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Mineralization of organic fertilizer for improved plant nutrient availability in hydroponics

Master´s thesis

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Declaration

I hereby declare that I have authored this master's thesis carrying the name „Mineralization of organic fertilizer for improved plant nutrient availability in hydroponics" independently under the guidance of my supervisor. Furthermore, I confirm that I have used only professional literature and other information sources that have been indicated in the thesis and listed in the bibliography at the end of the thesis. As the author of the master's thesis, I further state that I have not infringed the copyrights of third parties in connection with its creation.

In Prague on the 14th of April 2023 ____________________________

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Mineralization of organic fertilizer for improved plant nutrient availability in hydroponics

Summary:

There is an increasing interest in using organic fertilizer for hydroponic plant production, due to its lower greenhouse gas emissions, lower accumulation of harmful toxins in food, and greater resource recycling potential than mineral fertilizers. However, there are major limitations to the use of organic fertilizer in hydroponics, which mostly center around nutrient availability and nitrogen. Nitrogen in organic fertilizer is predominantly present in organic compounds, which need to undergo mineralization to become plant available. In soil, microorganisms are responsible for this and subsequent nitrification. The lack of microorganisms in hydroponics means that nutrients bound in organic compounds become available to plants only very slowly, limiting growth and inducing deficiencies. A parallel mineralization and nitrification method used in previous studies employs soil as a source of microorganisms and, through an incubation process, organic nitrogen compounds are broken down by the microorganisms. This thesis draws inspiration from this method and tests various sources of inoculum found in Denmark, including agricultural soils, compost soil, and seawater, to test the effect on ammonium and nitrate concentrations in organic fertilizer. In the incubation experiment of this thesis, successful nitrification was induced by the end of week 3, when almost all of the ammonium had been oxidized in the compost soil inoculum treatment. This inoculum, which produced the highest nitrate levels in week 3, was selected for the hydroponic plant growth experiment with *Brassica rapa* subsp. *chinensis*. After 27 days of treatment, plants grown with mineral fertilizer showed the highest biomass accumulation and no deficiency symptoms. Plants grown with the incubated organic fertilizer had a higher shoot and total fresh weight than the (non-inoculated) organic fertilizer treatment while displaying fewer visual symptoms of deficiency and having higher leaf concentrations of potassium and iron. Overall, this study shows that organic fertilizer, with the proper inoculation and mineralization, can be successfully used in hydroponic plant production.

Keywords: bok choy; *Brassica rapa* subsp. *chinensis*; Hoagland solution; hydroponics; microbial nitrification; mineralization; nitrate; organic fertilizer; vinasse

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1 Introduction

Modern agriculture has a sustainability problem. Over half of the anthropogenic non-CO_2 greenhouse gas (GHG) emissions are attributed to agricultural production with a significant portion of that being due to mineral fertilizer production and usage. Organic fertilizer, which originates from plant or animal residue or waste, produces fewer greenhouse gas emissions than mineral fertilizers and since its production is neither geographically concentrated nor energy intensive, it can be a more sustainable option that also promotes resource recycling.

Hydroponics is a plant production method that uses a nutrient solution in a soil-less growth medium. It is commonly employed alongside controlled-environment agriculture, allowing for year-round production with a significant reduction in land and water use, which is particularly pertinent in urban centers or desert regions. Due to this, hydroponics allows for shorter supply chains and fresher food for consumers. Hydroponics is particularly popular for leafy green and herb production.

There is both an environmental and economic incentive to using organic fertilizer in hydroponics. In some markets, such as the United States, hydroponically grown plants can be organically certified when using organic fertilizers, which allows producers to charge a higher price and satisfy consumer demand for organic produce. However, there are serious limitations around organic fertilizer use in hydroponics, mostly around nutrient availability. Plants are not generally able to uptake organic compounds, thus nutrients such as nitrogen are not available to plants when they are bound in proteins or nucleic acids.

Soil microorganisms can break down these organic compounds into plant-available forms, but the lack of these microorganisms in hydroponics leads to a very slow nutrient release and often a build-up of ammonium, which can be toxic for plants. The addition of soil microorganisms to organic fertilizer can help increase nutrient availability, specifically nitrogen availability, through microorganisms responsible for mineralization and nitrification. This thesis will focus on addressing the issue of nutrient availability in using organic fertilizer in hydroponics by inoculating the organic fertilizer with soil microorganisms.

2 Scientific hypothesis and aims of the thesis

The objective of this thesis is to utilize soil microorganisms to induce the mineralization of organic nitrogen compounds and nitrification of ammonium present in organic fertilizer to increase plant availability of nitrogen for use in hydroponic growing. First, an incubation experiment will be used to study the impact of several potential sources of microorganisms and their effect on nitrate and ammonium concentrations in organic fertilizer. Second, one inoculum will be selected for a hydroponic growth experiment with *Brassica rapa* var. *chinensis* (bok choy).

Overall, this thesis aims to optimize bok choy growth in hydroponics using organic fertilizer through the use of a soil inoculum as a source of microorganisms to mimic soil-based mineralization of organic nitrogen compounds into plant-available forms of nitrogen. It is expected that with the proper selection of inoculum, the nitrate concentration will increase and benefit plant growth.

Specifically, plants grown in the mineral fertilizer (MF) treatment are expected to have the highest biomass production and display no nutrient deficiency symptoms. Plants grown in the organic fertilizer (OF) treatment will have the lowest biomass production and display deficiency symptoms, especially of nitrogen. Plants grown in the inoculated organic fertilizer treatment (InocOF) will have greater biomass production than the OF treatment and display milder deficiency symptoms due to the increased availability of nutrients, specifically nitrogen.

3 Literature research

3.1 Plant nutrients

Plant nutrients are elements that are essential for plant growth and reproduction and must fulfill certain criteria: the element is necessary for the plant to complete its life cycle, the role of the element may not be substituted by any other element (the element is unique), and the necessity of the element must be universal among all plants (Barker and Pilbeam, 2007). However, not all identified plant nutrients fit all of these criteria, therefore there is some debate regarding the exact list. Of the seventeen elements making this list, three of them are C, H, and O, which are derived from the air or water. The remaining fourteen are presented in Table 1, with average concentrations of each nutrient in plant dry matter, although concentrations can differ by species. These elements are considered to be the macronutrients, with concentrations of 0.1 % and higher in plant dry matter, and micronutrients, with concentrations of less than 0.01 %, of plants (Barker and Pilbeam, 2007). Nitrogen is the nutrient that is needed in the highest concentrations by the plant and most commercial fertilizers will emphasize the N-P-K ratios (Taiz *et al*., 2018).

Table 1: *Average concentrations of elements in plant dry matter. Source: Kirkby, 2012; Taiz* et al., *2018; Munson, 1998; Hawkesford* et al.*, 2011.*

Macronutrients	**mg kg^{-1}**
N	10 000 – 50 000
P	2 000 – 5 000
K	10 000 – 50 000
Ca	1 000 – 50 000
Mg	1 500 – 3 500
S	1 000 – 20 000
Micronutrients	
Cl	100 – 500
B	10 – 200
Fe	100 – 500
Mn	20 – 300
Zn	27 – 100
Cu	5 – 30
Ni	0.1
Mo	0.1 – 2

Beneficial elements are an additional group that may provide a positive effect on the growth of the plant, or are only required by specific species of plants, but do not fulfill the three criteria listed above. Some of these elements may include Si, Co, Na, Se, Al, and V (Barker and Pilbeam, 2007). Plants primarily take up nutrients (other than C) via their roots, although it has been shown that plants can also intake nutrients through their leaves via so-called foliar fertilization (Bergstrand, 2022).

Deficiency symptoms in plants can be identified in four ways: visual symptoms, soil analysis, plant material analysis, and crop growth response (Bergstrand, 2022). The most accessible and low-cost method is observing the visual symptoms; however, many symptoms overlap between various nutrient deficiencies which, combined with symptoms of nutrient toxicity, makes this approach potentially problematic (Bergstrand, 2022). Using foliage for diagnosis can lead to a range of symptom identification: chlorosis (uniform or interveinal), necrosis (at the leaf tip, leaf margins, or interveinal), lack of new growth (death of axillary or terminal buds, leaf dieback), accumulation of anthocyanin (resulting in a purple/red color), or stunting with green or off-green/yellow color (Barker and Pilbeam, 2007).

Quantitative analysis, or laboratory testing of plant material, can be used to confirm visual diagnoses of deficiency; it can also help identify multiple concurrent deficiencies or toxicities or so-called hidden hunger, where visual symptoms do not occur but yield suppression does (Barker and Pilbeam, 2007). Analysis of plant tissue can also be used to confirm if an element has been absorbed by the plant, for example after the folical application of fertilizers or suspected heavy metal contamination (Barker and Pilbeam, 2007). Similar leaves or tissue should be collected between plants that will be compared, as concentrations of nutrients differ between the age or development of the leaf and between different organs (Barker and Pilbeam, 2007).

3.1.1 Role of nitrogen in plants

Nitrogen is the mineral nutrient required in the largest amounts by plants (Table 1) and the total dry matter of a plant usually consists of around 1 - 5 % N (Hawkesford *et al*, 2012). Nitrogen plays a key role in protein composition, chlorophyll, nucleic acids, amino acids, nucleotides, co-enzymes, phytohormones, and secondary metabolites, and has a major influence on plant growth (Hawkesford *et al*., 2012; Taiz *et al*., 2018). Around 85 % of the total N in the plant is in protein compounds (Barker and Bryson, 2007). The chlorophyll concentration, therefore the photosynthetic capacity of the plant, is closely associated with the leaf concentration of N (Bergstrand, 2022).

Nitrogen can be taken up by the plant as ammonium (NH_4^+) or nitrate (NO_3^-). Nitrate has to be reduced to ammonium in order to be assimilated into the plant (Hawkesford *et al*., 2012). The inorganic N is then assimilated into glutamate and glutamine, which is further synthesized into amino acids, amines, other amides, peptides, proteins, nucleic acids, and other compounds (Hawkesford *et al*., 2012). Mild N deficiency will lead to greater root development and therefore a reduction in the shoot:root ratio; specifically, lateral root growth is supported (Oldroyd and Leyser, 2020). Plant growth will be severely affected by N deficiency, with deficient leaves appearing stunted and narrow, chlorosis in older leaves (due to the mobility of N in the plant), and the overall crop will start to appear pale green or even yellow (Hawkesford *et al*., 2012). All plant organs will suffer from an N shortage and severe deficiency can lead to leaf death and senescence (Barker and Pilbeam, 2007; Barker and Bryson, 2007).

3.1.2 Macronutrients other than nitrogen

Many enzymes are dependent on potassium, such as the activation of ATPase and protein synthesis, and K plays a key role in CO_2 fixation, energy transfer during photosynthesis, RuBP carboxylase activity, osmotic pressure, and changing turgor pressure related to stomata aperture (Hawkesford *et al*., 2012; Mengel, 2007). Protein synthesis is also a likely function of K (Mengel, 2007). Potassium tissue concentrations are between 20 000 – 50 000 mg kg^{-1} dry weight (dw) for optimal growth (*Brassica* species tend to have 30 000 – 42 000 mg kg^{-1}), with deficiency symptoms appearing as slowed growth, flaccid leaves, weak stems, mature leaf chlorosis, and marginal or tip necrosis on older leaves (Hawkesford *et al*., 2012; Barker and Pilbeam, 2007; Mengel, 2007).

The ideal phosphorus concentration is 3 000 - 5 000 mg kg^{-1} in plant material and is typically absorbed in either $H_2PO_4^-$ or HPO_4^{2-}, with possible absorption of organic P compounds (Hawkesford *et al.,* 2012; Sanchez, 2007). Phosphorus plays a critical role as a structural component of nucleic acids and phospholipids, contributing to membrane structure, as well as in energy transfer (ADP and ATP) (Sanchez, 2007). Anthocyanosis is also closely associated with P deficiency due to anthocyanin overproduction, especially when the leaves are dark green or with a purple tint, instead of chlorotic (de Bang *et al.,* 2021; Taiz *et al*., 2018).

Phosphorus deficiency leads to the inhibition of leaf expansion but the concentration of chlorophyll does not decrease, which makes the leaves seem darker green (Hawkesford *et a*l., 2012). As with N deficiency, under low PO_4^- conditions, lateral root growth will be promoted, with overall root development encouraged at the expense of the shoot, therefore a decrease in the shoot:root ratio (Oldroyd and Leyser, 2020).

Calcium can be stored in high concentrations in the vacuoles, plays an important role as a signaling molecule, is often bound in pectate which strengthens cell walls, and contributes to membrane stability and cell integrity (Hawkesford *et al*., 2012). Calcium concentrations vary greatly between different plant species and growing conditions and can be between 1 000 and more than 50 000 mg kg^{-1}, with higher concentrations in dicotyledons (Hawkesford *et al*., 2012). Calcium deficiency presents itself as necrosis of the leaf tip and blossom end rot, as well as upper shoots that are yellow-green while lower shoots are dark green (Hawkesford *et al*., 2012; Barker and Pilbeam, 2007; Mengel, 2007).

Magnesium is the central atom of the chlorophyll molecule and can interact with strongly nucleophilic ligans; it also acts as a helper in the aggregation of ribosome subunits, is involved with many enzymes such as PEP carboxylase, and is a substrate for ATPase for ATP synthesis (Hawkesford *et al*. 2012). Under Mg deficiency, protein, and RNA synthesis slows down and affects chloroplast function including electron transfer in photosystem II (PSII) (Hawkesford et al., 2012). The optimal concentration of Mg in plants is 1 500 – 3 500 mg kg^{-1} with deficiency usually appearing as leaf tip yellowing or chlorosis in the intravenous regions of older, fully expanded leaves due to the translocation of Mg (Hawkesford *et al*., 2012; Merhaut, 2007).

Sulfur is taken up in the form SO_4^{2-} and forms a part of the amino acids cysteine and methionine; these amino acids can further form coenzymes or secondary plant products, including tripeptide glutathione, alliins, and glucosinolates (found in many *Brassicaceae* species) (Hawkesford *et al.,* 2012; Haneklaus *et al*., 2007). Sulfur should form 1 000 – 20 000 mg kg^{-1} of dw of plants and deficiency results in reduced shoot growth, reduced leaf area, decrease in shoot:root ratio, decrease in chlorophyll content, and chlorosis, which appears evenly distributed between old and young leaves; however, visual diagnosis needs to be accompanied with leaf analysis to confirm deficiency due to common deficiency symptoms (Hawkesford *et al.*, 2012; Haneklaus *et al*., 2007). Specifically in *Brassica* species, S deficiency develops as marbling of leaves, chlorosis starting at the edge while veins stay green, or anthocyanosis, with symptoms showing up in newer leaves due to limited mobility; in oilseed rape, S deficiency can affect the shape, size, and color of flowers, creating white flowers (Haneklaus *et al*., 2007).

3.1.3 Micronutrients

Iron, Mn, Fu, Zn, Ni, Mo, B, and Cl are considered to be micronutrients in terms of plant nutrition (Broadley *et al*., 2012). Copper plays an important role in catalyzing redox reactions and protein composition; 50 % of Cu in a plant is bound to plastocyanin, a component of the electron transport chain in Photosystem I (PSI) (Broadley *et al.,* 2012). Only about 2 % of copper is present in the free forms of Cu^{+2} and Cu^{+}. Copper deficiency leads to lower rates of photosynthesis which limits plant growth and the critical deficiency range is between 1 - 5 mg kg^{-1} dw (dependent on the plant species), but high levels of Cu are toxic to plants (Broadley *et al.,* 2012; Kopsell and Kopsell, 2007). Copper deficiencies appear to differ between species, while toxicity appears as reduced root growth and chlorosis on leaves (Kopsell and Kopsell, 2007).

Leaf iron concentrations in tomatoes are around 200 - 226 mg kg^{-1} dw in sufficient Fe conditions and around 20 mg kg^{-1} dw in low Fe conditions, with critical deficiency concentrations in the range of 50 - 150 mg kg^{-1} dw – with higher Fe need in C4 species over C3 species (Broadley *et al*., 2012). Iron is taken up by the plant in the forms of Fe^{2+} and Fe^{3+} and plays a role in electron transfer reactions while forming a part of heme proteins like cytochromes, catalase, and leghemoglobin, and Fe-S proteins, like ferredoxin, which is essential to *nitrite reductase* activity to reduce nitrite to ammonium (Broadley *et al*., 2012; Römheld and Nikolic, 2007). Catalase and peroxidase enzymes are sensitive to low Fe levels in the plant; increased levels of phenolics can be released and organic acids can be produced under low Fe conditions (Broadley *et al*., 2012). Due to the role of Fe in the biosynthesis of chlorophyll (chlorophyll *a* is more sensitive than chlorophyll *b*), visual symptoms of deficiency include chlorosis in young leaves, specifically in the laminae while veins remain dark green (Broadley *et al*., 2012; Römheld and Nikolic, 2007). Iron toxicity occurs above 500 mg kg^{-1} dw and can involve bronzing of the leaves and is most common in waterlogged soils (Broadley *et al*., 2012).

3.2 Bok choy

Bok choy (*Brassica rapa* subsp. *chinensis)*, also called pak choi, is a leafy green vegetable commonly consumed in many Asian countries, with 30 - 40 % of its worldwide production located in China, with consumption in Europe increasing (Suruban *et al*., 2022). The genus *Brassica* is economically the most important in the *Brassicaceae* family, which contains approximately 338 genera (Veazie *et al*., 2020; Šamec and Salopek-Sondi, 2019). Bok choy is a fast-growing long-day leafy green vegetable that is suitable for hydroponic systems, whose young leaves can be eaten as part of salads, and mature leaves can be steamed or cooked (Bergstrand *et al.*, 2020; Jeon *et al.,* 2018). Bok choy is an N-demanding plant, with optimum N application recommended around 200 kg N/ha and tissue concentrations of N around 5 - 6 % (Veazie *et al*., 2020). According to Barker and Bryson (2007), tissue concentrations of N for broadleaf vegetables, which include bok choy, are 3.5 - 5.1 %, with low concentrations being below 2.6 %.

