

Developing and evaluating a vermiliquer food-waste based hydroponic fertiliser derived from household waste.

Oscar Tse Wang

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Author Attribution

The work contained in the body of this thesis, except otherwise acknowledged, is original work resulting from my own investigations.

Chapter 1 is published in Agricultural Systems. Oscar Wang (OW) compiled, synthesised, and produced the manuscript. Floris Van Ogtrop (FvO) and Rosalind Deaker (RD) provided important feedback and provided intellectual inspiration.

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Chapter 6 was written by OW, and FvO provided feedback.

I certify that the above contributions accurately reflect the authorial contributions of each participant for each chapter in this thesis.

Oscar Tse Wang | 22 July 2025

As supervisor for the candidate, I can confirm the authorship attribution statements above are correct.

Floris Van Ogtrop | 22 July 2025

Preface

The chapters published below are written with the intention of standalone publication in peer-reviewed, scientific journals. Due to this, there is a degree of redundancy in the introductions across the manuscripts. Generally, this includes contextual information which is key in the justification, design, and discussion of each chapter. To minimise repetition, the contextual information usually provided by a “*General Introduction*” is instead found in the introduction of Chapter 1. Additionally, notation and heading titles have been altered from published forms for clarity. Finally, references have been reformatted and compiled at the end of the thesis for easier referencing.

This thesis did not utilise generative AI tools at any stage of research or writing.

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Thesis statement of originality

This thesis has not been submitted for any other degree or purposes. I certify that the intellectual content of this thesis is the product of my own work and that all the assistance received in preparing this thesis and sources have been acknowledged

Oscar Tse Wang | 22 July 2025

As supervisor for the candidature upon which this thesis is based, I can confirm that the statement of originality statement above is correct.

Floris Van Ogtrop | 22 July 2025

Abstract

Increases in climate variability, urban sprawl, and populations around the world have resulted in a global call-to-action in the development of sustainable, robust methods to challenge food insecurity. This thesis investigates the potential of re-utilising urban food-waste in urban horticulture systems by developing a food-waste based hydroponic fertiliser (FWBHF) from house-hold waste using vermicompost techniques. This approach aims to promote nutrient cycling by simultaneously addressing growing amounts of urban food-waste and providing sustainable nutrient sources in the emergent urban horticulture industry. An experimental FWBHF was developed after trialling a range of novel methods. A comprehensive nutritional profile was taken at each stage of the FWBHF development, aiming to enhance our understanding of plant macro-nutrient dynamics in response to potential processing methods. Finally, a combination of vermicompost and fermentation produced a solution which was trialled on lettuce (*Lactuca sativa*) in a nutrient film technique (NFT) system. In addition to the nutritional and yield effects, the FWBHF's effects on rhizosphere microbiome composition were also studied to determine the potential of plant growth promoting microbes (PGPM) and their role in hydroponic systems.

Chapter 1 explores existing research into FWBHF with a systematic literature review, identifying current research as well as existing challenges. Chapter 2 compares the nutrient composition of two experimental FWBHF development methods (fermentation and vermicompost) against a synthetic hydroponic control solution. Chapter 3 trials a vermiliquer derived FWBHF on lettuce (*Lactuca sativa*) in an NFT growth system. Chapter 4 compares the substrate microbiome between the FWBHF and a synthetic control fertiliser. Chapter 5 partially substitutes the control fertiliser with FWBHF, while also ascertaining the inoculative potential of the FWBHF by comparing yield outcomes against a sterilised treatment.

In 2022, a systematic literature review (PRISMA, 2022) identified 37 papers which included the development of a FWBHF using waste sourced from either farm, industry, or consumer/retail level waste. The primary findings of this review identified key issues pervasive to the use of consumer level food waste, primarily the lack of macro-nutrient species such as nitrogen, and calcium, coupled with the excess of other elements such as sodium. In addition, research papers were often unrelated to another and did not include similar reporting methods. This resulted in a mosaic of novel processing methodologies with limited details in methodology. The most common trend among literature was the use of bio-active conversion, usually this was done through fermentation (either aerobic or anaerobic), although some other methods such as vermicomposting or composting were also explored.

Initial development of FWBHF began with parallel fermentation and vermicompost trials. Fermentation trials focused on the reaction of individual foods in response to aerobic or anaerobic treatments over a period of 90 days. It was found that the nutrient dynamics of protein-rich (e.g beef and chicken) and fibre-rich (e.g assorted vegetative matter) food groups reacted similarly in response to both aerobic and anaerobic treatments. Carbohydrate-rich foods (e.g bread and pasta), however, did not show the same level of conformity – as we observed different changes in macro-nutrient levels between bread and pasta, as well as a greater level of variance within treatment groups. Results showed that no individual food

group could optimally produce the nutrient required to sustain crop growth, with a universal deficiency in nitrogen. The vermicompost treatment included a variety of household waste, and the resulting leachate was also deficient in nitrogen. Between the two treatments, vermiliquer demonstrated a greater capacity for nitrogen mineralisation, with a significantly greater nitrate: ammonia ratio compared to the fermentation trials.

Vermicompost techniques were then combined with anaerobic and aerobic fermentation techniques to produce a final FWBHF. Vermiliquer was collected and left to anaerobically ferment for 60 days and then aerated for 48 hours prior to application in an NFT system. A growth trial of lettuce (*Lactuca sativa*) found that vermiliquer could not replicate the yield outcomes produced by a commercial, synthetic A/B solution in lettuce. This was attributed to deficiency in nitrogen and calcium, as well as excesses in sodium and sulphur. This growth trial provoked two questions: i) what sort of microbiological impacts did FWBHF have on the rhizobiome of crop species, and ii) was there any implementable role for FWBHF in commercial hydroponic systems.

