Science of the Total Environment 771 (2021) 145263

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Chemically versus thermally processed brown shrimp shells or Chinese mitten crab as a source of chitin, nutrients or salts and as microbial stimulant in soilless strawberry cultivation

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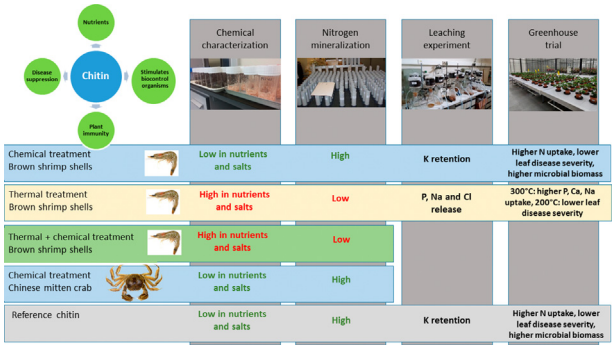
HIGHLIGHTS

- Production method rather than feedstock determines properties of the chitin source.
- Shrimp shells processed by torrefaction contained high amounts of nutrients and salts.
- Chitin sources with higher N mineralization resulted in higher total N plant uptake.
- Higher rhizosphere microbial biomass linked to disease suppression by chitin sources.
- Chemically treated shrimp shells retained K during exposure to fertigation solution.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 5 October 2020
Received in revised form 13 January 2021
Accepted 15 January 2021
Available online 20 January 2021

Editor: Charlotte Poschenrieder

Keywords:
Fertigation
Torrefaction
Growing media
Disease suppression

ABSTRACT

Brown shrimp (*Crangon crangon*) shells and Chinese mitten crab (*Eriocheir sinensis*) were chemically demineralized and deproteinized (denoted as M1 to M4 for the shrimp shells and M5 to M7 for the Chinese mitten crab), and shrimp shells were torrefied at 200 to 300 °C (denoted as R200, R255, R300), and were compared with a commercially available chitin source (denoted as reference chitin). Based on their chemical characteristics, a selection of chitin sources was tested for their N mineralization capacity. The N release was high for the chemically treated shrimp shells and Chinese mitten crab, but not for the torrefied shrimp shells with or without acid treatment, indicating that treatment at 200 °C or higher resulted in low N availability.

Interaction with nutrients was tested in a leaching experiment with limed peat for three thermally and two chemically processed shrimp shells and the reference chitin source. The K concentrations in the leachate for the chemically treated shrimp shells and the reference chitin were lower than for limed peat during fertigation. Irreversible K retention was observed for one source of chemically treated shrimp shells, and the reference chitin. The thermally treated shrimp shells had a significantly higher net release of P, Na and Cl than the treatment without chitin source.

Abbreviations: -aa, ammonium acetate extractable nutrients; C_{water}, water-extractable C; CCI, Chlorophyll Concentration Index; CMC, Chinese mitten crab; DM, dry matter; DW, dry weight; EC, electrical conductivity; FW, fresh weight; IC, inorganic carbon; M, method for chemical extraction; PLFA, phospholipid fatty acids; P_{water}, water-extractable P; R, processed by torrefaction.

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https://doi.org/10.1016/j.scitotenv.2021.145263

Mineral N release
Circular horticulture

Three shrimp shell based materials (M4, R200 and R300) and the reference chitin were tested in a greenhouse trial with strawberry at a dose of 2 g/L limed peat. A very positive and significant effect on *Botrytis cinerea* disease suppression in the leaves was found for the reference chitin, M4 and R200 compared to the unamended control. The disease suppression of the 3 chitin sources was linked with an increase of the microbial biomass in the limed peat with 24% to 28% due to chitin decomposition and a 9–44% higher N uptake in the plants.

1. Introduction

The need for environmental-friendly alternatives for mineral fertilizers and chemical plant protection in soilless strawberry cultivation is high. Chitin, a N-acetylglucosamine polymer, looks like a promising candidate (De Tender et al., 2021). Fishery wastes, and more specifically the crustacean fraction, are considered as a potential feedstock (El Knidri et al., 2018) for chitin production. Yearly, 25,000 t of brown shrimps are caught in Belgium and the Netherlands, resulting in 12,500 t of shrimp shells or 5000 t of chitin (Xu et al., 2008). Yearly, 5,465,000m^3 of growing media are produced in Belgium and the Netherlands for both the professional and hobby market (Schmilewski, 2017). If chitin would be mixed at a dose of 2 g/L growing medium (De Tender et al., 2021), 11 t of chitin would be yearly needed. Shells of brown shrimps thus are sufficiently available for being a potential source of chitin for this application.

1.1. Chitin production methods differ in environmental impact

Shellfish waste contains proteins, $CaCO_3$ and chitin and may thus serve in plant production as a source of nutrients, liming agent and/or plant protection alternative (Sharp, 2013; Aklog et al., 2016). Marine sources of chitin might be rich in salts as well, however. Today, the most common extraction methods in industrial production of chitin are chemical, due to its high productivity and practicality (Pighinelli et al., 2019). For processing these wastes into chitin by chemical treatment, a two steps approach is needed: a demineralization step by acid treatment followed by a deproteinization step by alkaline treatment, to eliminate the calcium carbonates and proteins, respectively (El Knidri et al., 2018). The chitin can be further transformed by deacetylation to chitosan. The degree of N-acetylation is basically employed to differentiate chitin (acetylation degree is higher than 50%) from chitosan (acetylation degree is less than 50%) (El Knidri et al., 2018). Decolorization, i.e. removal of pigments, is needed for some applications and is performed via peroxide treatment.

The preparation process of chitin based on chemical treatments, such as removal of protein and calcium carbonate with sodium hydroxide and hydrochloric acid, respectively, is not environmentally friendly (Aklog et al., 2016; El Knidri et al., 2018), and an (efficient) sustainable method is needed. Other production systems are mechanically, e.g., nanofibration (Egusa et al., 2019a, 2019b), thermal treatment, or biological treatment by enzymatic extraction/microbial fermentation (Ilangumaran et al., 2017; Zou et al., 2020).

1.2. Chitin characterization and application

Besides a range of industrial applications, including cosmetics, food packaging, biomedicine (Yadav et al., 2019), chitin can be applied in agriculture as a valuable soil or growing medium amendment. In soil or growing medium, chitin is degraded by inhabiting micro-organisms which leads to nitrogen (N) release (Sharp, 2013; De Tender et al., 2021), the production of small chitin oligomers and a stimulation of the below ground microbiome (e.g., Cretoiu et al., 2013; Debode et al., 2016). All three factors are subsequently related to plant growth promotion and activation of the plant's defense response against pathogens (e.g., Egusa et al., 2019a, 2019b; Debode et al., 2016; De Tender et al., 2019; Ilangumaran et al., 2017). Several methods focus on the presence and quantification of the amount of chitin in different sources (Fearghail et al., 2019), but besides the characterization, its degree of activity is an important topic in plant production with 3 different functionalities: nutrient source, liming agent and/or disease suppressing agent. The activity of the chitin source amended to soil or growing media can then be assessed by the stimulation of plant growth, nutrient release (e.g. N mineralization) and uptake by plants, an increase in disease suppression or stimulation of the microbial biomass or activity in the soil or growing medium. It is quite difficult to differentiate between these functionalities of chitin sources.

1.3. Chitin as nutrient source or liming agent

Chitin sources added to soil or growing media can be utilized as both a N and C source (i.e., energy source) by plants, bacteria and fungi (Sharp, 2013). Plants can access the N in chitin after microbial decomposition and the release of mineral N, or by directly taking up monomers as organic N (Sharp, 2013). During microbial degradation of the chitin in shrimp shells, fungi release proteolytic enzymes in order to deproteinize and demineralize the shrimp shell. This will result in the release amino acids that, in turn, would act as a N source for fungal growth, with these fungi being a source of chitin as well (Teng et al., 2001). Two distinct sources of chitin, the shrimp shell and fungal growth, would thus be obtained. Besides being a source of N, other macronutrients including Ca can be provided by some sources of chitin (Sharp, 2013), i.e. when these nutrients are not or only partly removed during processing shellfish waste. Chitin sources which still contain $CaCO_3$ can also act as a liming agent by increasing the pH of the soil or growing medium.

1.4. Chitin as disease suppressing agent

Digested lobster shell extracts induced disease resistance in *Arabidopsis* by induction of defense related genes upon *Pseudomonas syringae* and *Botrytis cinerea* infection (Ilangumaran et al., 2017). Chitin amendment raised the suppressiveness of soil toward *Verticillium dahliae*, and resulted in shifts in both the abundances and structures of the soil microbial communities (Cretoiu et al., 2013). In pot trials with soils from different fields, higher plant production and a lower root: shoot ratio was found when crab shell chitin was applied. In addition, the crab shell chitin also increased the populations of bacteria and fungi by 13-fold and 2.5-fold, respectively, and reduced the number of plant-parasitic nematodes (Sarathchandra et al., 1996). Biosolarization with chitin amendment resulted in the control of the plant pathogen *Fusarium oxysporum* f.sp. *lactucae* which was linked with an impact on the overall soil microbial community, with a higher impact on the fungal community than the bacterial community (Randall et al., 2020). *Botrytis cinerea* – the plant pathogen used in current study- is the most important fungal disease for strawberry and the second most significant fungal pathogen worldwide, with high risk for fungicide resistance (Petrasch et al., 2019). Therefore, the need for environmental friendly alternatives that increase the plant defense against *B. cinerea* is high.

1.5. Application in horticulture – soilless cultivation

In contrast to soil ecosystems as mentioned above, the knowledge on chitin use in soilless plant cultivation is limited. Use of chitin

has been reported for different soilless cultivation systems, i.e., micropropagated crops (Sharp, 2013), hydroponics (Egusa et al., 2019a) and growing media (Debode et al., 2016; De Tender et al., 2019). Significant increases in the biomass of aerial parts and concentration of chlorophyll following treatment with nanofibrillated crab shells or short-chain chitin oligomers were observed in hydroponically cultivated tomatoes under ultralow nutrient concentrations (Egusa et al., 2019a). In these trials, it was observed that nanofibrillation enhances the protective effect of crab shells against *Fusarium* wilt disease in tomato (Egusa et al., 2019b). For lettuce grown in limed peat, a higher lettuce yield, a strong increase in the microbial biomass in the growing medium, and a reduction of the survival of the zoonotic pathogen *Salmonella enterica* on lettuce leaves (Debode et al., 2016), was observed.

