caravaca, F., Roldán, A., Cegarra, J., Bernal, M.P., 2012. *Agricultural use of digestate for horticultural crop production and improvement of soil properties*. European Journal of Agronomy 43, 119–128. https://doi.org/10.1016/j.eja.2012.06.001

Alburquerque, J. A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Bernal, M.P., 2012. *Assessment of the fertiliser potential of digestates from farm and agroindustrial residues.* Biomass and Bioenergy 40, 181–189. https://doi.org/10.1016/j.biombioe.2012.02.018

APHA, 1998. *Standard methods for the examination of water and wastewater.* Washington: American Public Health Association, American Water Works Association, Water Environment Federation. ISBN: 978-0-87553-287-5

Assefa, K., 2013. *Biogas residues as source of sulfur to Pak choi Brassica rapa Var. chinensis (L.)*. J. Soil Sci. Environ. Manage. 4, 163–168. https://doi.org/10.5897/JSSEM13.0409

Bachmann, S., Uptmoor, R., Eichler-Löbermann, B., Bachmann, S., Uptmoor, R., Eichler-Löbermann, B., 2016. *Phosphorus distribution and availability in untreated and mechanically separated biogas digestates.* Scientia Agricola 73, 9–17. https://doi.org/10.1590/0103-9016-2015-0069

Bachmann, S., Wentzel, S., Eichler-Löbermann, B., 2011. *Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L.* Journal of Plant Nutrition and Soil Science 174, 908–915. https://doi.org/10.1002/jpln.201000383

Banks, C.J., Chesshire, M., Heaven, S., Arnold, R., 2011. *Anaerobic digestion of source-segregated domestic food waste: Performance assessment by mass and energy balance*. Bioresource Technology 102, 612–620. https://doi.org/10.1016/j.biortech.2010.08.005

Barrow, N., 1960. *A comparison of the mineralization of nitrogen and of sulphur from decomposing organic materials.* Aust. J. Agric. Res. 11, 960. https://doi.org/10.1071/AR9600960

Barzee, T.J., Edalati, A., El-Mashad, H., Wang, D., Scow, K., Zhang, R., 2019. *Digestate Biofertilizers Support Similar or Higher Tomato Yields and Quality Than Mineral*

Fertilizer in a Subsurface Drip Fertigation System. Front. Sustain. Food Syst. 3. https://doi.org/10.3389/fsufs.2019.00058

Bergstrand, K.-J., Hultin, S., 2014. *Development of strategies for hydroponic cultivation in vertical systems*. Acta Hortic. 149–156. https://doi.org/10.17660/ActaHortic.2014.1034.17

Bloomfield, C., McGrath, S.P., 1982. *A comparison of the extractabilities of Zn, Cu, Ni and Cr from sewage sludges prepared by treating raw sewage with the metal salts before or after anaerobic digestion.* Environmental Pollution Series B, Chemical and Physical 3, 193–198. https://doi.org/10.1016/0143-148X(82)90060-X

Bougnom, B.P., Niederkofler, C., Knapp, B.A., Stimpfl, E., Insam, H., 2012. *Residues from renewable energy production: Their value for fertilizing pastures.* Biomass and Bioenergy 39, 290–295. https://doi.org/10.1016/j.biombioe.2012.01.017

Brentrup, F., Palliere, C., 2008. *GHG emissions and energy efficiency in European nitrogen fertiliser production and use*. Proceedings, 639: 1–25. York, International Fertiliser Society.

Broadley, M., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Chapter 7 - Function of Nutrients: Micronutrients. In: *Marschner's Mineral Nutrition of Higher Plants* (Third Edition). Academic Press, San Diego, pp. 191–248.

Broyer, T.C., Carlton, A.B., Johnson, C.M., Stout, P.R., 1954. *Chlorine - A Micronutrient Element for Higher Plants.* Plant Physiol. 29, 526–532. https://doi.org/10.1104/pp.29.6.526

Båth, B., Elfstrand, S., 2008. *Use of Red Clover-Based Green Manure in Leek Cultivation.* Biological Agriculture & Horticulture 25, 269–286. https://doi.org/10.1080/01448765.2008.9755053

Båth, B., Rämert, B., 1999. *Organic Household Wastes as a Nitrogen Source in Leek Production.* Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 49, 201–208. https://doi.org/10.1080/713782027

c4energi, 2019. *Innehållsdeklaration för Karpalunds Biogödsel.* Available online at: https://www.c4energi.se/globalassets/dokument/biogodsel/2019-05-10--innehallsdeklaration-feb-mars.pdf

Calvo, P., Nelson, L., Kloepper, J.W., 2014. *Agricultural uses of plant biostimulants.* Plant Soil 383, 3–41. https://doi.org/10.1007/s11104-014-2131-8

Casimiro, I., Marchant, A., Bhalerao, R. P., Beeckman, T., Dhooge, S., Swarup, R., Graham, N., Inzé, D., Sandberg, G., Casero, P. J., & Bennett, M., 2001. *Auxin transport promotes Arabidopsis lateral root initiation.* The Plant cell, 13(4), 843–852. https://doi.org/10.1105/tpc.13.4.843

Chantigny, M.H., Angers, D.A., Bélanger, G., Rochette, P., Eriksen-Hamel, N., Bittman, S., Buckley, K., Massé, D., Gasser, M.-O., 2008. *Yield and Nutrient Export of Grain Corn Fertilized with Raw and Treated Liquid Swine Manure*. Agron. J. 100, 1303–1309. https://doi.org/10.2134/agronj2007.0361

Chantigny, M.H., Angers, D.A., Rochette, P., Bélanger, G., Massé, D., Côté, D., 2007. *Gaseous Nitrogen Emissions and Forage Nitrogen Uptake on Soils Fertilized with Raw and Treated Swine Manure.* Journal of Environmental Quality 36, 1864–1872. https://doi.org/10.2134/jeq2007.0083

Cheong, J.C., Lee, J.T.E., Lim, J.W., Song, S., Tan, J.K.N., Chiam, Z.Y., Yap, K.Y., Lim, E.Y., Zhang, J., Tan, H.T.W., Tong, Y.W., 2020. *Closing the food waste loop: Food waste anaerobic digestate as fertilizer for the cultivation of the leafy vegetable, xiao bai cai (Brassica rapa).* Science of The Total Environment 136789. https://doi.org/10.1016/j.scitotenv.2020.136789