Comparisons of rhizosphere microbiomes in lettuce grown in an NFT system found a high degree of elasticity in the functional population dynamics. Upstream sources greatly impacted the abundance and diversity of taxonomy found in lettuce rhizobiomes. Most notably, it was observed that functional groups tended towards a similar proportional composition – regardless of the original nutrient solution (vermiliquer FWBHF or A/B synthetic) or substrate type. By the end of the growth period, ANOVA did not detect any significant differences in the distribution of functional groups across any treatment groups. It is hypothesised that this gradual shift is the result of the crops influence over its own rhizosphere. Notably, PCoA and ANOVA analysis found that changes in bacterial taxonomies were more influenced by nutrient solutions than by substrate type. Conversely, fungal taxa showed greater response to substrate type compared to nutrient solutions. Plant pathogen populations were observed in all treatments, but all crops remained asymptomatic.

Utilising FWBHF as a partial substitute in commercial systems is beneficial in improving aspects of nutrient cycling. In addition to reducing the rate of synthetic fertiliser applications, the increased ecological diversity provided from FWBHF inoculation may influence yield outcomes. The final trial of this thesis compared a synthetic control fertiliser against two treatment groups: V1 (66% Control + 33% non-sterile FWBHF) and V2 (66% Control + 33% sterile FWBHF). The method of high-temperature sterilisation was carried out with an autoclave, which held a temperature of 121°C for 15 minutes. The primary intention of sterilisation was to test if the microbiology provided by the FWBHF had an effect on yield outcomes. This trial found that all three treatment groups (C, V1, and V2) had no significant differences in yield outcomes. Although, V1 had a notably reduced variance in fresh weight, plant height, and leaf count, potentially due to microbial inoculation provided by the FWBHF solution. In addition, high-temperature sterilisation improved nutrient availability across all macro-nutrient groups except nitrogen – although the benefits of these elevated macronutrients were partially nullified by a proportionate increase in sodium.

This study has found that the multi-step application of novel processing methods can produce a solution capable of sustaining plant growth, although not to the current yield standards of commercially available, synthetic hydroponics solutions. The outcome of this research challenges the sterile culture of conventional hydroponic systems by showing that growth

media in hydroponic systems can sustain complex and diverse ecologies, which are influenced by the rhizospheric activity of crop species. Future research should continue optimising processes which are robust and can handle seasonal fluctuations in food-waste composition, explore salt-tolerant crop varieties, further explore mechanisms of FWBHF induced rhizobiome dynamics, and address the risks of human pathogens in FWBHF systems.

This thesis has highlighted the primary obstacles to FWBHF development, specifically, the excess of sodium limiting the availability of plant-essential nutrients. The adoption of cutting-edge technologies such as selective reverse osmosis and plasmaphoresis as methods of salinity control may be viable in the future. In conjunction with the combined efforts of research groups studying wastewater remediation, soil salinity researchers, and desalination projects, we may see the adoption of affordable methods for the amelioration of excess sodium. Based on the experimental methods in this study, a household of three adults can generate enough food-waste to grow up to 700 heads of lettuce in a single year (Chapter 6.4). The conversion rate of food-waste into utilisable nutrients has many barricades to standalone viability, however even partial substitution of synthetic hydroponic solutions mitigates the nutrient leakage experienced in urban environments. We hope this thesis can lay foundational research for the integration of urban horticulture as a staple of sustainable, local food production in our cities.

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Chapter 1. Systematic literature review of food-waste based hydroponic fertilisers.

Abstract

Reutilising food-waste from urban populations presents a solution to increasing concerns surrounding food security while providing sustainable alternatives to the growing urban horticulture industry. This review seeks to improve nutrient cycling by identifying research which utilises food-waste at different stages of the food production chain (Farm, Industry, Consumer) and the range of novel techniques employed to transform them into organic hydroponic solutions. No clear trends in inputs or methods were identified, with the strongest relationship between an organic solution and success being its similarity to the corresponding synthetic control solution. Across 37 papers, a large range of inputs and processing methods have identified gaps in knowledge surrounding the sourcing, processing, application, and microbial aspects of developing a solution. The scale of research from Farm to Consumer greatly affected the primary inputs and methods used. The large quantities of homogenous waste led to research in this sector to favour energy efficient methods such as fermentation. As the production chain stage nears consumer levels, there is a distinct increase in both input variety and methods such as boiling or steaming. This lends itself to the increase in salinity related issues in consumer food-waste solutions. This study suggests that future reporting should include a standardised set of information, allowing for replication and comparison between literature.

1.1 Introduction

The 2021 United Nations Environment Programme Food Waste index reported that the world had wasted 931 million tonnes of food in 2018 – account for 17% of the total 5.3 billion tonnes of food produced throughout the year. The impacts of these losses are best appreciated when considering the resource-intensive processes that are involved in food production: deforestation, soil erosion, fertiliser inputs, greenhouse gas emissions, as well as energy and fuel used to add-value, store, and transport products. As the world population continues to grow, and food security is challenged by increasingly unpredictable climate patterns, it is important that alternative uses for food-waste are developed.

The concept of developing productive uses of food-waste is particularly important in cities. While it is inevitable that urban environments are net importers of food, countries such as Australia are seeing a surplus that towers well above the world average. The average Australian household generates 102kg of household waste per capita compared to the world average of 74kg/capita (FAO, 2021). Household waste in Australia only accounts for 34% of food-waste from the Australian food industry - the remaining waste being generated by primary production (31%), manufacturing (24%), and consumer facing services (10%), leading to the total amount of food-waste per capita in Australia to be 298kg (FIAL, 2021). With 86% of the population living in urban areas (ABS, 2020), it is clear that these high-density environments are producing large amounts of waste that have the potential to be transformed into productive sources of nutrients for urban horticulture.

