



Nutrient challenges with solid-phase anaerobic digestate as a peat substitute – Storage decreased ammonium toxicity but increased phosphorus availability

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ABSTRACT

The solid fraction (SD) obtained after liquid – solid separation of anaerobic digestate is interesting as a potential fertilizer as well as a peat substitute in horticultural growing substrates. We investigated the effect of incubation of the SD obtained by screw-press separation of digestate produced from food waste and plant residues on potentially plant available mineral nutrients and plant growth. The $\text{NH}_4\text{-N}$ concentration was initially $> 1000 \text{ mg L}^{-1}$ but rapidly decreased, probably due to NH_3 emission promoted by a high initial pH. No nitrate was detected during the first four weeks of incubation. The concentrations of potentially available P and Mg were closely related and strongly increased during incubation. The effect of adding 20 or 30 vol% of SD to a peat-based growing substrate on the growth of basil and lettuce was investigated before and after the incubation period. With the unincubated SD, the initial substrate $\text{NH}_4\text{-N}$ of 200–300 mg L^{-1} was potentially phytotoxic. Plant growth response ranged from inhibition to stimulation, probably reflecting variation in substrate ammonium status. After 96 days of incubation, ammonium concentrations had decreased with $> 50\%$ and basil growth was generally positively affected by addition of incubated SD. However, available P concentrations of 140–210 mg L^{-1} in the incubated substrates posed a high risk of P leakage. In conclusion, storage greatly reduced $\text{NH}_4\text{-N}$ concentrations and phytotoxicity when the SD was used as a partial substituent for peat in a horticultural growing substrate. Measures are needed, however, to limit available P concentrations in high-P solid digestate fractions.

1. Introduction

Returning mineral nutrients from agricultural and food waste back to crop production as fertilizers and soil amendments is a prerequisite for the development of a more sustainable society. Development of recirculated and resource-efficient fertilizers for food production provides an important step towards more sustainable food production.

Anaerobic digestion is utilised in many countries for biogas production from agro-industrial, food and municipal wastes (de Groot and Bogdanski 2013, Nkoa 2014), leaving a nutrient-rich residue as a side product of the biogas process. However, the composition of anaerobic digestates (AD) is very variable, depending on the feedstock (Alburquerque et al., 2012, Provenzano et al. 2011, Teglia et al. 2011, Lamolinara et al. 2022), as well as on the processing technology and the operating conditions (Lamolinara et al. 2022).

The original content of organic matter in the feedstock is commonly

reduced by about 25% during the AD process (Guilayn et al. 2020). There is usually little change in the total contents of macronutrients such as N, P, K, Ca and Mg. In contrast, the concentrations of plant available forms of N, P and K often increase after AD due to the degradation of organic material (Guilayn et al. 2020).

Anaerobic digestates can be used as secondary raw materials for fertilizer production (Lin et al. 2015, Vaneeckhaute et al. 2017) or applied directly as fertilizers (Möller & Müller 2012, Nkoa 2014, Wang & Lee 2021). Fertilizer effects of AD are often similar or higher in comparison to the undigested feedstock (Foeroid et al., 2021, Nkoa 2014) or to mineral fertilizer (Gunnarsson et al. 2010, Nkoa 2014, Riva et al. 2016, Grillo et al. 2021), but the response may differ between crops (Alburquerque et al., 2012).

While AD show a large potential as fertilizers, there are several challenges related to the use of AD in agricultural and horticultural plant production as reviewed by Nkoa (2014). Firstly, the high proportion of

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ammonium in the digestate combined with the pH increase during digestion favour the conversion of ammonium to ammonia, increasing the risk of ammonia emissions during digestion, digestate processing and handling (Nkoa 2014). Besides ammonia, nitrous oxide (N₂O) may also be emitted following soil application of anaerobic digestates (Nkoa 2014, Barduca et al. 2021), but the emission is generally lower in comparison with the undigested feedstocks (Nkoa 2014).

In addition to the negative environmental effects from NH₃ emission, phytotoxic effects of NH₃ as well as of NH₄⁺ have been reported for a large range of plant species (Britto & Kronzucker 2002, Pan et al. 2016, Frierichs et al. 2020). Phytotoxicity of digestates has also been related to other inorganic components such as heavy metals or high salt content, and to organic compounds such as volatile organic acids or phenolics (Nkoa 2014).

Solid-liquid separation is a common technique for the processing of AD for use in agriculture (Möller & Müller 2012, Tambone et al. 2017). Screw press solid-liquid separation of digestate from thirteen full-scale digestion plants during autumn, winter and spring resulted in 87% of total Kjeldahl N (TKN) and 71% of total P in the liquid fraction, while the solid fraction contained 13% and 29% of TKN and P, respectively (Tambone et al. 2017). Similarly, comparing six anaerobic digestates before and after solid-liquid separation, Mazzini et al. (2020) found about 20% of the TKN and 35% of the total P of the original digestate in the solid fraction. While the concentrations of available N were similar, the concentrations of phosphate and organic N were markedly higher in de-watered, solid fractions of digested cow slurry, pig slurry and mixed manures in comparison with the whole digestates. (Nkoa 2014). Hence, environmental concerns related to losses of N and P can be similar for the solid fraction and the original AD.

Nkoa (2014) suggested that the solid and liquid fractions of AD have the greater potential as soil amendment and fertilizer, respectively. This was supported by Tambone et al. (2017) finding that the N:P ratio in the solid fraction was similar to poultry manure and sewage sludge and proposing that the solid fraction could be used as an NP-organic fertilizer, while the liquid fraction could be used as a substitute for mineral N fertilizers.

Besides its potential as a soil amendment, promising results have been found for the solid fraction as a horticultural substrate amendment (Restrepo et al. 2013; Asp et al. 2022). While peat has traditionally been a dominating source for potting substrates, it has been questioned in many countries due to environmental concerns (Bullock et al. 2012). At the same time, due to its low pH, peat could be a valuable complement to digestates and other residues with a neutral to high pH. Hence, combining peat and residues could facilitate the reuse of nutrients in high-pH residues as fertilizers (Caspersen & Ganrot 2018), as well as reducing the amount of peat needed for substrate production. While organic residues will also contribute with plant nutrients, unbalanced substrate nutrient levels could be a challenge related to the use of organic residues as substrate components.

Few studies have been conducted on the effect of storage of the solid fraction on plant performance. Drennan & DiStefano (2010) reported that stabilization of organics and elimination of ammonia and phytotoxic volatile fatty acids occurred within 10–15 days of curing of the solid fraction of digestate from anaerobic digestion, while 15–20 days were required for reduction of volatile sulfur compounds. In contrast, Tambone et al. (2015) concluded that the solid fraction could be used directly as an organic amendment without the need for composting.

The aim of the present study was to investigate if storage could reduce the problems related to high ammonia concentrations and nutrient imbalances, thereby improving the suitability of the solid AD fraction as a substrate component and mineral nutrient source in potted plant production. We investigated the influence of storage/incubation of the solid fraction on the potential plant availability of mineral nutrients. We also evaluated the effect of partial substitution of peat with incubated or unincubated solid fraction on plant growth as well as on substrate physical and chemical characteristics.

2. Materials & methods

2.1. Digestate

The digestate was produced by Gasum AB in Örebro, Sweden. The main input to the anaerobic digestion process was food industry waste (56%), cultivated crops and cereal wastes (25%), water (17%) and FeCl₃ (1.0%) (Gasum AB, personal communication). After about three weeks of digestion, the digestate was divided into a liquid and a solid, de-watered fraction by screw press separation. The solid fraction (SD) was collected from the same batch in six separate 50 L bags and stored at 5 °C until use.

The concentrations of aqua regia-extractable nutrients in the SD were determined for three of the bags by ISO 11466 (Ca, Hg, K, Mg, P, S) and SS-EN 16,174 (Cd, Cu, Cr, Ni, Pb, Zn) and the total N content was determined by Kjeldahl + Dewardas (SS 028101) by Eurofins Environment Testing Sweden AB, Lidköping.

2.2. Incubation of the solid digestate fraction

The SD from each bag was incubated in a separate 50 L black plastic box (SmartStore™ Pro 45, Orthex Sweden AB, Tingsryd) in a climate controlled chamber at a temperature of 19–20 °C. The boxes were loosely covered with paper to allow gas exchange. The temperature development in the SD during the incubation period was followed by connecting two temperature sensors to a data logger (Tinytag, Gemini dataloggers, Chichester, UK) inserted at medium height in three of the boxes. The temperature decreased from the initial 25–26 °C to about 22 °C after the first week, followed by an increase to a maximum of 32–35 °C after two weeks. After four weeks, a stable temperature of 19–20 °C, similar to the ambient temperature, was obtained in all boxes.

The SD in the three other boxes was used for monitoring the release of potentially plant available nutrients during a 96 days incubation period. Samples of ca. 1.5 L were taken at Day 0, 14, 28, 56 and 96. Deionized water (1L) was sprinkled on top of the residue in each box at Day 46; i.e. 10 days before the 4th sampling. During the four weeks left before the 5th sampling, 1 L of water was added weekly. The contents of potentially plant available (CAT-extractable) mineral elements were determined after extraction of the samples in 0.01 M CaCl₂ + 0.002 M DTPA according to the European standard method for growing substrates (SS-EN 13651). The CAT-extractable elements, the laboratory compacted bulk density (BD) (SS-EN 13040), as well as substrate pH and electrical conductivity (EC) after 1:5 (v:v) substrate:water extraction (SS-EN 13037:2011, SS-EN 13038:2011), were determined by LMI AB, Helsingborg. Readily available Ca in the incubated SDs was estimated by LMI AB after extraction in 0.018 mol L⁻¹ of acetic acid according to a modified Spurway Lawton procedure (Spurway and Lawton 1949). Water soluble chloride was determined on the incubated SDs by SS-EN ISO 10304-1:2009 and the content of inorganic carbon was determined by SS-EN 15936:2022 by Eurofins Environment Testing AB, Lidköping.

2.3. Plant growth evaluation in SD-enriched peat substrates

The SD originating from three of the bags was used for two cultivation experiments: A *pre-incubation* experiment using unincubated SD at Day 0 of incubation and a *post-incubation* experiment using SD from Day 96 of the incubation described in section 2.2. Three different growing substrates were compared in both experiments: 100% peat (PC), 20:80 SD:peat v:v (SD20) and 30:70 SD:peat v:v (SD30). Three replicates per treatment were produced, one from each bag for the treatments containing SD. Each replicate consisted of four pots. The pre-incubation and the post-incubation experiments were conducted from February 13 to March 18 and from May 19 to June 13, respectively.