Demirel, B., Göl, N.P., Onay, T.T., 2013. *Evaluation of heavy metal content in digestate from batch anaerobic co-digestion of sunflower hulls and poultry manure.* J Mater Cycles Waste Manag 15, 242–246. https://doi.org/10.1007/s10163-012-0107-4

du Jardin, P., 2015. *Plant biostimulants: Definition, concept, main categories and regulation.* Scientia Horticulturae 196, 3–14. https://doi.org/10.1016/j.scienta.2015.09.021

Duan, N., Lin, C., Gao, R.Y., Wang, Y., Wang, J.H., Hou, J., 2011. *Ecological and economic analysis of planting greenhouse cucumbers with anaerobic fermentation residues*. Procedia Environmental Sciences 5, 71–76. https://doi.org/10.1016/j.proenv.2011.03.050

Elfstrand, S., Båth, B., Mårtensson, A., 2007. *Influence of various forms of green manure amendment on soil microbial community composition, enzyme activity and nutrient levels in leek.* Applied Soil Ecology 36, 70–82. https://doi.org/10.1016/j.apsoil.2006.11.001

Eriksen, J., 2005. *Gross sulphur mineralisation–immobilisation turnover in soil amended with plant residues.* Soil Biology and Biochemistry 37, 2216–2224. https://doi.org/10.1016/j.soilbio.2005.04.003

Eriksen, J., Mortensen, J.V., Kjellerup, V.K., Kristjansen, O., 1995. *Forms and plant-availability of sulfur in cattle and pig slurry*. Zeitschrift für Pflanzenernährung und Bodenkunde 158, 113–116. https://doi.org/10.1002/jpln.19951580125

Eriksen, J., Nielsen, M., Mortensen, J.V., Schjørring, J.K., 2001. *Redistribution of sulphur during generative growth of barley plants with different sulphur and nitrogen status*. Plant and Soil 230, 239–246. https://doi.org/10.1023/A:1010328115203

Eriksen, J., Thorup-Kristensen, K., Askegaard, M., 2004. *Plant availability of catch crop sulfur following spring incorporation*. Journal of Plant Nutrition and Soil Science 167, 609–615. https://doi.org/10.1002/jpln.200420415

Ertani, A., Pizzeghello, D., Baglieri, A., Cadili, V., Tambone, F., Gennari, M., Nardi, S., 2013a. *Humic-like substances from agro-industrial residues affect growth and nitrogen assimilation in maize (Zea mays L.) plantlets*. Journal of Geochemical Exploration 129, 103–111. https://doi.org/10.1016/j.gexplo.2012.10.001

Ertani, A., Pizzeghello, D., Baglieri, A., Cadili, V., Tambone, F., Gennari, M., Nardi, S., 2013b. *Humic-like substances from agro-industrial residues affect growth and nitrogen assimilation in maize (Zea mays L.) plantlets*. Journal of Geochemical Exploration 129, 103–111. https://doi.org/10.1016/j.gexplo.2012.10.001

FAO, 2012; Alexandratos, N., Bruinsma, J., 2012. *World agriculture towards 2030/2050: The 2012 revision.* ESA Working paper No. 12-03. Rome, FAO. Available online at: http://www.fao.org/3/a-ap106e.pdf

FAO, 2017. *The future of food and agriculture – Trends and challenges*. Rome. Available online at: http://www.fao.org/3/a-i6583e.pdf

FAO, 2019. *World fertilizer trends and outlook to 2022*. Rom.

Fascella, G., Montoneri, E., Francavilla, M., 2018. *Biowaste versus fossil sourced auxiliaries for plant cultivation: The Lantana case study.* Journal of Cleaner Production 185, 322–330. https://doi.org/10.1016/j.jclepro.2018.02.242

Fascella, G., Montoneri, E., Ginepro, M., Francavilla, M., 2015. *Effect of urban biowaste derived soluble substances on growth, photosynthesis and ornamental value of Euphorbia x lomi.* Scientia Horticulturae 197, 90–98. https://doi.org/10.1016/j.scienta.2015.10.042

Feng, H., Qu, G., Ning, P., Xiong, X., Jia, L., Shi, Y., Zhang, J., 2011. *The Resource Utilization of Anaerobic Fermentation Residue.* Procedia Environmental Sciences 11, 1092–1099. https://doi.org/10.1016/j.proenv.2011.12.165

Fixen, P., Brentrup, F., Bruulsema, T., Garcia, F., Norton, R., Zingore, S., 2015. *Nutrient/fertilizer use efficiency: measurement, current situation and trends*.

Fontaine, D., Feng, L., Labouriau, R., Møller, H.B., Eriksen, J., Sørensen, P., 2020. *Nitrogen and Sulfur Availability in Digestates from Anaerobic Co-digestion of Cover Crops, Straw and Cattle Manure.* J Soil Sci Plant Nutr 20, 621–636. https://doi.org/10.1007/s42729-019-00151-7

Fouda, S., Tucher, S. von, Lichti, F., Schmidhalter, U., 2013. *Nitrogen availability of various biogas residues applied to ryegrass*. Journal of Plant Nutrition and Soil Science 176, 572–584. https://doi.org/10.1002/jpln.201100233

Frankenberger, W.T., Arshad, M., 1995. *Phytohormones in Soils Microbial Production & Function*. CRC Press.

Freney, J.R., 1967. *Oxidation of sulphur in soils*. Mineral. Deposita 2. 181–187. https://doi.org/10.1007/BF00201914

Furukawa, Y., Hasegawa, H., 2006. *Response of Spinach and Komatsuna to Biogas Effluent Made from Source-Separated Kitchen Garbage*. Journal of Environment Quality 35, 1939. https://doi.org/10.2134/jeq2005.0482

Govasmark, E., Stäb, J., Holen, B., Hoornstra, D., Nesbakk, T., Salkinoja-Salonen, M., 2011. *Chemical and microbiological hazards associated with recycling of anaerobic digested residue intended for agricultural use.* Waste Management 31, 2577–2583. https://doi.org/10.1016/j.wasman.2011.07.025

Gruen, H.E., 1959. *Auxins and Fungi.* Annual Review of Plant Physiology 10, 405–440. https://doi.org/10.1146/annurev.pp.10.060159.002201

Guilayn, F., Benbrahim, M., Rouez, M., Crest, M., Patureau, D., Jimenez, J., 2020. *Humic-like substances extracted from different digestates: First trials of lettuce biostimulation in hydroponic culture.* Waste Management 104, 239–245. https://doi.org/10.1016/j.wasman.2020.01.025

Güngör, K., Jürgensen, A.J., Karthikeyan, K., 2007. *Determination of phosphorus speciation in dairy manure using XRD and XANES spectroscopy*. Journal of environmental quality. https://doi.org/10.2134/jeq2006.0563