The *Brassica* leafy greens are high in vitamins A, B, C, E, and K_1 as well as essential amino acids; sulfur compounds like amino acids methionine and cysteine but also glucosinolates and the related isothiocyanates are characteristic of the *Brassicaceae* family (Zou *et al*., 2021). Leafy green vegetables contain fiber and are rich in Fe, Mg, K, and Ca with an abundance of antioxidants (Zou *et al.,* 2021). One study found over 51 different flavonoids in bok choy, mostly including acylated and nonacylated glycosides of quercetin, kaempferol, and isorhamnetin, as well as five non-flavonoid phenolics hydroxycinnamoyl gentiobioses and their derivatives, and glucosides formed with caffeic, ferulic, and sinapic acids (Lin and Harnly, 2010). Glucosinolates and their hydrolyzed products (including isothiocyanates) are being researched as having potential anti-cancerogenic and anti-inflammatory properties and have been marketed as supplements (Šamec and Salopek-Sondi, 2019).

Brassica rapa contains many subspecies which include oilseed rape, turnip, Chinese cabbage, bok choy, chuy sum, mizuna, broccoli rabe, and others; the *chinensis* cultivar group includes bok choy (*B. rapa* subsp. *chinensis*), choy sum (*B. rapa* subsp. *parachinensis*) and tatsoi (*B*. *rapa* subsp. *narinosa*) (Bird *et al.,* 2017).

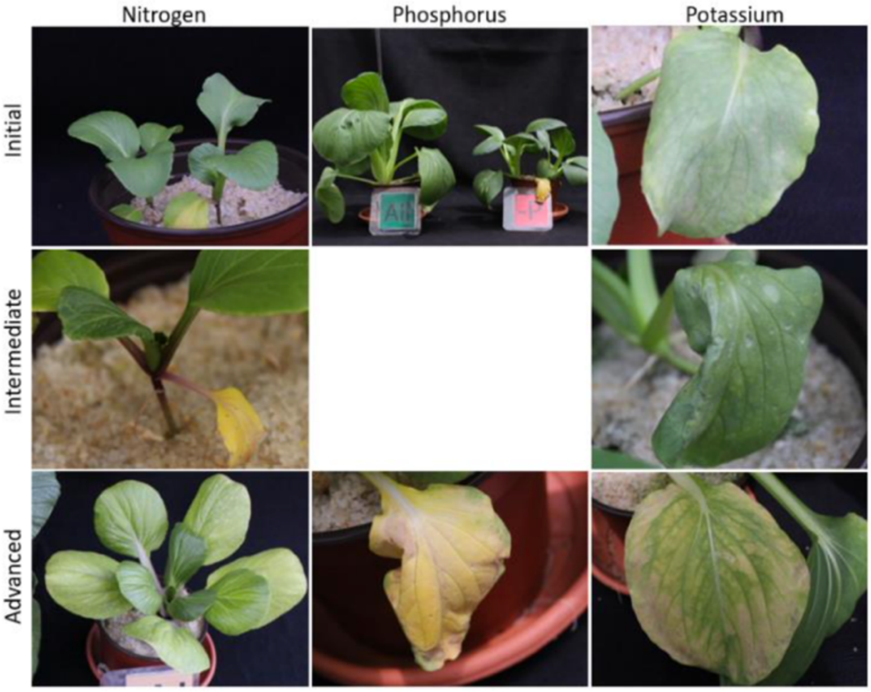
3.2.1 Deficiency symptoms

Veazie *et al*. (2020) induced nutrient deficiencies for two varieties of bok choy using a modified Hoagland solution missing the specific element and noted various stages of visual deficiency symptoms as well as tissue concentrations for all deficient and control bok choy plants. Visual symptoms of N deficiency in bok choy include stunted growth and chlorotic older leaves, with severe symptoms leading to entirely yellow and necrotic leaves (Figure 1a) (Veazie *et al.* 2020). The N-deficient plants in Figure 1 had leaf concentrations of 2 % ± 0.2 % N and had the greatest reduction in dw compared to the control (Veazie *et al.,* 2020). In *Brassica* species, N deficiency is also associated with purple leaves (Barker and Bryson, 2007). Severe N deficiency can lead to entirely chlorotic older leaves, then progressing to middle leaves; the total carotenoids and chlorophyll *a* and *b* concentrations were also significantly reduced (Taiz *et al.,* 2018; Veazie *et al*., 2020).

Phosphorus deficiency in bok choy manifests as yellowing of margins and irregular spotting of lower leaves, darker coloration in new leaves, and red or purple pigmentation in foliage (Figure 1a). Leaf concentrations of P were 0.37 - 0.55 % and 0.09 % in the control and deficient bok choy, respectively (Table 1) (Veazie *et al.,* 2020).

Potassium deficiency can be observed as the yellowing of leaf margins in older foliage, with chlorotic spotting developing which later turns to necrosis; plants that are K deficient have 70 % lower anthocyanin concentrations than the control (Veazie *et al*., 2020). Calcium deficiency exhibited itself as curling of the leaves and stunting of new leaves, later developing into overall new growth limitation and thicker leaves, with marginal necrosis. Magnesium deficiency appears as overall chlorosis and chloritic spotting in older leaves, particularly in the center of the leaf with leaf margins appearing darker (Veazie *et al.,* 2020). Iron deficiency is exhibited as a pale appearance and marginal chlorosis in younger leaves with some interveinal necrosis in the upper foliage (Veazie *et al*., 2020). No visual symptoms of S, Mo, or Cu deficiency were noted in this study.

a)



b)

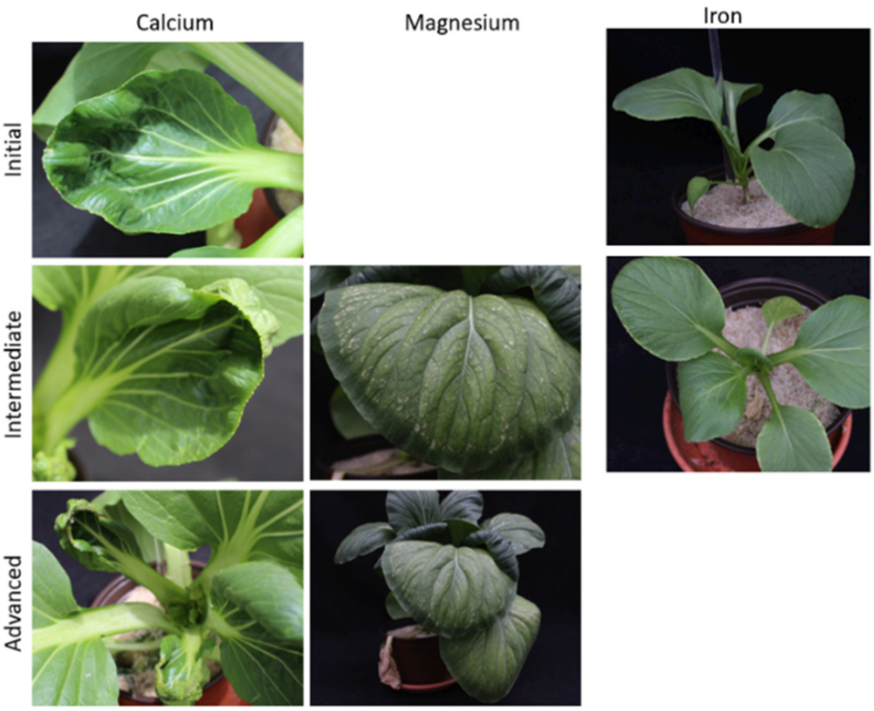


Figure 1: *Mild, intermediate, and advanced stages of N, P, and K-deficiency symptoms (a) and Ca, Mg, and Fe-deficiency symptoms (b) in* Brassica rapa *subsp.* chinensis in *Veazie* et al. *(2020).*

Leaf concentrations from Veazie *et al*. (2020) deficiency induction of bok choy experiment can be seen in Table 2 along with the optimal range based on Bryson and Mills (2015). The deficiency values represent the concentration of a specific element under deficiency stress with a modified Hoagland solution missing the specific element (Veazie *et al*., 2020).

Table 2: *Leaf tissue nutrient concentrations from Veazie* et al. *(2020*). Brassica rapa *subsp.* chinensis *'Black Summer' plants were grown under specific nutrient deficiencies for six weeks in a modified Hoagland solution with visual symptoms recorded and nutrient concentrations in the most recently matured leaves analyzed. *Optimal ranges are from Bryson and Mills (2015).*

	Nutrient concentration (%)						Nutrient concentration (mg kg-1)				
Element	**N**	**P**	**K**	**Ca**	**Mg**	**S**	**B**	**Cu**	**Fe**	**Mn**	**Zn**
Control	5.6	0.4	8.8	2.2	0.7	1.3	37.8	5.7	77.9	79.8	16.1
Deficiency	1.8	0.1	0.5	1	0.1	0.1	7.7	2.32	2.32	23.8	8.9
Optimal range*	2.39-5.51	0.36-0.8	2.86-5.74	1.29-3.21	0.19-0.35	0.41-0.77	19-38	3-7	85-363	35-52	14-38

3.3 Mineral fertilizers

Although the Haber-Bosch process for ammonia production has been credited with increasing food security and abundance for many people, thus allowing for the population boom we have seen in the last century, the annual carbon footprint from this production method is the highest among all industrial chemical-making reactions (Boerner, 2019). Synthetic fertilizer use increased by 200 - 300 % between 1970 and 2010 and even in recent years, demand for N, P, and K fertilizers increased annually between 1.5 - 2.4 % (Walling and Vanneeckhaute, 2020). Between 1908 and 2000, the number of people that could be fed by 1 hectare of arable land rose from 1.9 to 4.3, thanks to the advances in synthetic fertilizers (Barona *et al*., 2017). With only about half of the N fertilizer applied being taken up by the plant, the rest can leach away from agricultural lands, contaminating groundwater, feeding noxious algae blooms through eutrophication, and releasing greenhouse gases into the air (Stokstad, 2016).

3.3.1 Production of mineral fertilizers

According to the International Fertilizers Industry Association, 75 % of N fertilizers worldwide are produced through the Haber-Bosch process, which transforms N from the air and H into ammonia in a very energy-intensive process. The source of H is usually a fossil fuel, most often natural gas (CH_4) but also possibly coal or oil. The synthesis is performed at high pressure of 300 – 1 000 atm, a high temperature of 400 - 500 °C, and in the presence of a catalyst (Fe or other metal) (Barona *et al.,* 2017; Barker and Broadly, 2007). The production of ammonia is not concentrated geographically and is produced mostly in the areas where it will be consumed (Barona *et al*., 2017). Urea ($CO(NH_2)_2$) can be manufactured by combining CO_2 with ammonia under high pressure and together these processes make up 2 % of the world's total energy use (Barker and Pilbeam, 2007; Chen *et al*., 2020).

Phosphorus and K fertilizers are primarily produced through mining, which is concentrated geographically in a handful of countries. Only 9 % of N, 3 % of P, and 8 % of K fertilizers (in terms of global production) are produced in the EU (Fertilisers in the EU, 2019). China accounted for 50 % of global P rock mining in 2018, with Morocco and US accounting for about 10 % each, and K mining is led by Canada with approximately 30 % of global production, followed by Russia, Belarus, and China with about 15 % each (Walling and Vaneeckhaute, 2020). Due to the geographical concentration of production of P and K fertilizer, often there are long supply chains and shipping distances. Phosphorus is considered a limited resource and production in 2021 was estimated at 220 million tons while the global phosphate reserves were estimated to be 71 billion tons (Amar *et al.,* 2022). Nitrogen is not a limited resource due to its abundant presence in the air; however, the fixation of atmospheric N is a process heavily dependent on fossil fuels (Dawson and Hilton, 2011).

Rising fossil fuel prices, especially natural gas, have dramatically increased the cost of producing fertilizer and this has resulted in some fertilizer factories having to close (ECIU, 2022). Natural gas is used as raw material in the production of N fertilizers (as it supplies the H), thus price increases in natural gas will greatly influence N fertilizer production and prices (ECIU, 2022). The price of natural gas accounts for 60 - 80 % of the variable input costs of N fertilizer production (Fertilisers in the EU, 2019).

For example, in mid-March 2022, the price per tonne of ammonium nitrate was £925, which is more than quadruple 2020 prices (ECIU, 2022). This crisis is further fueled by geopolitical tensions between the West and Russia. In 2022, Russia limited natural gas exports and temporarily suspended urea exports. Sanctions against both Russia and Belarus in response to the Russian invasion of Ukraine also add to this crisis. The heavy reliance of the agricultural system on these mineral fertilizers makes the entire food system vulnerable.

3.3.2 Emissions from mineral fertilizers

The International Panel on Climate Change estimates around 56 % of all anthropogenic non-CO_2 greenhouse gas (GHG) emissions are attributed to agricultural production (IPCC, 2014). Carbon dioxide, methane, nitrous oxide, and ammonia are major GHGs emitted by agricultural production; among these, 80 % of anthropogenic nitrous oxide (N_20) emissions are tied to agricultural production (Walling and Vaneeckkhaute, 2020).

About 121 million tons of N are fixed by the Haber-Bosch process each year and 80 % of this goes into fertilizer production (Barona *et al*., 2018). The emissions from ammonia fertilizer production are more than 400 Mt of CO_2, which is 1.6 % of the global total CO_2 emissions (Walling and Vaneeckhaute, 2020; Barona *et al*., 2018). Almost half of the energy use by a conventional grain crop can be accounted for by synthetic N fertilizer production, while about 40% of the fertilizer applied will end up as atmospheric molecular N_2 again (Walling and Vaneeckhaute, 2020; Barona *et al*., 2018). The most common forms of N fertilizer are urea and ammonium nitrate, with ammonium nitrate production emitting between two and five times as many emissions as urea (Walling and Vaneeckhaute, 2020). When ammonia is lost to the air, it can bind with nitrogen oxides (NO_x) and sulfur dioxide to cause air pollution and premature human death when ingested; in fact, N-related air pollution is responsible for an annual cost in human life totaling 23.3 million years (Erisman, 2021).

3.3.3 Nitrate levels and human health

Leafy green vegetables constitute a major source of nitrate consumption in the human diet, with 72 - 94 % of total nitrate intake being caused by vegetables (Umar and Iqbal, 2006). High mineral fertilizer application leads to nitrate build-up in the soils and higher nitrate accumulation in leaves, presenting a potential health hazard (Umar and Iqbal, 2006; Zandvakili *et al*., 2019; Phibunwatthanawong and Riddech, 2019). When there is an accumulation of nitrate, plants can store the excess in vacuoles, which is remobilized when exogenous N levels drop (Umar and Iqbal, 2006). The enzyme *nitrate reductase* is responsible for reducing nitrate to the plant-assimilable ammonium and is sensitive to light intensity, therefore lower nitrate concentrations can be measured in the midday to the afternoon when light intensity is highest (Hawkesford *et al*., 2012). While nitrate accumulation is usually not toxic for plants, it is toxic for animals and humans who consume the plants; for example, methemoglobinemia can be caused in the liver if nitrate is converted into nitrite and binds to hemoglobin, thus rendering it unable to bind to oxygen, resulting in central nervous system depression, shock, or convulsions, or excess nitrate can be converted in the body to nitrosamines which are known carcinogens (Taiz *et al*., 2018; Umar and Iqbal, 2006). Oral, colon, and rectal cancers are also associated with nitrate toxicity, as well as respiratory tract infections in children (Umar and Iqbal, 2006). Additionally, high application of mineral N fertilizers has been shown to decrease levels of ascorbic acid in some plants, including kale (*Brassica oleracea* subsp. *acephala*) (Kano *et al*., 2021; Mozfar, 1993). Fertilizers with lower rates of nitrate as the source of nitrogen lead to less nitrate accumulation in leaves, thus some organic fertilizers can help limit excessive nitrate (Williams and Nelson, 2016).

Nitrate levels in plant material are limited by some countries when the product is aimed for human consumption (Taiz *et al*., 2018). The European Commission set maximum levels of nitrate in food, with a range present to account for harvesting period and growing conditions; fresh spinach has maximum levels of 2 500 – 3 000 mg NO_3 kg^{-1}, fresh lettuce has maximum levels of 2 500 - 4 500 mg NO_3 kg^{-1}, and baby food has a maximum level of 200 mg NO_3 kg^{-1} (Umar and Iqbal, 2006). The Acceptable Daily Intake (ADI) for nitrate according to the European Commission is 3.65 mg kg^{-1} body weight and the Environmental Protection Agency in the US sets the ADI at 1.6 mg kg^{-1} body weight (Umar and Iqbal, 2006).

3.4 Organic fertilizers

Organic fertilizers originate from plant or animal residues or wastes and are generally rich in C but fairly poor in most nutrients (Mengel and Kirkby, 2001). Organic fertilizers have most of the N in an organic form, such as amino acids or nucleic acids, which include carbon in their structure. Plants take up N in the form of ammonium (NH_4^+) and nitrate (NO_3^-), although some plants can also take up small amounts of amino acids (Matsumoto *et al.,* 2000; Wang *et al*., 2007; Hawkesford *et al.,* 2012). Studies have reported an average yield reduction of around 20 % in organic greenhouse production systems compared to conventional, with no difference between organic and conventional cultivation of leguminous crops; in Sweden, hydroponically grown tomatoes in organic conditions produce 50 - 80 % of the yield of conventionally hydroponically grown tomatoes (Bergstrand, 2022). Organic fertilizers also report unreliable nutrient values, as many of the compounds are organically bound and need to undergo mineralization by the soil microbiota, which then release the compounds into a plant-available form; however, this process is sensitive to external conditions and therefore the amount of plant available nutrients is difficult to predict (Bergstrand, 2022).

3.4.1 Production of organic fertilizers

Organic fertilizers originate from sources such as animal manure, plant matter, algae, seaweeds, slaughterhouse byproducts, composts, and anaerobic digestates (Bergstrand, 2022). Organic fertilizers, which can contain humic acids and other carbon molecules, can increase soil biodiversity, microbial activity, and nutrient availability (Walling and Vanneckhaute, 2020). However, many of the nutrients in organic fertilizers are inconsistent in amount and limited in availability due to their presence in organic compounds and require microbial degradation for plant access (Bergstrand, 2022). Nutrient availability can be improved in greenhouse production by inoculating the soil with beneficial soil microorganisms such as *Bacillus* and *Axotobacter*, whose presence can help support mineralization rates and therefore improve the uptake of nutrients like N and P (Bergstrand, 2022).