Generally, the extent of current technology has provided three potential avenues for food-waste: recycling, energy recovery, and disposal. Recycling involves the reutilization of waste in a functional manner, with examples ranging from using eggshell waste in pharmaceutical products, coffee grounds incorporated into cosmetic products, or simply the recovery of nutrients within food-waste to produce more food in the future. A 2014 census by *Food*

Innovation Australia (FIAL) found that 14% of waste generated by Australian households is composted in-house or commercially. Recycling for third party use is dominated by manufacturing and production sectors – with 72% of waste generated by industry being recycled. Energy recovery relies on stored nutrients in food-waste to feed methanogenetic microbes which produce biogases that can be used as a source of combustible energy. In 2021, only 28 tonnes of industrial waste was utilized as a source of energy. Finally, disposal is the least desirable outcome, as there is no additional benefit extracted from the food-waste before it is discarded. In Australia, 69% of consumer waste ends up in landfill, which comprises up to 95% of the total food-waste in landfill. The remaining 5% is produced by industry, which despite generating more than double the amount of waste compared to consumers, only sends 1.5% of its waste to landfill. This is likely attributed to value-adding or recycling processes which can be applied to the large amount of homogenous waste products produced by primary industries such as abattoirs, food refineries, or other intermediate processes products undergo prior to retail or wholesale availability. Primary industries in Australia do not utilize landfills (FIAL, 2021).

One proposed use for food-waste has been to transform it into liquid fertiliser for hydroponic systems to alleviate dependence on i) the importation of fresh produce to urban areas, ii) synthetic fertilisers, and iii) improve local food security. It is well known that overdependence on regional and international food imports can threaten overall food security (Luo Et al. 2021). This can be seen in Sydney, Australia, where in 2011, it was estimated the city imported 90% of its vegetables and 98% of its fruit from regional and international sources (SFF, 2011). Hydroponic systems are a budding solution to alleviating the pressure on regional and international importers to provide a secure source of affordable products to urban areas (Taghizadeh et al., 2021). While there has been extensive research performed to transform food-waste into effective on-field fertilisers, these solutions often rely upon microbial elements in soil to convert inaccessible minerals into plant available forms (Ouro-Salim et al., 2021). In hydroponics, the absence of soil removes a critical medium required for the regulation of appropriate nutrients. This initial obstacle is compounded by the non-homogenous inputs of “food-waste”, deviating from the sterile and precise dogma of hydroponic methods.

In recent years, research in this area has seen increased interest – likely due to growing adoption of commercial hydroponics in conjunction with protected systems. The growing popularity of protected hydroponics may be attributed to environmental and economic factors. These systems can offer optimal growing conditions throughout the year by emulating ideal soil and atmospheric conditions to bypass traditional cropping density limitations. In such conditions, systems are capable of doubling cauliflower yields, improving the yield of peas by 7 times, and tomatoes by 18 times when compared to traditional soil-based systems per acre (Sardare et al., 2013). Hence, recirculating water production systems, such as hydroponics, have been perceived as a potential solution to combat the loss of arable land due to soil degradation, urbanisation, and increasing rainfall variability (Wallace et al., 2015).

The improved spatial efficiency of hydroponic systems requires significant investment and is limited to a select range of crop species. Generally, a large amount of capital is required to purchase land, infrastructure, lighting, and machinery. Ongoing upkeep costs such as fertilisers, water, substrates, pH adjusters, pots, and electricity further limit the type of crops hydroponic systems can profitably cultivate. As such, the practicality of these systems excludes arboreal and broad-acre usage. Instead, high-value horticultural products are ideal species, including leafy greens, berries, fruits, and vegetables. The limitation of crop species,

however, may not significantly impact the urban or semi-urban viability of such hydroponic systems. Economic viability is compounded when considering the benefits of annually available and locally sourced fresh produce. This smooths seasonal supply while competing with imported goods – benefitting from a less extensive logistical network of refrigeration and atmospheric control to preserve food quality. (Armanda et al., 2019).

Supporting urban and peri-urban food production bolsters food security in both short- and long-term scenarios. Providing alternatives to regional or international imports eases the effects of seasonal and unexpected supply shocks – especially when disruptions in horticultural food chains tend to have a more profound effect on short-term availability compared to broadacre cereals or other long-life products. This is due to the fragility and short-shelf life of many horticultural products, evidenced by relatively more variable pricing of fresh produce compared to grains. In 2019, CSIRO reported that between 18 and 22% of all horticultural goods in Australia were wasted during production, processing, and transportation stages (CSIRO, 2019). Although no empirical evidence exists to quantify losses during transportation, causes of damage at this stage include disruptions in the cold chain, disease, and mechanical damage (Parfitt et al., 2010). By reducing transportation distance, and thus time spent in transport, urban hydroponic farms lower risk factors that contribute towards food-waste. There is also the additional benefit of the carbon footprint of food transportation, which accounts for 27% of all freight emissions (Li, 2022).

Research into improving hydroponic systems includes improving energy and water efficiency while maintaining optimal yield outcomes. In 2019, a life cycle analysis found that hydroponic systems have greater energy and input requirements when compared to conventional cropping systems (Martin et al. 2019). While there is constant research into optimising production methods, management, and energy efficiency, the crux of hydroponics relies on its nutrient fertiliser designed to emulate the mineral composition of fertile soils. Due to their synthetic origins, these solutions have constituent inputs sourced from unsustainable processes, such as extracting nitrates from the atmosphere with energy intensive Haber-Bosch systems or mining for phosphate rocks. These practices contribute heavily to greenhouse gas emissions and sometimes rely on the destructive exploitation of finite resources such as phosphates, which researchers believe will increase in price as accessible reserves are exhausted (Liu et al., 2020; Cordell et al., 2011). By reutilising food-waste into hydroponic solutions, three goals can be achieved, i) cities have an economically beneficial, local, and sustainable use for food-waste, ii) urban centres can improve their own food security by increasing local food production, and iii) Hydroponic systems can begin to develop in sustainable ways with potential long-term viability.