Gunnarsson, A., Bengtsson, F., Caspersen, S., 2010. *Use efficiency of nitrogen from biodigested plant material by ryegrass.* Journal of Plant Nutrition and Soil Science 173, 113–119. https://doi.org/10.1002/jpln.200800250

Gunnarsson, A., Norup, S., 2018. *Lokalproducerade gödselmedel från biogasrötning – steg 1 receptutveckling och teknikadaptering (No. 1)*. Hushållningssällskapet Spåne. Available

online at: https://www.biogas2020.se/wp-content/uploads/2018/06/lokalproduceradegodselmedeldelprfinal.pdf
Hammond, J.P., Bennett, M.J., Bowen, H.C., Broadley, M.R., Eastwood, D.C., May, S.T., Rahn, C., Swarup, R., Woolaway, K.E., White, P.J., 2003. *Changes in Gene Expression in Arabidopsis Shoots during Phosphate Starvation and the Potential for Developing Smart Plants.* Plant Physiol. 132, 578–596. https://doi.org/10.1104/pp.103.020941
Hawkesford, M., Horst, W., Kichey, T., Lambers, H., Schjoerring, J., Møller, I.S., White, P., 2012. Chapter 6, Functions of Macronutrients. In: *Marschner's Mineral Nutrition of Higher Plants (Third Edition).* Academic Press, San Diego, pp. 135–189.
Heckman, J.R., 2007. Chlorine. In: *Handbook of Plant Nutrition*. CRC Press: Boca Raton. CRC Press, pp. 362–381. https://doi.org/10.1201/b18458-13
Hinsinger, P., 2001. *Bioavailability of soil inorganic P in the rhizosphere as affected by root-induced chemical changes: a review.* Plant and soil 237, 173–195.
Hoffman, E., Cavigelli, M.A., Camargo, G., Ryan, M., Ackroyd, V.J., Richard, T.L., Mirsky, S., 2018. *Energy use and greenhouse gas emissions in organic and conventional grain crop production: Accounting for nutrient inflows.* Agricultural Systems 162, 89–96. https://doi.org/10.1016/j.agsy.2018.01.021
IPCC, 2007; Bernstein, L., Roy, J., Delhotal, K. C., Harnisch, J., Matsuhashi, R., Price, L., Tanaka, K., Worrell, E., Yamba, F., Fengqi, Z. Industry. In: *Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* [Metz, B., Davidson, O.R., Bosch, P.R., Dave, R., Meyer, L.A. (eds)], Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.
IPCC, 2018; Allen, M.R., Dube, O.P., Solecki, W., Aragón-Durand, F., Cramer, W., Humphreys, S., Kainuma, M., Kala, J., Mahowald, N., Mulugetta, Y., Perez, R., Wairiu, M., Zickfeld, K. Framing and Context. In: *Global Warming of 1.5°C. An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* [Masson-Delmotte, V., Zhai, P., Pörtner, H.-O., Roberts, D., Skea, J., Shukla, P.R., Pirani, A., Moufouma-Okia, W., Péan, C., Pidcock, R., Connors, S., Matthews, J.B.R., Chen, Y., Zhou, X., Gomis, M.I., Lonnoy, E., Maycock, T., Tignor, M., Waterfield, T. (eds.)]. In Press.
IPCC, 2019a; Arneth, A., Denton, F., Agus, F., Elbehri, A., Erb, K., Osman Elasha, B., Rahimi, M., Rounsevell, M., Spence, A., Valentini, R. Framing and Context. In: *Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems* [Shukla, P.R., Skea, J., Calvo Buendia, E., Masson-Delmotte, V., Pörtner, H.-O., Roberts, D.C., Zhai, P., Slade, R., Connors, S., van Diemen, R., Ferrat, M., Haughey, E., Luz, S., Neogi, S., Pathak, M., Petzold, J., Portugal Pereira, J., Vyas, P., Huntley, E., Kissick, K., Belkacemi, M., Malley, J. (eds.)]. In press.
IPCC, 2019b; Jia, G., Shevliakova, E., Artaxo, P., De Noblet-Ducoudré, N., Houghton, R., House, J., Kitajima, K., Lennard, C., Popp, A., Sirin, A., Sukumar, R., Verchot, L. Land–climate interactions. In: *Climate Change and Land: an IPCC special report on climate*

change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems In press
J. A. Field, J. S. Caldwell, S. Jeyanayagam, R. B. Reneau, Jr., W. Kroontje, E. R. Collins, Jr., 1984. *Fertilizer Recovery from Anaerobic Digesters.* https://doi.org/10.13031/2013.33060
Jain, S., Newman, D., Nzihou, A., Dekker, H., Le Feuvre, P., Richte, H., Gobe, F., Morton, C., Thompson, R., 2019. *Global Potential of Biogas*. World Biogas Association.
Jardin, P. du, 2012. *The Science of Plant Biostimulants – A bibliographic analysis,* Ad hoc study report.
Kamthunzi, V., 2015. *The potential for using anaerobic digester effluents in recirculating hydroponics system for lettuce production.* Malawi J Agric Nat Resour Dev Stud. 2015; 2015, 8–13.
Kertesz, M.A., 2004. *The role of soil microbes in plant sulphur nutrition*. Journal of Experimental Botany 55, 1939–1945. https://doi.org/10.1093/jxb/erh176
Kirchmann, H., Witter, E., 1992. *Composition of fresh, aerobic and anaerobic farm animal dungs.* Bioresource Technology 40, 137–142. https://doi.org/10.1016/0960-8524(92)90199-8
Klackenberg, L., 2019. *Produktion och användning av biogas och rötrester år 2018*. No. ER 2019:23. Energigas Sweden on behalf of the Swedish Energy Agency.
Kongshaug, G., 1998. *Energy Consumption and Greenhouse Gas Emissions in Fertilizer Production*. IFA Technical Conference, Marrakech, Morocco, 28 September- 1 October, 1998, 18pp.
Kostenberg, D., Marchaim, U., Watad, A.A., Epstein, E., 1995. *Biosynthesis of plant hormones during anaerobic digestion of instant coffee waste.* Plant Growth Regul 17, 127–132. https://doi.org/10.1007/BF00024172
Krishnasamy, K., Nair, J., Bäuml, B., 2012. *Hydroponic system for the treatment of anaerobic liquid.* Water Sci Technol 65, 1164–1171. https://doi.org/10.2166/wst.2012.031
Kupper, T., Bürge, D., Bachmann, H.J., Güsewell, S., Mayer, J., 2014. *Heavy metals in source-separated compost and digestates.* Waste Management 34, 867–874. https://doi.org/10.1016/j.wasman.2014.02.007
Kushkevych, I., Vítězová, M., Vítěz, T., Bartoš, M., 2017. *Production of biogas: relationship between methanogenic and sulfate-reducing microorganisms.* Open Life Sciences 12, 82–91. https://doi.org/10.1515/biol-2017-0009
Lasa, B., Frechilla, S., Aleu, M., Gonzalez-Moro, B., Lamsfus, C., Aparicio-Tejo, P.M., 2000. *Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate. Lasa, B., Frechilla, S., Aleu, M. et al. Effects of low and high levels of magnesium on the response of sunflower plants grown with ammonium and nitrate.* Plant and Soil 225, 167–174. https://doi.org/10.1023/A:1026568329860
Lavado, R.S., Rodríguez, M.B., Taboada, M.A., 2005. *Treatment with biosolids affects soil availability and plant uptake of potentially toxic elements*. Agriculture, Ecosystems & Environment 109, 360–364. https://doi.org/10.1016/j.agee.2005.03.010
Lee, I.-J., 2003. *Practical application of plant growth regulator on horticultural crops*. J. Hort. Sci 10, 211–217.