The peat for the pre-incubation experiment was 0–25 mm and consisted of 1/3 H2-H4 and 2/3 H5-H7 (SW Horto AB, Sweden). For the

post-incubation experiment, Solmull peat (Hasselfors Garden AB, Sweden), consisting of lightly humified (H2-H4) fine to medium peat, was used. The SD and the peat were mixed on a volume basis according to their BD. The SD20 and SD30 substrates as well as the PC were limed with 3 g L⁻¹ of CaCO₃ (ground limestone). CAT-extractable elements as well as pH and EC were determined on three samples from each substrate by LMI AB, Helsingborg, as described in sect. 2.2. The BD of the mixed substrates was determined according to SS-EN 13040.

Substrate corresponding to 0.5 L was weighed into each pot. Twenty seeds of sweet basil (*Ocimum basilicum*) or three seeds of *Lactuca sativa* cv. ‘Grand Rapids’ were sown in each pot. After emergence, the lettuce and basil seedlings were thinned to 1 and 15 plants, respectively. The pots were placed on plastic saucers and randomly distributed in a greenhouse chamber with heating and ventilation setpoints at 20 °C and 22 °C respectively. Additional light (high pressure sodium lamps, Philips GreenPower 400 W, Philips, Eindhoven, The Netherlands) was supplied for 16 h day⁻¹ at an intensity of 74 ± 10 μmol m⁻² s⁻¹.

The pots were watered to the same percentage of pot water holding capacity once or twice weekly. Supplemental watering was given as needed with the same amount of water to all treatments within each plant species. A mineral fertilizer solution containing 1 g L⁻¹ of each of the fertilizers Kristalon Indigo (8.5% N, 4.9% P, 24.7% K, 4.2% Mg, 5.7% S, 0.027% B, 0.004% Cu-EDTA, 0.2% Fe-DTPA, 0.06% Mn-EDTA, 0.004% Mo, 0.027% Zn-EDTA) and Calcinit (15.5% N, 19% Ca) (Yara, Sweden), giving a concentration in mg/L: N 240, P 49, K 247, S 57, Ca 190, Mg 42, B 0.27, Cu 0.04, Fe 2, Mn 0.6, Mo 0.04, Zn 0.27, was added weekly to the 100% peat pots from the second week. This resulted in a weekly addition to each pot of, in mg: N 4.8, P 1, K 4.9, S 1.1, Ca 3.8, Mg 0.8, and in μg: B 5.4, Cu 0.8, Fe 40, Mn 12, Mo 0.8, Zn 5.4. No additional nutrients were added to the SD-amended treatments. The total amounts of available nutrients in the respective treatments are shown in Table S1 Supplements. At harvest after 34 and 24 days for the pre- and post-incubation experiment respectively, fresh and dry (65 °C) weights of the aboveground material were determined.

2.4. Substrate mineral N dynamics

For determination of plant available NO₃⁻ and NH₄⁺ during the cultivation period, liquid from the substrates were sampled by installed Rhizon soil moisture samplers (Eijkelkamp Agriresearch Equipment, Giesbeek, the Netherlands). Two moisture samplers per pot in two basil pots from each combination of substrate treatment and SD bag were installed at eleven (pre-incubation experiment) or six days (post-incubation experiment) after sowing. Samples were collected at four (pre-incubation experiment) or three (post-incubation experiment) occasions by connecting a vacuum tube (BD Vacutainer, Becton, Dickinson and Co, Franklin Lakes, NJ, USA) to the sampler at 24 h after irrigation. The vacuum tubes were allowed to draw samples for 24 h before they were un-connected. Nitrate-N and ammonium-N were analysed with Hach test kits LCK303 and LCK340 using a Hach spectrophotometer (Xion 500, Hach, Loveland, CO, USA). The concentration of mineralised N was estimated as NH₄-N + NO₃-N.

2.5. Statistical methods

The results were analysed by the statistical program SAS (SAS Institute, Inc.). A one-way ANOVA with incubation time as a factor and the bags as replicates (n = 3) was performed both for the SD incubation and for the two cultivation experiments. A two-way ANOVA with substrate and bag as factors, with the pots as replicates, was also performed for the plant weight data to determine within-treatment variation (n = 4). Treatment means were separated by Duncan’s Multiple Range Test (p < 0.05). All results are presented as means ± SE.

3. Results and discussion

3.1. Chemical characterisation of the SD

The dry matter (DM) percentage, mineral nutrient contents and pH of the SD are presented in Table 1. The DM content of 27.1% was similar to the 25.6% DM reported for the solid fraction produced after digestion of cattle slurry and energy crops (Riva et al. 2016) but somewhat higher than the mean DM of 21–22 % for solid fractions separated by screw press from digestates originating from six (Mazzini et al. 2020) and 13 (Tambone et al. 2017) full-scale digestion plants.

Our observations of 9.47 g TKN (Kjeldahl-Dewardas) and 3.97 g NH₄-N (TAN) per kg of fresh SD were also higher in comparison with the values of Riva et al. (2016), Mazzini et al. (2020), and Tambone et al. (2017) reporting TKN of 5.5, 5.86 and 6.1 g kg⁻¹ FM and NH₄-N of 1.59, 1.53 and 2.14 g kg⁻¹ FM, respectively. Similarly, our TAN/TKN ratio of 41.9% was higher than the 25.7% and 35.1% found by Mazzini et al. (2020) and Tambone et al. (2017), respectively, but within the 26–50% range given for the solid fraction by Möller & Müller (2012).

The total P concentration of 16.0 ± 1.5 g kg⁻¹ DM was within the ranges from 5.6 to 22.7 g kg⁻¹ and 4–20 g kg⁻¹ observed by Tambone et al. (2017) and Jimenez et al. (2020), respectively, but higher than the mean value of 9.82 ± 2.40 g kg⁻¹ DM observed by Mazzini et al. (2020). Digestate P content varies with feedstock (Guilayn et al. 2019). A high total P content of the SD could be a problem as digestate amendments are often added to fulfil crop N requirement (Sogn et al. 2018), leading to excessive addition of P.

The heavy metal concentrations found in our study (Table 1) were lower than (Cd, Cu, Cr, Ni, Pb) or similar to (Zn) the mean values observed by Tambone et al. (2017) for 13 biogas plants. The Zn concentration of the SD was 25 % of the limit value, set by the Technical Research Institute of Sweden, for use of AD as a fertilizer.

3.2. Potentially available nutrients during SD incubation

The EC increased from 0.6 to 1.8 mS cm⁻¹ during the first 56 days of incubation (data not shown). A lack of change in EC during the first two weeks was surprising as the strong decrease in ammonium concentration was not matched by an increased concentration of nitrate. However, increased availability of other ions, such as phosphate, Mg and Fe ions in particular, might have contributed to the EC. A higher concentration of Cl⁻ ions, related to dissolution of Cl-containing precipitates, might be involved in the increase in EC observed at days 28 and day 56. While the potentially available Cl⁻ concentrations could not be followed by the CAT-analysis, the mean concentration of water soluble chloride of the incubated digestate was 8900 ± 1069 mg/kg DM, corresponding to 682

Table 1

Mineral nutrient contents in the solid-phase digestate (SD). FM = fresh matter, DM = dry matter. Means ± SE, n = 3.

pH	8.7 ± 0.1	
DM	27.1 ± 0.6	%
Total N	9467 ± 318	mg kg ⁻¹ FM
”	3.5 ± 0.1	% DM
NH ₄ -N	3967 ± 33	mg kg ⁻¹ FM
”	1.5 ± 0.1	% DM
P	16000 ± 1527	mg kg ⁻¹ DM
Ca	7533 ± 570	”
Mg	8433 ± 649	”
K	6767 ± 176	”
S	5533 ± 367	”
Cd	< 0.20	”
Cu	20.7 ± 0.33	”
Cr	2.1 ± 0.23	”
Hg	< 0.097	”
Ni	2.6 ± 0.15	”
Pb	< 0.97	”
Zn	210 ± 10	”

$\pm 95 \text{ mg L}^{-1}$ of digestate.

The decrease in pH from 8.0 to 6.5 during incubation ($p < 0.001$, Fig. 1) was similar to the results of Drennan & DiStefano (2010), reporting an initial pH of solid phase digestate of ca. 8.5 and a reduction to pH 6.5–7 during curing. The content of inorganic carbon in the incubated digestate was below 0.1% DM.

During the first two weeks of incubation, the concentrations of $\text{NH}_4\text{-N}$ as well as of total mineral N decreased dramatically ($p < 0.001$, Fig. 1). At the pH of 8.0 at the start of incubation, the formation of ammonia could be expected to be substantial (Sommer et al. 2004) and it is likely that the marked decrease in NH_4 and N_{min} concentrations before the first sampling after 14 days mainly was a consequence of ammonia emission. Indeed, for solid effluents, emission during storage has been reported to be the most important pathway for losses of N (Möller et al. 2010). Drennan & DiStefano (2010) reported a rapid decrease in the concentration of ammonia in a solid digestate:dried wood chips mix after 6–8 days, but at a higher temperature (35°C) than in our study. The formation and emission of ammonia could also, at least partly, be responsible for the strong reduction in pH observed during the first four weeks (Fig. 1) by increasing the concentration of H^+ in the soil (Sommer et al. 2004). The slight increase in the NH_4^+ concentration observed between Day 14 and Day 28 was possibly related to increased dissolution of struvite or other NH_4 -containing precipitates from the digestion process as the pH decreased below 7.

Declining pH and loss of NH_4^+ could also have been caused by nitrification of ammonium to nitrate and/or nitrite. However, the $\text{NO}_3\text{-N}$ was below the detection limit (0.55 mg L^{-1}) during the first month of incubation while the concentration increased markedly during the latter half of the storage period (Fig. 1). This suggests that nitrification was not a major contributor to the strong early decrease in NH_4^+ and pH. However, the initially high concentrations of NH_4^+ combined with a high supply of organic carbon and a high moisture content (Table 1) might favour accumulation of NO_2^- (Heil et al., 2016). Interestingly, Frerichs et al. (2020) observed notable NO_2^- accumulation and delayed production of NO_3^- by 3–4 weeks when the substrate pH was ≥ 7.0 during the onset of nitrification in peat spiked with amino acids. While nitrite was not measured separately in our study, the method used for the determination of $\text{NO}_3\text{-N}$ involved the reduction of NO_3^- to NO_2^- . Hence, any NO_2^- present in the SD would be included in the NO_3^- concentration reported. However, the possibility that nitrite/nitrate formed in the SD was lost by denitrification (Heil et al., 2016) cannot be excluded.