Organic fertilizers of animal origin can be from manure or slaughterhouse byproducts, whereas the material is then processed using various methods such as anaerobic digestion, drying, or pelleting (Bergstrand, 2022). Fish waste is also an important source of organic fertilizers, as 50 - 60 % of the weight of the fish goes to waste and can be converted to high-value fertilizers (Bergstrand, 2022). The amount of N in organic fertilizers varies significantly, with some animal-based fertilizers having 9 % (fish scraps), 2 - 3 % (poultry manure), or even 12 - 15 % (blood meal, feather meal, hair) of N in dry mass, while kelp and compost have more modest amounts of N (around 0.5 - 1 % N) (Barker and Broadley, 2007). Mineralization of organic N compounds in organic fertilizers has been found to be higher with increasing N concentration (Barker and Broadley, 2007).

3.4.1.1 Vinasse

Vinasse is a dark, liquid residue from sugar beet or sugar cane that originates from the distillation of alcohol and can be used as organic fertilizer due to its high content of certain mineral nutrients (up to 4.2 g N L^{-1}, 3 g P L^{-1}, 17.5 g K L^{-1}, as well as Ca and Mg) (dos Santos *et al.*, 2013; Bergstrand, 2022; Kusumaningtyas *et al.*, 2020). Vinasse is considered a pollutant generated by the ethanol industry (12 - 15 L of vinasse are generated for each liter of alcohol produced) which has high chemical oxygen demand and biological oxygen demand, high salinity and acidity, with a disagreeable odor (dos Santos *et al*., 2013; Kusumaningtyas *et al*., 2020). In some countries, it is common practice to integrate vinasse into agricultural soils to improve fertility; however, the high K content in vinasse can contribute to soil salinity (dos Santos *et al*., 2013). There have recently been issues with plant damage from pesticide residues present in vinasse fertilizers and therefore sourcing of the vinasse needs a closer look (Bergstrand, 2022). Vinasse from sugar cane has major organic components glycerol, lactic acid, ethanol, and acetic acid, while sugar beet-derived vinasse has mainly glycerol and betaine, which is rich in N; this could partially explain why vinasse from sugar cane has a significantly higher N concentration than that of sugar cane (Parnaudeu *et al*., 2008).

Due to the potentially toxic effects of vinasse discussed above, pretreatment is important and may involve anaerobic fermentation, which then can become liquid organic fertilizer (Kusumaningtyas *et al*., 2020). Fermentation often involves the addition of promoting microbes and filter cakes or other industrial wastes to promote the decomposition of organic matter (Kusumaningtyas *et al*., 2020).

One study compared the ion concentrations in pure and treated vinasse for use as organic liquid fertilizer in hydroponics; the treated vinasse was found to contain 37.1 mg Cl L^{-1}, 1458 mg SO_4^{2-} L^{-1}, 7 mg Na L^{-1}, 1760 mg K L^{-1}, 1642 mg Ca L^{-1}, 101.8 mg Mg L^{-1}, 380 mg PO_4^{3-} L^{-1}, 0.66 mg NO_3^- L^{-1}, 47.65 mg NH_4^+ L^{-1}, 27.92 mg Fe L^{-1}, 2.88 mg Mn L^{-1}, 0.75 mg Zn L^{-1}, and undetectable levels of BO_3^{3-}, Cu, and MoO_4^{-2} (Kusumaningtyas *et al*., 2020).

3.4.2 Greenhouse gas emissions from organic fertilizers

From an environmental perspective, organic fertilizers can also produce GHG emissions. Manure emissions come from enteric fermentation and manure storage and management, while emissions from compost are much lower and considered biogenic in that they do not contribute to global warming (Walling and Vaneeckhaute, 2020). The developing world is responsible for 80 % of emissions from manure deposited on pasture (IPCC, 2014). Approximately 70 % of anthropogenic ammonia emissions are due to agriculture and come from the application of both inorganic fertilizer and livestock manure, signaling significant emissions from inorganic as well as organic fertilizer sources; however proper storage and treatment can lower some of the emissions associated with manure (Walling and

Vanneckhaute, 2020). A significant part of lowering the environmental impact and GHG emissions from agriculture is decreasing mineral fertilizer production and instead utilizing compost, which can often come from industrial side streams, such as sugar beet production or fish byproduct, which further limits the environmental impact of fertilizer production (Kusumaningtyas *et al*. 2020).

3.5 Nitrogen cycle

Nitrogen exists in various forms in the environment. The majority of the air in our atmosphere (78 % by volume) is made up of nitrogen (N_2) (Fenice, 2022). The two N atoms present in this molecular form of N share a covalent triple bond that requires a lot of energy (945.3 kJ mol^{-1}) to break, thus this form of N is not readily available to living organisms (Taiz *et al*., 2018; Fenice, 2022). Nitrogen fixation is when the triple bond is broken by either natural or industrial processes and different biologically available molecules of N are formed like nitrate and ammonia (Taiz *et al*., 2018). Natural processes for the creation of bioavailable N compounds include lightning and biological N fixation – which accounts for about 90 % of all naturally fixed N – or photochemical reactions between nitric oxide (NO) and ozone (O_3) (Taiz *et al*., 2018). Industrial fixation utilizes the so-called Haber-Bosch process. Figure 2 shows a visual representation of the nitrogen cycle, including mineralization and nitrification.

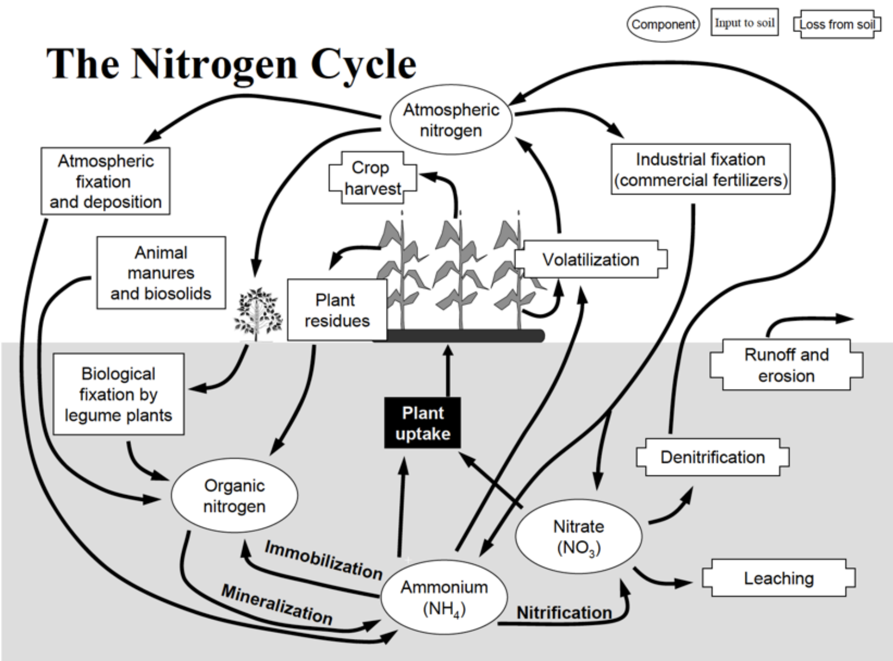


Figure 2: *Nitrogen cycle. Source: Schwab, G.J., 2008. https://www.agry.purdue.edu/CCA/2008/ Proceedings/Schwab.pdf*

It can be seen that the application of commercial mineral fertilizers leads to the direct addition of nitrate and ammonium to the soil, while N from plant or animal residue (including organic fertilizers) is first in the organic N form and must be mineralized to become plant available.

3.5.1 Soil microorganisms and nitrogen

Soil microorganisms play an absolutely critical role in soil composition and plant nutrient availability; arbuscular mycorrhizal associations can help scavenge for phosphate while symbiotic N-fixing bacteria will increase N availability for plants (Oldroyd and Leyser, 2020). Many of these microorganisms co-evolved with their plant companions, forming symbiotic relationships from the time of the earliest land plants (Oldroyd and Leyser, 2020).

3.5.1.1 Nitrogen fixation

Several genera of Bacteria and Archea, called diazotrophs, are able to convert N_2 into ammonia with the enzyme *nitrogenase*, eg. fix N; some are free-living bacteria in the soil while others form symbiotic relationships with plants (Fenice, 2022). The free-living organisms, which include photosynthetic bacteria, have low rates of N fixation and obtain energy from oxidating organic compounds (Fenice, 2022). There is also a category of free-living fixators that form associative relationships, but not nodules, with plants, including some cereal crops, and their fixation output is higher than those of free-living organisms (Fenice, 2022). In symbiotic relationships, usually characterized by nodule formation on the roots, the plant exchanges carbohydrates and other nutrients for the N fixed by the bacteria (Taiz *et al*., 2018). The most well-known association is probably between the legume family *Fabaceae* (alfalfa, beans, clover, peas, soybeans, etc.) and rhizobia bacteria (Taiz *et al*., 2018).

3.5.1.2 Mineralization

Soil microorganisms play a critical role in mineralizing organic N into plant-available forms. The mineralization of organic N compounds into ammonia can also be called ammonification and this process can be performed by practically all living organisms through the decomposition of nitrogenous compounds (Fenice, 2022). Of the various sources of organic N in the soil, amino-N present in amino acids is the most important for mineralization

(Mengel and Kirkby, 2001). Mineralization rates are highly variable and dependent on temperature, pH, water, and the microorganisms present (Taiz *et al*., 2018). Some sources describe the ideal pH for mineralization to be around 7.5 when using a nutrient solution (Hooks *et al.,* 2022; Saijai *et al.,* 2016).

3.5.1.3 Nitrification

Nitrifying bacteria are responsible for oxidizing ammonia and nitrite and are present in various ecosystems such as soils, water, oceans, and wastewater (Madigan *et al*., 2019). Ammonia-oxidizing bacteria (AOB) further convert ammonium into nitrite (NO_2) and nitrite-oxidizing bacteria (NOB) convert nitrite into nitrate (Prosser, 2005). Most nitrifying microbes can only catalyze one of these reactions; for example, ammonia oxidizers like *Nitrosomonas* spp. can oxidize ammonium to nitrite, while nitrite oxidizers like *Nitrobacter* spp. can oxidize nitrite to nitrate (Madigan *et al*., 2019). Only certain species of *Nitrospira* genera can catalyze reactions converting ammonium all the way to nitrate (Madigan *et al*., 2019).

The equations for ammonium oxidization to nitrite (a) and nitrite oxidization to nitrate (b) can be seen below (Wahman and Pressman, 2014):

a) $2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 2\ H_20 + 4\ H^+$

b) $2\ NO_2^- + O_2 \rightarrow 2\ NO_3^-$

Two enzymes, *ammonia monooxygenase* and *hydroxylamine oxidoreductase,* are needed to oxidize ammonium to nitrite, while a single enzyme, nitrite oxidoreductase, is responsible for nitrite oxidation to nitrate (Madigan *et al*., 2019). This process requires O, therefore nitrifying organisms are predominantly aerobic autotrophs; however, in forests that have acidic soil, heterotrophic fungi sometimes act as nitrifiers (Fenice, 2022). The so-called ANAMMOX (anaerobic ammonium oxidation) bacteria can oxidize ammonia under anaerobic conditions and produce gaseous molecular nitrogen N_2, which would be considered denitrification (Fenice, 2022).

The ideal conditions for ammonia oxidation are between pH 7.5 - 8.0 and 25 - 30 °C, and due to the low energy yields from this process, the growth rates for AOB tend to be slow (Wahman and Pressman, 2014).

3.5.1.4 Denitrification

Denitrification is a process under which higher oxidation states of N molecules are reduced to lower oxidation states, upon which molecules including NO, N_20, and N_2 can form; all of these molecules listed are gases and thus bring fixed N back into the atmosphere, often in the form of greenhouse gases or acid rain (Madigan *et al*., 2019). Some genera involved in N reduction and denitrification are *Proteobacteria*, *Pseudomonas*, and some species of *Archea*. It has been observed that under reduced oxygen presence, even nitrifying bacteria are capable of denitrification (Ward, 2008).

3.6 Hydroponics

Hydroponics is a technique for growing plants without soil in which the plants grow in a growth media (such as gravel, coconut husk, rockwool, or foam) with their roots submerged in a nutrient solution (Taiz *et al.,* 2018; Nanik and Muslikan, 2021). This technique emerged in the mid-1800s and by the 1900s it allowed for the experimentation of isolating specific nutrients, which is impractical and quite impossible to do in soil (Barker and Pilbeam, 2007). Within the conversation around more sustainable methods of food production, there has been a movement towards urban agriculture, local production, resource use efficiency, and circular economy (Rufí-Salís *et al*., 2020; Hemathilake and Gunathilake, 2022). Hydroponics allows producers to grow crops in otherwise challenging conditions, such as contaminated soil by heavy metals or desert climates, with basically no limitations on season or climate, allowing for year-round growth (Lee and Lee, 2015). Because of its space-saving potential and high water and nutrient use efficiency, hydroponics has become an increasingly popular method for environmentally conscious food cultivation (Zie *et al.,* 2022). The global hydroponic market size in 2021 was estimated at $2.6 billion and is expected to grow to $9.8 billion by 2028, with substantial growth in the Asia Pacific region (Grands View Research, 2021). Annual sales of organic leafy greens were nearly $600 million in the US in 2016, which presents an opportunity for hydroponic farmers (Hooks *et al*., 2022).

There are various systems for hydroponic growing as seen in Figure 3, but the core principles are the same: the plants are provided with a nutrient solution and aeration (Taiz *et al*., 2018; Lee and Lee, 2015). There are various benefits and drawbacks to each method of hydroponic growing systems. Among the most common commercially used methods are NFT, ebb-and-flow, and aeroponics (Figure 3), while the deep-water culture method is the simplest model and perhaps the easiest to set up (Lee and Lee, 2015). The methods which rely on constant immersion of roots in nutrient solution are more susceptible to fungal infections, and recirculating nutrient solutions can cause issues with pH change, while aeroponics requires fairly sophisticated machinery and monitoring of plants (Lee and Lee, 2015).

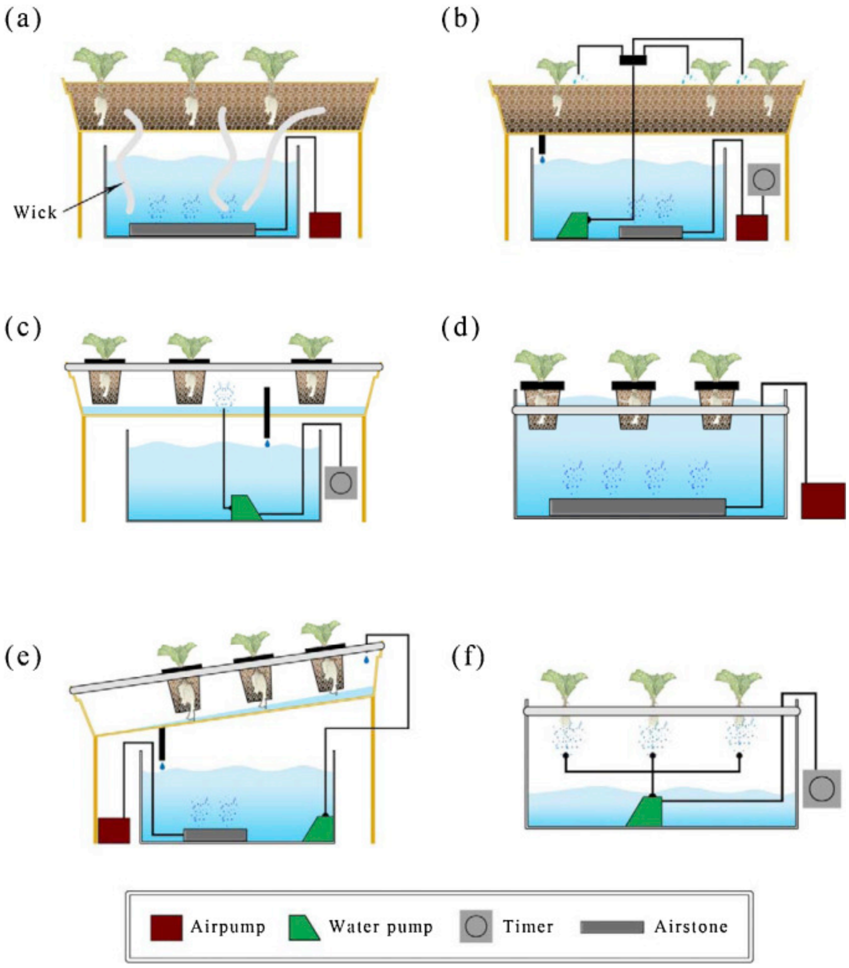


Figure 3: *Different methods of hydroponic growing systems. a) wick system, b) drip system, c) Ebb-Flow system, d) deep water culture system, e) nutrient film technique (NFT), and f) aeroponics. Source: Lee and Lee, 2015.*

Hydroponic growing systems primarily use mineral fertilizers, as the nutrients are already in a water-soluble mineral form that is bioavailable for the plant, thus plants can efficiently absorb the nutrients. Hydroponics is used globally to commercially grow plants for the food supply, especially in controlled-environment agriculture (CEA); leafy green vegetables are especially popular for hydroponics. Lettuce production in hydroponics can, for example, increase yield by 10x while using 90 % less water than soil-based cultivation (Hooks *et al*., 2022). Hydroponics can be an excellent method of increasing food security in urban areas or areas with limited land or water resources (Hooks *et al*., 2022).

An important environmental consideration with hydroponics is the processing of wastewater from growing facilities, possibly through nutrient recovery or membrane filtration, to reduce the effect of nutrient runoff into the water supply (Rufí-Salís *et al*., 2020).

3.6.1 Issues with using organic fertilizers in hydroponics

The release of nutrients from organic fertilizers is slower than directly applying the inorganic, plant-available form. The use of organic fertilizer is attractive to hydroponic growers, as it is a more environmentally friendly solution that promotes resource recycling and lower GHG emissions. However, using organic fertilizers in hydroponics has important limitations, including low bioavailability of nutrients, accumulation of phytotoxic compounds such as ammonium, and a decrease in dissolved oxygen which lead to limiting plant growth (Kano *et al*., 2021; Williams and Nelson, 2016). The bioavailability issue is related to much of the N present in organic fertilizers being bound in organic molecules and needing to undergo mineralization to a plant-available form. One method of overcoming the low availability of nutrients in organic fertilizer is to simply supplement with mineral fertilizer (Kerchasov *et al*., 2021). Other issues with organic fertilizer use in hydroponics involve ease of use – large pH fluctuations, biofilm formation, sedimentation, clogging of tubing, and low reproducibility of conditions (Hooks *et al*., 2022).