The objectives of this paper are to (i) Summarise the research performed on this topic by stage in the production chain; (ii) identify trends in successful trials and (iii) consolidate common limitations and proposed solutions.

1.2 Methodology

PRISMA protocol

This literature review was written utilizing the Preferred Reporting Items for Systematic Review and Meta-analysis protocols (PRISMA) (Page et al., 2020). The search criteria included papers published from between 1980 to 2022 across Web of Science, ProQuest, and Google Scholar. Only peer reviewed papers were considered in this paper. The relevance of reports was considered based on two main points. First, articles which involved the creation

of hydroponic fertilisers utilizing organic material. Second, the origin of the materials used to develop these fertilisers.

Identification and selection

Across three databases, three Boolean searches were carried out. At this stage, the filters used only excluded papers published before 1980, although the earliest papers relevant to this review was from 2001. The key words included: “Food-waste AND hydroponic AND fertiliser,” “Organic AND hydroponic AND fertiliser,” and “Organic AND Hydroponics.” All papers were screened for both Web of Science and ProQuest, however only the first 300 results were considered in Google Scholar. This yielded a total of 6840 results. From these, 308 were selected based on the relevance of their title and abstract. 93 were removed due to overlaps, and a new set of inclusionary criteria was then implemented, which required papers to be peer reviewed, have a full English version, and directly related to the research objective. Finally, 37 papers were selected to be used. These papers were then categorised as: Farm, Industry, or Consumer, based on the stage of the food chain they were produced at. It is evident that this area of research has only recently gained substantial interest, as 32 out of the selected 37 papers were written after 2018, highlighting the potential for a literature review on this subject.

1.3 Results and Discussion

The fledgling state of the food-waste based hydroponic fertilizer (FWBHF) research is highlighted by approaches from a range of disciplines, including horticulture, engineering, waste management, and microbiology. As a result, research of FWBHF's can be described as a collection of loosely related novel studies which build upon existing knowledge spanning across many fields of study. The over-arching findings of this literature review have identified 29 out of 37 papers performing growth trials comparing the efficacy of FWBHF's against synthetic controls. From the results provided, treatments with unique feedstock or methods were identified, and the most effective treatments were selected based on yield outcomes. To standardize results, yield outcomes were selected in the order of priority: *i) Fresh shoot/fruit weight, ii) Dry shoot/fruit weight, iii) Stem height, and iv) Leaf count*. Treatments were then categorized as follows:

Table 1.1: Definitions for quantifying treatment efficacy across literature in this review.

Outcome	Definition
Less yield (LY)	FWBHF yielded less than the control
Equal yield (EY)	The FWBHF yielded equal to was within 1 standard deviation (if given) of the control
Substituted yield (SY)	FWBHF was able to substitute a portion of a synthetic solution to produce a yield equal or greater than the control
More yield (MY)	The FWBHF was able to surpass the yield of the control

To quantify and compare, this review determines solution efficacy in two ways. The first is the direct comparison of nutrient availability within a solution, and the second is found in the reported performance against a control solution. In this review, “control” solutions are generally the synthetic, conventional nutrient solution that the respective researchers have used as a comparison. It should be taken into consideration that there are a variety of conventional solutions that vary in essential nutrient content but are generally designed to optimise growth in commercial settings. There is an underlying assumption that these commercial solutions provide an industry accepted standard of performance which are valid as a general indicator of treatment efficacy, with the understanding that they cannot strictly be used to compared between studies.

It should be noted that irregular reporting limits statistical analysis. Most notably, only 11 papers provide standard deviation, hindering attempts to determine whether a solution is statistically different from a control solution. Future research should include standard deviation, error, and other relevant statistical values, which is discussed further in *1.3.7 future recommendations and standardized reporting*.

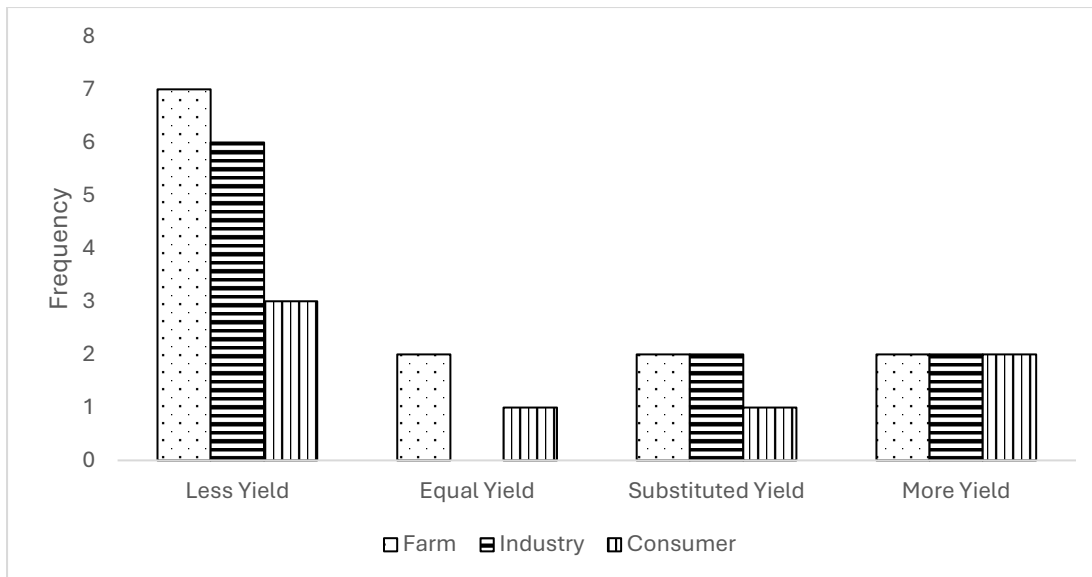


Figure 1.1: The distribution of treatment efficacy across papers, categorized by feedstock origin.