The large, early loss of N from the SD might be reduced by acidification of the digestate at the production site, for example by acid or peat amendment or by inducing more rapid nitrification. Alternatively, amendment of digestates with NH_4^+ adsorbing additives such as zeolites, biochar or activated carbon could reduce phytotoxicity as well as nitrogen loss during digestate composting (Manu et al. 2021, 2022).

The CAT-extractable concentrations of P, Mg, Fe, Zn (Fig. 1) as well as Mn and Al (data not shown) markedly increased during the 14 weeks incubation period ($p < 0.001$). The intimate relation between CAT-extractable P and Mg during the period of incubation ($r = 0.99$, $p < 0.001$), strongly suggest that the availability of both P and Mg in the SD was determined mainly by the dissolution of Mg-P containing compounds. This corresponds with the observation of Vanden Nest et al. (2021) that the phosphorus use efficiency (PUE) of digestates and mixes between digestates and animal manures and/or composts was positively related to their Mg/P ratio.

Besides Mg, pH was the most important factor for the release of potentially available P in the present study. The strong relationship observed between the decreasing pH and release of P in the SD (Fig. 1) is in accordance with the observations of Schoumans et al. (2017) that a decreasing pH in the 6.5–5.5 range strongly correlated with P release in the solid digestate fraction. The fraction of total P released, however, depended on the type of feedstock added to the digester (Schoumans et al. 2017). The close, negative correlations between pH and the

concentrations of both P and Mg ($r = -0.96$, $p < 0.001$) in the present study are in accordance with the observation of Degryse et al. (2017) that the dissolution of struvite strongly increased when pH decreased from 8.5 to about 6.0. Hence, pH was an important factor controlling the release of P in the present SD where the content of total Mg in relation total Ca, and hence the initial content of Mg phosphates, was high.

The strong positive associations ($p < 0.001$) between potentially available concentrations of P and Fe ($r = 0.90$), Al ($r = 0.87$) as well as Mn and Zn ($r = 0.8$) in the SD, combined with the negative correlations of Fe, Al, Mn and Zn with pH ($r = 0.8\text{--}0.9$, $p < 0.001$), suggested that even these metals were constituents of P-containing compounds potentially becoming more soluble as pH decreased. The low CAT-soluble concentrations of Zn and Al indicated that Al-P and Zn-P forms probably had a minor influence on the amount of P that was released. Hence, besides the contribution from Mg-P, the high availability of P in the SD might also be related to a relatively high occurrence Fe-P in comparison with Ca-P compounds (Grigatti et al. 2015). In the present study, FeCl_3 was added to the digester to prevent emission of H_2S (Gasum, personal communication). The presence of FeCl_3 during anaerobic digestion may lead to precipitation of ferrous phosphate (vivianite) (Mamais et al. 1994), which may be less stable when pH is reduced below 7 (Liu et al. 2018, Wilfert et al., 2015). Oxidation of Fe during SD incubation may also have contributed to the release of P from vivianite (Wilfert et al. 2015). Phosphate could also be liberated after chelation of ferric Fe by humic substances present in the organic matter (Wilfert et al. 2015).

A drawback of using the CAT extraction in the incubation study was that the relationship between potentially available Ca and P could not be evaluated. However, a negative relationship has been reported between soluble P in organic manures and their Ca:P ratio (Toor et al. 2005, Vanden Nest et al. 2021). Also, the availability of P has been suggested to be limited by apatite formation at a molar Ca:P ratio above a threshold value of 2–3 (Toor et al. 2005, Vanden Nest et al. 2021). Hence, a molar Ca:P ratio of 0.36 in the unincubated SD indicated that the Ca content was not a main determinant of the potential availability of P in our study. This was supported by a low molar Ca:Mg ratio (0.54), indicating that the formation of struvite was not restricted by the formation of less soluble Ca-P compounds (Daneshgar et al. 2018). As both P, Ca and Mg are preferentially allocated to the solid phase (Stoknes, 2020), it is likely that Ca:P and Ca:Mg ratios were low even in the original AD. In the incubated SD, the available Ca concentration of $320 \pm 28 \text{ mg L}^{-1}$ determined after Spurway-extraction was also lower in comparison with the 550 mg L^{-1} of CAT-Mg.

The positive relationship found by Vanden Nest et al. (2021) between $\text{NH}_4\text{-N}$ and PUE in digestates and digestate fractions was not mirrored in the relationship between CAT-extractable $\text{NH}_4\text{-N}$ and P during the incubation period (Fig. 1). The negative correlation found between CAT-P and CAT- NH_4^+ might be explained by the strong reduction in ammonium content during the first weeks of incubation. Hence, the most important effect of $\text{NH}_4\text{-N}$ on available P was probably related to pH reduction, affecting the solubility of P-containing compounds. It is also conceivable that a strong early loss of $\text{NH}_4\text{-N}$ via ammonia emission might have propelled the dissolution of $\text{NH}_4\text{-Mg-P}$ compounds. This is parallel to the suggestion of Caspersen & Ganrot (2018) that nitrification, by consuming ammonium, acted as a driver for further release of ammonium from nutrient-enhanced zeolite.

Phosphate adsorbents, such as P adsorbing clays, might be added to SD-containing growing substrates to control the concentrations of available P. A larger proportion of the P in the original digestate could also be allocated to the liquid fraction (Vaneckhaute et al. 2017). Alternatively, N and P in anaerobic digestates could be recovered and used for the production of inorganic fertilizers, e.g. by ammonia stripping, struvite precipitation or pyrochar production (Lin et al. 2015, Guilayn et al. 2020; Wang & Lee 2021).

The microelements Mn, Cu, B also increased markedly during the incubation period ($p < 0.001$, data not shown). Copper and Mn varied from 0.35 and 9.8 mg L^{-1} at start to 0.55 and 14 mg L^{-1} after 2 weeks.

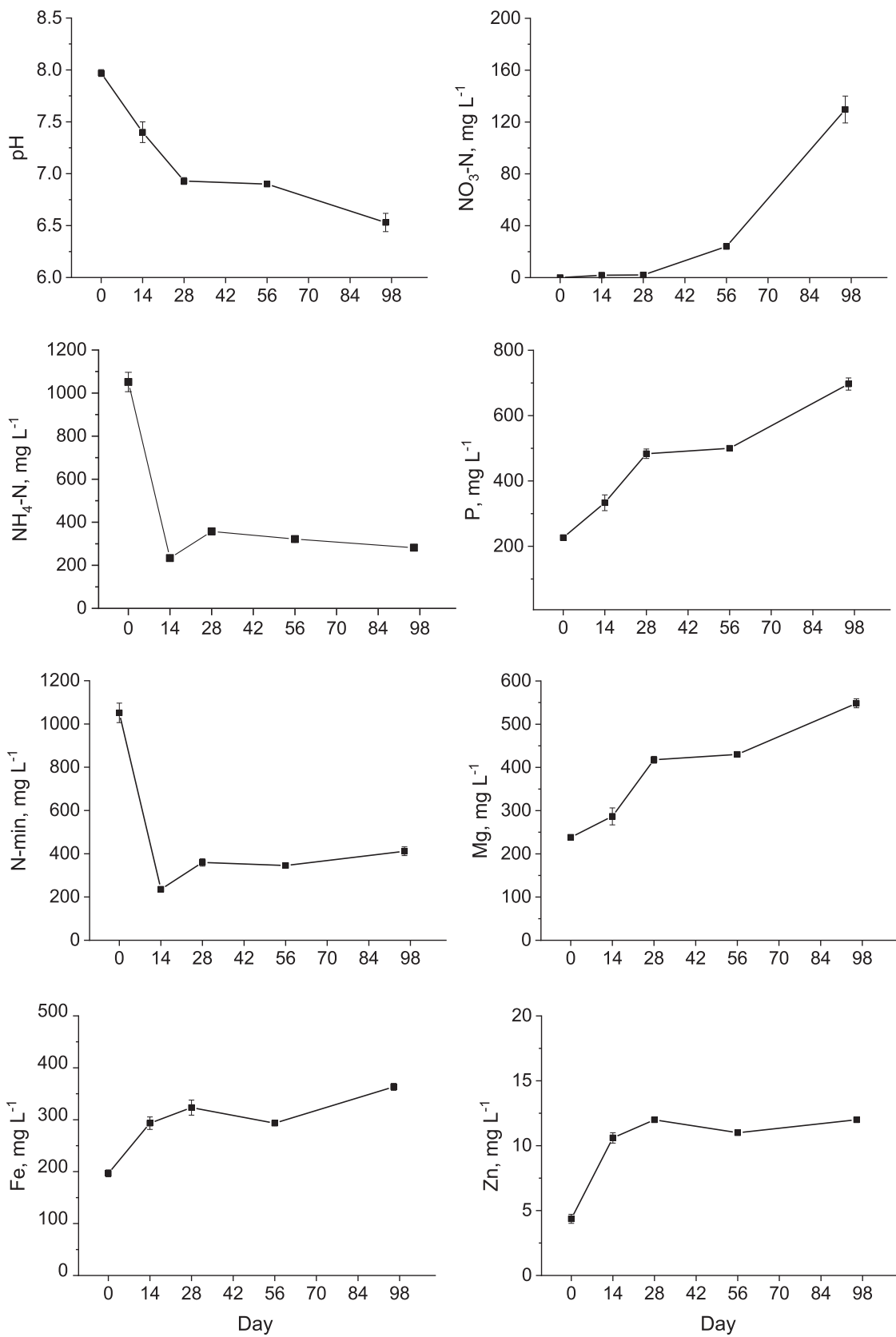


Fig. 1. pH and potentially available concentrations of NH₄-N, NO₃-N, N_{min}, P, Mg, Fe and Zn (mg L⁻¹) in the solid digestate fraction during 96 days of incubation. Means ± SE, n = 3.

Boron concentration ranged from 0.43 to a peak at 0.62 mg L⁻¹ after 4 weeks. The K concentration generally decreased during the incubation period from 530 to 457 mg L⁻¹ ($p < 0.05$) while silicone (Si) decreased from 21.3 at Day 0 to 11.7 mg L⁻¹ at the end ($p < 0.001$). Incubation had no significant effect on the CAT-extractable concentrations of S, Na, Cd and Ni, showing mean values of 199 ± 4.0 , 79.8 ± 1.2 , 0.02 ± 0.01 and 0.08 ± 0.01 mg L⁻¹ of substrate.