Lehmann, J., Kleber, M., 2015. *The contentious nature of soil organic matter*. Nature 528, 60–68. https://doi.org/10.1038/nature16069

Li, H., Chen, Z., Zhou, T., Liu, Y., Zhou, J., 2018. *High potassium to magnesium ratio affected the growth and magnesium uptake of three tomato (Solanum lycopersicum L.) cultivars.* Journal of Integrative Agriculture 17, 2813–2821. https://doi.org/10.1016/S2095-3119(18)61949-5

Li, X., Guo, J., Dong, R., Zhang, W., 2018. I*ndolic Derivatives Metabolism in the Anaerobic Reactor Treating Animal Manure: Pathways and Regulation.* ACS Sustainable Chem. Eng. 6, 11511–11518. https://doi.org/10.1021/acssuschemeng.8b01601

Li, X., Guo, J., Pang, C., Dong, R., 2016. *Anaerobic Digestion and Storage Influence Availability of Plant Hormones in Livestock Slurry.* ACS Sustainable Chem. Eng. 4, 719–727. https://doi.org/10.1021/acssuschemeng.5b00586

Liedl, B.E., Bombardiere, J., Chatfield, J.M., 2006. *Fertilizer potential of liquid and solid effluent from thermophilic anaerobic digestion of poultry waste*. Water Science and Technology 53, 69–79. https://doi.org/10.2166/wst.2006.237

Liedl, B.E., Cummins, M., Young, A., Williams, M.L., Chatfield, J.M., 2004a. *Liquid effluent from poultry waste bioremediation as a potential nutrient source for hydroponic tomato production*. Acta Hortic. 647–652. https://doi.org/10.17660/ActaHortic.2004.659.83

Liedl, B.E., Cummins, M., Young, A., Williams, M.L., Chatfield, J.M., 2004b. *Hydroponic lettuce production using liquid effluent from poultry waste bioremediation as a nutrient source.* Acta Hortic. 721–728. https://doi.org/10.17660/ActaHortic.2004.659.93

Liedl, Barbara E., Wilfong, K., Taylor, C., Mazzaferro, K., 2006. *Liquid Effluent from Poultry Waste Bioremediation as a Nutrient Source for Hydroponic Cucumber Production*. HortScience. 41. 997A-997. https://doi.org/10.21273/Hortsci.41.4.997A.

Lindsay, W.L., 1979. *Chemical equilibria in soils*. John Wiley and Sons Ltd., Chichester, Sussex, UK.

Liu, H., Hu, C., Sun, X., Tan, Q., Nie, Z., Hu, X., 2010. *Interactive effects of molybdenum and phosphorus fertilizers on photosynthetic characteristics of seedlings and grain yield of Brassica napus*. Plant Soil 326, 345–353. https://doi.org/10.1007/s11104-009-0014-1

Liu, W.K., Lianfeng, D., Qichang, Y., 2009a. *Biogas slurry added amino acids decreased nitrate concentrations of lettuce in sand culture.* Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 59, 260–264. https://doi.org/10.1080/09064710802029551

Liu, W.K., Yang, Q.-C., Du, L., 2009b. *Soilless cultivation for high-quality vegetables with biogas manure in China: Feasibility and benefit analysis.* Renewable Agriculture and Food Systems 24, 300–307.

Liu, W.K., Yang, Q.C., Du, L.F., Cheng, R.F., Zhou, W.L., 2011. *Nutrient supplementation increased growth and nitrate concentration of lettuce cultivated hydroponically with biogas slurry.* Acta Agriculturae Scandinavica, Section B - Soil & Plant Science 61, 391–394. https://doi.org/10.1080/09064710.2010.482539

Loide, V., 2004. *About the effect of the contents and ratios of soil's available calcium, potassium and magnesium in liming of acid soils.* 2.

López-Bucio, J., Hernández-Abreu, E., Sánchez-Calderón, L., Nieto-Jacobo, M.F., Simpson, J., Herrera-Estrella, L., 2002. *Phosphate Availability Alters Architecture and Causes*

Changes in Hormone Sensitivity in the Arabidopsis Root System. Plant Physiol. 129, 244–256. https://doi.org/10.1104/pp.010934

Loria, E.R., Sawyer, J.E., 2005. *Extractable Soil Phosphorus and Inorganic Nitrogen following Application of Raw and Anaerobically Digested Swine Manure.* Agronomy Journal 97, 879–885. https://doi.org/10.2134/agronj2004.0249

Loria, E.R., Sawyer, J.E., Barker, D.W., Lundvall, J.P., Lorimor, J.C., 2007. *Use of Anaerobically Digested Swine Manure as a Nitrogen Source in Corn Production.* Agron. J. 99, 1119–1129. https://doi.org/10.2134/agronj2006.0251

Lošák, T., Hlušek, J., Válka, T., Elbl, J., Vítěz, T., Bělíková, H., Von Bennewitz, E., 2016. *The effect of fertilisation with digestate on kohlrabi yields and quality.* Plant Soil Environ. 62, 274–278. https://doi.org/10.17221/16/2016-PSE

Lošák, T., Zatloukalová, A., Szostková, M., Hlušek, J., Fryč, J., Vítěz, T., 2011. *Comparison of the effectiveness of digestate and mineral fertilisers on yields and quality of kohlrabi (Brassica oleracea, L.).* Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 59, 117–122. https://doi.org/10.11118/actaun201159030117