In previous research with strawberry in soilless cultivation, we showed that, under low nutrient conditions, the amendment of the reference chitin based on crab shells induced the plant's shoot biomass, explained by elevated N concentration in the growing medium and/or a stimulation of fungal genera in the rhizosphere. Moreover, this chitin caused a clear defense priming effect on the strawberry leaves (De Tender et al., 2021). In practice, higher nutrient concentrations are usually supplied (Vandecasteele et al., 2018) than was the case in the aforementioned experiment, with these higher fertilizer doses potentially affecting the net effect of the chitin source.

1.6. Research questions and hypothesis

In our study, two types of shellfish waste were processed: brown shrimp (*Crangon crangon*) shells (waste after mechanically peeling the shrimps), and whole Chinese mitten crab (*Eriocheir sinensis*), being an invasive species in Belgium (Devisscher et al., 2015).

Besides chemical processing, we tested an alternative pretreatment of the shrimp shells by dry torrefaction. It is a relatively cheap and environmental-friendly low-tech alternative to the chemical chitin extraction process. The main aim is to preserve the chitin and to sanitize the material thereby making it easier to store for longer periods of time than fresh shrimp shells. A commercially available chitin from crab shell was included as a reference.

The research questions are:

- Do thermally and chemically treated shrimp shells have a similar microbial-mediated N release under controlled temperature and moisture conditions?
- Are there differences between the chitin sources for nutrient and salt leaching, interaction with fertigation, and plant uptake?
- Is the mode of action and the plant growth promotion of the reference chitin in limed peat similar under higher fertilizer application rates compared to low fertilizer application rates as described in De Tender et al. (2021)?
- Do thermally and chemically treated shrimp shells have the same effect on plant growth, nutrient uptake and disease suppression as the reference chitin from crab shells?
- If used after thermal treatment, i.e., without demineralization and deproteinization: is there a positive effect of the shrimp shell on plant growth as source of nutrients or liming agent and on disease suppression, or are there negative effects (i.e., salts)?
- Is the increase in microbial biomass in the rhizosphere/limed peat as observed by Debode et al. (2016) for the reference chitin higher for thermally processed shrimp shells versus the chemically processed feedstock?

We hypothesize that the feedstock (shrimp shells vs. Chinese mitten crab) and/or the production method (chemically vs. thermally) determines the properties of the chitin source and consequently its mode of action in peat-based growing medium. We evaluated this hypothesis by a 4-step approach: (a) chemical characterization, (b) N mineralization of the different chitin products, (c) a leaching test and (d) a greenhouse strawberry trial with peat blended with the different chitin products and with increasing mineral fertilizer doses. By applying different doses of this fertilizer, we combined the assessment of effects of increasing concentrations of salts and nutrients in the limed peat.

2. Materials and methods

2.1. Processing of shrimp shells and chemical characterization

Different chitin processing techniques were applied (Table S1): (a) brown shrimp shells and Chinese mitten crab were chemically demineralized and deproteinized, (b) shrimp shells were thermally treated at 200 to 300 °C, and (c) shrimp shells thermally treated at 200 to 300 °C were subsequently demineralized. First, the composition of both chitin sources was compared with the initial material and a commercially available chitin source (ref chitin). Second, a selection of these processed chitin sources were tested for their N mineralization capacity. Third, a leaching experiment was performed to test interaction between nutrients and limed peat for a selection of chitin sources. Finally, three shrimp shell based materials and the ref. chitin were tested in a greenhouse trial with strawberry. Their effect on plant growth and yield, water use and nutrient uptake, disease suppression and effects on microbial biomass in the growing medium were tested. The materials in each of these tests/experiment are summarized in Table S1, and full details are given below.

2.1.1. Chemical and thermal treatment

Two feedstocks and three processes were tested (Table S1):

- Chemical treatment at lab scale of brown shrimp shells (*Crangon crangon*) and Chinese mitten crab (*Eriocheir sinensis*) at ILVO (Oostende, BE), with the methods described below and in Table S2.
- Thermal treatment by torrefaction (shrimps) at 200, 255 and 300 °C at pilot scale at ECN > TNO (Petten, NL) for 1 h.
- Acid treatment for removing minerals from the torrefied shrimps at lab scale at ILVO (Oostende, BE) with method M4 (Table S2).

A commercially available chitin (from crab shells) from Biolog Heppe®GmbH was used as a reference (ref chitin) based on previous publications (Debode et al., 2016; De Tender et al., 2019; De Tender et al., 2021). The brown shrimp shells were obtained by a peeling machine at Brevisco (Oostende, BE) for a batch of shrimps caught at the Belgian coast. For chemical treatment, shrimp shells were thoroughly washed with tap water and then with distilled water, dried and ground (Hällde vertical cutter/blender VCB-61). The batch was then divided in four equal parts, each processed by another method (M1 to M4, Table S2). Each method consists of a demineralization step with HCl, followed by a deproteinization step with NaOH. For demineralization, 200 g dried shrimp shells were processed. After demineralization, the material was washed with deionized water to pH neutrality using a 250 μm sieve, dried overnight and stored for deproteinization. After deproteinization, the materials were washed to pH neutrality with deionized water over a 250 μm sieve, dried and stored for analyses and tests. For thermal treatment, shrimp shells were thermal treated by dry torrefaction, i.e., a mild temperature treatment at 200–300 °C in the absence of oxygen. The temperatures are based on the thermal decomposition of chitosan (initial decomposition temperature 254.6 °C and peak at 303 °C) and chitin (initial decomposition temperature 276.4 °C and peak at 380.4 °C) (Arora et al., 2011). The same batch of shrimp shells as for the chemical treatment were used. First the shrimp peels were dried at 105 °C overnight. Subsequently 20 L of peels were loaded into the batch size fixed bed torrefaction reactor at ECN > TNO (Petten, The Netherlands). This reactor typically handles 3–5 kg per feedstock/batch. The reactor consists of a vertical cylinder with an internal diameter of 16 cm and effective length of 1 m, and is directly heated by supplying preheated nitrogen through a distributor plate at the

bottom. The gas and tracing temperatures as well as the N flow are computer controlled and all temperatures, pressures and flows are logged. The reactor was heated to the desired temperature, being 200 (below the thermal decomposition of both chitosan and chitin), 255 (at the initial thermal decomposition of chitosan) or 300 °C (above both the initial thermal compositions of chitin and chitosan) (Arora et al., 2011). The heating up stage consisted of 1 h. The temperature was monitored by 3 thermocouples in the bottom, middle and top section. A continuous stream of nitrogen with 0.3% steam was applied during the whole period. After cooling down the shrimps were removed from the reactor.

The Chinese mitten crab (CMC) was caught as an invasive species in a canal in Grobbendonk (BE), the Province of Antwerp, and the crabs as such were processed. CMC were thoroughly washed with tap water and then with distilled water, dried and ground (Hällde vertical cutter/blender VCB-61), and processed by 3 methods (M5 to M7, Table S2). Each method consists of a demineralization step with HCl, followed by a deproteinization step with NaOH. After demineralization, the material was washed with deionized water to pH neutrality using a vacuum distillation pump with a Buchner funnel or a 250 μm sieve, dried overnight and stored for deproteinization. After deproteinization, the materials were washed to pH neutrality using a vacuum distillation pump with a Buchner funnel or a 250 μm sieve, dried and stored for analyses and tests.

2.1.2. Chemical characterization

The chemically or thermally treated shrimp shells, Chinese mitten crab and reference chitin were chemically characterized using methods based on European Standards developed by CEN, the European Committee for standardization. Dry matter content was determined according to EN 13040. Organic matter content and ash (= 100-% OM) was determined according to EN 13039. Total N content was determined according to the Dumas method (EN 13654-2) with a Thermo Scientific flash 4000 analyzer. Organic (OC) and inorganic (IC) C was measured with a Skalar Primacs SLC TOC analyzer, and C/N ratio was calculated based on OC and total N. Total Ca, K, Mg, P, Na, Al and Fe were measured by a 5110 VDV Agilent ICP-OES (Agilent, Santa Clara, CA) following digestion of 0.5 g chitin with 8 mL HNO_3 (p.a. 65%) and 4 mL H_2O_2 (p.a. 30%) in a 2:1 ratio using a Milestone ETHOS One high performance microwave digestion system (in 15 min to 200 °C, hold 15 min at 200 °C, max. 1500 W). The electrical conductivity (EC, EN 13038), pH-H_2O (EN 13037) and several water-extractable elements were measured (EN 13652) in a water extract (1:5 solid:water v/v): C_{water} (as a relative indicator for available C), Na and P_{water} by a 5110 VDV Agilent ICP-OES (Agilent, Santa Clara, CA), NO_3-N, Cl and SO_4 with a Dionex DX-3000 IC ion chromatograph (Dionex, Sunnyvale, CA) and NH_4-N with a Skalar SAN++ flow analyzer (Skalar Analytical B.V., Breda, The Netherlands). Ammonium acetate extractable (−aa) K, Ca, P, Mg, Fe and Mn were measured by ICP-OES after extracting the sample in ammonium acetate buffered at pH 4.65 (1:5 solid:water v/v). These methods were also used for characterizing the limed peat of the leaching and the peat blends in greenhouse trial. The cation exchange capacity (CEC) was determined by ammonium acetate at pH 7.0 and KCl, modified from the method by Rajkovich et al. (2012). Five gram material was extracted by 50 mL 1 M ammonium acetate at pH 7.0 on a shaker table overnight. After shaking, the suspension was transferred to a funnel with filter paper. The volume of the collected filtrate was made up to 250 mL by additionally slowly pouring 1 M ammonium acetate on top of the sample on the filter. After washing the sample on the filter three times with 60% ethanol, the NH_4^+ on the cation exchange sites of the sample was exchanged by K^+ by pouring 250 mL 10% KCl at pH 2.5 in 5 aliquots over the sample on the filter. The NH_4^+ concentration in the filtrate was determined with a Skalar SAN++ flow analyzer.