Root biofilm is the formation of microorganisms on the roots and can cause issues such as clogged equipment in hydroponics or oxygen depletion (Kano *et al*., 2021). However, biofilm formation on roots can have a positive and protective role in a hydroponics system as well. Pathogens can spread quickly in a hydroponic system through the circulated system in which there are plenty of nutrients (Lee and Lee, 2015). Root biofilm contains beneficial microorganisms which compete with pathogenic microorganisms and form a physical barrier on the root surface (Lee and Lee, 2015; Kano *et al*., 2021). Certain plant growth-promoting rhizobacteria (PGRP) that are commercially available have been shown to provide plants with a certain level of protection against pathogens (Lee and Lee, 2015).

pH management is a major issue with using organic fertilizers in hydroponics, as the addition of organic fertilizer, whether incubated or not, raises the pH to levels between 7 - 9 and requires a significant amount of acid to bring the pH to the optimum of 6.5 (Williams and Nelson, 2016). pH readings of up to 8.5 are common with organic fertilizers and are far from the optimum range of pH 5.5 - 6.2 for nutrient availability which is usually recommended for hydroponic growth (Williams and Nelson, 2016). Adjusting pH with organic grower-approved acids is a challenge and usually involves needing to use larger volumes of weaker acids (Williams and Nelson, 2016). Multiple studies also reported challenges managing pH levels in organic hydroponics (Hooks *et al*., 2022; Williams and Nelson, 2021).

Commercially available organic fertilizers often only list N, P, and K but the rest of the content is not provided (Zandvakili *et al.*, 2019). The content and, more importantly, the plant availability of nutrients in organic fertilizer represents a knowledge gap (Zandvakili *et al.*, 2019). There are some liquid organic fertilizers on the market which state that their products can be used in hydroponics, but the instructions only relate to soil-based applications (BioThrive Grow, General Hydroponics, USA; General Purpose Liquid Fertilizer, AgroThrive, USA). Most studies describing hydroponic plant growth experiments with organic fertilizers report decreased plant growth compared to mineral fertilizers (Williams and Nelson, 2021; Admed *et al*., 2021; Zandvakili *et al*., 2019).

3.6.2 Ammonium toxicity

While ammonium is a major source of N for plants and even acts as an intermediary in certain metabolic reactions, if it is the sole N source then ammonium toxicity symptoms can appear (Britto and Kronzucker, 2001). Ammonium is present in the majority of ecosystems and a range of factors can lead to ammonium dominance over nitrate, specifically low pH, low temperature, and poor oxygen supply, all of which limit the activity of nitrifying bacteria (Britto and Kronzucker, 2001). All plants react to ammonium toxicity but some families are more sensitive than others, for example, domesticated species like potato, tomato, barley, and pea belong among the more sensitive, while rice, onion, and blueberry are better adapted to higher ammonium concentrations (Britto and Kronzucker, 2001).

For ideal plant growth in hydroponics, ammonium needs to be a maximum of 50 % of total mineral N concentration; if ammonium is present in excessive quantities and is the primary source of N, the plants can suffer toxic effects and growth will be limited (Kechasov, 2021; Bergstrand, *et al*., 2020). Most organic fertilizers contain primarily organic N or ammonium, with very little nitrate, which causes issues with using organic fertilizers in hydroponics and can result in ammonium toxicity in plants (Williams and Nelson, 2016). Ammonium transporters are able to transport NH_4^+ down its energy gradient, and even at toxic concentrations in the external environment, there does not seem to be a downregulation in the transport of ammonium (Britto and Kronzucker, 2001). However, the uptake of nitrate requires energy in the form of a symport with two protons and is associated with an increase in the pH of the growing medium (Fang *et al.,* 2021; Mengel and Kirkby, 2001). Nitrate transport is regulated by enzymes that are induced in the presence of NO_3^-, suggesting the plant can modulate the intake and stop it if there is intracellular accumulation (Taiz *et al*., 2018). Excess ammonium can also contribute to the acidification of the root environment (via antiport with a proton), while nitrate-fed plants promote alkalinization (via symport with two protons) (Bergstrand *et al*., 2020; Fang *et al.,* 2021).

Ammonium is toxic to the plant in high concentrations and is therefore assimilated near the site of absorption or stored in the cellular vacuole. Symptoms of ammonium toxicity, including leaf chlorosis and stunted growth, usually appear at solution concentrations above 0.1 - 0.5 mmol L^{-1}; other symptoms can include a lower root:shoot ratio and lower yield, even death of the plant (Britto and Kronzucker, 2002). Other sources describe ammonium toxicity as more than 50 mg N-NH_4^+ kg^{-1} in soil or growing media, especially if it is the major source of N – these toxicity concentrations also apply to nitrite NO_2^- (Barker and Broadly, 2007). The toxicity stems from the ability of ammonium to disrupt transmembrane protein gradients which are necessary for many functions, including photosynthetic and respiratory electron transport, transmembrane nutrient transport, and metabolite sequestration in the vacuole (Taiz *et al*., 2018). Some toxicity can also be attributed to decreased cation uptake of K, Ca, and Mg, and on the other hand, an increase in tissue concentrations of phosphate, chloride, and sulfate (Britto and Kronzucker, 2002). The plant tries to respond to lower K levels by overexpressing K^+ channels, which only contributes to more NH_4^+ influx as the same channels can be used for NH_4^+ uptake (Britto and Kronzucker, 2001). It has been suggested that a method to alleviate NH_4^+ toxicity is to increase the external supply of K^+, or possibly to maintain a neutral or slightly alkaline environment (Britto and Kronzucker, 2001).

An interesting suggestion by Umar and Iqbal (2006) is to purposefully include ammonium in the nutrient solution in soilless cultivation, not only because its presence decreases nitrate accumulation, but also because ammonium lowers the pH of the nutrient solution, which can tend to increase. Thus, the high concentration of ammonium to nitrate is a major issue with using organic fertilizers for hydroponics, as N is mostly present in organic or reduced (ammonium, amide, amine) forms in organic fertilizers (Kechasov *et al*., 2021).

3.6.3 Vinasse and salinity

As mentioned above, there are many sources of organic fertilizer, including corn steep liquor, vermicompost tea, filter cake, vinasse, manure-based liquid digestate, and fish byproducts (Kano *et al*., 2021; Pant *et al*., 2011; Kechasov, 2021; de Mello Prado *et al.,* 2013; dos Santos *et al*., 2012; Shinohara *et al*, 2011). One interesting characteristic of vinasse-based organic fertilizer is its high sodium content. This could be because vinasse is made from sugar beet and sugar beet is a salt-tolerant crop (halophyte) for which sodium is a beneficial nutrient (Skorupa *et al*, 2019; Gorham, 2007). The benefit for sugar beet, a C3 plant, can be largely explained by the ability of Na to substitute K by more effectively maintaining turgor and cell expansion (Mengel and Kirkby, 2001).

Excessive sodium can inhibit the uptake of other nutrients, including K, Ca, and Mg, and can interfere with N and ammonium uptake (Gorham, 2007). When incorporated into the soil, as is common practice in Brazil, it can contribute to soil salinity due to the high levels of K (dos Santos *et al.,* 2013). In hydroponics specifically, excessive sodium can inhibit Ca uptake if the Ca levels are too low (Gorham, 2007). High Na^+ can also decrease K^+ uptake, while Cl^- can decrease NO_3^- uptake; respectively, low K^+ can increase the amount of Na^+ uptake; overall excessive sodium can upset the osmotic regulation of cells (Bergstrand, *et al*., 2020; Mengel, 2007; Gorham, 2007). A study by Madejón *et al*. (2001) found while vinasse application to agricultural soils increased the salinity moderately, there were no signs of salinization of the soil and the plants did not suffer in decreased yield.

3.6.4 Organic certification

There are major opportunities for certified organic hydroponic growing in the USA, where the court recently upheld the decision to include hydroponic farming in the USDA definition of organic farming (Court rules USDA, 2021). According to the USDA, non-GMO microbial inoculants and plant material residue are among the allowed fertilizers. In the EU, the definition of “organic farming” centers around soil growth medium and soil health, therefore hydroponic farming is not included (Regulation EU, 2018; Coleman, 2012). Since organic products are sold for a premium price (typically 10% to several times more) than conventional produce, there is a financial incentive for hydroponic farmers to produce high-yielding, high-quality food products that are organic (Winters, 2006). The sales of organic leafy greens in the US were $600 million in 2016 and the global organic market was valued at $97 billion in 2017 (Hooks *et al.*, 2022).

3.7 Parallel mineralization method

A possible solution to optimize the N availability of organic fertilizer for hydroponic growing would be to pre-process the organic fertilizer by initiating mineralization. Previous studies have separated the process into ammonification/mineralization and nitrification tanks, with nitrate generation efficiency at only 30 % (Strayer *et al.*, 1997). Shinohara *et al.* (2011) developed a parallel mineralization method that uses soil microorganisms to degrade organic fertilizer in a single-step process. The paper describes incubating 1 g L^{-1} fish-based organic fertilizer with 5 g L^{-1} nursery soil, field soil, bark compost, and seawater as sources of inoculum under aerobic conditions, with the final product showing nitrate generation. All inoculums generated some nitrate, as seen in Figure 4, with bark compost generating the highest levels.

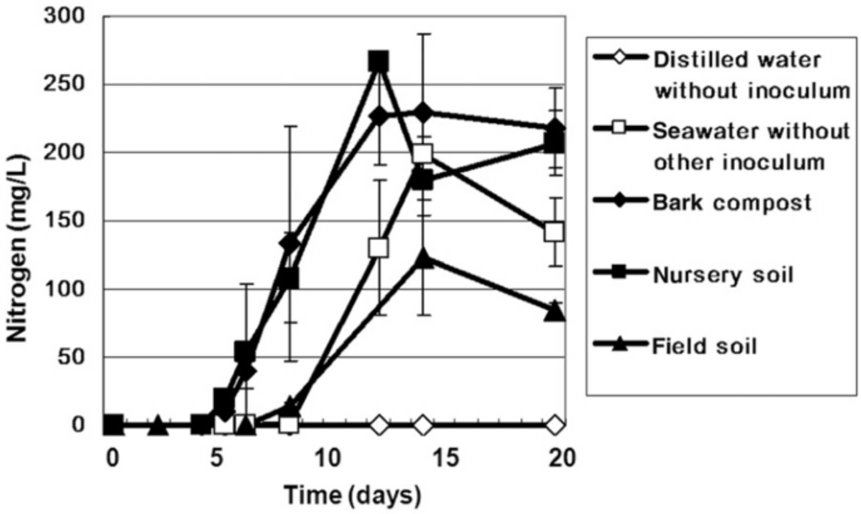


Figure 4: *Different sources of inoculum and the resulting nitrate-N concentrations after 20 days (Shinohara* et al.*, 2011).*

Previous tests by Shinohara *et al.* (2011), running between 12 - 14 days, had experimented with different amounts of inoculum and fertilizer and found that the highest nitrate production coupled with a drop in ammonium concentration was found with 5 g L^{-1} of inoculum (the highest amount tested) and 0.5 g L^{-1} day^{-1} of organic fertilizer, with an efficiency of nitrate generation from organic N to be 97.6 %, suggesting that excessive fertilizer will inhibit nitrification. Test using organic fertilizer with no inoculum produced only ammonium and no nitrate, suggesting that the nitrate increase is related to the addition of the soil inoculum (Shinohara *et al*., 2011).

Shinohara *et al.* (2011) further grew butterhead lettuce and tomato plants with a bark compost-inoculated organic fertilizer solution; butterhead lettuce grown with the organic hydroponics approach showed higher fresh weight (fw) than with conventional hydroponics and the tomato fruit fw was statistically similar.

3.8 Studies using organic fertilizer in hydroponics

While Shinohara *et al.* (2011) found that the yield and quality of lettuce and tomato grown with incubated organic fertilizer (OF) were not significantly different from conventional mineral fertilizer (MF) hydroponics, other studies have failed to produce plants with OF that are comparable to MF (Kano *et al.* 2021, Saijai *et al*., 2016, Hooks *et al*., 2022). One study found that in hydroponic cultivation using fish waste-based organic fertilizer, lettuce produced lower yield and leaf area with organic treatment than with inorganic, but higher phenolics, flavonoids, and antioxidant activity (using DPPH and ABTS) (Ahmet et *al.*, 2021). The same study found higher content of chlorophyll in the organic treatment which was complimented by a higher N leaf content (K, Mg, Mn, S, and Zn were lower and P, Ca, Na, and Fe were higher in organic than inorganic treatment) (Ahmed *et al*., 2021).

Meeboon (2022) compared fish-based fertilizer, corn steep liquor, and rapeseed oil cake, and found that the most successful mineralization was using fish-based fertilizer, which the study suggests could be related to the low C:N ratio of the fertilizer or the microbial community already present in the fertilizer. Shinohara *et al.* (2011) also used a fish-based fertilizer for a successful parallel mineralization method application. Both studies mention the speed of mineralization and nitrification, which perhaps circles back to the fertilizer origin.

A study by Hooks *et al*. (2022) using inoculated OF on lettuce was able to achieve similar shoot fw, dw, and growth index (GI) of the inoculated OF plants to the MF control, while non-inoculated OF plants had 17 % lower dw yield. The same study used a commercially available microbial inoculant which consisted of a blend of plant growth-promoting rhizobacteria (PGPR) and a commercially available organic fertilizer Pre-Empt (Hort Americas, USA) which is based on fermented molasses which the company markets for organic hydroponic use (Hooks *et al*, 2022). Kano *et al*. (2021) conducted an experiment on bok choy with the Shinohara parallel mineralization method using compost soil and corn steep liquor (CSL), a plant-based organic fertilizer. Additional fertilizer was added throughout the experiment, including a mineral fertilizer containing N, P, K, Mn, B, Cu, Zn, and Mo. The treatments were MF, OF, and OF2, which had double the concentration of N as OF. Nitrate and ammonium were measured in the hydroponic solution but nitrate was significantly higher in the MF than OF or OF2. It is unclear if the nitrate and ammonium were measured during plant growth or in the initial incubation. Plant growth was slower in OF and OF2 treatments but the dw of the shoot at harvest was similar in all treatments (in one of two replications).

Kerchasov *et al*. (2021) set up an experiment growing tomatoes in a drip irrigation hydroponic system using a bioreactor to induce mineralization of the liquid digestate produced by manure-based biogas production as can be seen in Figure 5. The results were that tomatoes produced larger fruits in the organic waste-based treatment compared to the high MF treatment, but there were fewer fruits per plant and low biomass of the plant, while the fruit yield of the organic treatment was lower but not significantly different (Kerchasov *et al*., 2021).

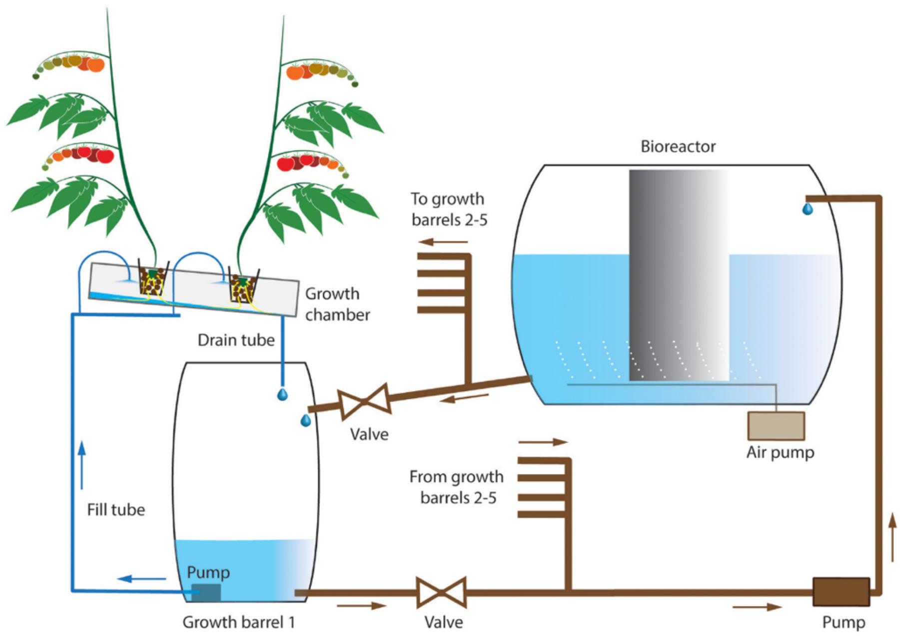


Figure 5: *Experimental setup for Kerchasov* et al. *(2021) for mineralization of organic waste-based fertilizer using an MBBR bioreactor.*

Nanik and Muslikan (2021) performed an experiment with an organic liquid fertilizer from goat manure on red spinach in a hydroponic system testing various growing media; they found that a 50 % concentration of OF with a mix of husk charcoal and cocopeat growing media was the treatment with the best growth, although the lack of a mineral fertilizer control limits the effect of this conclusion. A study by Churilova and Midmore (2019) using vermiliquer as organic fertilizer for the hydroponic growth of bok choy found that with the addition of a nitric acid pH buffer, the vermicompost treatment had 70 - 98 % of the fw of the mineral fertilizer control, but without the added pH buffer the results were much lower. A pH of 7 and 5.5 was tried, with the lower pH producing a much higher yield, which points to the key importance of pH management in organic hydroponics (Churilova and Midmore, 2019).

Suruban *et al.* (2022) focused on inducing the mineralization of organic amendments (including giant taro, mucuna, and erythrina) with added soil and measured NH_4^+-N and NO_3^--N over time, noting a much faster increase in NO_3^--N than the decline of NH_4^+-N. The same study also found that NH_4^+-N increased in the initial stage of mineralization before it started declining, probably due to the higher rate of mineralization of organic matter, surpassing the nitrification rates at this stage; by day 14 of the experiment, NO_3^--N had increased exponentially while NH_4^+-N decreased (Suruban *et al*., 2022).

Saijai *et al.* (2016) performed a similar inoculation of organic fertilizer with various soils and identified genera that were predominant at various stages. Genera *Bacillus* and *Pseudomonas* were dominant during the ammonification process, while genera *Nitrosomonas* and *Nitrobacter* were responsible for ammonium and nitrite oxidation, respectively. As both *Nitrosomonas* and *Nitrobacter* are aerobic and the desired reaction is oxidation, it is clear that sufficient aeration must be provided during the incubation process.