Mean BD of the SD increased with about 20% from 0.31 kg L⁻¹ at Day 0 to 0.37 kg L⁻¹ at Day 96, probably due to differences in substrate humidity. This could have contributed to underestimation of mineral concentrations at Day 96 in comparison with Day 0.

3.3. Characteristics of the SD-enriched growing substrates

The initial concentrations of CAT-extractable nutrients in the peat-based substrates amended with SD from Days 0 and 96 of SD-incubation (sect. 2.3), respectively, are shown in Table 2. The concentrations of all nutrients were generally higher when 30% compared to 20% SD was added, while pH was higher for 30% than for 20% at Day 0 only. Both NH₄-N and N_{min} were markedly lower while K, Na, Mn and Cu concentrations were slightly reduced in the substrates containing the incubated SD. In contrast, the concentrations of NO₃-N, P, Mg and Zn were higher in the substrates containing the incubated SD. The porosity was similar in SD20 and SD30, but increased slightly when the incubated SD was used (data not shown).

In the present study, the substrates were mixed and limed the day before sowing. At the pH of 6.6 in the 30% treatment using SD from Day 0 in particular, a notable ammonia emission was likely. Frerichs et al. (2020) observed higher NH₄⁺ accumulation from mineralization of an amino acid-based fertilizer in peat substrates at pH 5.5 than at pH 6.5, and ascribed this to a higher emission of NH₃ at the higher pH level. In fact, Frerichs et al. (2020) suggested that the initial substrate pH in organically fertilized, peat-based substrates should be in the 5.5–6.0 range to avoid ammonia emission and reduce the risk of ammonium toxicity. However, they used a liquid amino acid fertilizer that was rapidly ammonified, leading to a pH increase in the substrate. Using SD as a fertilizer, the main part of the ammonification of organically bound N to ammonium would already have occurred during anaerobic digestion and the more recalcitrant organic matter would be left in the residue (Gunnarsson et al. 2010). Hence, a further rapid increase in pH due to ammonification is less likely when SD are used as fertilizers.

The greatly improved availability of nitrate in the substrates containing the incubated in comparison with the unincubated SD (Table 2) was in agreement with the increased NO₃-N content observed during the latter part of SD incubation (Fig. 1). Nitrification in the growing substrates with the unincubated SD, however, may have proceeded faster than it did during the incubation study due to the aeration provided by the initial mixing of the substrates and the higher temperature in the greenhouse (≥ 20 °C) in comparison with the incubation chamber

(19–20 °C) (Verhagen 2021). However, the substrate pH of 6.2–6.3 (Table 2) probably had a negative influence on nitrification (Verhagen 2021). Increasing the pH from 6.1 to 6.7 in a peat-based medium amended with 20% nutrient-enriched zeolite strongly increased nitrification (Caspersen & Ganrot, 2018). Similarly, Frerichs et al. (2020) observed higher and faster nitrification at pH 6.5 than at pH 5.5 in a peat-based medium. In the present study, the NH₄-N/NO₃-N ratio in the SD was still high after 96 days, indicating that the SD was not yet fully stabilised (Brewer & Sullivan 2003).

The high potentially available P concentrations obtained in the SD after incubation (Fig. 1) as well as in the peat:SD substrates (Table 2) pose a problem due to the high risk of leakage of P. Losses of P from agriculture is an important contributor to eutrophication and algal blooms in lakes and estuaries/coastal sea areas (Ulén et al., 2007; Elser et al., 2007; Schindler et al., 2016). For soilless media, controlling the availability of P is particularly important as they have a limited capacity to retain P and hence a high risk for P loss (Marconi and Nelson, 1984; Prasad & Woods, 1971). Leakage of P from peat has been related to exchangeable Al and Fe (Prasad & Woods 1971). Similarly, positive relationships between available P and Fe, as well as between P and Al, were found in our study. A markedly lower initial concentration of P in the substrate, combined with supplemental additions of P during the cultivation period, could reduce the risk of P leakage. For example, Caspersen & Bergstrand (2020) concluded that 18–24 mg P L⁻¹ as a starter fertilization in the peat-based substrate as well as in the weekly fertilizer solution was sufficient for poinsettia and chrysanthemum.

3.4. Plant growth evaluation in the SD-enriched peat substrates

3.4.1. Pre-incubation experiment

Fresh and dry weights of basil and lettuce did not differ significantly between the three substrates mixed using the unincubated SD (Day 0) when the bags were used as replicates ($n = 3$) (Fig. 2 Total). This was probably due to the large SE for SD30. The %DM of basil, however, was markedly higher for PC than for the two treatments containing SD (Fig. 2). Oppositely, lettuce showed significantly higher %DM for both SD20 and SD30 than for PC.

When the within-treatment variation for plant weights was evaluated, using the four pots from each bag as replicates, it was clear that the differences in response to the SD from individual bags were remarkably large (Fig. 2 Bag 1–3). This was primarily due to a large variation for the SD30 treatment where both plant species generally showed a growth depression for Bag 1 but a growth increase for Bag 3. For Bag 2, lettuce responded negatively but basil positively to SD amendment.

The concentration range of 230–313 mg L⁻¹ for NH₄-N in the substrates containing unincubated SD (Table 2) was above the critical level of 50–100 mg L⁻¹ reported for basil in the early developmental phase (7–21 DAS) in the absence of nitrate (Frerichs et al. 2020). Hence, NH₄⁺/NH₃ toxicity was probably a main explanation for the growth depression

Table 2

Bulk density (BD), pH, and potentially available nutrients in the peat-based substrates at the start of the cultivation experiments conducted before (Day 0) and after (Day 96) incubation of the solid digestate (SD), respectively. In both experiments, the substrates contained SD:peat in the ratios 20:80 (SD20) and 30:70 (SD30). Means \pm SE, $n = 2$, *** = $p < 0.001$, ** = $p < 0.01$, * = $p < 0.05$, ns = not significant, after two-way ANOVA with substrate (Sub) and incubation time (T) as factors. Within each experiment, significantly different treatments are separated by different letters (Duncan's Multiple Range Test, $p < 0.05$).

	BD kg L ⁻¹	pH	NH ₄ -N	NO ₃ -N	Nmin	SO ₄ -S	P	K	Mg mg L ⁻¹	Na	Fe	Mn	Zn	Cu	B
<i>Day 0</i>															
SD20	0.35	6.2a	230a	1.7	231a	44	123a	113	175a	49	117	5.3	2.4	0.24	0.13
SD30	0.35	6.6b	313b	0.6	314b	65	170b	163	207b	53	137	6.8	3.5	0.31	0.16
<i>Day 96</i>															
SD20	0.27	6.3	95A	23A	118A	44	143A	107	185A	42	113	4.8	2.9	0.14	0.11
SD30	0.29	6.3	131B	35B	166B	66	213B	153	245B	47	133	6.4	4.3	0.25	0.15
T	***	ns	***	***	***	ns	***	*	***	**	ns	*	**	*	ns
Sub	ns	**	***	**	***	***	***	***	***	*	***	***	***	*	**
T × Sub	ns	**	***	**	**	ns	**	ns	**	ns	ns	ns	ns	ns	ns

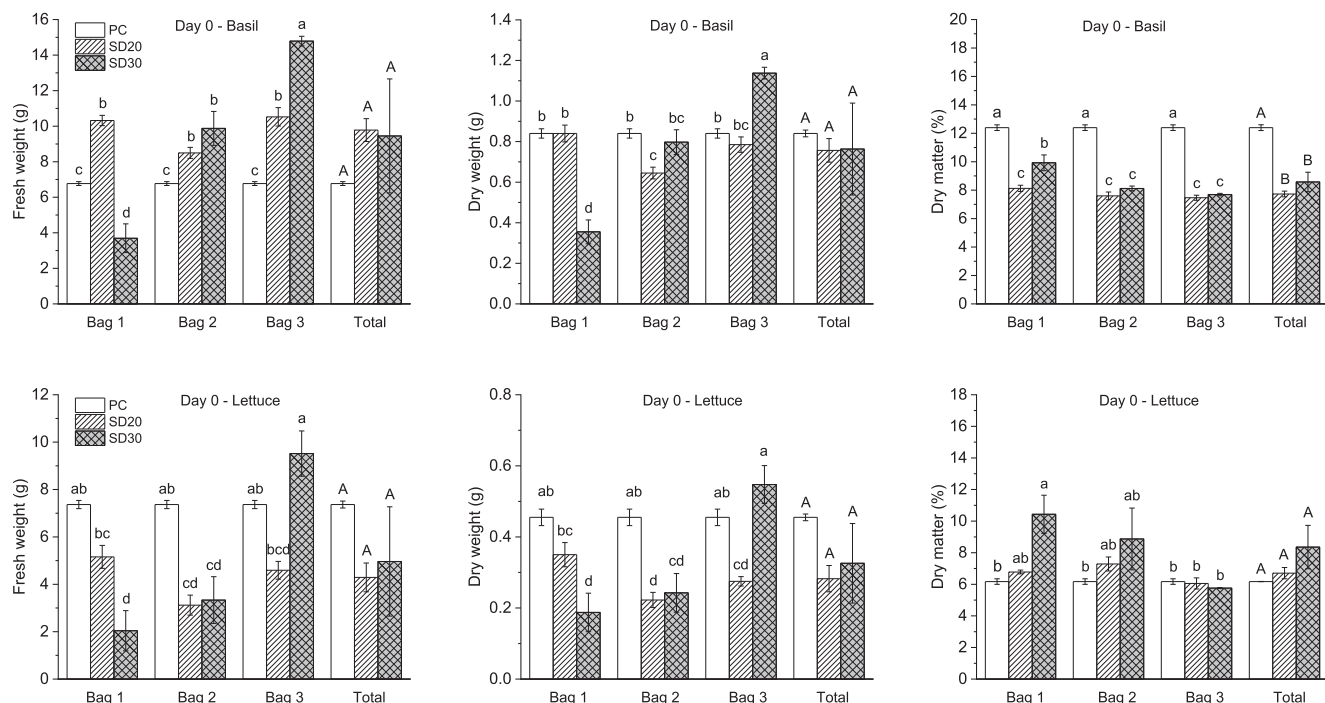


Fig. 2. Shoot fresh and dry weights and % dry matter (DM) of basil and lettuce in the pre-incubation experiment. Data are presented as means \pm SE, for each bag separately using the pots as replicates (Bag 1–3, $n = 4$), and also using the bags as replicates (Total, $n = 3$). PC = peat, SD20 = SD:peat 20:80, SD30 = SD:peat 30:70. Significantly different treatments are separated by different letters (Duncan's Multiple Range Test, $p < 0.05$).

observed for both basil and lettuce grown in SD30 from Bag 1, and for lettuce grown in SD20 and SD30 from Bag 2. We suspect that the absence of nitrate during the first weeks of incubation aggravated plant susceptibility to phytotoxicity, as plants have frequently been shown to tolerate lower concentrations of ammonium in the absence of nitrate (Britto & Kronzucker 2002, Frerichs et al. 2020). Consequently, an early onset of nitrification could reduce the risk of NH_4^+ toxicity (Frerichs et al. 2020) and might shorten the incubation time needed for obtaining a SD suitable for use in plant production.