2.2. N mineralization

For assessing the N mineralization based on a 100 day-incubation trial (Vanden Nest et al., 2021), mineral soil (top soil from an arable field, 0.9% organic C; pH-KCl: 6.2; 5.3% clay, ammonium lactate extraction: 230 mg P, 170 mg K, 150 mg Mg and 900 mg Ca per kg of air-dried soil) thoroughly mixed with the processed materials and with moisture content of 50% water filled pore space was put in PVC tubes (h = 12 cm, r = 2.3 cm) at a bulk density of 1.4 g cm^{-3}, covered with a single layer of gas permeable Parafilm® and incubated at 15 °C and 70% relative humidity. Soil mineral N was extracted in a 1:5 extraction (w/v) with 1 M KCl and measured with a Skalar SAN++ flow analyzer (ISO 14256-2). The cumulative net N mineralization on each sampling date was calculated as the difference between the amount of mineral N released in the soil amended with processed materials and the amount of N released in the unamended soil, and the net N mineralization after 100 days was expressed as % of the total N content in the product (Vanden Nest et al., 2021).

2.3. Leaching experiment

2.3.1. Input materials for the leaching test

The peat used was Prelvex white peat 100% (AVEVE Lammens, Wetteren, Belgium). Given the low pH of the peat (4.25 in water), the peat was limed one week before the start of the leaching experiment with 1.4 g RHP magnesium lime per liter of peat (RHP, MG's-Gravenzande, The Netherlands). Besides the pure limed peat, six blends were tested (Table S1): three thermally treated shrimp shells (R200, R255 and R300), two chemically treated shrimp shells (M1 and M4) or the reference chitin. These were blended with the limed peat in a ratio of 5 g dry matter (DM) per liter limed peat.

2.3.2. Set-up

A leaching experiment (Fig. S1) was performed with the pure limed peat and the six blends (in duplicate) with the method described by Amery et al. (2021). A volume of 1 l of peat (blend) was put in a leaching column (ROBU, diameter 125 mm and height 110 mm). At the bottom of the leaching columns there was a Macherey-Nagel GF/D filter (2.7 μm) and a glass fiber filter (10–16 μm). On top of the peat (blend), a Macherey-Nagel 640w paper filter was placed to ensure a uniform distribution of the incoming solution and total sample wetting. The incoming solution was pumped onto the sample by a peristaltic pump (Watson Marlow 503S/RL) at an average solution addition speed of 2.8 mL per hour or 0.067 L per day onto one column (0.012 m^2). This is comparable to drip fertigation rates for strawberry in greenhouse culture during low evaporation conditions, as applied in experiments in Vandecasteele et al. (2018). After percolating through the peat (blend), the leaching solution was collected in a leachate bottle, and the composition of the solution was analyzed twice a week. First, the collected volume was recorded. The pH was measured by a Metrohm 785 DMP Titrino pH meter with temperature correction, EC by a Consort C832 EC meter with temperature correction. Anion concentrations (NO_3^-, SO_4^{2-}) were measured by a Dionex DX-3000 IC ion chromatograph (Dionex, Sunnyvale, CA), elemental concentrations (Fe, Al, Mg, Mn, Ca, K, Na, P) were measured by a 5110 VDV Agilent ICP-OES (Agilent, Santa Clara, CA) and NH_4^+ by a Skalar SAN++ flow analyzer (Skalar Analytical B.V., Breda, The Netherlands).

During the first 25 days of the leaching experiment, a fertigation solution was dripped upon the leachate columns to investigate the interaction between the incoming nutrients and the peat (blend). This fertigation solution was made by solving 1 g of N-P-K-Mg 20-5-10-2 fertilizer in 1 L of demineralized water. After 25 days, the incoming solution was switched to demineralized water to verify if the accumulated nutrients could be leached out. After 21 days of dripping demineralized water, the experiment was stopped.

2.3.3. Data handling

The concentrations in the leachate and the net leached mass of the elements, i.e. the cumulative leached mass subtracted by the total mass added by fertigation, were compared between the pure peat and

the blends by ANOVA and Dunnett's test with pure peat as control group. Only 2 replicates were measured per treatment, so homogeneity of variances and data normality could not be checked for this experiment. However, in Amery et al. (2021), the conditions of normal distribution and/or homogeneity of variance of data in a previous leaching experiment with peat and biochar performed with 3 replicates were verified and confirmed. Data analysis was carried out with the statistical program JMP Pro 14.1.0 (SAS, Cary, NC, USA).

2.4. Greenhouse trial with strawberry

2.4.1. Rationale

The chitin sources were tested at a dose of 2 g DM/L limed peat. This low dose was selected for avoiding hydrophobicity and/or too high salt concentrations in the root zone. As fertilizer, we selected Haifa Multimix Potting Soil 14 + 16 + 18 (+micronutrients) PGMix fertilizer (Haifa North West Europe) as it is a combination of several nutrients and the related salts. By applying different doses of this fertilizer, we combined the assessment of effects of increasing concentrations of salts and nutrients in the growing medium. Nutrient or salt losses by leaching from pots was avoided by the dish underneath each pot.

2.4.2. Methodology

2.4.2.1. Experimental set-up: 4 nutrient levels (fertilizer doses) and 4 types of chitin. We included four different fertilizer doses (0.70, 1.05, 1.40 and 1.75 g PGMix/L) in the greenhouse experiment to (a) assess the effect of increasing concentration of salts and nutrients on plant growth and (b) to assess the nutrient release by the four tested chitin sources relative to these fertilizer doses (Table S1): 2 chemically treated (commercially (Reference chitin) and M4) and 2 thermally treated shrimps at two different temperatures (R200 and R300). These chitin sources were tested at the reference nutrient dose of 1.05 g PGMix/L peat (De Tender et al., 2021).

Strawberry plants were grown in 1.5 L pots containing Prelvex white peat 100% (AVEVE Lammens, Wetteren, Belgium), mixed with different PGMix fertilizer doses (see above) and 1.43 g/L lime (RHP, MG's-Gravenzande, The Netherlands). For the chitin source treatments, the peat substrate with 1.05 g PGMix/L was mixed with 2 g dry matter (DM) chitin source/L peat substrate (De Tender et al., 2021). All mixtures were wetted to obtain 40% water-filled pore space (WFPS), and were put in a closed bag to pre-incubate at 15 °C for 1 week (De Tender et al., 2021). No additional fertilizer was applied during plant growth. After pre-incubation, cold-stored bare-root strawberry transplants (cultivar Elsanta) were planted in all pots. The pots were arranged in a semi-randomized design in the greenhouse and plants were grown for 11 weeks at 20 °C. In total, 6 biological replicates per treatment were sampled at the end of the experiment (except for treatment M4, with only 5 biological replicates included).

2.4.2.2. Water use and plant physiological parameters. Every week, the moisture content of the substrate was adjusted to 40% WFPS based on mass loss recorded for each pot separately, by adding water to the dish underneath the pot (De Tender et al., 2021). The water volume added per pot was recorded during the whole experiment. The total water use during the experiment was expressed per pot, thus expressing the net effect of both transpiration by the strawberry plant and evaporation from the growing medium.

From week 6 onwards, fruits started to appear on the plants. Ripe fruits were picked per plant, counted and weighed (FW), and this on 6 sampling times. Part of these fruits were inoculated with *B. cinerea* (see below) and the remaining fruits were used for chemical characterization (see below). Strawberry plants (leaves, petioles + unripe fruits) were sampled after 11 weeks of plant growth and weighed for fresh weight (FW) and dry weight (DW, 48 h at 70 °C) determination in a ventilated oven.

The leaf chlorophyll content was estimated as described in detail in Debode et al. (2018). At the end of the experiment, the total leaf area (TLA) measured with a high resolution flatbed photo scanner (Konica Minolta Bizhub C224e, Konica Minolta, Tokyo, Japan) and analyzed using ImageJ. Furthermore, chlorophyll content was estimated for each of the three separate leaflets of two fully grown compound leaves of these six plants per treatment using a CCM-200 chlorophyll content meter (Opti-Sciences Inc., Hudson, NH,USA). The output was expressed in Chlorophyll Concentration Index (CCI), defined as the ratio of transmission at 931 to 653 nm through a leaf (Opti-Sciences Inc., USA). Total plant CCI was calculated as a weighted average based on individual leaf area. For the root development, depending on the number of visible roots (lateral roots and root hairs) on the surface, a 1–3 developmental score was given, with 1 = a few roots; 2 = roots all over the substrate surface; and 3 = substrate surface fully covered with roots. Statistical analysis of the data on plant leaves and fruits was done using a general linear model (lm), as described in De Tender et al. (2021). Homogeneity of variances was checked by means of boxplots and data normality was checked by QQplots.

2.4.2.3. Botrytis cinerea bio-assay to test plant defense. Plant leaves of 4 plants per treatment were inoculated after ten weeks of plant growth with *B. cinerea* isolate PCF895 (Debode et al., 2013) according to the method of Harel et al. (2012) and described in detail in De Tender et al. (2016). Two plants per treatment were inoculated with sterile potato dextrose agar plugs. The resulting lesions on the leaflets were recorded one week after inoculation using a 0–4 disease scale. This scoring was used to calculate the disease severity index (DSI) per plant (i), used as input for statistical analysis.

$$DSI_i = 100\, x\, \frac{1\, x\, n_{i1} + 2\, x\, n_{i2} + 3\, x\, n_{i3} + 4\, x\, n_{i4}}{4\, x\, n_{i.}}$$

where $n_{i1}, \ldots, n_{i4}$ represent the number of leaves of each infection score and $n_i = \sum_{l=0}^{4} n_{il} = 9$ is the number of leaves measured for each plant. This index has values in the interval [0,100], with a minimum index if all leaves score 0 and a maximum when all leaves score 4. Inoculated leaves remained on the plant until the end of the experiment. For plant leaf infection, the DSI was used as response variable for disease score. A linear mixed effect model was fitted with DAI (7 days) and chitin treatment as fixed main effects, and the plant as random effect.