Shinohara *et al.* (2011) found that organic fertilizers with a C:N ratio of less than 11 were the best at N mineralization, while those with higher C:N ratios did not generate much nitrate, possibly due to starving the microorganisms of N. The same could be said about soil-based nitrification, where more N is usually released in soils that have more organic matter and higher microbial biomass (Loynachan, 2012). Mengel and Kirkby (2001) confirm that a lower C:N ratio produces a higher proportion of mineralized N.

In summary, recent studies have found that plants grown with organic fertilizer have lower growth compared to mineral fertilizer. Increasing the concentration of OF, as by 2x in Kano *et al*. (2021), can help offset the limited nutrient availability of organic fertilizers.

Shinohara *et al*. (2011) utilized the parallel mineralization method to increase the nutrient availability in organic fertilizer, leading to improved plant growth compared to non-incubated organic fertilizer. This method has been repeated by other studies with varied results, signaling challenges in reproducibility due to incubation conditions and inocula sources.

4 Methodology

In the experimental part, various sources of inoculum were collected and incubated along with organic fertilizer to initiate mineralization and nitrification. The inoculum that led to the highest nitrification rate was chosen for a further plant growth experiment in hydroponics. The incubated (mineralized) organic fertilizer was compared to a mineral fertilizer control and a non-incubated organic fertilizer treatment in a bok choy hydroponics growth experiment. The experiments were conducted in Copenhagen, Denmark between March and July 2022 using materials sourced from Denmark and all analyses were done at the University of Copenhagen.

4.1 Materials

4.1.1 Inoculum collection

Compost soil (C) was collected from Borgervænget Recycling Center in Østerbro, Copenhagen, Denmark. Compost soil was taken from various parts of the compost soil pile to create a homogenous sample. The compost soil arrived the day prior from the composting facility and was a mix of compost, topsoil, and sand/gravel. The compost was made from plant material sourced from gardens, parks, and green spaces in and around Copenhagen and is usually composted for 8 - 18 months. Seawater for the incubation study was collected from Nordhavenstippen park near Nordhavn, Copenhagen. Organically farmed sandy-clay/clay agricultural soil AG1 (Danish soil type JB 6 or 7) was collected from topsoil on the University of Copenhagen Taastrup campus. This soil had been farmed organically with a crop rotation that included red clover. Organically farmed clay-sandy agricultural soil AG2 (Danish soil type JB 4) was collected from topsoil on the University of Copenhagen Taastrup campus. This soil had been farmed organically. Organic clay-sandy agricultural soil AG3 (Danish soil type JB 4 or 5) was collected from the top 15 cm of soil on Mangholm organic farm in Hillerød, north of Copenhagen. Mangholm farm was in the process of converting to biodynamic farming.

4.1.2 Organic fertilizer

BioBizz Bio Grow organic fertilizer (BioBizz Worldwide, Basque Country, Spain) (OF) is a vinasse-based organic liquid fertilizer from Dutch sugar beet extract. It is certified for use in organic farming by Control Union and the Organic Materials Review Institute and is recommended for use in both indoor and outdoor settings in all kinds of substrates and mediums. This NPK 4-3-6 fertilizer contains, according to the manufacturer, 40 000 mg L^{-1} total nitrogen, 4 640 mg L^{-1} ammonium, and 2 000 mg L^{-1} nitrate. Organic carbon percentage was listed as 27.7 % with a C:N ratio of 6.65. The actual concentrations of nutrients were measured and are listed further in Table 3.

4.1.3 Mineral fertilizer

Mineral fertilizer (MF) was prepared using a modified Hoagland solution which consisted of five solutions:

Solution A = 0.2M KH_2PO_4, 0.2M K_2SO_4.

Solution B = 0.3M $MgSO_4$, 0.1M NaCl.

Solution N = 0.3M $Mg(NO_3)_2$, 0.9M $Ca(NO_3)_2$, 0.6M KNO_3.

Solution N0 = 1M $CaCl_2$, 0.2M $MgCl_2$.

Solution M = 0.054M Na-EDTA, 0.001M $MnCl_2$, 0.0008M Na_2MoO_4, 0.0007M $ZnCl_2$, 0.0008M $CuSO_4$, 0.002M H_3BO_3, 0.001M $NiSO_4$, 0.05M $Fe(NO_3)_3$.

4.1.4 Inoculum preparation

Various soils (AG1, AG2, AG3, C) were used as inoculum in this experiment and are pictured in Figure 6. In order to use the same amount of dry matter among the various inoculants, the moisture content of the soils had to be calculated. The moist soils (AG1, AG3, C) were first roughly homogenized in plastic bags and then sieved using an 8 mm sieve. Due to the high content of clay in AG1, it was challenging to sieve and break up the clay into smaller pieces. Therefore, the entire bag of soil was set out to dry for approximately 2 hours so that the pieces could be broken into smaller chunks. Following sieving, the soils were homogenized by hand in the trays and 300 g of subsample was collected into an aluminum tray to be dried in an oven for 40 hours at 50 °C until a constant weight was achieved. The dry samples were weighed and soil moisture content was calculated for AG1, AG3, and C to be 10.4 %, 20.1 %, and 27.7 %, respectively. AG2 had been collected, air-dried, and sieved before the start of this experiment. The 5 g L^{-1} to be used as inoculum was therefore calculated from dry weight. All soils except for AG2 were stored in a cooling room (4 - 5 °C) in sealed bags. AG2 was stored at room temperature in a 60 L plastic bin. Seawater was not prepared in any way and was used directly in the incubation experiment in place of deionized water.

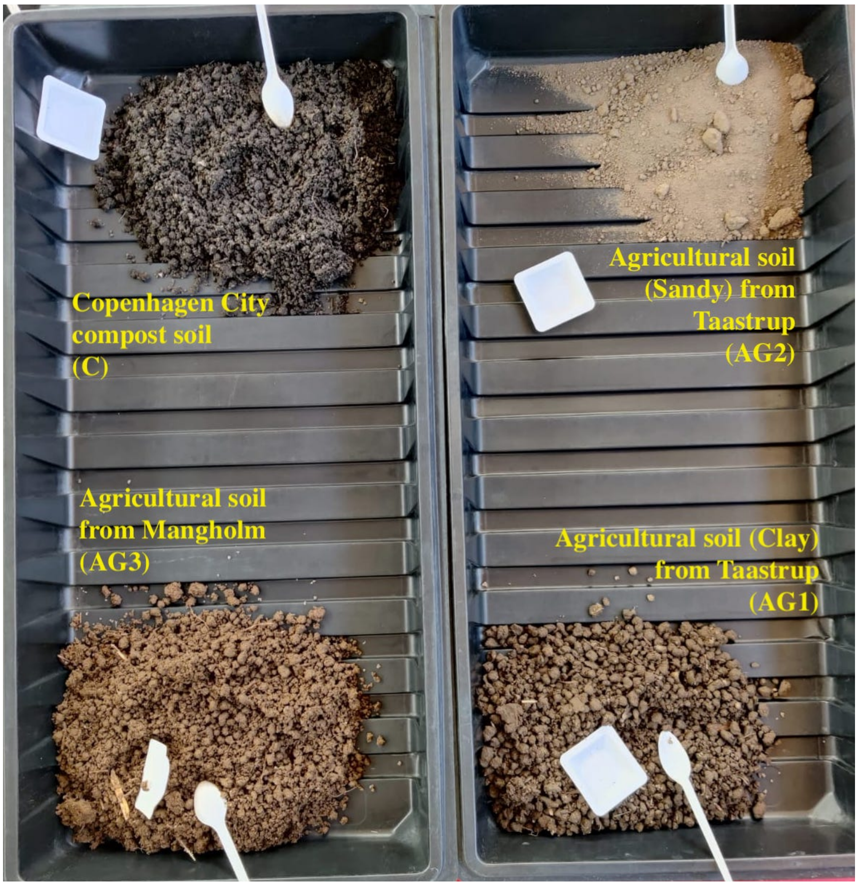


Figure 6: *Soils used as inoculum.*

4.2 Incubation experiment

Organic fertilizer was incubated for 21 days with 5 different sources of inoculum: seawater (S), agricultural soils 1, 2, and 3 (AG1, AG2, and AG3), and compost soil (C) (*n=3*). 250 ml glass flasks covered with punctured parafilm and aerated with needles were used. Treatments had 1 g L^{-1} OF and 5 g L^{-1} dw of inoculum. Controls for each inoculum included deionized water and 5 g L^{-1} dw of inoculum (no OF). Seawater was used as an inoculum with 1 g L^{-1} OF (diluted directly into seawater) and the control was 250 ml seawater. Two additional controls of organic fertilizer diluted in water (1 g L^{-1}) and water were used. The incubation study took place in a dark room and samples were exposed to light only during weekly sampling. The average temperature was 18.5 °C and the average humidity was 34.6 %. This was measured on a data logger of temperature and humidity (Testo 184 H1). The setup of the incubation experiment can be seen in Figure 7. Incubation study samples (10 ml) were collected from each flask on days 1, 7, 14, and 21. Samples were stored in Falcon Tubes in the freezer (-20 °C) until the end of the 21 days. Following the conclusion of the incubation period, pH was measured for all samples and then the samples were filtered using Whatman 5 filter papers to prepare for FIA analysis.

a) b)

Figure 7: *Set up of incubation experiment. Aeration setup and bottle placement are visible. The temperature and humidity meter is seen on the left side of Figure 7b.*

4.3 Plant growth experiment

The bok choy plants were grown in a hydroponic deep water culture system. After a 10-day adjustment period in low-dose mineral fertilizer, the treatments were initiated. There were 3 treatments: mineral fertilizer (MF), organic fertilizer (OF), and the organic fertilizer that had been incubated with compost soil (InocOF). The nutrient solution was changed weekly.

4.3.1 Organic fertilizer incubation

A 60 L plastic box was filled with 319 g of compost soil and 267 ml of BioBizz fertilizer (Figure 8). The compost soil concentration was 5 g L^{-1} dw to maintain the same concentration as the incubation study. The OF concentration during incubation was 5.34 ml L^{-1}, corresponding to 6.4 g L^{-1}. Double-distilled water was added (50 L) and the contents were stirred. Aeration using 8 needles was set up, with weights on the aeration tubes to distribute the aeration to the bottom of the bin. Figure 8 shows the setup of the bin with aeration tubes. The bin was left to incubate in a dark, enclosed room. The average temperature was 20.0 °C and the average humidity was 40.8 %. This was measured on a data logger of temperature and humidity (Testo 184 H1). The incubation was set up on April 25th and continued until the end of the plant growth experiment. At the time of the experiment setup, the incubated fertilizer was 32 days old. A part of the contents was taken and diluted with double-distilled water to be used for the InocOF treatment each week, with the rest left to incubate further. The concentration of OF in the incubation tank was 5.34 ml L^{-1} and the treatment concentration was 1.48 ml L^{-1}. This was done to achieve the desired N concentration (0.081 g N L^{-1}) at 2x the N concentration of MF treatment (0.041 g N L^{-1}).

Figure 8: *Set up of incubation of fertilizer for the hydroponic growing experiment. The bin contains 50 L of double-distilled water mixed with soil-inoculated organic fertilizer mixed.*

4.3.2 Plant material

Bok choy has been mentioned in the literature under two names interchangeably: *Brassica rapa* subsp. *chinensis* and *Brassica rapa* subsp. *pekinensis.* These two subspecies of *B. rapa* are closely related but *chinensis* more commonly refers to bok choy and *pekinensis* to Chinese cabbage. Throughout this report, both names are used, depending on the source, with a preference for *B. rapa* subsp. *chinensis*.

Bok choy seeds (Weibulls) (*Brassica rapa* subsp. *chinensis*) were sown in vermiculite in the greenhouse and then were transferred to the hydroponic system with 0.45 ml L^{-1} of each nutrient solution A, B, N, and Micro. pH was adjusted every other day with HCl or KOH. After 10 days in the adjustment period for mineral fertilizer, the plants were moved to the treatment buckets. Four bok choy seedlings were placed in each hydroponic bucket, with three buckets per treatment, making twelve plants per treatment.

4.3.3 Treatments

MF = 1 ml L^{-1} of each A, B, N, Micro mineral fertilizer solution per bucket (0.041 g N L^{-1})

OF = 1.48 ml L^{-1} of BioBizz organic fertilizer per bucket (0.081 g N L^{-1}) (2x N of MF)

InocOF = 1.48 ml L^{-1} of incubated BioBizz organic fertilizer per bucket (0.081 g N L^{-1}) (2x N of MF) and (theoretical) 1.39 g L^{-1} of compost soil

For MF treatment in the hydroponic plant growth study, 1 ml L^{-1} of each nutrient solution A, B, N, and Micro was used. pH adjustments were made with 1M HCl and 1M KOH. MF was adjusted to 5.5 pH and OF and InocOF treatments were adjusted to 6.5 (Oakton EcoTestr pH 2). Adjustments were made every other day. KOH was chosen to limit Na^+ toxicity, which had been reported in previous hydroponic experiments using organic fertilizers. Nutrient solutions were replaced every week. Each bucket contained 4.5 - 4.8 L of total contents. Buckets were topped up with double-distilled water throughout the week to compensate for evapotranspiration.

4.3.4 Harvest

Plants were harvested on day 27 after the initiation of treatment. A representative bucket was chosen for each treatment and photographed from the top and side. Fresh weight (fw) for shoots, flowers, and roots was measured for each bucket. The roots and the rest of the shoots were placed into paper bags and dried in a Heraeus oven at 60 °C for 72 hours. Dry weight (dw) was then measured.

4.4 Elemental analysis

4.4.1 Liquid analysis

Ammonium and nitrate concentrations by flow injection analysis

Ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N) concentrations of BioBizz fertilizer and all incubation samples were measured by flow injection analysis (FIA, Fiastar 5000 Analyzer, FOSS, Hillerød, Denmark). The fertilizer sample was prepared with 2.05 g of fertilizer and diluted with 1023 g of milli-Q water. The mixture was magnetically mixed at medium speed (Bee mixer) for 4:30 minutes and filtered with Whatman 5 filter papers into three 10 ml falcon tubes. Incubation study samples (10 ml) were filtered using Whatman 5 filter papers to prepare for FIA analysis.

Nitrogen and carbon content

Total N and C content were analyzed by Dumas combustion using a MacroCube CNS Elemental Analyzer (Elementar, Germany). Fertilizer was analyzed undiluted, diluted with milli-Q water 1 : 1, and 1.5 : 1. Liquid samples were weighed (10 µg) into tin capsules containing chromosorb. The N and C content was calculated using sulfanilamid as a calibration standard.

Multi-elemental analysis with ICP-OES

Multi-element analysis of the organic fertilizer was conducted with inductively coupled plasma–optical emission spectroscopy (ICP-OES, Agilent 5100 ICP-OES, Agilent Technologies, Santa Clara, USA). The fertilizer was diluted 1 : 10 with milli-Q water and filtered with filter papers. Then the sample was diluted 1 : 1 with 7 % ultrapure HNO_3 to form 2 times 10ml samples. Two blanks were included (milli-Q water filtered with Whatman filter paper and diluted 1 : 1 with 7 % ultrapure HNO_3).

4.4.2 Plant material analysis

Multi-elemental analysis with ICP-OES

Multi-element analysis was conducted with inductively coupled plasma–optical emission spectroscopy (ICP-OES, Agilent 5100 ICP-OES, Agilent Technologies, Santa Clara, USA). The youngest fully developed leaves were harvested separately into 250 ml plastic containers for ICP-OES analysis. Containers were placed on ice immediately after harvesting and placed into a freezer at -20 °C for 18 hours. For 1 hour before freeze-drying, samples were placed into a freezer at -80 °C. A freeze-dryer (Scanvan CoolSafe; LaboGene, Denmark) was used to dry samples for 49 hours. Freeze-dried plant material was ground, and homogenized weighed to 100 mg, and digested in 2.5 ml of 70 % ultrapure HNO_3 and 1 ml of 15 % H_2O_2. The samples were then diluted to 3.5 % HNO_3 with milli-Q water. A microwave (Ultrawave single reaction chamber digestion system, Milestone, Sorisole, Italy) was used to digest the samples. NIST1515 (apple leaves, National Insitute of Standards and Technology, MD, UDA) were used as standard reference material for data quality assurance.

Nitrogen and carbon content

Total N and C were analyzed by Dumas combustion using a MacroCube CNS Elementar Analyzer (Elementar, Germany). Freeze-dried, ground, and homogenized plant material was weighed (30 mg) into tin capsules. The N and C content was calculated using sulfanilamind as a calibration standard.

4.5 Statistical approach

Data was analyzed in RStudio version 2022.02.3 and in Microsoft Excel version 16.61.1. All statistical models were tested for Gaussian distribution with QQ Plots. The variance between groups was tested using ANOVA and further by Tukey test (α=0.05). Standard deviation was calculated in Excel using ST.DEV function.

5 Results

The following section presents the results from the incubation experiment, including nitrate, ammonium, and pH measurements, and the plant growth experiment, with photos of plants and leaves from each treatment, graphical representations of harvest biomass data, and tables presenting leaf concentrations and plant uptake of nutrients. Included in these results is also an elemental analysis of the organic fertilizer used in this thesis.

5.1 Incubation experiment

The incubation study was set up to test various sources of inoculum and their effect on the organic fertilizer regarding ammonium and nitrate concentrations during a 3-week period. Samples were taken each week, including the initial day (week 0), and analyzed for ammonium (Figure 9) and nitrate (Figure 10) concentrations.

5.1.1 Ammonium concentration

As seen in Figure 9, ammonium is present in all samples containing organic fertilizer, which points to the fertilizer being the primary source of N. There is little to no ammonium in the control treatments, therefore almost no ammonium is being released from the soil particles or present in the seawater. In treatments OF, OF + AG1, OF+AG3, and OF+S, there is a fairly stable concentration of ammonium, with no significant differences within each treatment between the weeks. While it looks for treatments OF, OF+AG1, and OF+AG3 to be trending upwards, it is due to a large standard deviation. OF+S shows stable ammonium concentrations throughout the 3 weeks with a fairly small standard deviation. Treatment OF+AG2 shows an increase in the ammonium concentration (Figure 9), which could be the mineralization of organic N compounds in the fertilizer. In OF+C treatment, the ammonium concentration stays stable until the final week, when there is a sharp decrease in the amount of ammonium.