The large variation between the pots in the occurrence of toxicity symptoms was surprising. However, the $\text{NH}_4\text{-N}$ concentration in the analysed SD bags varied from 974 to 1130 mg L^{-1} at the start of incubation, i.e. a difference of 150 mg L^{-1} (Fig. 1). Hence, the $\text{NH}_4\text{-N}$ concentration in the 30% SD substrate could potentially differ with 45 mg L^{-1} depending on the bag used. At a toxicity limit for basil of 50–100 mg L^{-1} of NH_4^+ (Frerichs et al. 2020), the variation inherent in the SD could potentially be decisive for the outcome of cultivation. In addition to toxic effects of high NH_4^+ concentrations in the root zone, emission of NH_3 might also have contributed to the growth depression and toxicity symptoms observed. Frerichs et al. (2020) reported adverse effects on basil growth at $> 50 \text{ mg L}^{-1}$ of $\text{NH}_4\text{-N}$ when aerial concentrations of NH_3 was 0.2 ppm. At lower levels of NH_3 , basil could tolerate up to 300 $\text{mg NH}_4\text{-N}$ in the substrate.

It is likely that the growth depression and toxicity symptoms observed for Bag 1 (basil, lettuce) and Bag 2 (lettuce) was due to an interplay between high substrate concentrations of ammonium, chloride and sodium. The Na concentration was moderately high as indicated by CAT-Na in the 40–50 mg L^{-1} range. Water soluble chloride ion concentrations of 566–871 mg L^{-1} strongly suggests that Cl^- toxicity was also an important contributor to the phytotoxicity observed in the present study. Increasing concentrations of NaCl or Cl^- can reduce NO_3^- uptake and growth of lettuce (Carillo & Rouphael 2022). For basil, Corrado et al. (2020) concluded that reduced growth at high levels of Cl^- was due partly to $\text{Cl}^-/\text{NO}_3^-$ antagonism and partly to the induction of oxidative stress. For *Arabidopsis thaliana*, it has even been suggested that NH_4^+ as the sole source of N can aggravate salt stress by inducing over-

accumulation of Cl^- (Liu et al. 2020). Furthermore, inhibited nitrification at high Cl^- concentrations (Magde et al. 2014) might have contributed to the late onset of nitrification in the SD (Fig. 1).

The stronger negative response of lettuce than of basil to substrate amendments with unincubated SD (Fig. 2) indicates that lettuce was more sensitive than basil to $\text{NH}_4^+/\text{NH}_3$ and/or Cl^- toxicity. Lettuce has been described as sensitive to moderately sensitive both to salt stress (Maas & Hoffman, 1977) and to acute ammonia injury (Krupa 2003). Frerichs et al. (2017) suggested that basil was relative sensitive to high ammonium.

Volatile sulfur compounds may also be produced under anaerobic digestion (Drennan & DiStefano, 2010). However, the stable S concentration during incubation (Fig. 1) indicated that there was limited formation and loss of volatile sulfur compounds from the SD.

3.4.2. Post-incubation experiment

In the experiment using the incubated SD (Day 96), both SD20 and SD30 produced significantly higher basil FW and DW in comparison with PC when the bags were used as replicates (Fig. 3 Total). In contrast, %DM was markedly higher for PC than for the two SD-containing treatments (Fig. 3 Total). For lettuce, the same pattern as for basil was evident for the plant weights (Fig. 3 Total); however, the difference between PC and the other two treatments was significant for %DM only (Fig. 3 Total).

We observed a similar within-treatment variation for the substrates containing incubated SD (Fig. 3 Bag 1–3) as for the substrates with unincubated SD (Fig. 2); however with a lower amplitude. For basil, both SD20 and SD30 showed increased growth in comparison with PC, irrespective of the bag used (Fig. 3 Bag 1–3). For lettuce, however, there was no significant effect of SD addition for Bag 1, a positive effect of SD30 on fresh weight using Bag 2, and increased fresh and dry weights for both SD20 and SD30 with Bag 3 as the source of SD. The % DM was markedly higher for basil than for lettuce and was also higher for PC than for SD20 and SD30 (Fig. 3 Total).

The strong positive growth response observed using the incubated (Fig. 3) compared to the unincubated (Fig. 2) SD indicate that storage

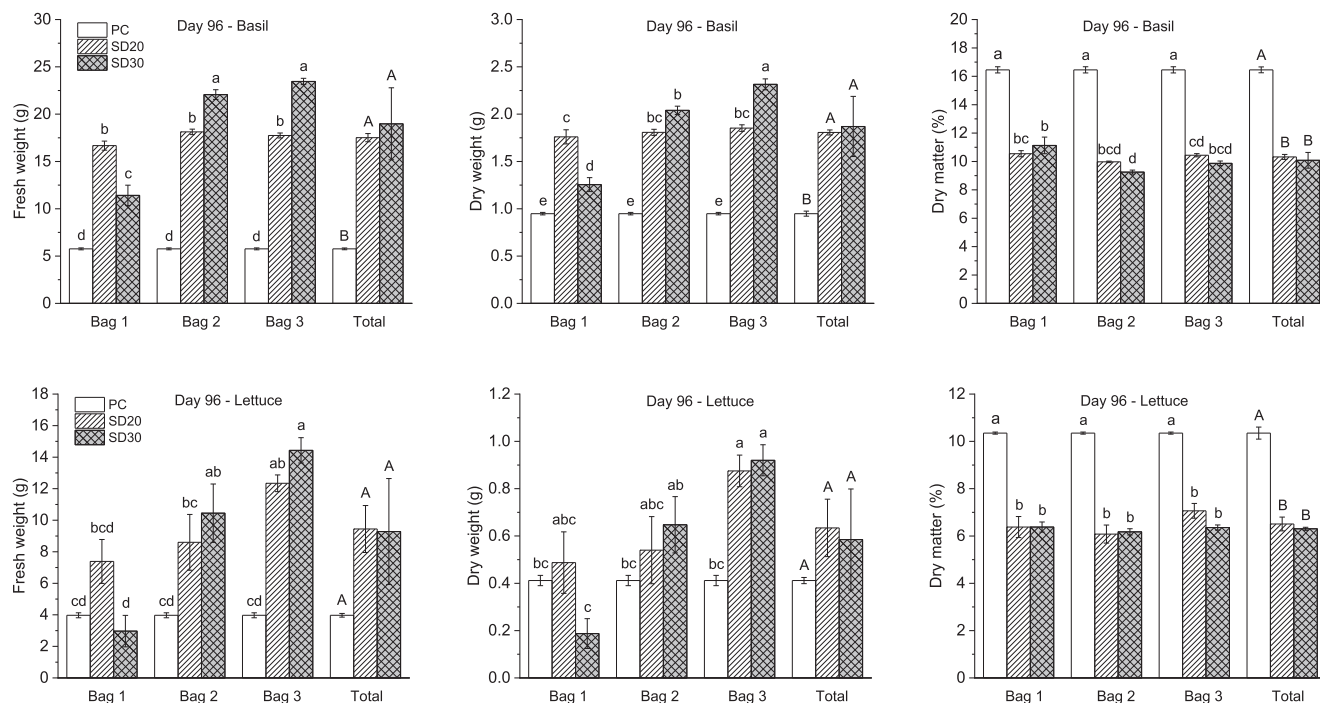


Fig. 3. Shoot fresh and dry weights and % dry matter (DM) of basil and lettuce in the post-incubation experiment. Data are presented as means \pm SE, for each bag separately using the pots as replicates (Bag 1–3, $n = 4$), and also using the bags as replicates (Total, $n = 3$). PC = peat, SD20 = SD:peat 20:80, SD30 = SD:peat 30:70. Significantly different treatments are separated by different letters (Duncan's Multiple Range Test, $p < 0.05$).

could avert or strongly reduce the problems with phytotoxicity observed for the fresh SD. Our results are in accordance with the results of Restrepo et al. (2013), observing no phytotoxicity for tomato, pepper or muskmelon after amending peat with 25% of composted digestate solid fraction. Similar results were shown for basil with 20–50% of composted SD mixed with peat (Asp et al. 2022).

The amounts of nutrients added as mineral fertilizers to the PC treatment were low in both experiments (Table S1, Supplements). The restricted growth of basil in PC, in the post-incubation experiment in particular was probably related to limiting tissue N concentrations, leading to carbohydrate accumulation and a decreased rate of photosynthesis (Araya et al. 2010). Carbohydrate accumulation could also explain the increased %DM in comparison with SD20 and SD30. The comparison of plant growth in the pre-incubation (Fig. 2) and post-incubation (Fig. 3) experiments, however, is complicated by the longer days and higher temperature and light as well as the lower total amount of fertilizer added in the latter experiment. The differences in climate probably contributed to the stronger plant growth and reduced symptoms of phytotoxicity for the substrates containing the incubated SD.

3.5. Substrate mineral N dynamics

3.5.1. Pre-incubation experiment

The concentrations of mineralised N (N_{\min}) and NH_4-N in the soil solution were significantly affected by both substrate type and the time of sampling ($p < 0.001$). N_{\min} differed between the substrates in the order SD30 > SD20 > PC. For NH_4-N , even the interaction between the substrate type and time was significant ($p < 0.01$). For the SD-containing pots, the concentrations of both N_{\min} and NH_4-N significantly decreased during the period of measurement. However, the strong initial decrease in NH_4-N was not accompanied by a corresponding increase in NO_3-N (Fig. 4). For the SD30 treatment, the large loss of N from the soil solution from 15 to 21 days after the start of cultivation could not be accounted for by plant uptake only, suggesting that substantial amounts of N were lost during the first three weeks. As previously discussed (sect. 3.3), at

the substrate pH of 6.6 of SD30 at Day 0 (Table 2), ammonia emission could be an issue (Verhagen 2021). For PC, the N_{\min} concentration close to zero between days 21 and 46 of cultivation supports the conclusion that the supply of N was not sufficient for plant growth in this treatment (sect. 3.4).