Figure 1.1 shows that of 29 papers, six unique treatments were identified from six papers as MY. Proportionally, consumer-based feedstock performs best, however this may not be representative of feedstock superiority – the papers included under “consumer” wastes include a combination of animal manure and kitchen waste (Loera-Muro et al., 2021), biogas (Bergstrand et al., 2020), and a variety of novel processing methods including vermiculture (Churilova et al., 2019), powdering (Kawamura et al., 2014), and boiling (Yusuf et al., 2021). The spread of feedstock and methodology limits the accuracy of a formal meta-analysis. Instead, the most powerful correlation between a treatment and success was identified as the similarity of NPK between a FWBHF and its corresponding control solution. The impacts from specific feedstocks or methodologies were limited when considered as individual variable. It may be more important for a processing method to be selected to accommodate for the available feedstock.

1.3.1 Feedstock versus fertiliser production methods

After reading the available literature it was found that there were opportunities to categorise these papers in two distinct ways – by feedstock or by methodology. At first glance, either option provided a logical foundation for understanding existing trends in research – however closer examination identified weaknesses which may have compromised the rigidity of this review. For example, both terms are broad – requiring additional sub-categorisation into groups such as: *i) Farm waste, ii) Industrial waste, or iii) Consumer waste* for feedstock, and *i) Anaerobic fermentation, ii) Aerobic fermentation, iii) Novel methods of fermentation, and iv) non-fermentation processes* for methodology. Additionally, both feedstocks and methods found that even within the same categories, there was great variation in inputs and processing techniques across literature.

It is difficult to identify and synthesise common practices across literature when confounding factors, such as methods and feedstock have limited replications. Within similar sub-

categories, there is still variation, expressed by table 1. which looks at three papers which have been categorized as “anaerobically fermented vegetative biogas digestate.”

Table 1.2: Comparison between three research papers that are categorised as “vegetative biogas digestate.”

	Lind et al., (2019)	Ronga et al., (2018)	Ntinis et al., (2021)
Feedstock composition	Crop residue (85.5%), plant-based food-waste (12.5%), and iron chloride (2%)	Maize silage (43%), triticale silage (22%), Cow slurry (27%) and grape stalks (8%)	“Co-digested Livestock manure and other agricultural residues”
Growth trial species	Bok Choy (<i>Brassica rapa subsp. chinensis</i>)	Baby Lettuce (<i>Lactuca Sativa</i>)	Baby Lettuce (<i>Lactuca Sativa</i>)
System type	Nutrient Film Technique (NFT)	Drip irrigation	Floating system
Grow period	21 days	21 days	31 days
Control	Kristalon Indigo + Calcinit inorganic commercial solution. (NPK ~19-4-20)	Hydrofood macro and micronutrient inorganic commercial solution (NPK 17-16-11), and 20mL acidifying agent	Hoaglands solution (pH 6.5)
Dosage method	6L added 1-3 times a week, 2L added 3 times a week, and 20mL added when pH<5.8	Diluted to 6.25% strength. 20L replaced each week for the first 2 weeks. Last week of crop cycle is tap water only.	Diluted with water by 5%, 10%, and 20% strength with and without pH adjusted trials
Substrate used	Pumice	Perlite and solid fraction of biogas waste	Polystyrene plugs
Production method	Filtered (0.8mm), diluted, and nitrified	Filtered (200 microns)	Filtered (1mm) and frozen
Microbial inoculation	Yes	No	No
NPK composition (mg/L)	182-41-250	34 – 0** – 95	330 – 154 - 470

*Hoaglands solution may refer to one of several iterations of the original recipe.

**P2O5 absent in liquid extract, but available in solid byproduct at rate of 60 mg/L

From *Table 1.2*, despite having the same “biogas digestate” input, there are differences in the compositions which greatly impact the final product. Differences in feedstock can be observed with the inclusion of nitrate rich livestock manures, or unspecified “plant-based food-waste”. Additional differences include the species used in growth trials to the presence of microbial inoculation. This can confound comparisons between solutions, as different systems produce different effects on a given solution. It should also be noted that there is variation in control treatments across studies, this creates a moving baseline for yield which is a common metric used to determine the viability of a given solution. Ultimately, this

provides insight into how categorisation by feedstock may be sub-optimal for identifying trends.

Outside of feedstocks sourced from aquaponics, no two papers were alike across all the reviewed literature. The high level of variation in method and inputs is a testament to the infancy of this research area. The absence of identical feedstock replication at consumer level contributes to the difficulty of performing a comparative analysis of variables which impact measured outcomes. Instead of categorising papers based on feedstock, it was determined that categorisation by methodology would be a more effective way to identify overall trends across studies. By isolating the processes utilised there are three benefits. First, it is hoped that the characteristics of each method begin to reveal themselves across studies while being minimally impacted by inputs, system types, or crop species. Second, it refocuses the review away from grading and comparing potential feedstock and onto the processes that may be applied to develop fertilisers. Finally, it aims to recommend factors relevant to report when conducting research – a standardised structure when reporting hydroponics research.

In this review, the first part of the discussion will describe the broad trends found in feedstock and methodology. The second section will explore common obstacles faced across research, correlating factors which may have contributed to these problems, and solutions that have been developed in response. The third and final section of this review will seek to identify the role of microbial activity and ascertain its impact in the efficacy of an organic hydroponic solution.

1.3.2 Discussion of feedstock

While the synthesis of literature is distinguished through methodology, understanding the properties and potential of inputs is relevant to a comprehensive perspective of organic hydroponic solutions. The feedstock of a fertiliser is essential - nutrients that do not exist in the material of origin will not be available in the output in any form. A more nuanced perspective could also include considerations toward salinity-nutrient ratios, carbon-nitrogen ratios, threats of contamination, as well as overall solubility of organic materials within a given timeframe. As such, categorisation can provide insight into the potential strengths and challenges that may be faced when using a variety of inputs. These categories are: i) Farm waste, ii) Industrial waste, and iii) Consumer waste, which are further sub-categorised into groups of similar nutritional composition.