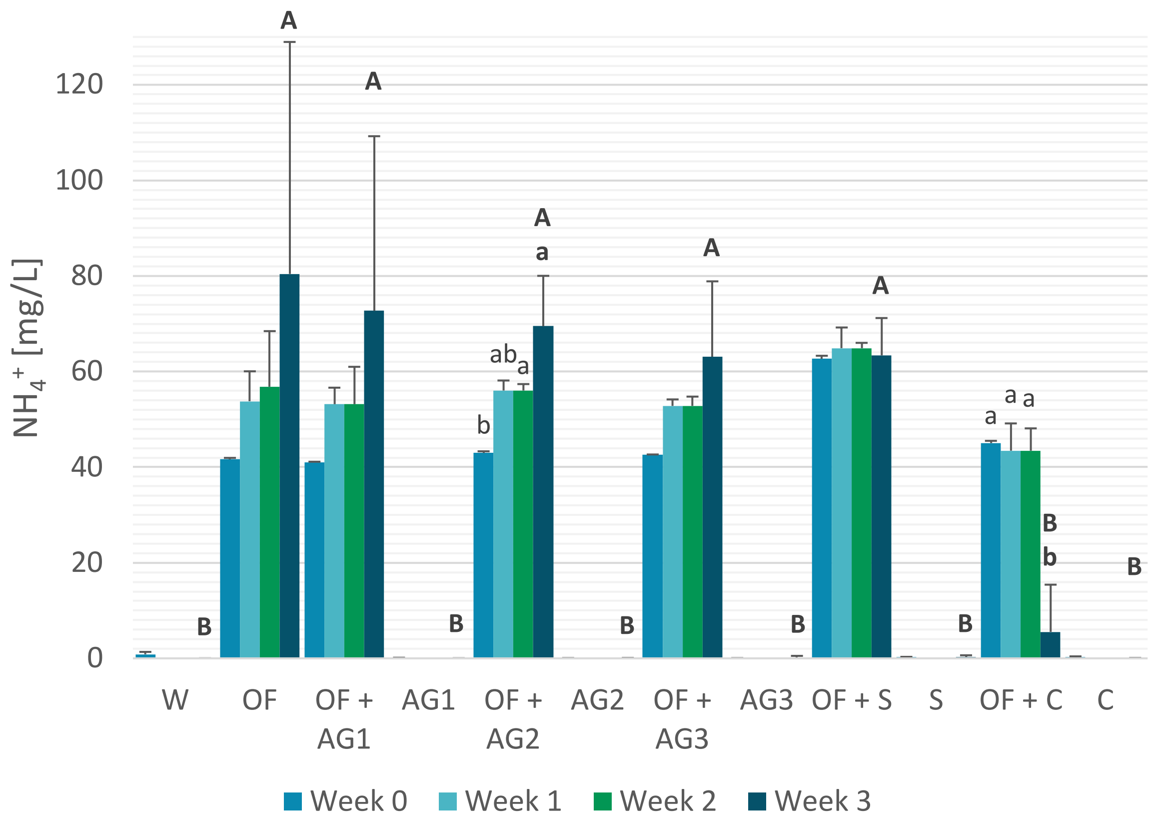


Figure 9. *Ammonium concentrations from incubation study. Treatments: water (W), organic fertilizer (OF), organic fertilizer and agricultural soil 1 (OF + AG1), agricultural soil 1 (AG1), organic fertilizer and agricultural soil 2 (OF + AG2), agricultural soil 2 (AG2), organic fertilizer and agricultural soil 3 (OF + AG3), agricultural soil 3 (AG3), organic fertilizer in seawater (OF + S), seawater (S), organic fertilizer and compost soil (OF + C), compost soil (C). Error bars indicate standard deviation in the positive direction (n=3). Lowercase letters indicate significant differences within a treatment (no letters mean no difference within a treatment). Uppercase letters indicate significant differences between treatments in week 3 measurements.*

5.1.2 Nitrate concentration

The nitrate concentrations throughout the incubation study are presented in Figure 10. In treatments OF+AG1, OF+AG2, and OF+AG3 we see a significant increase in nitrate concentration in week 3. In OF+C we see a significant increase in week 2 and week 3. While the treatments OF+AG1, OF+AG2, and OF+AG3 all showed an increase in ammonium in week 3, their ammonium concentrations (Figure 9) remain high which suggests the start of the nitrification process. While the nitrate concentration in OF+S is increasing in weeks 2 and 3, the overall concentration is significantly lower than all other treatments with fertilizer and is comparable to control treatments with no fertilizer. The coupling of lower ammonium and higher nitrate concentrations in OF+C was an encouraging result, as this suggests advanced nitrification and a major goal of incubation was to avoid ammonium toxicity in plants usually associated with hydroponic organic fertilizer use. Therefore, compost soil was chosen as the inoculum for the plant growth experiment.

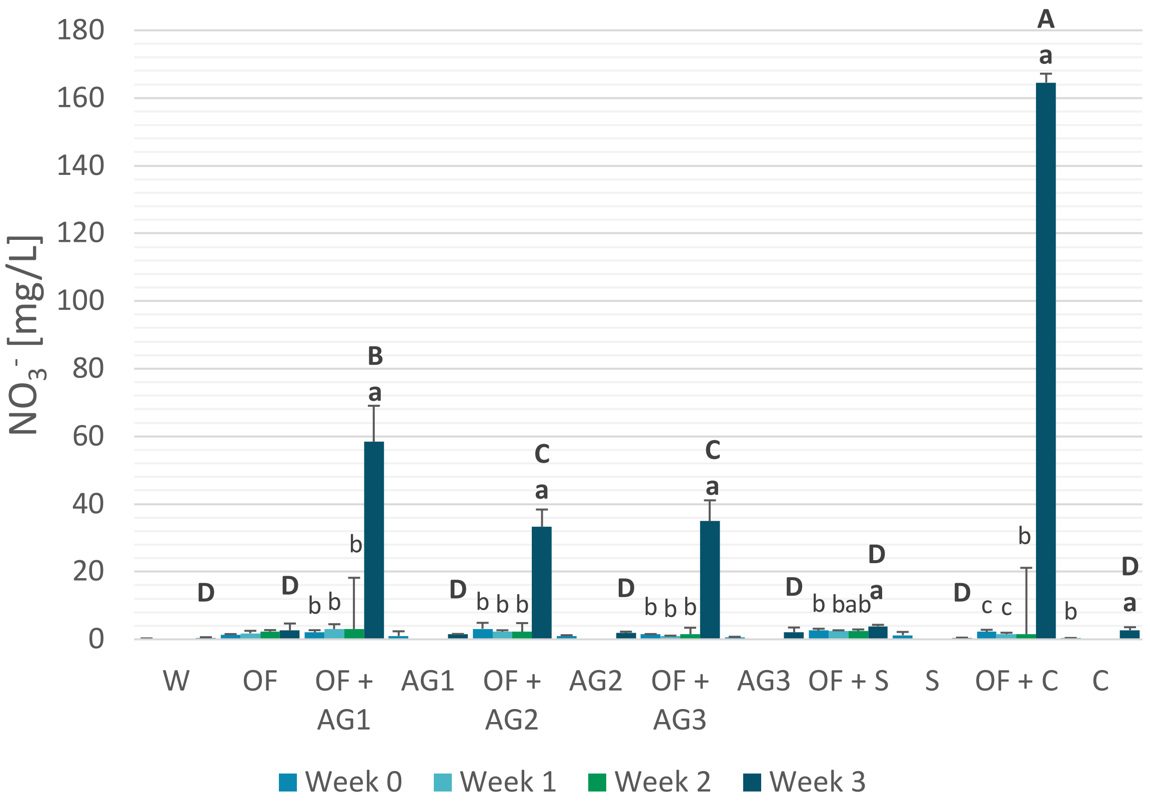


Figure 10. *Nitrate concentrations from incubation study. Treatments: water (W), organic fertilizer (OF), organic fertilizer and agricultural soil 1 (OF + AG1), agricultural soil 1 (AG1), organic fertilizer and agricultural soil 2 (OF + AG2), agricultural soil 2 (AG2), organic fertilizer and agricultural soil 3 (OF + AG3), agricultural soil 3 (AG3), organic fertilizer in seawater (OF + S), seawater (S), organic fertilizer and compost soil (OF + C), compost soil (C). Error bars indicate standard deviation in the positive direction (n=3). Lowercase letters indicate significant differences within a treatment (no letters mean no difference within a treatment). Uppercase letters indicate significant differences between treatments in Week 3 measurements.*

5.1.3 Changes in pH during incubation

The pH of samples was measured at all sample dates and is presented in Figure 11. All treatments that showed an increase in nitrate concentration in Figure 10 followed a similar decreasing trend with their pH values in Figure 11. Figure 11a presents control treatments without fertilizer added and we can see either a flat trend or an upwards trend. Figure 11b presents treatments containing organic fertilizer. The treatment with no inoculum (OF) follows an upwards trend in the first week and then stays stable (Figure 11b). All remaining lines in Figure 11b are treatments with inoculum and OF, and we see a trend towards a pH increase in weeks 1 and 2 and a subsequent decrease in week 3. Seawater treatments S and OF+S have high pH throughout the study. In treatments OF+AG1, OF+AG2, OF+AG3, and OF+C, the pH started low in week 0, rose to a maximum value in weeks 1 and 2, and decreased again in week 3, when the nitrate concentration increased. OF+C had the highest pH values in the middle weeks (except for treatments with seawater), reaching close to a pH of 8 (Figure 11b).

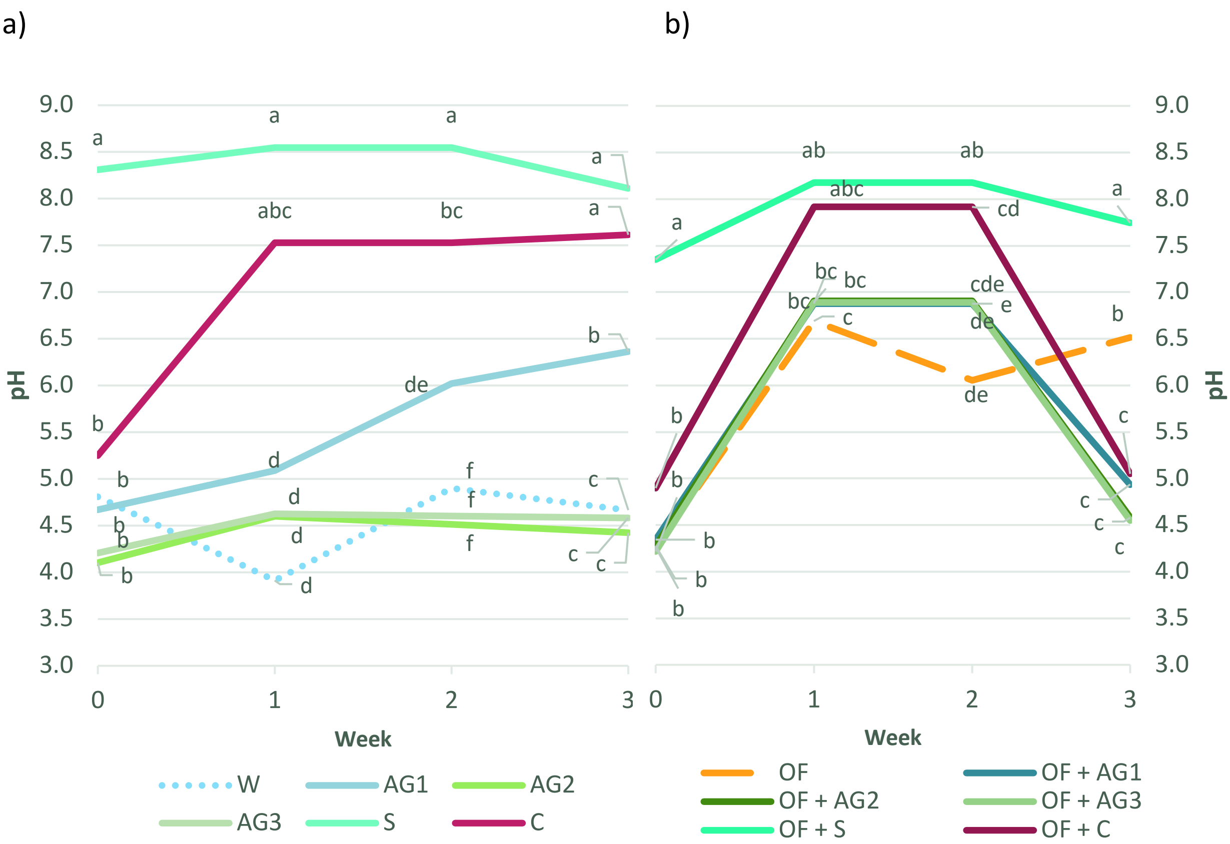


Figure 11. *pH measurements of incubation study samples. Treatments in 11a: water (W), agricultural soil 1 (AG1), agricultural soil 2 (AG2), agricultural soil 3 (AG3), seawater (S), and compost soil (C). Treatments in 11b: organic fertilizer (OF), organic fertilizer and agricultural soil 1 (OF + AG1), organic fertilizer and agricultural soil 2 (OF + AG2), organic fertilizer and agricultural soil 3 (OF + AG3), agricultural soil 3 (AG3), organic fertilizer in seawater (OF + S), and organic fertilizer and compost soil (OF + C). Letters indicate significant differences between treatments in a given week (n=3).*

5.1.4 Concentrations in BioBizz organic fertilizer

The organic fertilizer was analyzed for nutrient concentrations (Table 3). The samples were not digested beforehand, therefore do not reflect the total content of nutrients, as some nutrients are bound in organic compounds. The concentrations listed on the fertilizer label were not confirmed with analysis. The total N was 5.5 %, higher than the 4 % listed. While the label claims 2 000 mg NO_3^- L^{-1} and 4 640 mg NH_4^+ L^{-1}, the analysis found these concentrations to be 419 mg NO_3^- L^{-1} and 32 513 mg NH_4^+ L^{-1} (Table 3).

Besides K and P, no other nutrient values were listed on the label. The K concentration was measured at 35 167 mg L^{-1}, while the label listed the K concentration as 60 000 mg L^{-1}. The P concentration was even more varied, with the label concentration at 30 000 mg L^{-1} and the measured only 708 mg L^{-1}. Overall, this analysis showed that organic fertilizer label concentrations cannot be fully trusted in most cases. For the plant growth experiment calculations, the measured concentrations of the organic fertilizer were used.

Table 3: *Concentrations in BioBizz Organic Fertilizer. Label concentrations as listed on the bottle. Total N and C were measured by Dumas combustion. C:N ratio was calculated from these results. Nitrate and ammonium were measured with FIA. The rest of the nutrients were measured on ICP-OES.*

	Label concentrations	Measured concentrations
Total N	4%	5.5% ± 0.3
Nitrate	2 000 mg L^{-1}	419 ± 23 mg L^{-1}
Ammonium	4 640 mg L^{-1}	32 513 ± 5 869 mg L^{-1}
Total C	28%	18.2% ± 0.9
C/N ratio	6.65	3.3
Al		0.75 ± 0.04 mg L^{-1}
B		14 ± 0.04 mg L^{-1}
Ca		3 784 ± 7 mg L^{-1}
Cu		0.31 ± 0.02 mg L^{-1}
Fe		67 ± 0.4 mg L^{-1}
K	60 000 mg L^{-1}	35 167 ± 166 mg L^{-1}
Mg		1 067 ± 2 mg L^{-1}
Mn		25 ± 0.05 mg L^{-1}
Ni		3.2 ± 0.04 mg L^{-1}
P	30 000 mg L^{-1}	708 ± 5 mg L^{-1}
S		2 674 ± 10 mg L^{-1}
Zn		6.8 ± 0.04 mg L^{-1}

5.2 Plant growth experiment

Bok choy plants grown in organic fertilizer (OF), mineral fertilizer (MF), and incubated organic fertilizer (InocOF) treatments were harvested, photographed, weighed, and analyzed for nutrient contents.

5.2.1 Photos of plants at harvest

Photos of a representative plant from each treatment were taken at harvest and are presented in Figure 12. All three treatments can be seen side by side in Figure 12g. The OF plant (Figures 12c and d) shows significant yellowing of leaves. Both MF (Figures 12a and b) and InocOF plants (Figures 12e and f) show no significant nutrient deficiency symptoms.

a) b)

c)

d)

e)

f)

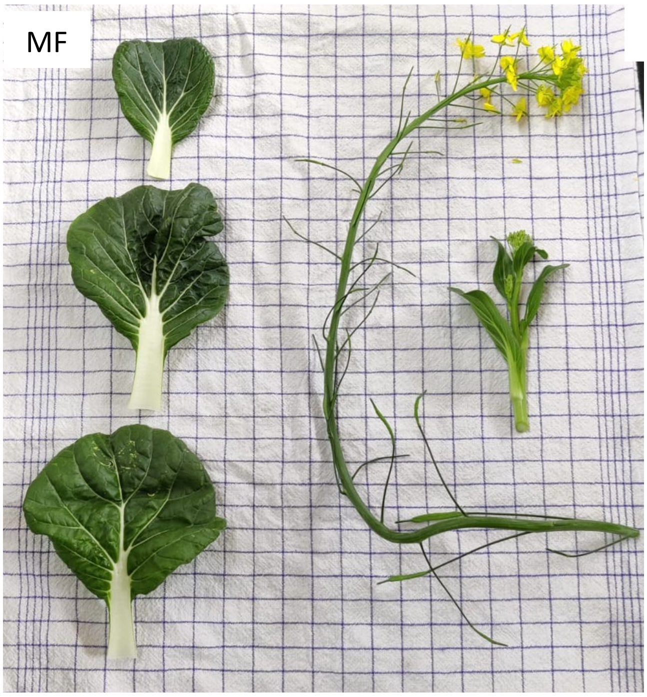
g)

Figure 12: *Side and top view of plants at harvest. Figures 12a-g show the side and top views of a representative plant from each treatment at harvest. Figures 12a and b show the side and top view of the MF treatment plant. Figures 12c and d show the side and top view of the OF treatment plant. Figures 12e and f show the side and top view of the InocOF treatment plant. Figure 12g shows all three plants side by side.*

5.2.2 Photos of leaves at harvest

In Figure 13, detailed photos of leaves and flowers at harvest of each treatment are presented. All treatments had flowers develop. In Figure 11a, dark green leaves and some slight wrinkling can be seen in MF. In OF (Figure 11b), the oldest leaf is entirely chlorotic with necrotic spots and some necrosis along the leaf edges. The middle leaf displays chlorosis, with increasing intensity towards the outer edge of the leaf, and the youngest leaf looks very dark green. The flower looks less developed and smaller. In InocOF, the oldest leaf looks smoother with slight chlorosis on the leaf edge while the middle and young leaves look slightly wrinkled and dark green. The flower looks more developed than the OF treatment.

a)



b)

c)

Figure 13. *Leaves, flowers, and buds at harvest. Figures 13a-c show an old leaf, a young leaf, a middle-aged leaf, a flower, and a flower bud from the representative plant of each treatment – MF (a), OF (b), and InocOF (c).*

5.2.3 Harvest data analysis

At harvest, the plant material was separated and weighed while it was fresh. Figure 14 presents the plant biomass data, including shoot, root, flower, and total plant fresh weight (fw). In the shoot (Figure 14a) and total plant (Figure 14c) fw, there were significant differences between all treatments. In the root fw (Figure 14b) and flower fw (Figure 14d), there were no significant differences between treatments.