From the moment strawberry fruits were formed, these were picked and on average ten fruits per treatment were inoculated with *B. cinerea* according to the method of Reddy et al. (2000) and described in detail in De Tender et al. (2016). The area under the disease progress curve (AUDPC) was calculated based on the relative infection scores (Campbell and Madden, 1990). In total, the inoculation was repeated independently six times. For *B. cinerea* infection on fruits, the AUDPC value is used as disease index. A generalized linear model was used with chitin treatment and repeat (infection was scored on four independent time points) as main effects, according to Schandry (2017).

2.4.3. Analyses input materials

2.4.3.1. Chemical characterization of the growing media, plant leaves and strawberry fruits. The peat substrate was sampled at the beginning and end of the greenhouse experiment. The six biological replicates per condition were sampled separately at the end of the experiment, mixed and one composite sample per treatment was analyzed. Methods for chemical characterization of the peat blends were described above.

For leaf sampling, six biological replicates were studied per treatment. "Leaf" is defined as the aboveground vegetative biomass, including the stalks and the three separate leaflets of the compound leaves. Leaves were dried at 70 °C and ground, and the material of one plant (one pot) was considered as one biological replicate. Fruits were collected during the trial, and a sample per plant for the whole trial was freeze-dried before grinding. Total N was determined by Thermo scientific – flash 4000 N analyzer (ISO 16634-1), total concentrations of P, K, Mg and Ca

were determined by 5110 VDV Agilent ICP-OES in the extract following digestion of 0.5 g dried and ground material with 6 mL HNO_3 (p.a. 65%) and 2 mL H_2O_2 (p.a. 30%) in a 3:1 ratio using a Milestone ETHOS One high performance microwave digestion system (in 15 min to 200 °C, hold 15 min at 200 °C, max. 1500 W). Total uptake in the leaves and fruits was calculated by multiplying the measured concentration with the dry mass of leaves/fruits. Total uptake was compared with one-way ANOVA.

2.4.3.2. Microbial analysis of the growing medium using total phospholipid fatty acids (PLFAs). Total phospholipid fatty acids (PLFAs) were used to measure the microbial biomass of the peat substrates at the end of the greenhouse experiment (4 pots were analyzed per treatment). The peat substrate of each pot was mixed and a subsample of 150 mL was frozen at −20 °C and then freeze-dried. PLFAs were isolated from 0.75 g freeze-dried material using phosphate buffer, chloroform and methanol at a 0.9:1:2 ratio. Phospholipids separated by solid phase extraction were saponified to obtain free fatty acids, which were subsequently methylated using 0.2 M methanolic KOH to form fatty acid methyl esters (FAME), which were analyzed with a capillary gas chromatograph-flam ionisation detector (Perkin Elmer Clarus 600, Perkin Elmer, Waltham, USA) with a col-elite-2560 column (100 m length x 0.25 mm ID, 0.25 μm film thickness, Perkin Elmer). PLFAs were identified by retention time using an external FAME and bacterial acid methyl ester (BAME) mix (Sigma Aldrich, St Louis, MO, USA) and quantified by a C19:0 internal standard. Seventeen PLFAs were selected because of their use of biomarker fatty acids for six distinct microbial groups: Gram-positive bacteria (i-C15:0, a-C15:0, i-C16:0, i-C17:0), Gram-negative bacteria (C16:1c9, C17:0cy, C19:0cy), bacteria (non-specific) (C14:0, C15:0, C16:0, C17:0, C18:0), actinomycetes (10Me-C16:0, 10Me-C18:0), fungi (C18:2n9,12) and mycorrhiza (C16:1c11), and summed up together with C18:1c9 to calculate total microbial biomass. Total biomass and biomass for six groups was compared with one-way ANOVA.

3. Results

3.1. Processing of shrimp shells and chemical characterization

3.1.1. Chemical treatment versus thermal treatment

In comparison with the initial mass of dried shrimp shells, the yield for the chemically processed shrimp shells was on average 11%, and was much lower than the yield for the thermally processed shrimp shells (94% at 200 °C, 85% at 255 °C and 74% at 300 °C). The yield for the chemically processed CMC was on average 10% (starting from dried material). The different temperatures during torrefaction gave a distinctly different colour, i.e. darker colors at higher temperatures. The mass loss of the material was: 6% at 200 °C, 15% at 255 °C and 26% at 300 °C (starting from dried shrimp shells). The materials may differ in their bulk density due to differences in ash and (residual) moisture content, and to allow for a better comparison between the materials, pH, EC and extractable nutrients and salts are expressed on a volume basis. Both feedstocks of shrimp shells and CMC are characterized by high carbonate (high inorganic C contents), salt and nutrient contents. The results of the chemical characterization (Tables 1 and S3) confirm the effect of the different treatment methods: the materials after chemical treatment only have lower IC, nutrient and ash contents, pointing at the removal of minerals and carbonates, while only torrefaction does not remove salts or nutrients and carbonates. This is also reflected in the high EC values for the torrefied materials, indicating high salt concentrations. The materials that were first torrefied at 200, 255 or 300 °C and then acidified with method M4, have a very low pH, low carbonate contents but still contain high Cl concentrations (Tables 1 and S3). The total N content is lower for the CMC than for the shrimp shells, both for the feedstock and for the processed materials: all materials based on shrimp shells and Chinese mitten crab had N contents >6.9 and >5.2% N/DM, respectively. Only acidification after thermal treatment results in a higher total N content, indicating a higher removal of mineral components by this sequence of treatments. The chemically treated materials do not contain any mineral N, while the feedstocks have higher mineral N than the thermally treated materials. The effect of the treatment on the total nutrient and salt content is also reflected in the nutrient availability (as measured in the water or the -aa extract). Feedstocks and thermally treated materials have high extractable salt and nutrient concentrations, while chemically treated materials were low in available salts and nutrients. Method M6 was not successful in removal of minerals from CMC as carbonates are still present (both higher IC and Ca concentrations). The water-extractable C (C_{water}) can be both organic and inorganic C. High range is observed for C_{water}, with higher values for feedstocks and for torrefied materials. Although there are large differences in total P and P-aa, these differences are not reflected in P_{water}, indicating that all materials have a similar easily available P concentration.

Table 1
pH, electrical conductivity (EC), total content of C and nutrients, and N mineralization of shrimp shells and Chinese mitten crab (CMC) before processing, the thermally treated shrimps and the chemically treated chitins, including the ref. chitin (commercial chitin based on crab shells). M1–4: methods used for chemical treatment of shrimp shells, M5–7: methods used for chemical treatment of Chinese mitten crab, see m&m for full details. Values in parentheses are standard deviations for 3 replicates. NA = not assessed.

Process	pH-H2O	EC	ash	OC	IC	N	C/N	Ca	K	Mg	Na	P	N mineralization
	–	μS/cm	%/DM				–	g/kg DM					% /total N
Shrimp shells													
Feedstock	9.0	4510	33	37	2.7	7.7	4.8	98	4.4	3.4	9.4	13.1	NA
Chemical M1	8.1	110	7	48	< 0.1	7.6	6.4	2.9	0.12	0.2	0.0	0.8	NA
Chemical M2	8.1	92	4	48	< 0.1	7.0	6.8	4.4	0.12	0.2	0.2	0.7	NA
Chemical M3	8.3	91	8	47	< 0.1	7.1	6.6	6.7	0.17	0.4	0.6	0.7	NA
Chemical M4	8.9	96	6	49	< 0.1	7.6	6.4	9.2	0.16	0.5	0.5	1.5	65.6 (2.5)
Thermal, 200 °C	9.4	2580	35	37	2.6	8.6	4.3	105	3.9	3.5	9.8	13.4	29.1 (1.0)
Thermal, 255 °C	9.4	2550	40	37	2.9	7.3	5.1	125	4.1	3.9	10.1	15.2	NA
Thermal, 300 °C	10.2	2790	47	36	3.2	6.9	5.2	142	5.1	4.5	12.6	18.2	7.9 (0.4)
Thermal 200 °C + chemical M4	2.6	1085	1	52	< 0.1	13.1	4.0	1.2	0.16	0.1	0.1	2.7	18.1 (1.8)
Thermal 255 °C + chemical M4	2.7	1561	5	52	< 0.1	10.9	4.8	2.5	0.24	0.2	0.5	2.1	NA
Thermal 300 °C + chemical M4	2.6	1251	1	59	< 0.1	10.6	5.6	0.9	0.15	0.1	0.2	1.7	1.5 (0.1)
Chinese mitten crab													
Feedstock	8.5	3590	40	31	2.33	6.5	4.7	159	5.3	3.8	8.6	11.3	NA
Chemical M5	7.6	76	2	51	< 0.1	6.9	7.4	6.1	<0.1	0.3	0.4	0.2	NA
Chemical M6	9.0	108	26	36	5.29	5.2	7.0	106	0.1	2.3	1.8	7.4	NA
Chemical M7	8.6	113	2	48	0.12	6.6	7.3	7.6	<0.1	0.2	0.2	1.2	53.8 (1.8)
Reference													
Ref chitin	8.8	157	1	44	<0.1	7.0	6.3	2.0	<0.1	0.2	1.1	0.5	60.9 (1.6)

3.1.2. N mineralization

Although C/N ratios are low for feedstocks and processed materials (Table 1), we observe large differences in the amount of the total N that has been mineralized: between 2 and 66% of the N was mineralized after 100 days. The three chemically treated materials (reference chitin, one based on shrimp shells and one on CMC) had a N mineralization >50%, while the shrimps after torrefaction at 300 °C (both with or without acid treatment for removing minerals) had values <8%. The shrimps torrefied at 200 °C had a value in between, and for both torrefied materials, acid treatment resulted in a decrease of the N mineralization. In summary, the tested chitin sources thus clustered in 2 groups: the chemically treated materials (including the commercially chitin) with high mineralization rate, and the thermally treated materials with low mineralization rates. The N incubation takes 12 weeks, however after 4 weeks we already observed a high N release, being at least 2/3th of the total N release measured after 12 weeks (results not shown). This indicates that the screening of different chitin sources based on N release can be reduced to 4 weeks.