Farm waste

Farm waste categorises low-grade horticultural products, aquaponic wastes, and animal manure as forms of “nutrient leakage”. There are three main sources of farm waste: i) Animal waste ii) Aquaponic by-product and iii) Farm residue. While not strictly considered “food-waste”, these inputs are by-products of food production and can be a rich source of nutrients. The concept of transporting rurally sourced farm waste for usage in urban hydroponic systems may seem inefficient, however, protected cropping is still a popular method of production in rural areas, particularly for fragile horticultural crops which require shelter from variable or unfavourable climactic conditions. Furthermore, protected systems themselves will produce waste – in the form of vegetation or defective produce which can be immediately reutilised in growing the next crop. Farm waste can be characterised as large amounts of homogenous organic matter. As a result, scalability becomes a priority when considering methods to process such vast amounts of waste. Evidence supporting a strong consideration of scalability is shown in this review, which found that 17 of the 18 papers in

this review utilise some form of fermentation – a relatively energy efficient method of bioconversion relative to some of the other techniques found in this review.

Animal Manure

Animal manure has been a significant source of plant nutrients throughout history, as such it has been extensively studied in on-field use, and recently interest has spread to using it as a source of nutrients for hydroponic systems. Manure is typically favoured as an on-field fertiliser as it has a rich cation exchange capacity and has all the necessary micro- and micro-nutrients required for crop growth – many studies use this as a basis for the development of hydroponic solutions utilising manure as the primary input (Tikasz, 2019). The infancy of this research is reflected by a large variety of variables with few replications across studies. These include the presence of additional inputs such as sugars, the presence of microbial inoculation, as well as the primary inputs themselves – a trend that repeats itself through all categories of food-waste. This leads a lot of research that is largely incomparable. This review identified 10 papers that fit within the definition of “animal manure,” found that nine of them utilised manure sourced from goats (3), poultry (4), pigs (1), or unspecified livestock (1), while only one paper utilised urine (rabbit) as its primary input – justified by citing the high nitrogen content of rabbit urine (2% N), as well as “other nutrient contents” (Guntara et al., 2018).

As a whole animal manure solutions did not produce consistent results, although the spread of methods limits the reliability of comparisons between papers. *Table 3* shows that of the seven papers that utilised a mineral control solution, one was able to be considered superior to a significant degree (Tikasz et al., 2019), two were able to substitute a portion of the control without significant yield loss (Sunaryo et al., 2018, Wang et al., 2019), and one other was able to match the control without significant difference. Between these findings, it was found that there was no common factor in feedstock that consistently produced superior yields. Instead, solutions that could most replicate the nutrient profiles of the commercially available standard solutions performed the best.

Table 1.3: Comparison between the most effective treatments relative to a synthetic control. Only papers which trialled animal manure based FWBHF against a synthetic control are included.

Author	Feedstock	Substitution rate (v/v)	Treatment	Crop species	Average control fresh weight yield (g)	Average treatment fresh weight yield(g)
Abd-Elmoniem et al., (2001)	Chicken	FWBHF + AB-mix (1:1)	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	873	839
Kechasov et al., (2021)	Pig manure	na	Active bioconversion of anaerobic fermentation	Tomato (<i>Solanum lycopersicum</i>)	4700±900	3700±1300
Liedl et al., (2006)	Broiler litter	na	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	170.27	99.92

Ntinas et al., (2021)	Animal and plant based biogas	na	Biogas slurry (anaerobic)	Lettuce (<i>Lactuca sativa</i>)	249	175
Sunaryo et al., (2018)	Goat manure	FWBHF + AB-mix (1:1)	Anaerobic Fermentation	Lettuce (<i>Lactuca sativa</i>)	75.33	79.03
Tikasz et al., (2019)	Turkey manure	na	Aerobic Fermentation	Lettuce (<i>Lactuca sativa</i>)	17.5±0.7	19.1±2.7
Wang et al., (2019)	Poultry manure	FWBHF + AB-mix (1:1)	Biogas slurry (anaerobic)	Lettuce (<i>Lactuca sativa</i>)	204.46±13.46	190.66±15.47

Aquaponic by-product

Aquaponic systems are a variation of the organic hydroponics system. By utilising aquatic species, often fish or crustaceans, as a source of nutrients for crops in soilless growing systems. During operation, plants extract solubilised nutrients from the water column, produced by fish and mineralised by microbial populations which favour porous and well aerated locations. In addition to the solubilised ammonia produced, aquaponic systems produce a solid by-product comprised of insoluble fish and feed waste that has collected at the bottom of tanks or in filter systems. This sludge is rich in ammonia and requires aeration to begin the mineralisation of its ammonium deposits (Zhang et al., 2020). Nitrate rich sludge may not be usable in aquaponics system, as nitrates are toxic to many aquatic species, however they may be an effective nitrogen supplement in other organic hydroponic systems.

Six research papers have been found to incorporate aquaponic by-products as the primary feedstock. Five of the six papers utilise sludge, while the last utilises biofloc – the semi-solid bacterial colonies which appear at the top of aquaponic reservoirs (Raju et al., 2020). All papers utilised either aerobic or anaerobic digestion as the processing method to optimise the nutrient content and separate solid and liquid parts. Two papers compared a FWBHF against a synthetic control solution, with results showing that there was potential in replicating, but not surpassing, the efficacy of the control (Ezzidine et al., 2021).