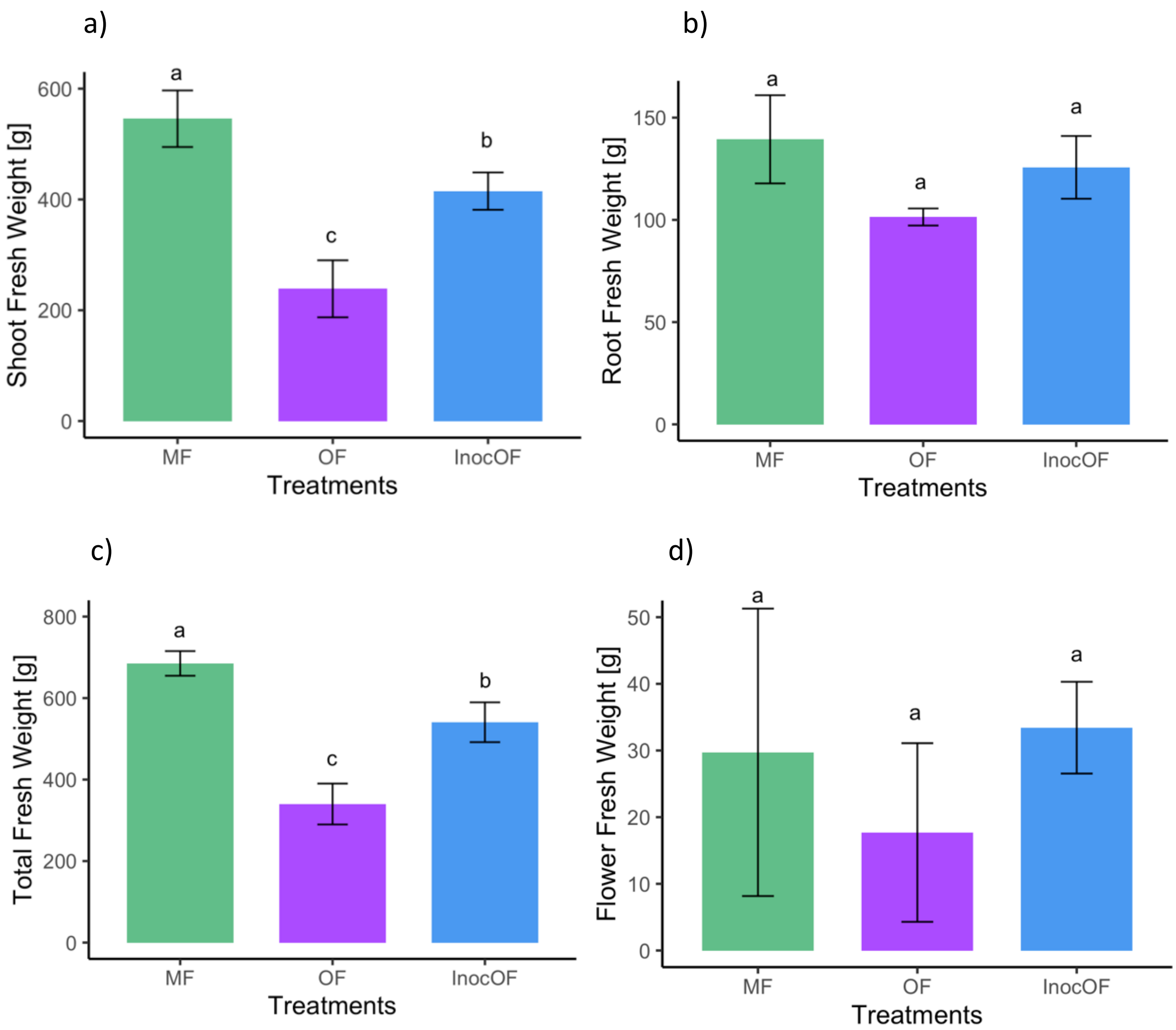


Figure 14. *Fresh weight measurements. Shoot fw (a), root fw (b), total fw (shoot + root) (c), and flower fw (d) for each treatment. n=3. Standard deviation bars are shown. Letters indicate differences between treatments.*

After harvesting and weighing of fresh plant material, the plant material was dried. Figure 15 presents the biomass data for the dry weight (dw) of plant material. For shoot (Figure 15a) and total plant (Figure 15c) dw, there are significant differences between the MF and OF treatments, but no differences between InocOF and the other two treatments. For root (Figure 15b) and flower (Figure 15d) dw, there are no differences between treatments. The root:shoot ratio (RSR) is presented in Figure 15e with no significant difference between treatments.

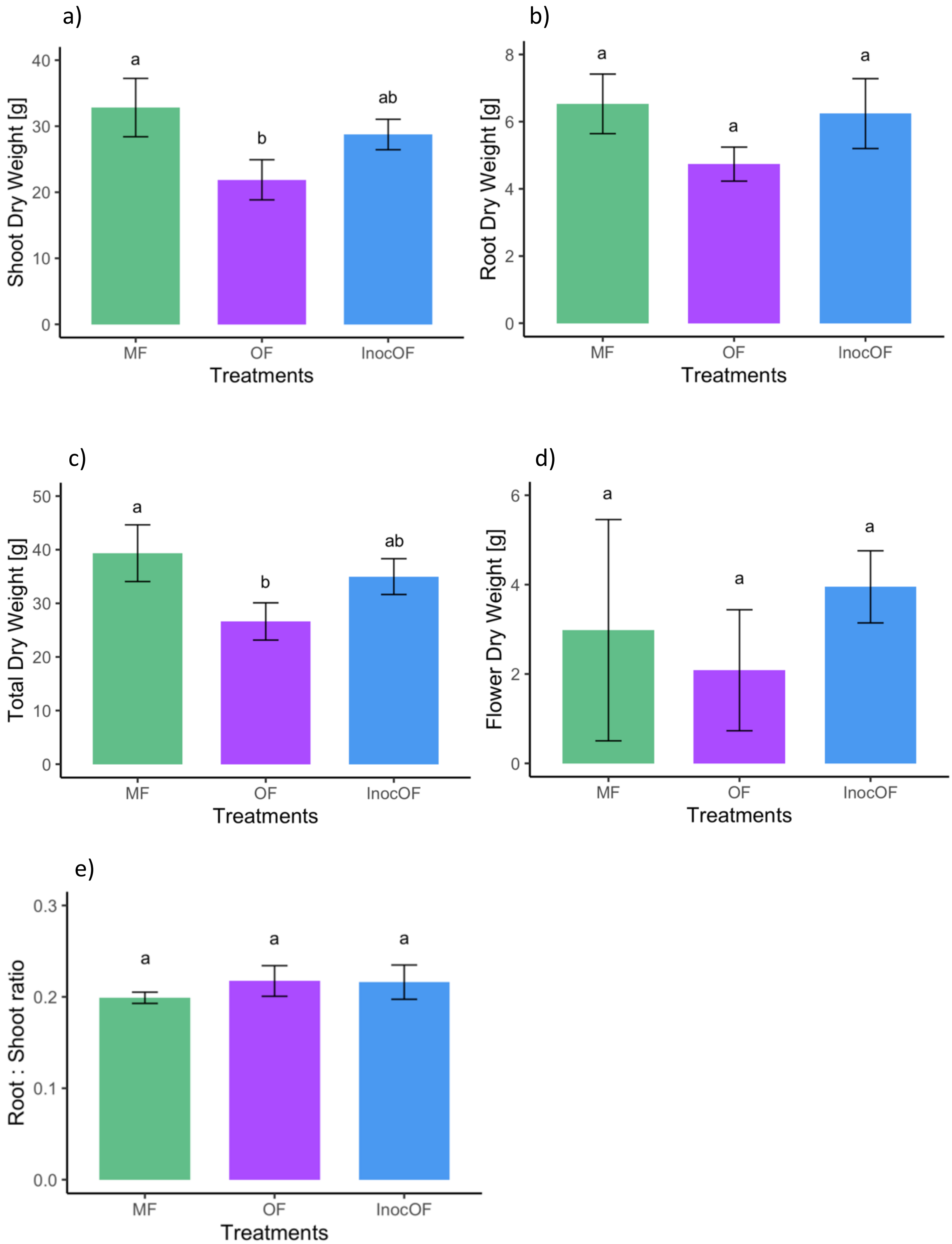


Figure 15: *Dry weight measurements at harvest. Shoot dw (a), root dw (b), total dw (shoot + root) (c), flower dw (d), and Root:Shoot ratio (e) for each treatment. Standard deviation bars are shown. Letters indicate differences between treatments.*

5.2.4 Leaf nutrient concentrations

The measured leaf concentrations of nutrients in bok choy after harvest are presented in Table 4. The youngest fully developed leaves and middle leaves were analyzed. Included are most of the essential nutrients as well as the beneficial nutrients Na and Al. For elements N, P, Mg, S, and Cu, the MF treatment has significantly higher values than the OF and InocOF treatment plants. For N, none of the leaf concentrations fall below the optimal range provided for bok choy. For P and Cu, the OF and InocOF leaf concentrations fall below the optimal range. For Mg, the InocOF plants are still in the optimal range but the OF plants seem to be deficient. For S, both the InocOF and OF plants are at the lower edge of the optimal range. For K, there is no significant difference between MF and InocOF, suggesting there was an improvement in K availability during the inoculation. For Ca, there is a significant difference between MF and OF, but not with InocOF and the other treatments. However, both OF and InocOF Ca values fall below the optimal range for bok choy. There are significant differences between all treatments for Fe concentration. For Zn, B, Mn, and Al, there are no significant differences between any of the treatments. The InocOF has the highest concentrations of Na, with no difference between the MF and OF treatments, however, the MF treatment seems to suggest the lowest concentrations.

Table 4: *Leaf concentrations measured in leaves of each treatment. N was measured with Dumas and all other nutrients with ICP-OES. NA for Cl, Ni, and Mo indicates that the nutrient was not measured or that results were not certifiable. Na and Al are not essential plant nutrients and therefore do not have an optimal range. Letters indicate the significance between treatments. Optimal range source: * Bryson and Mills, 2015, ° Kirkby, 2012. The optimal range listed for bok choy when available (*).*

	Element	Optimal range	MF	OF	InocOF
%	N	2.39 - 5.51 *	4.98 ± 0.18 a	3.07 ± 0.53 b	3.51 ± 0.12 b
	P	0.36 - 0.8 *	0.64 ± 0.06 a	0.16 ± 0.03 b	0.24 ± 0.03 b
	K	2.86 - 5.74 *	3.01 ± 0.24 a	1.96 ± 0.18 b	3.31 ± 0.24 a
	Ca	1.29 - 3.21 *	1.87 ± 0.80 a	0.42 ± 0.20 b	0.98 ± 0.32 ab
	Mg	0.19 - 0.35 *	0.57 ± 0.12 a	0.14 ± 0.02 b	0.23 ± 0.04 b
	S	0.41 - 0.77 *	1.17 ± 0.19 a	0.41 ± 0.07 b	0.41 ± 0.02 b
mg kg $^{-1}$	B	19 - 38 *	61.4 ± 23.1 a	57.3 ± 17.8 a	64.2 ± 14.3 a
	Cu	3 - 7 *	6.9 ± 0.8 a	2.11 ± 0.10 b	2.03 ± 0.19 b
	Fe	85 - 363 *	83.0 ± 3.1 a	31 ± 4.6 c	59.7 ± 6.3 b
	Mn	35 - 52 *	47.1 ± 14.9 a	25.9 ± 7.7 a	63.1 ± 21.6 a
	Zn	14 - 38 *	49.9 ± 14.6 a	29.1 ± 21.2 a	20.6 ± 1.4 a
	Cl	100 °	NA	NA	NA
	Ni	0.1 °	NA	NA	NA
	Mo	0.1 °	NA	NA	NA
	Na	NA	476 ± 23 b	2962 ± 750 b	6757 ± 2061 a
	Al	NA	10.3 ± 4.3 a	11.5 ± 3.6 a	14.3 ± 4.9 a

5.2.5 Uptake of nutrients

In Table 5 are presented the uptake values, which were calculated from the shoot dw and leaf concentrations of nutrients, to better present the actual amount of nutrients in the plant material. Table 5 takes into account the biomass of the plant and therefore more accurately presents the amounts of nutrients taken up by the plants. The results suggest that the mineralization of OF (InocOF plants) increased the N uptake by 40 % and P uptake by 100 % compared to untreated organic fertilizer (OF plants). The MF plants had about 3x the N uptake and 6x the P uptake as OF. Overall, this table highlights the differences in uptake between OF and InocOF, which often has similar leaf concentrations but the InocOF had greater shoot biomass accumulation.

Table 5: *Uptake of nutrients into plants. The below values are calculated from the shoot dw and leaf concentrations of the nutrients. Values used are from Table 4 and from the shoot dw measurements.*

Unit	Nutrient	MF	OF	InocOF
g	**N**	1.49 ± 0.34	0.61 ± 0.12	0.87 ± 0.11
	P	0.19 ± 0.05	0.03 ± 0.01	0.06 ± 0.01
	K	0.9 ± 0.22	0.39 ± 0.05	0.82 ± 0.11
	Ca	0.56 ± 0.27	0.08 ± 0.04	0.24 ± 0.09
	Mg	0.17 ± 0.05	0.03 ± 0.01	0.06 ± 0.01
	S	0.35 ± 0.1	0.08 ± 0.02	0.1 ± 0.01
mg	**B**	1.83 ± 0.81	1.13 ± 0.37	1.59 ± 0.4
	Cu	0.21 ± 0.05	0.04 ± 0.00	0.05 ± 0.01
	Fe	2.48 ± 0.57	0.61 ± 0.11	1.48 ± 0.23
	Mn	1.4 ± 0.55	0.51 ±0.16	1.56 ± 0.57
	Zn	1.49 ± 0.55	0.58 ± 0.4	0.51 ± 0.07
	Na	14.2 ± 3.31	58.7 ± 15.8	168 ± 55
	Al	0.31 ± 0.15	0.23 ± 0.07	0.35 ± 0.13

6 Discussion

This section aims to interpret the results of this study and put them into context with other studies. An evaluation of the methodology used and takeaways will also be covered.

6.1 Incubation experiment

6.1.1 Nitrate and ammonium concentrations

The incubation experiment showed that the addition of soil microorganisms to organic fertilizer under the right conditions does lead to nitrate production. Compost soil as an inoculum proved to be the highest nitrate generator, which was coupled with a decrease in ammonium (Figure 9, 10), pointing to successful nitrification. Suruban *et al.* (2022) observed that ammonium concentration increased during the initial stages of mineralization before it started decreasing and that the nitrate concentration increased faster than the ammonium concentration decreased. Similar results can be seen in Figures 9 and 10, where treatments OF+AG1, OF+AG2, and OF+AG3 show a trend towards increasing ammonium concentrations from week to week, while in the final week, all three of the treatments showed a significant increase in nitrate concentration. These results suggest that in treatments OF+AG1, OF+AG2, and OF+AG3 the nitrification process was just starting and that if the incubation had continued, a decrease in ammonium could have been expected in another week or two.

6.1.2 Effect of pH on incubation

The compost soil inoculated fertilizer (OF+C) had the highest pH values in weeks 2 and 3 (besides seawater treatments), reaching close to pH 8 (Figure 11). All other inoculated fertilizer treatments (besides seawater treatments) reached a maximum pH of between 6.5 and 7.2. This could suggest that the higher pH in weeks 1 and 2 was favorable to nitrification and that the pH decrease in the final week reflects the release of protons during the oxidation of ammonium. pH is one of the most important factors influencing the rate of mineralization, with temperature and the composition of organic fertilizer being other factors (Hooks *et al.,* 2022). The ideal pH for nitrifying bacteria is between 7.5 and 8.0 and OF+C (Figure 11b) was the only treatment to reach that range (Wahman and Pressman, 2014; Saijai *et al.,* 2016; Tyson *et al.*, 2008). Perhaps this was part of the reason that this inoculum had initiated the strongest nitrate generation, which could suggest that pH adjustment should be used in future incubation studies.

6.1.3 Inoculums

Another possible reason that the compost soil proved to be the best source of soil microorganisms for mineralization and nitrification could be the varied source of material in the compost soil itself. As explained in section 4.1.1, the compost soil was collected from a city recycling center where the compost is made from plant material collected from a wide source of gardens, parks, and public green spaces. The compost was then mixed with topsoil and sand or gravel. This diverse input could lead to a more diverse array of microorganisms. Additionally, the compost soil underwent an 8 – 18 months composting process, which also has a significant impact on the microorganisms present. In contrast, the agricultural soils AG1, AG2, and AG3, while all from organically farmed production, most likely had less varied inputs.

Microorganisms responsible for mineralization and nitrification are fairly common in soil and would be expected to be present in all soils used as inoculum. If the incubation experiment had continued for another week or two, it is possible that the same effect of lower ammonium and higher nitrate would be seen in all OF+AG1/2/3 treatments. Since all of these treatments showed a decrease in pH, it does indicate that ammonium had started to be oxidized and protons were being released into the solution (Figure 10). However, if the optimum pH for nitrification is higher than the pH reached in these treatments, this would cause a slowdown of nitrate production.

Seawater (S) was included as an inoculum as it had been used in the study by Shinohara *et al*. (2011), where it was a successful inoculum for nitrification. It could be expected that runoff from agricultural fields would be present in seawater, thus bringing with it soil microorganisms; additionally, nitrifying bacteria are reported to be present in aquatic ecosystems including oceans (Madigan *et al*., 2019). In this experiment, it was not shown to be a successful inoculum, as levels of ammonium and nitrate remained the same through the entire three-week experiment. Very little activity was also reflected in the stable pH values seen for seawater in Figure 10, thus, it seems that neither mineralization nor nitrification was happening under seawater conditions.