3.2. Leaching experiment

3.2.1. Course of the leaching experiment

The composition of the peat (Table S4) differed significantly from the chemically treated and thermally treated shrimp shells (Tables 1 and S3). The regular sampling of the leachate of the columns allowed detailed analysis of the interaction between the leaching solution and the peat or blend. The leachate composition varied during both the fertigation and the water irrigation phase (Fig. 1). During the fertigation phase, on average 2.2 l of fertigation solution percolated through the columns. During the percolation, elements in the fertigation solution could be accumulated by the peat and/or chitin sources. This is e.g. the case for K as can be seen from the lower K concentration in the leachate compared to the concentration in the fertigation solution (Fig. 1 and Table 2). Alternatively, elements present in the peat, chemically treated and/or thermally treated shrimp shells can be leached out by the percolating solution, resulting in a higher concentration in the leachate compared to the fertigation solution. Leaching of Ca, Na and Cl is very clear from Table 2. The EC of the leachate was in general similar or slightly lower in the leachate compared to the fertigation solution (Table 2). The fertigation phase was followed by a phase with water addition, with on average 1.7 l of water irrigation. During this phase, elements initially present in the peat blends, or accumulated during the fertigation phase could be washed out. The net nutrient or salt release or accumulation by the peat blends at the end of the column experiment can be evaluated by subtracting the total mass added by fertigation from the cumulative leached mass (Table 3). One of the two columns filled with the blend of peat and M1 clogged in the beginning of the water irrigation phase. This treatment has therefore only duplicate results for the fertigation phase.

3.2.2. Interaction of peat with percolating solution

Peat interacted with the percolating solution resulting in a leachate composition deviating from the fertigation solution composition (Table 2). During percolation concentrations increased for Fe, Al, Mg, Ca, Na and Cl, and pH decreased. During the fertigation phase, concentrations of Mn, K, P, NO_3-N, NH_4-N and SO_4 were lower in the leachates compared to the fertigation solution suggesting accumulation of these elements by the peat. During the subsequent water irrigation, these accumulated components could possibly be leached out again (Table 3). Part of the added mass of Mn, K and NH_4-N was still present, i.e. there was net accumulation by the peat at the end of the water irrigation phase (prolonged accumulation). However for P, NO_3-N and SO_4 the accumulation was only temporary, as no net accumulation was observed after the water irrigation phase.

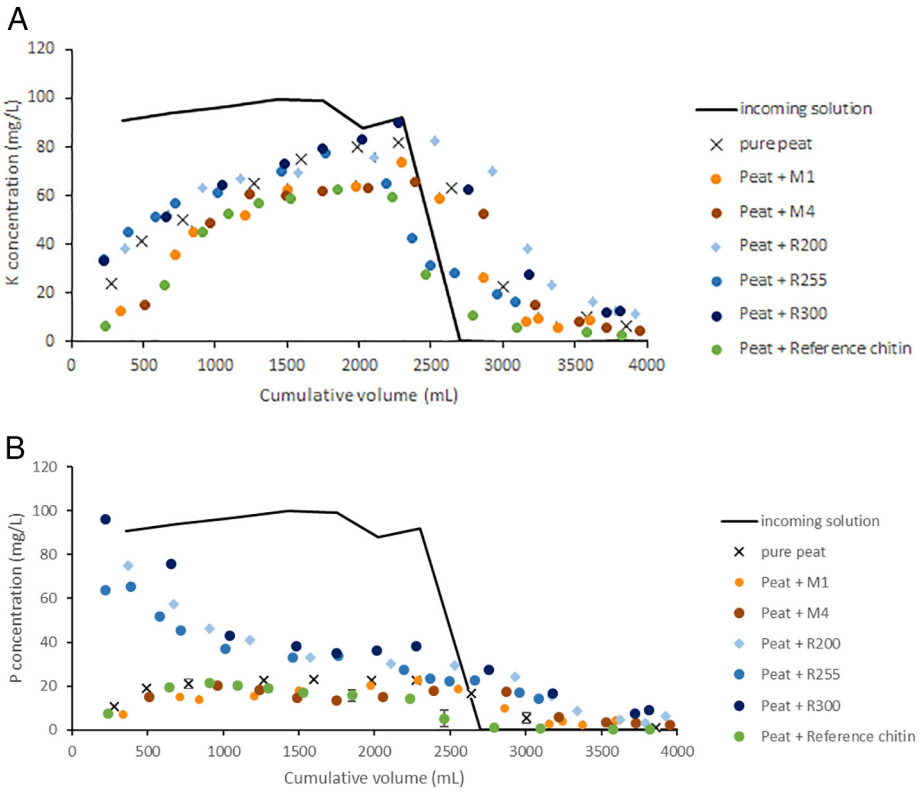

Fig. 1. Concentration of K (above) and P (below) in the leachate of the peat and peat blend columns (average of 2 replicates). The solid black line represents the incoming solution concentration (first phase: fertigation solution, second phase: water). M1–4: methods used for chemical treatment of shrimp shells, R200, R255 and R300: shrimp shells thermally treated at 200 °C, 255 °C and 300 °C, reference chitin: commercial chitin based on crab shells.

Table 2
Composition of the fertigation solution and the leachates of the leaching experiment during the fertigation phase (weighed mean), average of two replicates with standard deviation in parentheses. The composition of the leachates between treatments is compared by ANOVA. The average composition of the leachate of the treatments with chitin are compared with the control treatment of pure limed peat by Dunnett (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). M1–4: methods used for chemical treatment of shrimp shells, R200, R255 and R300: shrimp shells thermally treated at 200 °C, 255 °C and 300 °C, Ref chitin: reference chitin.

	Fe (mg/L)	Mn (mg/L)	Mg (mg/L)	Ca (mg/L)	K (mg/L)	Na (mg/L)	P (mg/L)	NO_3-N (mg/L)	NH_4-N (mg/L)	SO_4 (mg/L)	Cl (mg/L)	pH	EC (μS/cm)
Fertigation solution	0.5	0.50	15	0	94	1.2	23.4	74	125	310	1.5	5.0	1567
100% limed peat	1.1 (0.0)	0.05 (0)	19 (1)	52 (2)	62 (1)	3.7 (0.3)	20.8 (0.7)	68 (0)	83 (2)	284 (0)	2.5 (0.1)	4.7 (0.1)	1385 (5)
Peat + R200	0.6 (0.0)***	0.07 (0.02)	20 (5)	83 (20)	65 (8)	24.3 (8.8)**	43.2 (7)**	67 (2)	94 (9)	292 (8)	27.0 (9.5)***	6.6 (0.3)***	1583 (15)
Peat + R255	0.7 (0.0)***	0.06 (0.01)	22 (5)	89 (12)	59 (7)	34.9 (4.1)***	43.8 (2.2)**	65 (3)	74 (13)	284 (15)	38.1 (5.5)***	6.4 (0.1)***	1521 (40)
Peat + R300	0.6 (0.1)***	0.09 (0.02)*	25 (6)	119 (30)*	68 (1)	35.6 (2.5)***	52.0 (9.0)**	66 (2)	78 (8)	288 (3)	35.2 (3)***	6.7 (0.3)***	1639 (147)*
Peat + Ref chitin	1.1 (0.1)	0.05 (0.00)	19 (1)	52 (2)	42 (8)*	7.8 (1.8)	17.2 (1.8)	62 (4)	72 (9)	259 (19)	3.5 (0.7)	4.8 (0.2)	1253 (83)
Peat + M1	1.0 (0.0)	0.03 (0.00)	12 (1)	34 (3)	50 (5)	3.9 (0.1)	16.6 (0.7)	63 (2)	94 (3)	273 (3)	3.1 (0.0)	4.7 (0.1)	1350 (31)
Peat + M4	1.1 (0.0)	0.03 (0.01)	13 (2)	41 (6)	50 (4)	4.5 (0.2)	17.1 (1.5)	65 (1)	95 (14)	266 (8)	2.8 (0.0)	4.6 (0.1)	1338 (51)
p (ANOVA)	<0.0001	0.008	0.08	0.006	0.019	0.0001	0.0003	0.18	0.15	0.099	0.00012	0.000011	0.0062

3.2.3. Interaction of thermally or chemically treated shrimp shells with percolating solution

Blending limed peat with chemically treated or thermally treated shrimp shells altered the composition of the leachates of the column experiment for most of the elements (Fig. 1 and Table 2). The pH during the fertigation phase was significantly higher in the leachates of the peat blended with the three thermally treated shrimp shells compared to the pH in the leachates of the pure limed peat. The EC was only for the R300 significantly increased. Compared to the pure limed peat, some blends showed an accumulation of Fe and K, and release of Ca, Mn (limited), Na, P, SO_4 and Cl during the fertigation phase (Table 2). Release and accumulation characteristics differed strongly between the chemically treated (including the reference chitin) and thermally treated shrimp shells, therefore they will be discussed separately.

The thermally treated shrimp shells showed only for Fe an accumulation. Since the net leached Fe mass after both phases was still smaller for the thermally treated shrimp shells blends compared to the pure peat (Table 3), this accumulation was not temporary. The thermally treated shrimp shells contain smaller total Fe amounts compared to the pure peat (Tables S3 and S4). For NO_3-N, NH_4-N, SO_4 and Al, there was no accumulation nor release from the thermally treated shrimp shells (Tables 2 and 3). Limited N release was expected given the low N mineralization from the thermally treated shrimps (Table 1). For Mn, Mg and K (Fig. 1) there was a tendency to a small release from the thermally treated shrimp shells. The release of Ca, Na, P (Fig. 1) and Cl from the shrimps was most clear (Tables 2 and 3). The release was especially large in the beginning, but concentrations of especially Ca and P in the blend leachates at the end of the column experiment were still elevated compared to the pure peat leachates (details not given). The release was presumably related to the large available amounts of Ca, Na, P and Cl in the thermally treated shrimps shells (Table S3) and not to the increase in pH. The net released amount of Na and Cl was similar to the water available and total Na and Cl mass present in the blend, and therefore probably completed by the end of the column experiment. The net released mass of Ca was only 10% to 20% of the ammonium acetate available Ca mass in the blend, and the net released P mass was larger than the water available P mass, similar to the ammonium acetate available P mass and smaller than the total P mass present in the thermally treated shrimps shells. The release of Ca and P could probably go on for a longer period at a limited level.