Lu, J., Muhmood, A., Czekała, W., Mazurkiewicz, J., Dach, J., Dong, R., 2019. *Untargeted Metabolite Profiling for Screening Bioactive Compounds in Digestate of Manure under Anaerobic Digestion.* Water 11, 2420. https://doi.org/10.3390/w11112420

Lynch, J.M., 1985. *Origin, Nature and Biological Activity of Aliphatic Substances and Growth Hormones Found in Soil*, in: Vaughan, D., Malcolm, R.E. (Eds.), Soil Organic Matter and Biological Activity, Developments in Plant and Soil Sciences. Springer Netherlands, Dordrecht, pp. 151–174. https://doi.org/10.1007/978-94-009-5105-1_5

Magnusson, M., Rölin, Å., Ögren, E., 2006. *Förslag till riktvärden för jord- och plantanalyser i ekologisk grönsaksodling*. Hushållningssällskapet. Available online at: http://www.margaretamagnusson.se/PDF/PUBLICERING/FOURAPPORT2006.pdf

Marcato, C.-E., Mohtar, R., Revel, J.-C., Pouech, P., Hafidi, M., Guiresse, M., 2009a. *Impact of anaerobic digestion on organic matter quality in pig slurry*. International Biodeterioration & Biodegradation 63, 260–266. https://doi.org/10.1016/j.ibiod.2008.10.001

Marcato, C.-E., Pinelli, E., Cecchi, M., Winterton, P., Guiresse, M., 2009b. *Bioavailability of Cu and Zn in raw and anaerobically digested pig slurry.* Ecotoxicology and Environmental Safety 72, 1538–1544. https://doi.org/10.1016/j.ecoenv.2008.12.010

Marcato, C.E., Pinelli, E., Pouech, P., Winterton, P., Guiresse, M., 2008. *Particle size and metal distributions in anaerobically digested pig slurry.* Bioresource Technology 99, 2340–2348. https://doi.org/10.1016/j.biortech.2007.05.013

Markensten, T., Bodin, P., Andersson, J., Hagerberg, A., Samuelsson, L., Loberg, J., Söderberg, T., Bång, M., Hjulfors, L.N., Frid, G., Franke, U., 2018. *Hur kan den svenska jordbruks- sektorn bidra till att vi når det nationella klimatmålet? Sammanställning av pågående arbete och framtida insatsområden.* (No. Rapport 2018:1). Swedish Board of Agriculture.

Marschner, H., 1995. *Mineral nutrition of higher plants*, 2. ed. ed. Academic Press, London.

Massa, D., Lenzi, A., Montoneri, E., Ginepro, M., Prisa, D., Burchi, G., 2018. *Plant response to biowaste soluble hydrolysates in hibiscus grown under limiting nutrient availability.* Journal of Plant Nutrition 41, 396–409. https://doi.org/10.1080/01904167.2017.1404611

Massa, D., Prisa, D., Montoneri, E., Battaglini, D., Ginepro, M., Negre, M., Burchi, G., 2016. *Application of municipal biowaste derived products in Hibiscus cultivation: Effect on leaf gaseous exchange activity, and plant biomass accumulation and quality.* Scientia Horticulturae 205, 59–69. https://doi.org/10.1016/j.scienta.2016.03.033

Massé, D.I., Croteau, F., Masse, L., 2007. *The fate of crop nutrients during digestion of swine manure in psychrophilic anaerobic sequencing batch reactors*. Bioresource Technology 98, 2819–2823. https://doi.org/10.1016/j.biortech.2006.07.040

Menezes-Blackburn, D., Paredes, C., Zhang, H., Giles, C.D., Darch, T., Stutter, M., George, T.S., Shand, C., Lumsdon, D., Cooper, P., Wendler, R., Brown, L., Blackwell, M., Wearing, C., Haygarth, P.M., 2016. *Organic Acids Regulation of Chemical–Microbial Phosphorus Transformations in Soils.* Environ. Sci. Technol. 50, 11521–11531. https://doi.org/10.1021/acs.est.6b03017

Mengel, K., 1987. *Principles of plant nutrition,* 4. ed. ed. International Potash Institute, Worblaufen-Bern.

Moestedt, J., Nilsson Påledal, S., Schnürer, A., 2013. *The effect of substrate and operational parameters on the abundance of sulphate-reducing bacteria in industrial anaerobic biogas digesters.* Bioresource Technology 132, 327–332. https://doi.org/10.1016/j.biortech.2013.01.043

Mohammed, N., Onodera, R., Or-Rashid, M.M., 2003. *Degradation of tryptophan and related indolic compounds by ruminal bacteria, protozoa and their mixture in vitro.* Amino Acids 24, 73–80. https://doi.org/10.1007/s00726-002-0330-8

Molinuevo-Salces, B., Gómez, X., Morán, A., García-González, M.C., 2013. *Anaerobic co-digestion of livestock and vegetable processing wastes: Fibre degradation and digestate stability.* Waste Management 33, 1332–1338. https://doi.org/10.1016/j.wasman.2013.02.021

Möller, K., 2015. *Effects of anaerobic digestion on soil carbon and nitrogen turnover, N emissions, and soil biological activity. A review*. Agron. Sustain. Dev. 35, 1021–1041. https://doi.org/10.1007/s13593-015-0284-3

Möller, K., Müller, T., 2012. *Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review.* Engineering in Life Sciences 12, 242–257. https://doi.org/10.1002/elsc.201100085

Möller, K., Stinner, W., 2010. *Effects of organic wastes digestion for biogas production on mineral nutrient availability of biogas effluents.* Nutr Cycl Agroecosyst 87, 395–413. https://doi.org/10.1007/s10705-010-9346-8

Möller, K., Stinner, W., 2009. *Effects of different manuring systems with and without biogas digestion on soil mineral nitrogen content and on gaseous nitrogen losses (ammonia, nitrous oxides).* European Journal of Agronomy 30, 1–16. https://doi.org/10.1016/j.eja.2008.06.003

Möller, K., Stinner, W., Deuker, A., Leithold, G., 2008a. *Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems.* Nutr Cycl Agroecosyst 82, 209–232. https://doi.org/10.1007/s10705-008-9196-9

Möller, K., Stinner, W., Leithold, G., 2008b. *Effects of biogas digestion of clover/grass-leys, cover crops and crop residues on nitrogen cycle and crop yield in organic stockless* *farming systems.* European Journal of Agronomy 29, 125–134. https://doi.org/10.1016/j.eja.2008.04.006

Montoneri, E., Mainero, D., Boffa, V., Perrone, D.G., Montoneri, C., 2011. *Biochemenergy: a project to turn an urban wastes treatment plant into biorefinery for the production of energy, chemicals and consumer's products with friendly environmental impact*. Ijgenvi 11, 170. https://doi.org/10.1504/IJGENVI.2011.043528

Moorhead, K.K., Graetz, D.A., Reddy, K.R., 1987. *Decomposition of Fresh and Anaerobically Digested Plant Biomass in Soil.* Journal of Environmental Quality - J Environ Qual. 16. 10.2134/jeq1987.00472425001600010031x.