Only one paper performed a comparison between processed sludge-waste against a conventional aquaponics system, Goddek et al (2016) found that both anaerobic and aerobic treatments yielded equal to, or more effectively, than the control aquaponics system. The utilisation of an aquaponic system as a benchmark is tenuous as a reference for yield performance, as aquaponic systems tend to yield less than conventional hydroponic systems (Ayipio et al., 2019). Additionally, the cost of lowered yields is generally made up from the husbandry of aquatic species – although majority of profits from aquaponic systems are from the cultivation of crops, not fish (Greenfield et al., 2019). In the case of Goddek et al, lettuce has been identified as one of the few species where aquaponic systems can compete in yield efficacy relative to conventional systems (Ayipio et al., 2019) – hence, the level of performance derived from the sludge-based FWBHF highlights the potential for re-utilising aquaponic byproduct, due to its capacity to replicate the efficacy of an aquaponic system.

Table 1.4: The comparative performance between a conventional aquaponic system and anaerobically fermented aquaponic sludge.

Author	Input	Treatment	Crop species	Average shoot weight (g)
Goddek et al., (2016)	Recirculating aquaponic system (Nile Tilapia)	na	Lettuce (<i>Lactuca sativa</i>)	92.4±9
Goddek et al., (2016)	Aquaponic sludge (Nile Tilapia)	Aerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	104.8±15
Goddek et al., (2016)	Aquaponic sludge (Nile Tilapia)	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	137.4±15

Notably, all five papers based on aquaponic sludge provided nutrient analysis on the final product (Delaide et al., 2021; Ezzidine et al., 2020; Panana et al., 2020, Ezzidine et al., 2021; Goddek et al., 2016). Their research is consistent in showing that there is an adequate density of nitrogen, however macro- and micro-nutrient ratios are vastly different from the standard control solutions – with potassium deficiency being a particularly defining aspect of aquaponically based FWBHF. This nutrient imbalance is further discussed in section 1.3.4 *Nutrient deficiencies and Solutions*.

Table 1.5: Comparison between the most effective treatments relative to a synthetic control. Only papers which trialled aquaponic-waste based FWBHF against a synthetic control are included.

Author	Feedstock	Substitution	Treatment	Crop species	Average control fresh weight yield (g)	Average treatment fresh weight yield(g)
Ezzidine et al., (2021)	Aquaponic sludge (Brown Trout)	Na	Aerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	243±30	203±40
Delaide et al., (2021)	Aquaponic sludge (Pikeperch)	Na	Aerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	650	500
Delaide et al., (2021)	Aquaponic sludge (Pikeperch)	Supplemented NPK to 1, 1.8, and 4.5 mmol/L	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	600	580

Vegetative residue

Vegetative residue from farms is classified as the organic waste by-products generated from crop production. Research into the use of raw vegetative waste from farms is limited, with three of the four papers in this section utilising biogas digestates derived from vegetative

matter. This is likely due to farm residue being regularly reincorporated back into the land it is derived from (FIAL, 2021), or that it is too costly to transport waste materials to produce hydroponic solutions without prior value extraction, such as biogas production. However, this research can be applied to reutilise vegetative by-products from hydroponic production of fruit bearing crops, such as tomatoes, which produce a large amount of unmarketable vegetative matter which must be disposed of. Thus, local, or in-house bioconversion facilities could potentially be used to reconstitute such waste into future crops.

Across the four papers included, vegetative residue included cane reed residue, pasture silage, and grape vines as a source of nutrients for a hydroponic solution. One of three treatments utilising biogas residue matched or surpassed the control. The methodology transformed the solid waste from liquid fertiliser into a substrate for hydroponic systems. The pelletised waste significantly increased fresh shoot weight compared to the rockwool (control) substrate, demonstrating the potential of solid fertilisers (Ronga *et al.*, 2019).

Table 1.6: Comparison between the most effective treatments relative to a synthetic control. Only papers which trialled vegetative farm waste based FWBHF against a synthetic control are included.

Author	Feedstock	Substitution rate (v/v)	Treatment	Crop species	Average control fresh weight yield (g)	Average treatment fresh weight yield(g)
Ntinas <i>et al.</i> , (2021)	Animal and plant-based biogas	Na	Biogas slurry (anaerobic)	Lettuce (<i>Lactuca sativa</i>)	249	175
Pelayo Lind <i>et al.</i> , (2021)	Crop residue, plant-based food waste, iron chloride	Na	Biogas slurry (anaerobic)	Bok choy (<i>Brassica rapa var. chinensis</i>)	175	140
Ronga <i>et al.</i> , (2019)	Maize silage, triticale silage, cow slurry, grape stalks	Substrate replacement	Biogas slurry (anaerobic)	Lettuce (<i>Lactuca sativa</i>)	0.64 (dry weight)	0.82 (dry weight)

Industry waste

Industry waste includes the by-products of food processing productions that are discarded without further use. This section includes 10 papers that includes wastewater, mushroom media waste, distillery slop, and seafood residue. Similar to the farm section, majority of the methods used are conscious of scalability, utilising non-energy intensive methods of processing. This can be attributed, again, to the homogeneity of inputs as well as the large availability of inputs. The main inputs in this section can thus be broken down into i) Wastewater and ii) Solid wastes. Within the *solid wastes* category, there is a greater level of variance in both inputs and methods compared to other sections – aligning with the expectation of increasingly individualised products that exist as the food-chain approaches consumer levels.

Wastewater

Industrial wastewater papers were selected based on the predominant input being in liquid form. This category was distinguished from solid wastes as the state of matter may influence the rate of precipitation, mineralisation, or a range of other processes that can influence nutrient availability, equipment functionality, and mineralisation rate during any treatments between waste collection and hydroponic use (Gyrzyb et al., 2020). Of the five papers included, two source their constituent wastewater from tofu production, one from a brewery, the other from molasses production, and the last is by-product from corn wet milling. Like farm-based treatments, most wastewater-based treatments utilised anaerobic digestion to mineralise nutrients into inorganic forms. There is limited understanding as to whether the liquid state of these by-products alters the pathways organic nutrients undergo to transform into plant available form.