Despite the different origin of the chemically treated shrimp shells and the reference chitin, similar behavior in peat blends could be observed in the column experiment. The chemically treated shrimp shells and the reference chitin contain relatively small amounts of total and available elements, sometimes even smaller amounts than present in the peat (Table S3 and S4). Only the reference chitin showed some release of Na (Table 3), related to the somewhat larger Na content of this chitin (Table S3 and S4). There was a tendency of NH_4-N release, but this was not significant given the large variation between the duplicates. Ammonium release can be induced by mineralization (see above). The average net leached mass of NO_3-N and NH_4-N was for the blend with M4 37 mg N larger than for the pure peat, in the same order of magnitude of the theoretical N mineralization of 23 mg N, as calculated from the total N content and N mineralization (Table 1). However, for the reference chitin the difference of the leached N mass with the pure peat was only 7 mg N, compared to the theoretical mineralization of 21 mg N. For most other elements an accumulation by the chemically treated shrimp shells and the reference chitin was observed,

Table 3
Net leached mass (in mg) of the elements in the leaching experiment, i.e. the cumulative leached mass subtracted by the total mass added by fertigation, average of two replicates with standard deviation in parentheses (except for Peat + M1: only one replicate). Numbers are compared by ANOVA, and for the blends compared with control treatment of pure limed peat by Dunnett (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). M1–4: methods used for chemical treatment of shrimp shells, R200, R255 and R300: shrimp shells thermally treated at 200 °C, 255 °C and 300 °C, Ref chitin: reference chitin.

	Fe (mg)	Mn (mg)	Mg (mg)	Ca (mg)	K (mg)	Na (mg)	P (mg)	NO_3-N (mg)	NH_4-N (mg)	SO_4 (mg)	Cl (mg)
100% limed peat	2.1 (0.3)	−1.00 (0.20)	14 (2)	135 (24)	−31 (9)	7.5 (0.2)	4.2 (0.3)	19 (4)	−43 (21)	64 (16)	4.4 (1.9)
Peat + R200	0.7 (0.2)*	−1.03 (0.37)	15 (9)	226 (23)*	−22 (11)	56.5 (0.8)***	64.8 (5.6)***	19 (4)	−12 (10)	95 (3)	61.0 (0.1)***
Peat + R255	0.8 (0.1)*	−0.72 (0.07)	19 (7)	190 (0)	−13 (11)	63.1 (1.9)***	63.2 (3.7)***	22 (7)	−30 (19)	86 (21)	65.6 (1.8)***
Peat + R300	0.7 (0.5)*	−0.87 (0.13)	28 (12)	302 (44)**	−6 (1)	80.1 (2.5)***	91.9 (8.4)***	16 (6)	−44 (24)	78 (0)	77.6 (0.2)***
Peat + Ref Chitin	1.8 (0.4)	−0.83 (0.22)	10 (1)	107 (20)	−59 (5)*	13.6 (0.2)*	−3.5 (0.0)	13 (3)	−30 (8)	36 (12)	5.3 (1.4)
Peat + M1	1.7 (−)	−1.01 (−)	−6 (−)	77 (−)	−56 (−)	7.7 (−)	−3.0 (−)	3 (−)	−6 (−)	−27 (−)**	3.7 (−)
Peat + M4	2.0 (0.2)	−1.10 (0.17)	−3 (6)	102 (1)	−70 (1)**	9.3 (1.8)	−2.4 (1.9)	11 (1)	2 (24)	28 (4)	4.5 (1.5)
p (ANOVA)	0.010	0.62	0.043	0.001	0.001	<0.0001	<0.0001	0.111	0.26	0.0016	<0.0001

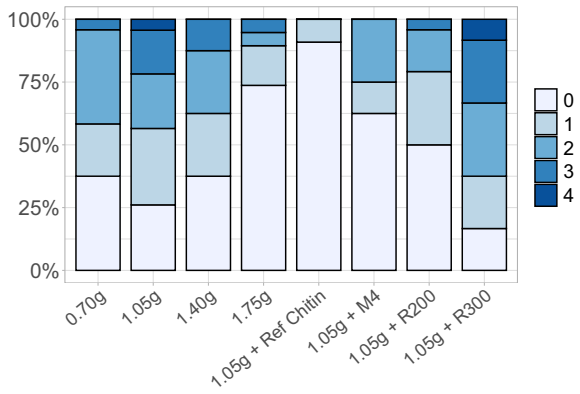

Fig. 2. *Botrytis cinerea* infection score on strawberry plant leaves for 4 treatments with increasing fertilizer doses and 4 treatments with different chitin sources. The infection was scored with a value of 0 (no infection) to 4 (100% infected leaf) 7 days after infection (DAI). 0.70 g = 0.70 g PGMix/L, 1.05 g: 1.05 g PGMix/L (reference fertilizer dose), 1.40 g = 1.40 g PGMix/L, 1.75 g = 1.75 g PGMix/L, M4: method used for chemical treatment of shrimp shells, R200 and R300: shrimp shells thermally treated at 200 °C or 300 °C, Ref chitin: reference chitin.

although mostly not statistically significant (Mg, Ca, P, NO_3-N and SO_4). The amounts present in the chitins are for most of these nutrients smaller or similar compared to the pure peat, but for P some release could have been expected given the somewhat larger P-aa content in the chemically treated shrimp shells and the reference chitin than in the peat. The observation that the P-aa was released from the thermally treated but not from the chemically treated shrimps shells could be related to the more than 10 times higher P content of the thermally treated shrimp shells (Table S3), to other P binding characteristics and the higher pH in the thermally treated than in the chemically treated shrimp shells blends (Table 2). Pronounced K accumulation by the chemically treated shrimp shells and the reference chitin could be observed. These materials contain very low K amounts.

3.3. Greenhouse trial with strawberry

3.3.1. Water use, plant physiological parameters and disease resistance

The average water use per pot during the experiment was 6.7 L. Although no significant differences in water use between treatments were observed, some trends were found: water use increased with increasing fertilizers levels, and was higher for the chemically treated shrimp shells versus the torrefied ones. Increasing the fertilizer dose did not result in significant higher yields, but there was a tendency of a higher total chlorophyll content when higher fertilizers doses were added, with a significant lower total plant CCI for the lowest fertilizer dose (0.70 g PGMix/L) than the reference fertilizer dose (1.05 g PGMix/L) (Table S5). Adding chemically or thermally processed shrimps shells to the limed peat had no or very little effect on the strawberry plant growth and the disease susceptibility of the fruits (Table S5). Only the plant DW of M4 is higher than the same treatment without M4 (= reference fertilizer dose of 1.05 g PGMix/L). However, M4 did not had a significant effect on the total fresh biomass of the plant.

The lowest (0.70 g PGMix/L) and highest (1.75 g PGMix/L) fertilizer dose caused a significant lower disease severity on the leaves than the reference fertilizer dose (B), ($p < 0.05$ and $p < 0.01$, respectively). All shrimps shell treatments decreased the disease severity, except for the R300 treatment for which the disease severity was increased ($p < 0.01$ and $p < 0.05$, respectively) (Fig. 2).

3.3.2. Nutrient uptake

The different fertilizer doses resulted in on average higher N, P, K, Mg, Ca and Na uptake in the aboveground biomass for a higher fertilizer dose, but only the K uptake was significantly higher for the 1.75 g PGMix/L versus the reference fertilizer dose (Table 4). Total plant uptake in the aboveground vegetative biomass was significantly affected by the commercial chitin, M4 and R300: higher N uptake was only observed for the commercially available chitin and M4, and a higher Ca, P and Na uptake was found for R300. Ca and Mg uptake was significantly higher for the shrimp shells processed with M4 versus the reference (Table 4).

Data for pH, EC and the available nutrients in the peat blends at the start of the trial illustrate the effect of the different fertilizers doses for EC, mineral N, SO_4, P (both confirmed for P_{water} and P-aa) and K, with higher values for these characteristics at higher fertilizer doses (Table S6 and S7). The thermally treated shrimp shells are an important source of Cl, P, Ca and Na, resulting in higher values for these characteristics in the peat blends at the start of the trial (Table S6 and S7). Due to nutrient uptake by the plants, all peat blends at the end of the trial have lower values for EC, mineral N, SO_4, and P_{water} than at the start of the trial. Only the treatment with the highest fertilizer dose has a residual mineral N concentration > 20 mg N/L (Table S6). This illustrates that nutrients released by the fertilizer and the chitin sources were used by the plants during the trial.

3.3.3. Microbial biomass (PLFA) in the limed peat

Three chitin sources (ref chitin, M4 and R200) caused an increase of 4 microbial groups in the limed peat, with a significant increase of the total microbial biomass with 24% to 28% (Table 5). Remarkably, these are also the three chitin sources which caused a decrease of disease symptoms on the leaves (Fig. 2). The increase in microbial biomass in these treatments is mainly related to the increase in Non-specific bacteria, Gram+ bacteria and Gram- bacteria, while no change in the fungal biomass is found (Table 5). In contrast, the chitin source R300 did not increase in microbial biomass nor did it cause a decrease in disease symptoms on the leaves (Table 5, Fig. 2).

4. Discussion

The hypothesis of this study, i.e., the feedstock (brown shrimp shells vs. Chinese mitten crab) and/or the production method

Table 4
Total N, P, K, Mg, Ca and Na uptake (mg/pot) in the aboveground vegetative biomass in the strawberry pot trial of 6 replicates with standard deviations in parentheses. For treatment M4, 5 replicates were used (one outlier was removed from the dataset). Bold values indicate significant differences compared with reference treatment (limed peat +1.05 g PGMix/L) by Dunnett (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). M4: method used for chemical treatment of shrimp shells, R200 and R300: shrimp shells thermally treated at 200 °C or 300 °C, Ref chitin: reference chitin, PGMix: Multi-mix Potting Soil 14 + 16 + 18 mineral fertilizer.