The NO_3-N concentrations at day 15 varied from 26 (SD20) to 69 (PC) $mg\ L^{-1}$, but did not differ significantly between the substrate types. The concentration significantly decreased in PC while it increased in SD20 during the period of measurement. After 46 days, NO_3-N was 277, 124 and 8 $mg\ L^{-1}$ for SD30, SD20 and PC, respectively. Even for the N-values in the soil solution, there was a large within-treatment variation.

3.5.2. Post-incubation experiment

The concentrations of mineral N-forms in the soil solution were affected both by the substrate treatment ($p < 0.001$) and by time ($p < 0.01$) but not significantly by their interaction. The SD-enriched substrates contained higher concentrations of NH_4-N than did PC (Fig. 4). For both NO_3-N (Fig. 4) and N_{\min} (Fig. 4), all three substrates differed significantly in the order SD30 > SD20 > PC. The NH_4-N concentration was generally lower after 22 than after 8 or 15 days of cultivation. NO_3-N was generally higher at 15 than at 8 or 22 days, while N_{\min} was generally higher at 15 than at 22 days. SD30 showed the highest concentrations of NH_4-N and NO_3-N with 193 $mg\ L^{-1}$ and 343 $mg\ L^{-1}$ respectively, after 15 days. The soil solution NH_4-N concentrations were markedly lower in the experiment with incubated SD (Fig. 4) in comparison with the highest concentrations observed in the experiment with the unincubated SD (Fig. 4). Still, the NH_4-N of the pots containing incubated SD was sufficiently high to pose a risk of phytotoxicity as suggested by the results of Frerichs et al. (2020).

For the SD containing pots, the peak NO_3-N concentration observed in the soil solution after two weeks suggested that nitrification increased early in the cultivation period, followed by decreasing NO_3-N concentrations as the plant uptake proceeded. Even for NH_4-N and N_{\min} , the reduced concentrations after three weeks probably reflected uptake by the rapidly growing basil plants.

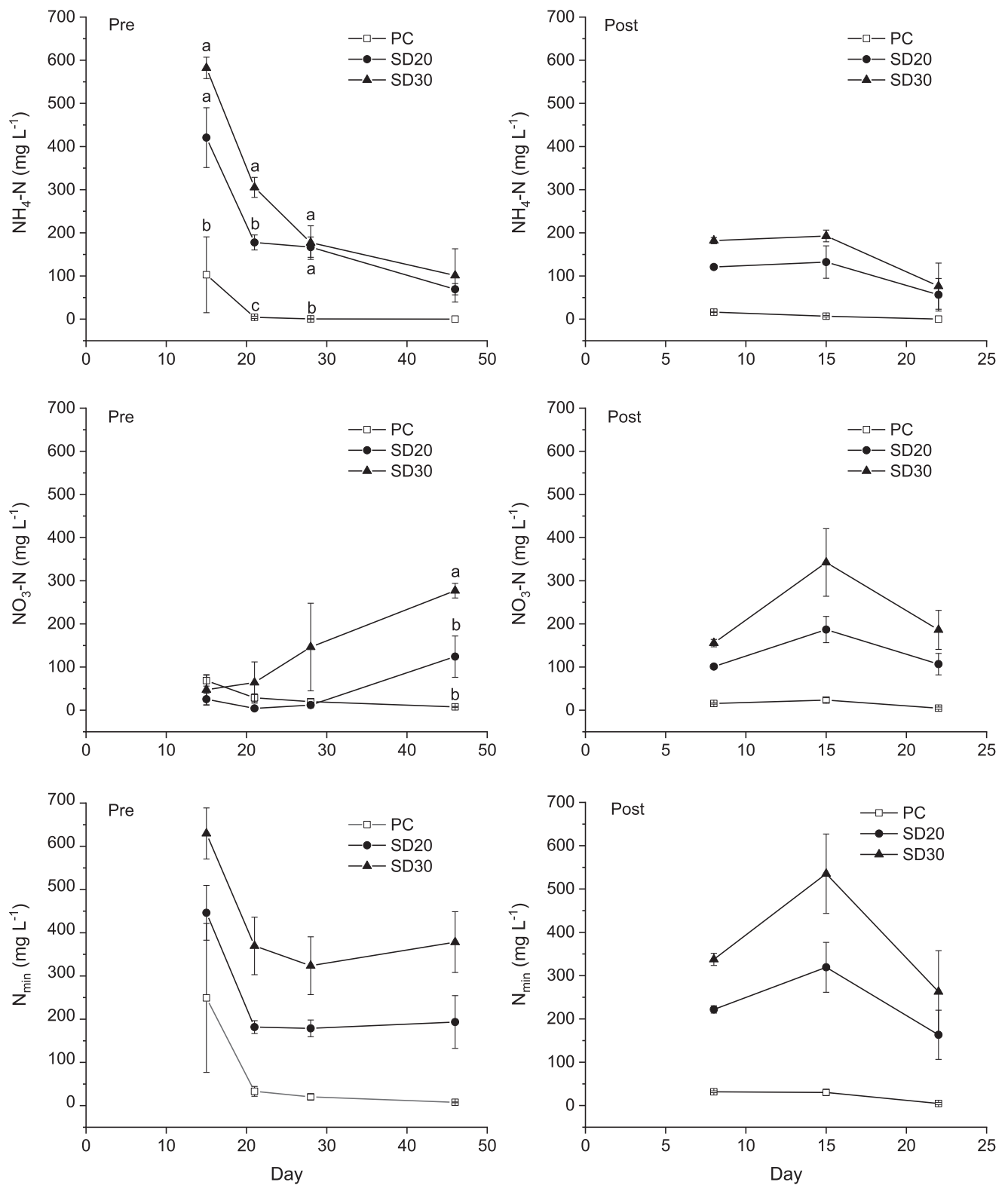


Fig. 4. Concentrations of NH_4-N , NO_3-N and N_{min} ($mg L^{-1}$) in the soil solution of basil pots in the pre-incubation (Pre) and post-incubation (Post) experiments as a function of time (Day = day after the start of cultivation). Data are presented as means \pm SE, using the bags as replicates ($n = 3$). PC = peat, SD20 = SD:peat 20:80, SD30 = SD:peat 30:70. Significantly different treatments are separated by different letters (Duncan's Multiple Range Test, $p < 0.05$).

3.5.3. Nutrient uptake efficiency

Nutrient uptake efficiency (the amount of nutrient in plant divided with the amount of nutrient added), was estimated from the sufficient nutrient concentration ranges for basil leaves (Owen et al. 2018) combined with the largest dry biomass observed in our experiments, divided

with the total amounts of nutrients added with the unincubated SD per pot (Table 3).

After a relative short cultivation period as for basil and lettuce, a substantial amount of the nutrients is left in the spent SD-based substrate, as indicated by the soil solution mineral N concentrations (Fig. 4)

Table 3

Estimated nutrient uptake efficiency for basil grown in the SD20 and SD30 substrate with the *unincubated* SD. The sufficiency range for basil (newly matured leaves) was based on Owen et al. (2018). The maximum sufficient content of each nutrient is estimated using the values obtained after Kjeldahl-Dewardas (N) and aqua regia extraction (P, K, Mg, Ca) (Table 1) together with the maximum amount of shoot dry matter observed (2.4 g) (Fig. 3) plus root weight estimated using a root:shoot ratio of 0.17 from Germano et al. (2022).

Element	Basil leaf sufficiency range (%)	Max sufficient content (mg)	Amount of nutrient per pot, SD20 (mg)	Amount of nutrient per pot, SD30 (mg)	Nutrient uptake efficiency, SD20 (%)	Nutrient uptake efficiency, SD30 (%)
N	4.00 – 6.00	112 – 168	301	451	37 – 56	25 – 37
P	0.62 – 1.00	17 – 28	138	207	12 – 20	8 – 14
K	1.55 – 2.05	43 – 57	58	87	74 – 98	50 – 66
Mg	0.60	17	73	109	23	16
Ca	1.25 – 2.00	35 – 56	65	97	54 – 86	36 – 58

as well as the difference between plant requirement and the total nutrient content in the fresh SD (Table 3). The maximum estimated P uptake efficiency in the 8–20% range confirmed that excess total P content in relation to plant requirement was a main challenge related to the use of the present SD as a substrate component and fertilizer (Table 3). The available P content of 71–107 mg per pot in the incubated substrates (Table 2) was also greatly in excess of the estimated plant P need in the 17–28 mg range (Table 3). Even Mg and Ca were added in excess with the SD (Table 3) as well as with the subsequent liming of the peat-based substrate. For K, however, the estimated nutrient uptake efficiency was relatively high with > 50% in SD30 and 74–98% in SD20 (Table 3).

In relation to the total N content of the fresh, unincubated SD, N uptake efficiency was low with 37–56% for SD20 and 25–37% for SD30 (Table 3). The incubation study, however, showed that a substantial part of the N was lost as gaseous emissions, in particular during the first two weeks of incubation (Fig. 1). For the substrates produced with the incubated SD, the concentrations of mineral N (Table 2) of 118 mg L⁻¹ (SD20) and 166 mg L⁻¹ (SD30) were within or close to the ranges of 100–150 mg L⁻¹ and 150–200 mg N L⁻¹ considered as optimal for basil and lettuce, respectively. As the total mineral N present in the 0.5 L of substrate per pot at the start of the experiment was 157 mg for unincubated and 83 mg for incubated SD30, the estimated maximum N uptake of 112 – 168 mg per pot (Table 3) indicated that at least for the pots containing incubated SDs, uptake of organic N compounds such as amino acids and/or further N mineralization would be needed during the cultivation period to fulfill the requirement of the largest basil plants. The mineral N concentrations of 160–260 mg L⁻¹ in the soil solution close to harvest indicated that ample mineral N was available during the experimental period even for the pots containing the incubated SD (Fig. 4).

To increase the sustainability of digestate use in horticultural production, the spent substrate could be reused in food production, e.g. as soil amendments in the field or as growing substrate for mushroom cultivation (Hultberg et al., 2022). Substrates containing SD may also be used for longer cultivation periods as a way to utilise a larger part of the nutrient content, as shown in a study with tomatoes (Stoknes et al. 2018). Alternatively, spent substrates could be processed to facilitate recycling. For example, Vandecasteele et al. (2018) showed that spent peat- and perlite-based growing media that had been steamed, acidified and reused were able to supply the subsequent chrysanthemum culture with P and K. Biochar production from spent growing media has also been tested. Amery et al. (2021) reported that when added in small amounts to new peat-based growing media, biochars produced from spent growing media were equivalent to biochars based on lignocellulosic biomass in relation to plant growth, but released more P, K, S, Na and Cl. Spent, peat-based growing media have also been included as bulking material during composting of N-rich residues (Viaene et al. 2017). While the spent SD20 and SD30 substrates from the present study might be good sources of P, Ca and Mg in particular, initial concentrations of water-soluble chloride in the 70–100 mg L⁻¹ range indicate that

the Cl content might narrow the number of options available for recycling of the spent substrate.