Mulder, E.G., 1956. *Nitrogen-magnesium relationships in crop plants.* Plant Soil 7, 341–376. https://doi.org/10.1007/BF01394322

Myhre, G., Shindell, D., Bréon, F.-M., Collins, W., Fuglestvedt, J., Huang, J., Koch, D., Lamarque, J.-F., Lee, D., Mendoza, B., Nakajima, T., Robock, A., Stephens, G., Zhang, H., 2013. Anthropogenic and Natural Radiative Forc-ing. In: *Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* [Stocker, T.F., Qin, D., Plattner, G.-K.M., Tignor, Allen, S.K., Boschung, J., Nauels, A., Xia, Y., Bex V., Midgley, P.M. (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA.

Nacry, P., Canivenc, G., Muller, B., Azmi, A., Van Onckelen, H., Rossignol, M., Doumas, P., 2005. *A Role for Auxin Redistribution in the Responses of the Root System Architecture to Phosphate Starvation in Arabidopsis.* Plant Physiol. 138, 2061–2074. https://doi.org/10.1104/pp.105.060061

Nardi, S., Pizzeghello, D., Schiavon, M., Ertani, A., 2016. *Plant biostimulants: physiological responses induced by protein hydrolyzed-based products and humic substances in plant metabolism.* Sci. agric. (Piracicaba, Braz.) 73, 18–23. https://doi.org/10.1590/0103-9016-2015-0006

Neal, J., Wilkie, D.A.C., 2014. *Anaerobic Digester Effluent as Fertilizer for Hydroponically Grown Tomatoes.* Available online at: https://ufdc.ufl.edu/UF00091523/00710

Nicoletto, C., Galvao, A., Maucieri, C., Borin, M., Sambo, P., 2017. *Distillery anaerobic digestion residues: A new opportunity for sweet potato fertilization.* Scientia Horticulturae 225, 38–47. https://doi.org/10.1016/j.scienta.2017.06.048

Nicoletto, C., Santagata, S., Sambo, P., 2013. *Effect of anaerobic digestates application on qualitative traits in early and late cauliflower.* Acta Horticulturae. International Society for Horticultural Science (ISHS), Leuven, Belgium, pp. 463–469. https://doi.org/10.17660/ActaHortic.2013.1005.56

Nicoletto, C., Santagata, S., Zanin, G., Sambo, P., 2014. *Effect of the anaerobic digestion residues use on lettuce yield and quality*. Scientia Horticulturae 180, 207–213. https://doi.org/10.1016/j.scienta.2014.10.028

Nkoa, R., 2014. *Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review.* Agronomy for Sustainable Development 34, 473–492. https://doi.org/10.1007/s13593-013-0196-z

Odhner, P., Sernhed, K., Svensson, S.-E., Juhlin, M., 2015. *Biogödsel i Skåne en inventering och marknadsanalys.* Sveriges lantbruksuniversitet, Alnarp, Sweden. Available online at: https://pub.epsilon.slu.se/12649/17/svensson_et_al_150929_1.pdf

Odlare, M., Arthurson, V., Pell, M., Svensson, K., Nehrenheim, E., Abubaker, J., 2011. *Land application of organic waste – Effects on the soil ecosystem.* Applied Energy 88, 2210–2218. https://doi.org/10.1016/j.apenergy.2010.12.043

Pandey, V., Bhatt, I.D., Nandi, S.K., 2019. *Role and Regulation of Auxin Signaling in Abiotic Stress Tolerance*, in: Plant Signaling Molecules. Elsevier, pp. 319–331. https://doi.org/10.1016/B978-0-12-816451-8.00019-8

Pelayo Lind, O., Hultberg, M., Bergstrand, K.-J., Larsson-Jönsson, H., Caspersen, S., Asp, H., 2020. *Biogas Digestate in Vegetable Hydroponic Production: pH Dynamics and pH Management by Controlled Nitrification*. Waste Biomass Valor. https://doi.org/10.1007/s12649-020-00965-y

Peu, P., Sassi, J.-F., Girault, R., Picard, S., Saint-Cast, P., Béline, F., Dabert, P., 2011. *Sulphur fate and anaerobic biodegradation potential during co-digestion of seaweed biomass (Ulva sp.) with pig slurry.* Bioresource Technology 102, 10794–10802. https://doi.org/10.1016/j.biortech.2011.08.096

Pitts, J., 2019. Digestates from food waste and lignocellulosic materials: effects on plant growth. Available online at: http://hdl.handle.net/10125/63523

Pokhrel, B., Sorensen, J.N., Moller, H.B., Petersen, K.K., 2018. *Processing methods of organic liquid fertilizers affect nutrient availability and yield of greenhouse grown parsley.* Renewable Agriculture and Food Systems 1–9. https://doi.org/10.1017/S1742170517000771

Psomopoulos, C.S., Bourka, A., Themelis, N.J., 2009. *Waste-to-energy: A review of the status and benefits in USA.* Waste Management 29, 1718–1724. https://doi.org/10.1016/j.wasman.2008.11.020

Rickard, D., Luther, G.W., 2007. *Chemistry of Iron Sulfides.* Chem. Rev. 107, 514–562. https://doi.org/10.1021/cr0503658

Risberg, K., 2015. *Quality and function of anaerobic digestion residues*. Doctoral Thesis. Faculty of Natural Resources and Agricultural Sciences Department of Microbiology Uppsala, Swedish University of Agricultural Sciences.