Treatment: limed peat +	N	P	K	Mg	Ca	Na
0.70 g PGMix/L	108 (13)	21 (2)	74 (16)	43 (5)	109 (9)	0.33 (0.09)
1.05 g PGMix/L	135 (10)	28 (5)	99 (16)	47 (5)	118 (14)	0.35 (0.08)
1.40 g PGMix/L	141 (11)	28 (3)	130 (10)	47 (8)	122 (21)	0.36 (0.10)
1.75 g PGMix/L	157 (23)	36 (6)	**147 (20)****	48 (8)	116 (20)	0.43 (0.11)
1.05 g PGMix/L + Reference Chitin (2 g/L)	**173 (16)***	26 (4)	111 (19)	59 (4)	126 (12)	0.4 (0.05)
1.05 g PGMix/L + Shrimps M4 (2g/L)	**195 (21)*****	35 (6)	130 (34)	**67 (13)*****	**150 (26)***	0.48 (0.15)
1.05 g PGMix/L + Shrimps R200 (2 g/L)	147 (29)	35 (12)	100 (39)	55 (12)	146 (35)	0.43 (0.07)
1.05 g PGMix/L + Shrimps R300 (2 g/L)	136 (20)	**42 (8)****	99 (17)	58 (8)	**169 (27)****	**0.68 (0.20)*****

Table 5
Absolute concentrations phospholipid fatty acids (PLFAs) of the limed peat at the end of the strawberry experiment (Average of four replicates with standard deviation in parentheses). Bold values indicate significant differences compared with reference treatment (limed peat +1.05 g PGMix/L) by Dunnett (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$). M4: method used for chemical treatment of shrimp shells, R200 and R300: shrimp shells thermally treated at 200 °C or 300 °C, Ref chitin: reference chitin, PGMix: Multi-mix Potting Soil 14 + 16 + 18 mineral fertilizer.

Treatment	Non-specific bacteria	Gram+ bacteria	Actino-mycetes	Gram- bacteria	AM fungi	Fungi	Total biomass
	nmol/g DM						
1.05 g PGMix/L = reference fertilizer	40 ± 6	33 ± 7	3 ± 1	12 ± 1	3 ± 1	20 ± 6	122 ± 16
1.40 g PGMix/L	39 ± 6	29 ± 3	3 ± 1	12 ± 3	2 ± 1	21 ± 3	116 ± 14
1.05 g PGMix/L + Ref Chitin (2 g/L)	48 ± 5	**47 ± 6** ***	**5 ± 1** ***	**18 ± 2** ***	4 ± 1	22 ± 5	**155 ± 18** *
1.05 g PGMix/L + M4 (2g/L)	47 ± 11	**45 ± 9** **	**5 ± 1** ***	**18 ± 2** ***	3 ± 1	24 ± 9	**152 ± 32** *
1.05 g PGMix/L + R200 (2 g/L)	**56 ± 13** ***	37 ± 11	**4 ± 1** **	**17 ± 3** **	3 ± 1	23 ± 8	**157 ± 36** *
1.05 g PGMix/L + R300 (2 g/L)	36 ± 13	28 ± 9	3 ± 1	12 ± 4	3 ± 1	14 ± 7	105 ± 37

(chemically vs. thermally) determines the properties of the chitin source and consequently its mode of action in peat-based growing medium, was confirmed by the differences observed during (a) chemical characterization, (b) N mineralization, (c) the leaching test and (d) the greenhouse trial with strawberry.

4.1. N mineralization: moisture content of the peat or mineral soil versus N release and "chitin" effect

Thermally and chemically treated shrimp shells did not have a similar microbial-mediated N release under controlled temperature and moisture conditions (first research question). Microbial-mediated N release, depending on temperature and moisture content of the mineral soil or growing medium, may be used to assess the activity of chitin sources, with higher N release reflecting higher microbial decomposition activity (Vanden Nest et al., 2021). We observed large differences in N release through mineralization between the different products, with higher values for the chemically treated chitin sources. In the leaching experiment, no significant differences in leached N mass between the treatments was observed because of the relative high variation between replicates. However, there was a tendency of larger N leaching from the chemically treated shrimps compared to the thermally treated shrimps. The difference in leached N mass between the blends with chitin sources and pure limed peat were of the same order of magnitude as the theoretically expected mineralized N mass, based on the total N content and N mineralization, which was largest for M4. There was no visual observation of fungal growth in the limed peat with chitin in the leaching test.

For the two chemically processed chitins tested in the greenhouse trial, the high N mineralization observed in the incubation trial was confirmed by a significantly higher N uptake in the aboveground biomass versus the treatment with the same fertilizer dose but without chitin amendment. In contrast to the chemically treated chitin sources, we observed no additional N uptake in the aboveground biomass due to the thermally treated shrimp shells amendment, which is in agreement with the low N mineralization rate observed during incubation. The higher N release from the two chemically processed chitins did not result in higher aboveground biomass for these amendments. This indicates that this additional N supply was low compared with the N supplied by the treatment with 1.05 g PGMix/L, or that other factors than N supply were limiting plant growth. Previously, the N release from the reference chitin (De Tender et al., 2021) was quantified in a time series experiment with chitin amended to limed peat in pots without a strawberry plant, while the effective N uptake was calculated for pots with strawberry plants. At the end of their experiment, 63% of the total N in the chitin was mineralized (De Tender et al., 2021), which is very similar to the 61% N mineralization observed in the incubation trial here. In our trial, for the reference and M4 chitin treatment 46 and 66% of this extra released N was taken up in the aboveground vegetative plant parts (comparison between the released N and the extra net N uptake by the plants). These calculations indicate that the released N is mainly taken up by the aboveground biomass, the remaining N may be in the roots, the harvested fruits, or is fixed in the growing medium, e.g., by the microbial biomass. As a closed system with a controlled water balance was used in the greenhouse trials, no nutrients were lost by leaching.

The incubation trial, the leaching experiment and the greenhouse trial differ in moisture content of the mineral soil and the peat used: i.e. 40% WFPS in the greenhouse (De Tender et al., 2021), 50% WFPS in the incubation trial (Vanden Nest et al., 2021) versus saturation in the leaching experiment. Although the moisture content of the peat or soil appears to be a key factor in determining the development of the microbial biomass and the related N mineralization, the difference in N release between chemically versus thermally treated shrimps was observed in each trial. In soils, the microbial transformation of chitin significantly depends on the soil moistening (Yaroslavtsev et al., 2009).

4.2. Effects of salts and interaction with other nutrients

We conclude that there were differences between the chitin sources for nutrient and salt leaching, interaction with fertigation, and plant uptake (second research question). Chitin sources may interact with plant growth in different ways: N release, hydrophobicity, as source of other nutrients, salts, $CaCO_3$ as a liming agent, microbial community and chelating effect (El Knidri et al., 2018; Sharp, 2013). By separately testing the interaction with nutrients (i.e. both sorption and release) in the leaching experiment (open system), and the nutrient uptake by strawberry in the greenhouse experiment (closed system), these two aspects were differentiated. However, in a commercial setting, these two aspects are integrated (Vandecasteele et al., 2018) but based on the outcome of the two experiments we can extrapolate the net effect of these two aspects. Interaction of chitin sources with nutrients may be direct or indirect. Chitin sources can directly act as a source of available macronutrients other than N (Aklog et al., 2016; Teng et al., 2001), and may release N through microbial degradation (De Tender et al., 2021). Examples of indirect interaction of chitin sources with nutrients are sorption or complexation (Sharp, 2013), or interaction with microbiology (including nutrient uptake in the microbial biomass) (Teng et al., 2001). Although a low chitin dose of 2 to 5 g/L limed peat was tested, we found clear changes for some chitin sources versus the limed peat without chitin amendment as source of nutrients or interaction with other fertilizers. For K, the concentration pattern for the chemically treated shrimp shells deviated from the pattern for limed peat during the leaching experiment, with distinct and prolonged K accumulation by M4 and the reference chitin source. Previously De Tender et al. (2021) observed interaction between this reference chitin product and the P availability of the amended peat for a lower fertilizer application rate, while we observed this indirect effect for K but not for P. For P, the pattern for the three thermally treated shrimp shells deviated from the pattern for limed peat, with pronounced P release from R200, R255 and R300.

The reason for the K retention by the chemical treated chitin sources is not clear. The CEC of the reference chitin and other chitin sources is very low compared to the limed peat and the amount of K accumulated

by the chemically treated shrimp shells and reference chitin (25–39 mg K/L peat during the leaching experiment) is larger than the extra CEC added by blending the chitin (approximately 0.5 $mmol_c$, or 20 mg K). It is therefore not likely that the CEC of the chitins is responsible for this K accumulation, especially since this would imply that the chitin CEC, which was largely occupied by Ca at the start of the experiment (details nog given), should have exchanged Ca for K, but no Ca release was observed.

We included 4 different fertilizer doses in the greenhouse experiment to (a) assess the effect of increasing concentration of salts and nutrients on plant growth and (b) to assess the nutrient release by the tested products relative to these fertilizer doses. Although effects were not significant, there was a trend for higher water use, aboveground vegetative biomass and nutrient uptake with increasing fertilizer doses, and for higher plant biomass for the treatments with chemically treated chitin sources. We found higher Na and Cl leaching for the thermally treated shrimp shells in the leaching test, but no clear effects of these salts on plant growth were observed for the thermally treated shrimp shells versus the other treatments in the greenhouse trial. The EC, representing the integrated effect of all salts, was only significantly higher for R300 than for the limed peat without chitin source. Torrefied shrimp shells still contain high nutrient contents and salts. These nutrients are susceptible to leaching as shown by the leaching experiment (especially Na, P and Cl), and plant available as e.g. P and Mg uptake by strawberry plants was higher for these chitin sources than for the reference. The nutrients in these chitin sources thus had more effect than the salts. For screening of chitins as nutrient source, the leaching test and the plant uptake both showed that the -aa extraction of the pure chitin gives an indication on the release of other nutrients than N, as the higher extracted amounts of P, K and other nutrients resulted in higher plant uptake and/or more leaching. For N release, a time-consuming incubation during several weeks is needed. In practice, high nutrient doses supplied by fertigation in greenhouse cultivation systems (Vandecasteele et al., 2018) may mask sorption effects of chitin sources, with a reduced risk for observable chemical or biological interaction.