4. Conclusion

Utilization of the solid fraction of anaerobic digestates as a substrate component could increase the sustainability of horticultural plant production both through the reuse of nutrients as fertilizers and by reducing the amount of peat required for growing substrates. However, our results have shown that unbalanced nutrient contents can be a main challenge related to the use of the solid fraction as a substrate component and/or fertilizer.

The high concentrations of N in the form ammonium found in the fresh SD pose a high risk of NH₃ emission as well as of phytotoxicity. Our results showed that even at 20 or 30% of the unincubated SD in the substrate, plant growth was inhibited and visual symptoms confirmed the presence of phytotoxicity. The NH₄-N concentrations of 200–300 mg L⁻¹ in these substrates were within the toxicity range, but high substrate concentrations of Cl and Na ions probably also contributed to toxicity symptoms and growth depression. The low levels of NO₃-N early in the growing period might have aggravated plant stress and growth inhibition.

We conclude that storage of the SD reduced both the NH₄⁺ concentration and the occurrence of phytotoxicity. Ammonium emission was probably the main reason for the strong, early reduction observed in NH₄⁺ concentration during SD incubation (Fig. 1). After 96 days of incubation, NH₄-N concentrations in the SD had decreased with >50%. The large variation observed in plant response within the SD30 treatment in particular was probably related to local variation in NH₄⁺/NH₃ concentrations and pH between microsites. Due to the high concentrations of NH₄⁺ and Cl⁻ in particular, the risk for growth depression was too high using 30% of the present SD as a substrate component, even after incubation. With 20% amendment using the incubated SD, nutrient uptake efficiency was higher and there was less variation in plant growth in comparison with the 30% amendment, but the potential for growth increase was smaller.

The high concentrations of potentially available P in the SD as well as in the SD-amended substrates also pose an environmental risk. The marked increase in the P concentration in the SD during incubation was strongly related to the reduction in substrate pH. The close correlation between CAT-extractable P and Mg indicate that struvite or other P-Mg forms were the most important sources of P, dissolving as pH decreased.

High concentrations of available P in SD-amended growing substrates might be avoided by using digestates with a lower P content, by limiting the amount of SD added and/or by including phosphate adsorbents, e.g. P adsorbing clays, to reduce the risk of losses of P to the environment. The effects of pH on N-transformations as well as on P availability are complex and research is needed on the optimisation of solid digestate pH with the aim of limiting losses of N as well as controlling the concentrations of available P.

Our results suggest that the present, N- and P-rich SD should

preferably be used in smaller proportions as a fertilizer dose according to plant nutrient requirement and not as a bulk substrate component. To reduce the negative environmental effects and health risks associated with the large losses of N as ammonia emission during storage, more N could be preserved in the fresh digestate or digestate fraction by addition of pH-lowering and/or NH_4^+ adsorbing materials such as peat, wood fibres or sawdust, zeolites or biochar. Increased nitrification during digestate storage could also contribute to a lower pH. Techniques such as struvite precipitation, ammonia stripping or pyrochar production could be alternative solutions for nutrient recovery from high P and N containing digestates and digestate fractions.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.wasman.2023.04.032>.

References

- Albuquerque, 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. *Europ. J. Agron.* 43, 119–128.
- Amery, F., Debode, J., Ommeslag, S., Visser, R., De Tender, C., Vandecasteele, B., 2021. Biochar for circular horticulture: Feedstock related effects in soilless cultivation. *Agronomy* 11, 629. <https://doi.org/10.3390/agronomy11040629>.
- Araya T, Noguchi K, Terashima I, 2010. Effect of nitrogen nutrition on the carbohydrate repression of photosynthesis in leaves of *Phaseolus vulgaris* L. *J. Plant. Res.* 123, 371–379. [10.1007/s10265-009-0279-8](https://doi.org/10.1007/s10265-009-0279-8).
- Asp, H., Bergstrand, K.-J., Caspersen, S., Hultberg, M., 2022. Anaerobic digestate as peat substitute and fertilizer in pot production of basil. *Biol. Agric. Hortic.* <https://doi.org/10.1080/01448765.2022.2064232>.
- Barduca, L., Wentzel, S., Schmidt, R., Malagoli, M., Joergensen, R.G., 2021. Mineralisation of distinct biogas qualities directly after application to soil. *Biol. Fertil. Soils* 57, 235–243.
- Brewer, L.J., Sullivan, D.M., 2003. Maturity and stability evaluation of composted yard trimmings. *Compost Sci. Util.* 11, 96–112.
- Britto, D.T., Kronzucker, H.J., 2002. NH_4^+ toxicity in higher plants: a critical review. *J. Plant Phys.* 159, 567–584.
- Bullock, C.H., Collier, M.J., Convery, F., 2012. Peatlands, their economic value and priorities for their future management — The example of Ireland. *Land Use Policy* 29, 921–928.
- Carillo, P., Roupheal, Y., 2022. Nitrate uptake and use efficiency: Pros and cons of chloride interference in vegetable crops. *Front. Plant Sci.* 13, 899522 <https://doi.org/10.3389/fpls.2022.899522>.
- Caspersen, S., Bergstrand, K.-J., 2020. Phosphorus restriction influences P efficiency and ornamental quality of poinsettia and chrysanthemum. *Sci. Hortic.* 267, 109316 <https://doi.org/10.1016/j.scienta.2020.109316>.
- Caspersen, S., Ganrot, Z., 2018. Closing the loop on human urine: Plant availability of zeolite-recovered nutrients in a peat-based substrate. *J. Environ. Manag.* 211, 177–190.
- Corrado, G., Lucini, L., Miras-Moreno, B., Chiaiese, P., Colla, G., De Pascale, S., Roupheal, Y., 2020. Metabolic insights into the anion-anion antagonism in sweet basil: Effects of different nitrate/chloride ratios in the nutrient solution. *Int. J. Mol. Sci.* 21, 2482. <https://doi.org/10.3390/ijms21072482>.
- Daneshgar, S., Buttafava, A., Capsoni, D., Callegari, A., Capodaglio, A.G., 2018. Impact of pH and ionic molar ratios on phosphorous forms precipitation and recovery from different wastewater sludges. *Resources* 7, 71. <https://doi.org/10.3390/resources7040071>.
- de Groot, L., Bogdanski, A., 2013. Bioslurry = brown gold? A review of scientific literature on the co-product of biogas production, Food and Agriculture Organization of the United Nations (FAO), Rome.
- Degryse, F., Baird, R., da Silva, R.C., McLaughlin, M.J., 2017. Dissolution rate and agronomic effectiveness of struvite fertilizers – effect of soil pH, granulation and base excess. *Plant Soil* 410, 139–152.
- Drennan, M.F., DiStefano, T.D., 2010. Characterization of the curing process from high-solids anaerobic digestion. *Bioresour. Technol.* 101, 537–544.
- Elser, J.J., Bracken, M.E., Cleland, E.E., Gruner, D.S., Harpole, W.S., Hillebrand, H., Ngai, J.T., Seabloom, E.W., Shurin, J.B., Smith, J.E., 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. *Ecol. Lett.* 10, 1135–1142.
- Foeroid, B., Szocs, J., Patinvoh, R.J., Sárvári, I., Horváth, I.S., 2021. Effect of anaerobic digestion of manure before application to soil – benefits for nitrogen utilization? *Int. J. Recycl. Org. Waste Agric.* 10, 89–99.
- Frerichs, C., Daum, D., Koch, R., 2017. Ammonium toxicity - one cause for growth and quality impairments on organic fertilized basil? *J. Kult.* 69, 101–112.
- Frerichs, C., Daum, D., Pacholski, A.S., 2020. Ammonia and ammonium exposure of basil (*Ocimum basilicum* L.) growing in an organically fertilized peat substrate and strategies to mitigate related harmful impacts on plant growth. *Front. Plant Sci.* 10, 1696. <https://doi.org/10.3389/fpls.2019.01696>.
- Germano, R.P., Melito, S., Cacini, S., Carmassi, G., Leoni, F., Maggini, R., Montesano, F., Pardossi, A., Massa, D., 2022. Sweet basil can be grown hydroponically at low phosphorus and high sodium chloride concentration: Effect on plant and nutrient solution management. *Sci. Hortic.* 304, 111324 <https://doi.org/10.1016/j.scienta.2022.111324>.
- Grigatti, M., Boanini, E., Cavani, L., Ciavatta, C., Marzadori, C., 2015. Phosphorus in digestate-based compost: Chemical speciation and plant-availability. *Waste Biomass Valor.* 6, 481–493.
- Grillo F, Piccoli I, Furlanetto I, Ragazzi F, Obber S, Bonato T, Meneghetti F, Morari F., 2021. Agro-environmental sustainability of anaerobic digestate fractions in intensive cropping systems: Insights regarding the nitrogen use efficiency and crop performance. *Agronomy* 2021, 11, 745.
- Guilayn, F., Jimenez, J., Martel, J.-L., Rouez, M., Crest, M., Patureau, D., 2019. First fertilizing-value typology of digestates: A decision-making tool for regulation. *Waste Manag.* 86, 67–79. <https://doi.org/10.1016/j.wasman.2019.01.032>.
- Guilayn, F., Rouez, M., Crest, M., Patureau, D., Jimenez, J., 2020. Valorization of digestates from urban or centralized biogas plants: a critical review. *Rev. Environ. Sci. Bio-technol.* 19, 419–462.
- Gunnarsson, A., Bengtsson, F., Caspersen, S., 2010. Ryegrass mineral N use efficiency of effluent from biodigested plant material. *J. Plant Nutr. Soil Sci.* 173, 113–119.
- Heil J, Vereecken H, Brüggemann, 2016. A review of chemical reactions of nitrification intermediates and their role in nitrogen cycling and nitrogen trace gas formation in soil. *Eur. J. Soil Sci.* 67, 23–39.
- Hultberg, M., Oskarsson, C., Bergstrand, K.-J., Asp, H., 2022. Benefits and drawbacks of combined plant and mushroom production in substrate based on biogas digestate and peat. *Environ. Technol. Innov.* 28, 102740 <https://doi.org/10.1016/j.eti.2022.102740>.
- Jimenez, J., Grigatti, M., Boanini, E., Patureau, D., Bernet, N., 2020. The impact of biogas digestate typology on nutrient recovery for plant growth: Accessibility indicators for first fertilization prediction. *Waste Manag.* 117, 18–31.
- Krupa, S.V., 2003. Effects of atmospheric ammonia (NH_3) on terrestrial vegetation: a review. *Environ. Pollut.* 124, 179–221.
- Lamolinará, B., Pérez-Martínez, A., Guardado-Yordi, E., Fiallos, C.G., Diéguez-Santana, K., Ruiz-Mercado, G.J., 2022. Anaerobic digestate management, environmental impacts, and techno-economic challenges. *Waste Manag.* 140, 14–30.
- Lin H, Gan J, Rajendran A, Reis CER, Hu B 2015 Phosphorus removal and recovery from digestate after biogas production. In: Biernat K (ed) Biofuels—status and perspective. InTech. <https://doi.org/10.5772/60474>.
- Liu, J., Cheng, X., Qia, X., Li, N., Tian, J., Qiu, B., Xu, K., Qu, D., 2018. Recovery of phosphate from aqueous solutions via vivianite crystallization: Thermodynamics and influence of pH. *Chem. Eng. J.* 349, 37–46.
- Liu, X.X., Zhu, Y.X., Fang, X.Z., Ye, J.Y., Du, W.X., Zhu, Q.Y., Lin, X.Y., Jin, C.W., 2020. Ammonium aggravates salt stress in plants by entrapping them in a chloride over-accumulation state in an NRT1.1-dependent manner. *Sci. Tot. Environ.* 746, 141244 <https://doi.org/10.1016/j.scitotenv.2020.141244>.
- Maas, E.V., Hoffman, G.J., 1977. Crop salt tolerance - current assessment. *J. Irrig. Drain. Div. Am. Soc. Civ. Eng.* 103, 115–134.
- Magde, M.X.V., Mariano, E., Leite, J.M., Megda, M.M., Trivelin, P.C.O., 2014. Chloride ion as nitrification inhibitor and its biocidal potential in soils. *Soil Biol. Biochem.* 72, 84–87.
- Mamais, D., Pitt, P.A., Cheng, Y.W., Loiacono, J., Jenkins, D., 1994. Determination of ferric chloride dose to control struvite precipitation in anaerobic sludge digesters. *Water Environ. Res.* 66, 912–918.
- Manu MK, Li DY Liwen L, Jun Z, Varjani S, Wong JWC 2021 A review on nitrogen dynamics and mitigation strategies of food waste digestate composting 334, 125032. [10.1016/j.biortech.2021.125032](https://doi.org/10.1016/j.biortech.2021.125032).
- Manu, M.K., Wang, C., Li, D., Varjani, S., Wong, J.W.C., 2022. Impact of zeolite amendment on composting of food waste digestate. *J. Clean. Prod.* 371, 133408 <https://doi.org/10.1016/j.jclepro.2022.133408>.
- Marconi, D.J., Nelson, P.V., 1984. Leaching of applied phosphorus in container media. *Sci. Hortic.* 22, 275–285.
- Mazzini, S., Borronovo, G., Scaglioni, L., Bedussi, F., D'Imporzano, G., Tambone, F., Adani, F., 2020. Phosphorus speciation during anaerobic digestion and subsequent solid/liquid separation. *Sci. Total Environ.* 734, 139284 <https://doi.org/10.1016/j.scitotenv.2020.139284>.