Ronga, D., Pellati, F., Brighenti, V., Laudicella, K., Laviano, L., Fedailaine, M., Benvenuti, S., Pecchioni, N., Francia, E., 2018. *Testing the influence of digestate from biogas on growth and volatile compounds of basil (Ocimum basilicum L.) and peppermint (Mentha x piperita L.) in hydroponics.* Journal of Applied Research on Medicinal and Aromatic Plants 11, 18–26. https://doi.org/10.1016/j.jarmap.2018.08.001

Ronga, D., Setti, L., Salvarani, C., De Leo, R., Bedin, E., Pulvirenti, A., Milc, J., Pecchioni, N., Francia, E., 2019. *Effects of solid and liquid digestate for hydroponic baby leaf lettuce (Lactuca sativa L.) cultivation.* Scientia Horticulturae 244, 172–181. https://doi.org/10.1016/j.scienta.2018.09.037

Saha, B., Saha, S., Roy, P.D., Padhan, D., Pati, S., Hazra, G.C., 2018. *Microbial Transformation of Sulphur: An Approach to Combat the Sulphur Deficiencies in Agricultural Soils,* in: Meena, V.S. (Ed.), Role of Rhizospheric Microbes in Soil: Volume

2: Nutrient Management and Crop Improvement. Springer, Singapore, pp. 77–97. https://doi.org/10.1007/978-981-13-0044-8_3

Sarwar, M., Frankenberger, W.T., 1994. *Influence of L-tryptophan and auxins applied to the rhizosphere on the vegetative growth of Zea mays L*. Plant Soil 160, 97–104. https://doi.org/10.1007/BF00150350

Scaglia, B., Pognani, M., Adani, F., 2017. *The anaerobic digestion process capability to produce biostimulant: the case study of the dissolved organic matter (DOM) vs. auxin-like property*. Science of The Total Environment 589, 36–45. https://doi.org/10.1016/j.scitotenv.2017.02.223

Scaglia, B., Pognani, M., Adani, F., 2015. *Evaluation of hormone-like activity of the dissolved organic matter fraction (DOM) of compost and digestate.* Science of The Total Environment 514, 314–321. https://doi.org/10.1016/j.scitotenv.2015.02.009

Scherer, H.W., 2001. *Sulphur in crop production — invited paper*. European Journal of Agronomy 14, 81–111. https://doi.org/10.1016/S1161-0301(00)00082-4

Schievano, A., D'Imporzano, G., Salati, S., Adani, F., 2011. *On-field study of anaerobic digestion full-scale plants (Part I): An on-field methodology to determine mass, carbon and nutrients balance.* Bioresource Technology 102, 7737–7744. https://doi.org/10.1016/j.biortech.2011.06.006

Schnug, E., Haneklaus, S., 1998. *Diagnosis of Sulphur Nutritio*n, in: Sulphur in Agroecosystems, Nutrients in Ecosystems. Springer Netherlands, Dordrecht, pp. 1–38. https://doi.org/10.1007/978-94-011-5100-9_1

Sensel, K., Wragge, V., 2008. *Pflanzenbauliche Verwertung von Gärrückständen aus Biogasanlagen unter besonderer Berücksichtigung des Inputsubstrats Energiepflanzen.*

Sheets, J.P., Yang, L., Ge, X., Wang, Z., Li, Y., 2015. *Beyond land application: Emerging technologies for the treatment and reuse of anaerobically digested agricultural and food waste.* Waste Management 44, 94–115. https://doi.org/10.1016/j.wasman.2015.07.037

Shen, D., 2001. *Beneficial Microorganisms and Metabolites Derived from Agriculture Wastes in Improving Plant Health and Protection.* Journal of Crop Production 3, 349–366. https://doi.org/10.1300/J144v03n01_29

Šimon, T., Kunzová, E., Friedlová, M., 2016. *The effect of digestate, cattle slurry and mineral fertilization on the winter wheat yield and soil quality parameters.* Plant, Soil and Environment 61, 522–527. https://doi.org/10.17221/530/2015-PSE

Sims, J.T., Sharpley, A.N. (Eds.), 2007. *Phosphorus: agriculture and the environment*. American Society of Agronomy, Madison, Wis.

Smith, G.S., Johnston, C.M., Cornforth, I.S., 1983. *Comparison of Nutrient Solutions for Growth of Plants in Sand Culture.* New Phytologist 94, 537–548. https://doi.org/10.1111/j.1469-8137.1983.tb04863.x

Sogn, T.A., Dragicevic, I., Linjordet, R., Krogstad, T., Eijsink, V.G.H., Eich-Greatorex, S., 2018. *Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching.* Int J Recycl Org Waste Agricult 7, 49–58. https://doi.org/10.1007/s40093-017-0188-0

Song, W., Liu, S., Meng, L., Xue, R., Wang, C., Liu, G., Dong, C., Wang, S., Dong, J., Zhang, Y., 2015. *Potassium deficiency inhibits lateral root development in tobacco*

seedlings by changing auxin distribution. Plant Soil 396, 163–173. https://doi.org/10.1007/s11104-015-2579-1
Sonneveld, C., Straver, N.B., 1994. *Nutrient solution for vegetables and flowers grown in water or substrates.* Research Station for Floriculture and Glasshouse Vegetables. Aalsmeer, Naaldwijk, The Netherlands, Series: Voedingspolossingen Glastijnbouw, 8.
Sortino, O., Montoneri, E., Patanè, C., Rosato, R., Tabasso, S., Ginepro, M., 2014. *Benefits for agriculture and the environment from urban waste.* Science of The Total Environment 487, 443–451. https://doi.org/10.1016/j.scitotenv.2014.04.027
Spurway, C.H. 1883-, 1949. *Soil testing : a practical system of soil fertility diagnosis*. Agriculture Experiment Station, Michigan State College.
Stewart, W.M., Dibb, D.W., Johnston, A.E., Smyth, T.J., 2005. *The Contribution of Commercial Fertilizer Nutrients to Food Production.* Agronomy Journal 97, 1–6. https://doi.org/10.2134/agronj2005.0001
Stoknes, K., Wojciechowska, E., Jasińska, A., Gulliksen, A., Tesfamichael, A.A., 2018. *Growing vegetables in the circular economy; cultivation of tomatoes on green waste compost and food waste digestate.* Acta Hortic. 389–396. https://doi.org/10.17660/ActaHortic.2018.1215.71
Straka, F., Jenicek, P., Zabranska, J., Dohanyos, M., Kuncarova, M., 2007. *Anaerobic fermentation of biomass and wastes with respect to sulfur and nitrogen contents in treated materials*. Proceedings of the 11th International Waste Management and Landfill Symposium, Sardinia 2007). 33.
Svehla, P., Caceres, L.M.V., Michal, P., Tlustos, P., 2020. *Nitrification of the liquid phase of digestate can help with the reduction of nitrogen losses*. Environmental Technology & Innovation 17, 100514. https://doi.org/10.1016/j.eti.2019.100514
Svensson, K., Odlare, M., Pell, M., 2004. *The fertilizing effect of compost and biogas residues from source separated household waste.* J. Agric. Sci. 142, 461–467. https://doi.org/10.1017/S0021859604004514
Swaminathan, B., Sukalac, K.E., 2004. *Technology transfer and mitigation of climate change: The fertilizer industry perspective*. Presented at the IPCC Expert Meeting on Industrial Technology Development, Transfer and Diffusion, Tokyo, Japan, 21-23 Sept. 2004.
Swedish Board of Agriculture, 2018. *Hur kan den svenska jordbruks- sektorn bidra till att vi når det nationella klimatmålet? Sammanställning av pågående arbete och framtida insatsområden.* Report 2018:1.
Swedish Climate Policy Council, 2019. *2019, Swedish Climate Policy Council report*. (Summary in English). Available online at: https://www.klimatpolitiskaradet.se/wp-content/uploads/2019/09/kprrapport190426.pdf
Swedish Energy Gas (2018). *Förslag till nationell biogasstrategi 2.0.*
Swedish Environmental Protection Agency, 2019. *National Inventory Report Sweden 2019. Greenhouse Gas Emission Inventories 1990-2017.* Submitted under the United Nations Framework Convention on Climate Change and the Kyoto Protocol. Available online at: https://www.naturvardsverket.se/upload/miljoarbete-i-samhallet/internationellt-miljoarbete/miljokonventioner/FN/nir-sub-15-april.pdf
Syers, J.K., Johnston, A.E., Curtin, D., 2008. *Efficiency of soil and fertilizer phosphorus use: reconciling changing concepts of soil phosphorus behaviour with agronomic information*.