4.3. Microbial effects of the products and impact of the processing

The increase in microbial biomass in the rhizosphere/limed peat as observed by Debode et al. (2016) was lower for thermally processed shrimp shells versus the chemically processed feedstock (third research question). Based on PLFA, we observed an increase in total microbial biomass for 3 of the 4 tested chitin sources, and an increase in fungal biomass for 2 chitin sources. Growth promoting effects of chitin are related to microbial interaction with the chitin source. Due to this microbial interaction, application of chitin results in changes in the microbial community for field soils (Cretoiu et al., 2013; Zegeye et al., 2019) and growing media (Debode et al., 2016; De Tender et al., 2019). The pretreatment of chitin sources may affect the interaction with microbial activity, i.e., during the microbial degradation, as was observed by Teng et al. (2001) for washed versus demineralized shrimp shells. Microbial growth is driven by nutrient and C availability for bacteria and fungi. If we relate a lower N mineralization rate with a lower decomposition of the chitin source in case of the thermally treated shrimp shells, this may indicate that the other minerals present in higher concentrations in the thermally treated shells do not improve the decomposition process nor the microbial biomass, in contrast to the hypothesis for washed versus demineralized shrimp shells by Teng et al. (2001). These authors hypothesized that washed shrimp shells could possibly supply more minerals and other trace nutrients in addition to being a N source for fungal growth than demineralized shrimp shells, which may explain the higher fungal growth observed for the washed shells in their study (Teng et al., 2001). Based on the measured C_{water} concentrations, these thermally treated shrimp shells are more important as a C source than the chemically treated shrimp shells, and thus C availability is not the limiting factor for thermally treated shrimp shells. The fungal growth on the decomposing chitin source is a key element in the effect on plant growth and disease resistance (De Tender et al., 2019), but both the initial amendment and the fungal mycelium formed during its decomposition may act as a source of chitin (Teng et al., 2001).

4.4. Disease suppression and plant growth promotion

The positive effect on disease suppression in the leaves of the reference chitin in limed peat is similar under higher fertilizer application rates in this study compared to low fertilizer application rates as described in De Tender et al. (2021), but we did not observe plant growth promotion for this chitin source (Fourth research question). Chemically treated shrimp shells have the same effect on plant growth, nutrient uptake and disease suppression as the reference chitin from crab shells, while this is not the case for thermally treated shrimp shells at 300 °C (Fifth research question). Shrimp shells after thermal treatment at 300 °C, i.e., without demineralization and deproteinization, did not have a positive effect on plant growth as source of nutrients or liming agent and disease suppression, while distinct negative effects (i.e., salts) were observed neither (sixth research question). For the shrimp shells torrefied at 200 °C, there was a positive effect on disease suppression, while this chitin source had a rather low N mineralization rate. This effect of torrefaction temperature may confirm the lower thermal decomposition temperature of chitin, being 276.4 °C (Arora et al., 2011). Due to the N released by the chitin, chitin-treated plants may have higher N concentrations (Winkler et al., 2017) and total N uptake in plants (De Tender et al., 2021), eventually resulting in a higher plant biomass, i.e., plant growth promotion. We hypothesize that chitin-induced growth promoting effects are not only due to the release of plant-available N (ammonium-N or nitrate-N) in the peat substrate by chitin-degrading species of the microbial community. If the effect is merely a nutrient effect, higher concentrations of mineral fertilizers in the limed peat would then reduce the chitin effect. We aimed with the reference mineral fertilizer dose in this experimental set-up for measuring the effect on disease suppression without subsequent changes/differences in plant growth or nutrient/N uptake. In the experiment in this paper, the mineral fertilizer dose was three times higher than in a previous experiment (De Tender et al., 2021). In our experiment, the N released by the chemically treated chitins resulted in significantly higher N uptake, but not in significantly higher plant biomass, except for M4. The chitin source M4 resulted in higher vegetative aboveground biomass (on dry weight basis), and in higher total N uptake in this biomass, while the reference chitin source only resulted in higher total N uptake in the aboveground biomass. The higher disease suppression measured in the leaves is thus observed for plants with equal biomass for the chitin-treated plants and the control treatment without chitin, but the chitin-treated plants have a higher N concentration in the leaves. None of the treatments with higher fertilizer doses had a similar high N uptake as for the chemically treated chitins.

The nutritional status of a plant is known to influence its susceptibility to pathogens (Huber and Haneklaus, 2007; Précigout et al., 2017; Vandecasteele et al., 2018). An excessive N and/or K supply resulted in higher susceptibility for plant pathogens in case of anthracnose caused by *Colletotrichum gloeosporioides* (Nam et al., 2006) and powdery mildew caused by *Podosphaera aphanis* (Xu et al., 2013) on strawberry. In tomato, overall disease severity caused by *B. cinerea* was lower on plants with higher N inputs (Lecompte et al., 2010). In the current study, both low and high fertilizer doses increase disease resistance against *B. cinerea* on the strawberry leaves, pointing at the role of unbalanced fertilizer supply to crops for diseases. Different types of chitin also caused different levels of *B. cinerea* disease severity. The disease suppressive activity of 3 chitin sources was linked with a higher microbial biomass in the growing medium as compared to the control treatment without chitin. Similar as in De Tender et al. (2021), the reference chitin caused a reduced disease severity on the leaves. Similar as in Debode

et al. (2016) the reference chitin clearly increased the microbial biomass around the roots. For lettuce, this was linked with a lower incidence of *Salmonella enterica* on the lettuce leaves. In the current study, this was linked with a lower disease severity caused by *B. cinerea* on the strawberry leaves. *B. cinerea* is seen as a model fungal necrotrophic pathogen. Reasons for this are the worldwide economic importance of the fungus, the availability of its genome sequence and molecular tools (knock-out mutants, ease of gene silencing and transformation), the exceptionally wide host range and its continuously increasing resistance to a wide range of fungicides (Petrasch et al., 2019). Previous research showed that members of the fungal family *Mortierellaceae* were highly enriched after chitin amended to mineral soil (Zegeye et al., 2019) or peat and *Mortierellacieae* were thus suggested as the main drivers for the disease suppressive activity of chitin (De Tender et al., 2019; De Tender et al., 2021). Further research is needed however to confirm this suggestion.

5. Conclusions

Brown shrimp shells and Chinese mitten crab were chemically demineralized and deproteinized, and shrimp shells were torrefied at 200 to 300 °C, and their composition was compared with the initial material and a commercially available chitin source. From this study we conclude that the production method (chemically vs. thermally) rather than the feedstock (shrimp shells vs. Chinese mitten crab) determines the properties of the chitin source and consequently its mode of action in a peat-based growing medium. Chemically processed brown shrimp shells produced according to method 4 performed similar as the reference chitin, the positive control in this study.

After thermal treatment of the shrimp shells by torrefaction, the material still contained high amounts of other nutrients and salts. These nutrients were highly potentially plant-available based on the -aa extraction. Chemical processing of shrimp shells and Chinese mitten crab resulted in removal of salts and nutrients. All materials based on shrimp shells and Chinese mitten crab had N contents >6.9 and > 5.2% N/DM, respectively, but strongly differed for their microbial-mediated N release. The N mineralization rate in the incubation trial was used as an indicator for the "chitin" effect. The high N mineralization for the chemically treated chitin sources was confirmed by the higher total N plant uptake in the greenhouse trial for these two chitins. Based on the total aboveground nutrient uptake, only the chemically treated chitin sources resulted in a higher N uptake in the plants, and the shrimp shells torrefied at 300 °C resulted in a higher P, Ca and Na uptake versus the treatment with the same fertilizer dose without chitin amendment.

In the leaching experiment, the pH during the fertigation phase was significantly higher in the leachates of the peat blended with the three torrefied shrimp shells compared to the pH in the leachates of the pure limed peat, while no difference in pH was observed for the leachates with the chemically processed shrimp shells. The EC was only for the R300 significantly increased. All chemically treated chitin sources had a higher mineral N release in the leaching experiment, as confirmed by the observed N mineralization during incubation for the chemically processed shrimp shells. For P, the pattern for the three torrefied shrimp shells deviated from the pattern for limed peat, with higher P concentrations in the leachate for R200, R255 and R300.

There were no significant effects in the greenhouse trial of the chitin sources on water use, plant growth, fruit yield and disease suppression on the fruits when treatments with chitin sources were compared with the same fertilizer dose without chitin source. A very positive and significant effect on disease suppression in the leaves was found for 3 chitin sources (ref chitin, M4 and R200) compared to the control treatment without chitin. For these chitins, an clear and significant increase of the microbial biomass in the limed peat was measured. Stimulation of the microbial biomass in the rhizosphere is linked and thus assumed to be (at least partially) responsible for the disease suppressive activity of chitin sources. The torrefaction temperature affected the activity of the chitin source, with higher N release and higher disease suppression in the leaves for the shrimp shells torrefied at 200 °C versus 300 °C.

CRediT authorship contribution statement

Bart Vandecasteele: Conceptualization, Data curation, Formal analysis, Funding acquisition, Project administration, Methodology: chemical characterization and plant uptake, Writing - original draft; **Fien Amery:** Methodology: leaching experiment and data processing, Writing - review & editing; **Sarah Ommeslag:** Methodology: greenhouse experiment, inoculation test and data processing, Writing - review & editing; **Kaitlyn Vanhoutte:** production of chemically treated chitin sources, Writing - review & editing; **Rian Visser:** Conceptualization, Funding acquisition, Methodology: production of thermally treated chitin sources, Writing - review & editing; **Johan Robbens:** Conceptualization, Funding acquisition, Methodology: supervision of production of chemically treated chitin sources, Writing - review & editing; **Caroline De Tender:** Funding acquisition, Methodology: supervision of the statistics of the greenhouse experiment, Writing - review & editing; **Jane Debode:** Conceptualization, Funding acquisition, Methodology: greenhouse experiment and inoculation test, Writing - review & editing

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.

Acknowledgements

This research was funded and executed within the Horti-BlueC project. This project received funding from the Interreg 2 Seas programme 2014-2020 co-funded by the European Regional Development Fund under subsidy contract N° 2S03-046. Both the Province of Antwerp and the Province of East-Flanders are co-funding ILVO for Horti-BlueC. Caroline De Tender received a grant of the Research Foundation Flanders (FWO) with application number 12S9418N. We are grateful to Jonas Schoelynck (University Antwerp) for providing Chinese Mitten Crab feedstock.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.145263.

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