- Möller, K., Müller, T., 2012. Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Eng. Life Sci.* 12, 242–257.
- Möller, K., Schulz, R., Müller, T., 2010. Substrate inputs, nutrient flows and nitrogen loss of two centralized biogas plants in southern Germany. *Nutr. Cycl. Agroecosys.* 87, 307–325.
- Nkoa, R., 2014. Agricultural benefits and environmental risks of soil fertilization with anaerobic digestates: a review. *Agron. Sustain. Dev.* 34, 473–492.
- Owen, W.G., Cockson, P., Henry, J., Whipker, B.E., Currey, C.J., 2018. *Basil. Nutritional Monitoring Series 1*, 14. <https://urbanagnews.com/wp-content/uploads/2018/05/Nutritional-Factsheet-Basil.pdf>.
- Pan, W.L., Madsen, L.J., Bolton, R.P., Graves, L., Sistrunk, T., 2016. Ammonia/ammonium toxicity root symptoms induced by inorganic and organic fertilizers and placement. *Agron. J.* 108, 2485–2492.
- Prasad, M., Woods, M.J., 1971. Leaching and nutrient uptake of tomatoes in peats. *J. Sci. Fd Agric.* 22, 564–568.
- Provenzano, M.R., Iannuzzi, G., Fabbri, C., Senesi, N., 2011. Qualitative characterization and differentiation of digestates from different biowastes using FTIR and fluorescence spectroscopies. *J. Environ. Prot.* 2, 83–89. <https://doi.org/10.4236/jep.2011.21009>.
- Restrepo, A.P., Medina, E., Pérez-Espinosa, A., Agulló, E., Bustamante, M.A., Mininni, C., Bernal, M.P., Moral, R., 2013. Substitution of peat in horticultural seedlings: Suitability of digestate-derived compost from cattle manure and maize silage codigestion. *Commun. Soil Sci. Plant Anal.* 44, 668–677. <https://doi.org/10.1080/00103624.2013.748004>.
- Riva, C., Orzi, V., Carozzi, M., Acutis, M., Boccasile, G., Lonato, S., Tambone, F., D'Imporzano, G., Adani, F., 2016. Short-term experiments in using digestate products as substitutes for mineral (N) fertilizer: agronomic performance, odours, and ammonia emission impacts. *Sci. Tot. Environ.* 547, 206–214.
- Schindler, D.W., Carpenter, S.R., Chapra, S.C., Hecky, R.E., Orihel, D.M., 2016. Reducing phosphorus to curb lake eutrophication is a success. *Environ. Sci. Technol.* 50, 8923–8929.
- Schoumans, O.F., Ehlert, P.A.I., Regelink, I.C., Nelemans, J.A., Noij, I.G.A.M., van Tintelen, W., Rulkens, W.H., 2017. Chemical phosphorus recovery from animal manure and digestate. Wageningen University & Research. Report 2849 Wageningen Environmental Research.
- Sogn, T.A., Dragicevic, I., Linjordet, R., Krogstad, T., Eijnsink, V.G.H., Eich-Greatorex, S., 2018. Recycling of biogas digestates in plant production: NPK fertilizer value and risk of leaching. *Int. J. Rec. Org. Waste Agric.* 7, 49–58.
- Sommer, S.G., Schjoerring, J.K., Denmead, O.T., 2004. Ammonia emission from mineral fertilizers and fertilized crops. *Adv. Agron.* 82, 557–622.
- Spurway, C.H., Lawton, K., 1949. Soil testing: a practical system of soil fertility diagnosis. Agriculture Experiment Station, Michigan state University, USA, East Lansing.
- Stoknes, K., 2020. Circular food; crops from digested waste in a controlled environment. Series of dissertations submitted to the Faculty of Mathematics and Natural Sciences. University of Oslo, No. 2263.
- 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.* 1215, 389–396.
- Tambone, F., Terruzzi, L., Scaglia, B., Adani, F., 2015. Composting of the solid fraction of digestate derived from pig slurry: Biological processes and compost properties. *Waste Manag.* 35, 55–61.
- Tambone, F., Orzi, V., D'Imporzano, G., Adani, F., 2017. Solid and liquid fractionation of digestate: mass balance, chemical characterization, and agronomic and environmental value. *Biores. Technol.* 243, 1251–1256.
- Teglia, C., Tremier, A., Martel, J-L 2011 Characterization of solid digestates: Part 2, assessment of the quality and suitability for composting of six digested products. *Waste Biomass Valor.* 2, 113–126. [10.1007/s12649-010-9059-x](https://doi.org/10.1007/s12649-010-9059-x).
- Toor, G.S., Peak, J.D., Sims, J.T., 2005. Phosphorus speciation in broiler litter and turkey manure produced from modified diets. *J. Environ. Qual.* 34, 687–697.
- Ulén, B., Bechmann, M., Fölster, J., Jarvie, H.P., Tunney, H., 2007. Agriculture as a phosphorus source for eutrophication in the north-west European countries, Norway, Sweden, United Kingdom and Ireland: a review. *Soil Use Manag.* 23, 5–15.
- Vandecasteele, B., Debode, J., Willekens, K., Van Delm, T., 2018. Recycling of P and K in circular horticulture through compost application in sustainable growing media for fertigated strawberry cultivation. *Eur. J. Agron.* 96, 131–145. <https://doi.org/10.1016/j.eja.2017.12.002>.
- Vanden Nest, T., Amery, F., Fryda, L., Boogaerts, C., Bilbao, J., Vandecasteele, B., 2021. Renewable P sources: P use efficiency of digestate, processed animal manure, compost, biochar and struvite. *Sci. Total Environ.* 750, 141699 <https://doi.org/10.1016/j.scitotenv.2020.141699>.
- Vaneckhaute, C., Zeleke, A.T., Tack, F.M.G., Meers, E., 2017. Comparative evaluation of pre-treatment methods to enhance phosphorus release from digestate. *Waste Biomass Valor.* 8, 659–667. <https://doi.org/10.1007/s12649-016-9647-5>.
- Verhagen JBG 2021 Using organic fertilisers in growing media for young plant production. *Acta Hortic.* 1317, 141–147. [10.17660/ActaHortic.2021.1317.17](https://doi.org/10.17660/ActaHortic.2021.1317.17).
- Viaene, J., Reubens, B., Willekens, K., Van Waes, C., De Neve, S., Vandecasteele, B., 2017. Potential of chopped heath biomass and spent growth media to replace wood chips as bulking agent for composting high N-containing residues. *J. Environ. Manage.* 197, 338–350.
- Wang, W., Lee, D.-J., 2021. Valorization of anaerobic digestate: A prospect review. *Biores. Technol.* 323, 124626 <https://doi.org/10.1016/j.biortech.2020.124626>.
- Wilfert, P., Kumar, P.S., Korving, L., Witkamp, G.-J., van Loosdrecht, M.C.M., 2015. The relevance of phosphorus and iron chemistry to the recovery of phosphorus from wastewater: A review. *Environ. Sci. Technol.* 49, 9400–9414. <https://doi.org/10.1021/acs.est.5b00150>.