6.1.4 Effect of fertilizer on mineralization and nitrification

Vinasse-based organic fertilizer was used in this thesis; however, some previous studies with a similar experimental setup used fish-based fertilizer (Shinohara *et al*., 2011; Meeboon *et al*., 2022). Both studies suggested that the success of mineralization and nitrification with fish-based fertilizer could be due to the low C:N ratio or the microbial community in the fertilizer. In fact, the C:N ratio of the organic fertilizer used in this thesis was measured to be 3.3, which is closer to the fish fertilizer C:N ratio (2.9) in the study by Meeboon *et al.* (2022) than the other two fertilizers examined: corn-steep-liquor (4.8) and rapeseed oil cake (6.9). A similar study by Saijai *et al*. (2016) which produced successful nitrification of organic fertilizer used a fish-based fertilizer with a C:N ratio of 2.9. The C:N ratio for the organic fertilizer used in this experiment was lower than the label value of 6.7 (Table 3). It could be interesting to look at the effect that organic fertilizer origin has on the rates of mineralization and nitrification in further studies.

The quantity of fertilizer and soil inoculum in the incubation experiment in this study was the same as in the study by Shinohara *et al*. (2011), in which for the initial selection of various inoculums used 1 g L^{-1} of fertilizer and 5 g L^{-1} of inoculum. In that study, once the most successful inoculum was chosen, different concentrations of both fertilizer (0.5, 2.5, and 5 g L^{-1}) and soil (0, 0.5, and 5 g L^{-1}) were examined (Shinohara *et al*., 2011). It is noted that the fish-based fertilizer used in that study contained 4.5 mol N kg^{-1}, which is about 6.3% N. Therefore, since the BioBizz fertilizer used in this study had a measured N concentration of 5.5% (Table 3), in the incubation experiment there was approximately 8 mg N L^{-1} less than in the Shinohara *et al.* (2011) experiment. It is unknown how much ammonium or nitrate was already available in the fish-based fertilizer used in Shinohara *et al.* (2011) experiment, as only the concentration of total N is provided.

When determining the optimum concentration of fertilizer and soil, Shinohara *et al*. (2011) found that the lowest dose of fertilizer (0.5 g L^{-1}) added daily for a week (total of 3.5 g L^{-1}) generated the most nitrate, while no nitrate formed under the higher concentrations, suggesting that nitrification can be inhibited under high organic fertilizer concentrations (2011). It was also reported by Shinohara *et al*. (2011) that the highest concentrations of nitrate were formed when bark compost was added at 5 g L^{-1} at a fertilizer concentration of 2.5 g L^{-1}.

6.2 Plant growth experiment

6.2.1 Effect of treatments on plant growth parameters

Incubation of the organic fertilizer had a positive effect on plant growth measured by multiple biomass parameters. InocOF plants had a significantly higher shoot fw (Figure 14a) and total fw (Figure 14c) than OF plants. This can also be seen in Figure 12g with a side-by-side comparison of the plants. The InocOF plant (Figures 12e and f) looks more green, healthy, and larger than the OF plant. The OF plant (Figures 12c and d) appears to have stunted growth with significant yellowing and wilting of leaves. The roots of both plants appear brown in comparison to the roots of the MF treatment (Figure 12a) which is due to the organic fertilizer, possibly due to the formation of biofilm on the roots or due to the brown color of the vinasse fertilizer used. The brown biofilm on roots, as well as greater root hair growth, was also seen in other experiments with organic fertilizer (Shinohara *et al.,* 2011).

The root:shoot ratio (RSR) as calculated in Figure 15e was not significantly different for the treatments, while Kano *et al*. (2021) found that plants grown with mineral fertilizer in the autumn season had a lower RSR, meaning comparatively more shoot growth and less root growth.

6.2.2 Plant nutrient deficiencies

The OF plants have the most striking visual deficiency symptoms of all the treatments (Figure 12g). The oldest leaves are entirely chlorotic with necrotic spots (Figure 13b) and the leaves are wilting (Figure 12c). The middle leaves have moderate chlorosis, specifically concentrated on the leaf tips and the youngest leaves are underdeveloped and dark green (Figure 13b). In Figure 12c purple stems can be seen in one of the OF plants. These symptoms could represent a variety of nutritional deficiencies in bok choy, including N, P, and K. As described by Veazie *et al*. (2020), N deficiency in bok choy presents itself as stunted growth and chlorotic older leaves, eventually necrotic leaves, with some purple discoloration possible in *Brassica* species; P deficiency manifests as yellow margins and irregular spotting on lower leaves with dark young leaves and purple pigmentation; K deficiency shows up as yellow leaf margins in older leaves, with chlorotic and necrotic spots. In Figure 1a, we see these symptoms on experimental plants and both the advanced nitrogen and phosphorus deficiency symptoms look like the OF plants. Flaccid leaves and weak stems can also be caused by K deficiency, and

N, P, and K are all mobile elements that would exhibit their deficiency symptoms in older leaves first, as the plant would, under deficiency stress, translocate the necessary element into older leaves, leaving the older leaves with more prominent symptoms (Hawkesford *et al*., 2012; Taiz and Zeiger, 2018).

An important consideration when looking at the measured nutrient concentrations in leaf material is that the leaves analyzed were the youngest fully developed leaves and middle leaves for all plants so that similar ages and developmental stages of leaves could be compared; however, this could misrepresent deficiencies that would be more severe in other leaves, such as older leaves (Barker and Pilbeam, 2007). Therefore, it is useful to pair visual symptoms with measured leaf concentrations, as each source can give you slightly different information. According to the measured nutrient leaf concentrations shown in Table 4, the OF plants do not seem to be N deficient, with a leaf concentration of 3.07 % while Bryson and Mills (2015) describe the optimal range for bok choy to be between 2.39 and 5.51 %. The nitrogen concentration in deficient plants in Veazie *et al*. (2020) experiment was 1.8 % N. Kano *et al*. (2021) found the N concentration in bok choy from the summer cultivation period to be 3.27, 2.89 and 5.09 % for the OF, OF2, and CF treatments, respectively, and in the autumn cultivation period 4.4, 5.1, and 5.57 %, respectively. These N concentrations in this experiment followed a similar trend, with MF having the highest N concentration (4.98 %) and OF and InocOF having 3.07 and 3.51 %, respectively (Table 4).

As explained above, there could still be an N deficiency in the oldest leaves due to the mobility of the element. However, for P and K the deficiency is clearer from the leaf concentrations, with OF plants having 0.16 and 1.96 % P and K, respectively, while the optimal ranges are 0.36 – 0.8 % for P and 2.86 – 5.74 % for K (Table 4). The OF plants have the lowest concentrations of these elements, although the P concentration is not significantly different from the InocOF plants. Veazie *et al*. (2020) P-deficient plants had leaf tissue concentrations of 0.1 %, compared to 0.4 % in the control group; K-deficient plants had 0.5 % concentration of K compared to 8.8 % in the control group as seen in Table 2. These results, paired with the visual symptoms seen in Figures 12 and 13, seem to confirm a P and K deficiency in the OF treatment plants. Of the other macronutrients, OF plants seem to be slightly deficient in Mg and deficient in Ca, although neither deficiency can be clearly confirmed with visual analysis (Figure 13). In Table 2, Ca-deficient bok choy has tissue concentrations of 1 % which would support that both OF and InocOF plants were Ca deficient, with concentrations of 0.42 and 0.23 %, respectively (Table 4).

The S concentration for all treatments was sufficient, with OF and InocOF plants at the lower end of the optimal range and MF plants above the upper limit (Table 4). Sulfur is important for amino acids, coenzymes, and secondary plant metabolites; specifically for *Brassica* species, glucosinolates and isothiocyanates are S-containing compounds that have potential anti-cancerogenic and anti-inflammatory properties and could be considered an important nutritional benefit from *Brassica* consumption (Šamec and Salopek-Sondi, 2019). Sulfur deficiency in bok choy can lead to decreased yield and lower concentration of nutrients (N, Mg, and P) and glucosinolates (Hu *et al*., 2011).

Looking at micronutrients, OF plants have lower leaf concentrations of Cu, Fe, and Mn (Table 4) than the optimal range for bok choy provided by Bryson and Mills (2015). Iron deficiency in bok choy specifically can manifest as a pale appearance and marginal chlorosis in younger leaves with interveinal necrosis in newer leaves. Iron and copper are both immobile elements, so the deficiency symptoms would be present predominantly in younger leaves, which we did not see in the OF plants (Figure 13) (Taiz and Zeiger, 2018). While visual deficiency symptoms are not clear for Fe, the element is an important component of ferredoxin, a protein that is essential to *nitrate reductase* activity, therefore iron deficiency could limit nitrogen assimilation in the plant (Broadley *et al*., 2012).

Copper-deficient plants in Table 2 have a concentration of 2.32 mg kg^{-1}, which is higher than the tissue concentrations in the OF and InocOF treatments in Table 4. Therefore, it is very likely that these two treatments were copper deficient. The visual symptoms of Cu deficiency differ among species but include leaf chlorosis (Kopsell and Kopsell, 2007).

Plants in the MF treatment had no clear visual deficiency symptoms besides wrinkled leaves (Figure 13a). The overall plant size was the largest, as seen in Figure 12g and confirmed in Figure 14a. Wrinkled leaves with little to no chlorosis could be a sign of Ca deficiency, but this is not confirmed in the leaf concentration data, with leaf concentration of Ca at 1.87 % (Table 4) and the optimal range being 1.29 – 3.21 %, according to Bryson and Mills (2015).

The InocOF treatment plants did not have many deficiency symptoms either, with only minor leaf edge chlorosis visible in the smooth oldest leaf (Figure 13c). The middle and youngest leaves look similar to the MF treatment plant, although perhaps the size of the leaves is slightly smaller (Figures 13a and c). The overall size of the plant, as determined by shoot fw, is larger than the OF plant but smaller than the MF plant (Figure 14a). The P concentration in InocOF was 0.24 % (Table 4), below the optimal range of 0.36 – 0.8 % (Bryson and Mills, 2015). However, the K concentration was 3.31 %, the highest of all the treatments and similar to the MF treatment (Table 4). Therefore, we can see an improvement in the K concentration thanks to the incubation of organic fertilizer, which is even clearer in Table 5, where we see the uptake of the nutrient in relation to the plant fw. The InocOF plants took up almost 3x the amount of K as the OF plants (Table 5). This stark difference can also be seen in the N concentration, where there is no significant difference between the OF and InocOF treatments in Table 4, but the uptake is almost double in the InocOF plants in Table 5. The Ca concentration in the InocOF treatment was higher than the OF treatment, but not significantly different from neither OF or MF plants (Table 4). The Fe concentration in InocOF was significantly higher than OF plants at 59.7 mg kg^{-1} compared to 31 mg kg^{-1}, and the uptake was nearly 3x (Table 4 and 5), thus again pointing to the incubation as the reason for improvement.

Hooks *et al*. (2022) found that plants with inoculated organic fertilizer had 10 % and 24 % higher N concentrations than mineral fertilizer and organic fertilizer treatments, respectively, in their first experiment, while there was no difference between experiments in the second experiment. For other nutrients, in the first experiment, the inoculated organic fertilizer treatment had higher concentrations of P, Mg, S, and Fe than the mineral fertilizer and organic fertilizer treatments, while in the second experiment, B, Cu, Mn, and S were greater in both the organic fertilizer treatments, regardless of incubation, compared to the mineral fertilizer treatments (Hooks *et al*., 2022). Overall, Hooks *et al.* (2022) found the inoculation of organic fertilizer increased nutrient availability for plants, which is in line with the results of this study.

Kano *et al.* (2021) had lower Ca tissue concentrations for all treatments and lower Mg concentrations in all treatments except OF in autumn cultivation period than the results from this experiment; meanwhile, the K tissue concentrations in the summer cultivation were similar to this experiment and the values from the autumn cultivation were significantly higher (4.8 - 5 % in autumn and 2.2 – 3 % in summer with no difference between treatments).

The incubation seemed to have the greatest impact on K, Ca, and Fe when looking at only the tissue concentrations (Table 4). This could be due to the addition of compost to the incubation, not necessarily the mineralization of organically bound nutrients. The compost soil was not analyzed for elemental analysis, but it can be expected that some nutrients from the soil would be released into the nutrient solution. According to Marschner and Rengel (2012), concentrations of elements such as Ca, Mg, S, K, Al, and Fe in soil can be significant. To get a clearer picture of the effect of the incubation on nutrient mineralization, it would be better to use a microbial inoculum directly, without the additional nutrients present in the soil. However, this study focused on the ease of use of adding agricultural soil directly to organic fertilizer and was modeled after Shinohara *et al*. (2011) method of parallel mineralization method.

6.2.3 pH in nutrient solution

In the plant growth experiment, pH management was challenging in the OF and InocOF treatments. The pH in the InocOF buckets was around 8.5 - 9 before adjustment and 1M HCl was used to bring the pH down to 6.5. A few days after changing the nutrient solution and adjusting the pH, the pH was back at around 8, therefore multiple adjustments of pH were required each week. Similar pH changes were seen in the OF treatment, however, it was more manageable to adjust the pH, possibly due to the incubated fertilizer having a higher level of organic matter (carbon) from the soil which acted as a buffer (Williams and Nelson, 2016). Saijai *et al*. (2016) specifically added $CaCO_3$ to help buffer the organic nutrient solution.

Kerchasov *et al*. (2021) used nitric acid (HNO_3) as a pH adjustor in their experiment (shown in Figure 5) to keep the pH around 7 in the bioreactor; this pH could have been selected to be low enough to ensure proper availability of plant nutrients while being high enough to ensure bacterial growth, which is mentioned in the study. However, this pH adjustment method also added nitrate into the circulation, thus this would affect the total plant available nitrogen in the circulating system, which is no longer all originating from the organic waste-based fertilizer. Additionally, the pH of 7 is lower than the ideal for nitrification, so it could be expected that the nitrification potential in the bioreactor was diminished, thus leading to nitrogen-deficient plants (Kerchasov *et al*., 2021). Because this thesis focused on nitrogen availability, extra care was taken to not input any external sources of nitrogen into the system.

As explained by Tyson *et al*. (2008), the ideal pH for nitrification (7.5 - 9.0) is different from the ideal pH for hydroponic plant growth (5.5 - 6.0) and management of pH is critical to reconcile these two optimums. In the experiment by Tyson *et al*. (2008), three different pH targets (6, 7, and 8) were used in an aquaponic system, and pH 6 was used in a hydroponic system to grow cucumber, with no difference in the final fruit yield. Williams and Nelson

(2016) noted that pH management was one of the major challenges to using organic fertilizer in hydroponics, not only because it requires constant monitoring, but because large shifts in pH are not good for the plants, and the mineral fertilizers result in much smaller changes in pH in hydroponics systems.

6.2.4 Nutrient concentrations

The concentration of organic fertilizer used in the plant growth experiment was 2x the concentration of N of the mineral fertilizer. This was to account for much of the N in the OF being bound in an organic form, thus lowering the plant available N. Kano *et al*. (2021) also used a 2x N concentration of OF in one treatment in his experiment. The concentration of N was listed as 4 % on the BioBizz fertilizer bottle (Table 3) but the measured content was 5.5 % and this was the value that was used for calculating fertilizer addition.

6.2.5 Flowering

All plants in the experiment flowered during the treatment period, which is due to long days inducing flowering in bok choy plants. As the experiment was conducted in June in Copenhagen, the day length was over 17 hours. Day length longer than 12 to 14 hours induces bolting in bok choy (Cabi, 2019). While there was a tendency for more flower biomass in the InocOF and MF treatments (Figures 14d and 15d) compared to the OF treatment, there were no significant differences between the treatments.

6.2.6 Microorganisms

This study focused on microorganism inoculation through soil addition without a specific focus on the type of organisms, but rather on the effect they had on N compounds. However, for future studies into organic fertilizer mineralization, it could be beneficial and more reproducible to focus on specific species of microorganisms. Saijai *et al.* (2016) analyzed the microbial communities present during the oxidation of NH_4^+ and NO_2^-, finding that *Nitrosomonas nitrosa* performed most ammonium oxidation while *Nitrobacter winogratskyi* was predominantly responsible for nitrite oxidation. Therefore, a study in the future could focus on the addition of these specific species to the incubation of organic fertilizer. The presence of microorganisms is directly related to biofilm formation in the treatments containing organic fertilizer. While the biofilm can cause issues with the absorption of other nutrients and oxygen, the thin layer of microorganisms can actually enable the mineralization of organic N compounds directly on the plant root for direct accessibility (Kano *et al*., 2021). Here, we see the role of microorganisms not just in the preliminary incubation of organic fertilizer but also during the growth of plants in the hydroponics system.

7 Conclusion

- Five sources of inoculum (three agricultural soils, compost soil, and seawater) were tested for their ability to induce mineralization and nitrification of organic nitrogen compounds present in the organic fertilizer.
- The compost soil inoculum showed the highest rates of nitrification and mineralization, as shown by the decrease in ammonium and increase in nitrate and nitrite in week 3 of the incubation study.
- Vinasse-based organic fertilizer was incubated with the compost soil inoculum for 32 days and subsequently used in a hydroponic plant growth experiment with *Brassica rapa* subsp. *chinensis* with mineral fertilizer and (non-inoculated) organic fertilizer controls.
- Plants grown with mineral fertilizer (MF) had the highest biomass production and displayed no deficiency symptoms.
- The incubation of organic fertilizer had a positive effect on plant growth parameters. Plants grown with the inoculated organic fertilizer (InocOF) had greater shoot fresh weight and total fresh weight and displayed fewer deficiency symptoms than the organic fertilizer treatment (OF).
- The plants grown with organic fertilizer (OF) had the lowest biomass production and significant chlorosis and wilting of leaves. While the leaf concentrations of nitrogen do not suggest a deficiency, the vidual symptoms suggest that the oldest leaves were nitrogen deficient and this was not shown in the analysis due to the translocation of this mobile nutrient.
- The inoculated organic fertilizer plants (InocOF) had higher leaf concentrations of potassium and iron compared to organic fertilizer plants (OF). When considering the total uptake of the nutrient by the plant, the inoculated organic fertilizer plants (InocOF) took up more than 2x the amount of potassium, calcium, magnesium, and iron and more than 3x the amount of manganese than organic fertilizer plants.
- This thesis shows that soil can be used as an inoculum for the successful mineralization of organic fertilizer for increased nutrient availability in hydroponics.

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