FAO fertilizer and plant nutrition bulletin. Food and Agriculture Organization of the United Nations, Rome.
Taiz, L., Zeiger, E., Møller, I.M., Murphy, A.S., 2015. *Plant physiology and development*.
Tambone, F., Orzi, V., Zilio, M., Adani, F., 2019. *Measuring the organic amendment properties of the liquid fraction of digestate.* Waste Management 88, 21–27. https://doi.org/10.1016/j.wasman.2019.03.024
Tambone, F., Scaglia, B., D'Imporzano, G., Schievano, A., Orzi, V., Salati, S., Adani, F., 2010. *Assessing amendment and fertilizing properties of digestates from anaerobic digestion through a comparative study with digested sludge and compost.* Chemosphere 81, 577–583. https://doi.org/10.1016/j.chemosphere.2010.08.034
Tampio, E., Salo, T., Rintala, J., 2016. *Agronomic characteristics of five different urban waste digestates.* Journal of Environmental Management 169, 293–302. https://doi.org/10.1016/j.jenvman.2016.01.001
Torrey, J.G., 1950. *The induction of lateral roots by indoleacetic acid and root decapitation.* American Journal of Botany 37, 257–264. https://doi.org/10.1002/j.1537-2197.1950.tb12192.x
Trzcinski, A.P., Stuckey, D.C., 2011. *Parameters affecting the stability of the digestate from a two-stage anaerobic process treating the organic fraction of municipal solid waste.* Waste Management 31, 1480–1487. https://doi.org/10.1016/j.wasman.2011.02.015
Uchimura, K., Sago, Y., Kamahara, H., Atsuta, Y., Daimon, H., 2014. *Treatment of anaerobic digestion effluent of sewage sludge using soilless cultivation*. Presented at the The Irago Conference 2013, Aichi, Japan, pp. 59–63. https://doi.org/10.1063/1.4866619
UN, 2015. *Transforming our world: The 2030 agenda for sustainable development*. Available online at: https://sustainabledevelopment.un.org
UN, 2019. *World Population Prospects: The 2019 Revision*. Available online at: https://esa.un.org/unpd/wpp
UNFCCC, 2015. *Paris Agreement on Climate Change*. Available online at: https://unfccc.int/sites/default/files/english_paris_agreement.pdf
Wahid, R., Feng, L., Cong, W.-F., Ward, A.J., Møller, H.B., Eriksen, J., 2018. *Anaerobic mono-digestion of lucerne, grass and forbs – Influence of species and cutting frequency*. Biomass and Bioenergy 109, 199–208. https://doi.org/10.1016/j.biombioe.2017.12.029
Webb, A.R., Hawkes, F.R., 1985. *Laboratory scale anaerobic digestion of poultry litter: Gas yield-loading rate relationships.* Agricultural Wastes 13, 31–49. https://doi.org/10.1016/0141-4607(85)90010-1
Wege, S., Gilliham, M., Henderson, S.W., 2017. *Chloride: not simply a 'cheap osmoticum', but a beneficial plant macronutrient.* Journal of Experimental Botany 68, 3057–3069. https://doi.org/10.1093/jxb/erx050
Williams, G., Al-Ansari, F., 2007. *IFA Benchmarking of Global Energy Efficiency in Ammonia Production.* Workshop on Energy Efficiency and CO2 Reduction Prospects in Ammonia Production, IN: IFA Technical Committee Meeting, 12-14 March Ho Chi Minh City, Vietnam.
Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., Brown, P.H., 2017. *Biostimulants in Plant Science: A Global Perspective.* Front. Plant Sci. 7. https://doi.org/10.3389/fpls.2016.02049

Yekta, S.S., 2014. *Chemical Speciation of Sulfur and Metals in Biogas Reactors: Implications for Cobalt and Nickel Bio-uptake*. Journal of Hazardous Materials, 324, 110-116. https://doi.org/10.1016/j.jhazmat.2015.12.058

Yokoyama, M.T., Carlson, J.R., 1979. *Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole*. The American Journal of Clinical Nutrition 32, 173–178. https://doi.org/10.1093/ajcn/32.1.173

Yu, F.-B., Luo, X.-P., Song, C.-F., Zhang, M.-X., Shan, S.-D., 2010. *Concentrated biogas slurry enhanced soil fertility and tomato quality.* Acta Agriculturae Scandinavica, Section B - Plant Soil Science 60, 262–268. https://doi.org/10.1080/09064710902893385

Zhang, H., Rong, H., Pilbeam, D., 2007. *Signalling mechanisms underlying the morphological responses of the root system to nitrogen in Arabidopsis thaliana.* Journal of Experimental Botany 58, 2329–2338. https://doi.org/10.1093/jxb/erm114

Zirkler, D., Peters, A., Kaupenjohann, M., 2014. *Elemental composition of biogas residues: Variability and alteration during anaerobic digestion.* Biomass and Bioenergy 67, 89–98. https://doi.org/10.1016/j.biombioe.2014.04.021