Table 1.7: Comparison between the most effective treatments relative to a synthetic control. Only papers which trialled industrial wastewater based FWBHF against a synthetic control are included.

Author	Feedstock	Substitution rate (v/v)	Treatment	Crop species	Average control comparison metric	Average treatment comparison metric
Anggraini et al., (2020)	Tofu wastewater	na	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	14.8 cm (stem height)	14 cm (stem height)
Chaorlina et al., (2021)	Tofu wastewater	FWBHF + AB-mix (1:2)	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	3.46 g (fresh weight)	5.73 g (fresh weight)
Figueroa et al., (2015)	Anchovy fishmeal wastewater	na	Anaerobic fermentation	Red Bean	6 cm (stem height)	7 cm (stem height)
Kano et al., (2021)	Corn steep liquor	na	Raw	Bok choy (<i>Brassica rapa</i> var. <i>chinensis</i>)	6.51 g (dry leaf weight)	6.12 g (dry leaf weight)
Li et al., (2020)	Molasses wastewater	FWBHF + AB-mix (1:19)	Anaerobic fermentation + reduction	Canola (<i>Brassica napus</i> L. 'Zhongshuang 11')	0.14 g (dry weight)	0.22 g (dry weight)
Riera-Viela et al., (2019)	Brewery wastewater	na	Raw	Lettuce (<i>Lactuca sativa</i>)	2.83±0.1 (dry plant weight)	0.46±0.1 (dry plant weight)
Riera-Viela et al., (2019)	Brewery wastewater	na	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	2.83±0.1 (dry plant weight)	2.09±0.5 (dry plant weight)

Overall, wastewater-based solutions performed well relative to their respective controls. Of the four papers with synthetic control solutions, all included at least one treatment which, on

its own or substituting a portion of the conventional solution, statistically matched or surpassed the efficacy of the control. Differences in reporting methods are apparent in this grouping of papers. The metrics utilised to measure the yield outcomes of a solution include the dry leaf or shoot weight, fresh weight, and stem height. In addition, liquid waste suffers from an unfavourable salt to nutrient ratio, which will be further discussed in section 1.3.5 *Salinity and Sodicity*.

Solid Wastes

Solid wastes in the context of industrial waste encompasses a wide range of sources. It can be distinguished from liquid waste by the presence of a substantial amount of solid material when dried. Six papers were collected in this section. Two utilise fishery waste, one uses sugar cane leaves and ethanol slop, another uses mushroom substrate waste, another uses cow paunch waste, and the final paper utilises a wheat bran-based compost to release nutrients over time. There are deviations from traditional digestate methodologies here, with the drying and powdering of crab shells (Sawain et al., 2018), as well as the development of a hydroponically suitable nutrient substrate (Tong et al., 2021). The solid waste section marks a transition in processing techniques, where scalability is often deprioritised for more novel, exploratory methodologies.

Table 1.8: Comparison between the most effective treatments relative to a synthetic control. Only papers which trialled industrial solid-waste based FWBHF against a synthetic control are included.

Author	Feedstock	Substitution rate (v/v)	Treatment	Crop species	Average control comparison metric	Average treatment comparison metric
Churilova et al., (2019)	Paunch waste	na	Vermiculture	Pak Choi (<i>Brassica chinensis</i> L. cv. Shanghai)	9394 g/m ² (fresh shoot weight)	7244 g/m ² (fresh shoot weight)
Phibunwatthanawong et al., (2019)	Molasses, distillery slop, sugarcane leaves	na	Anaerobic fermentation	Lettuce (<i>Lactuca sativa</i>)	2.33 g (shoot fresh weight)	2.42 g (shoot fresh weight)
Tong et al., (2021)	Wheat bran, oil cake, chicken manure	na	Compost	Bell peppers (<i>Capsicum annuum</i> , L.)	7.6±6.5 cm (plant height)	6.1±2.4 cm (plant height)

Despite the absence of shared feedstocks and methodology, both trials with synthetic controls matched the efficacy of their relative solutions. These findings highlight the importance of processing steps between obtaining food-waste and utilising it as FWBHF. Replication in this area is limited, as *solid industrial waste* hosts a wide array of novel inputs and methodologies which define the transition of FWBHF development as food-waste sources transition towards consumer level waste.

Consumer waste

Consumer waste includes the by-products and waste produced by retail, food industry, or end-users of the food production chain. In high-income countries, *UNEP* (2021) reported that 61% of waste at this level was produced by households, with retail and food services generating the remaining 39% of waste. This section includes nine papers that study a wide range of treatments applied to food-waste that come from a variety of sources. These papers are rarely similar, and unlike previous sections, are much more varied in both treatments and inputs. Feedstock specificity ranges from homogenous end-stage food, like sea lettuce and tomatoes, to non-specific ratios of food-waste from restaurants. A key distinction between food-waste at consumer levels and previous levels is the unique blend of processed and unprocessed products that have been altered from their original form with salts, oils, seasoning, and sauces. This poses as the crux of transforming consumer food-waste into a viable hydroponic solution. The transformation of such non-homogenous, fatty, and salty produce into a solution fit for plant uptake has proven to be a challenge – often presenting as overly saline solutions (Siddiqui et al., 2021).

Generally, research in this category is largely incomparable due to the difference in methodology and inputs. The methodologies included tend towards more novel techniques which do not prioritise scalability. These methods produced a range of results; of the 5 papers which included a suitable control, one surpassed the control, while two more were able to substitute a portion of the solution without losing effectiveness.

Table 1.9: Comparison between the most effective treatments relative to a synthetic control. Only papers which trialled consumer-based FWBHF against a synthetic control are included.

Author	Feedstock	Substitution rate (v/v)	Treatment	Crop species	Average control comparison metric	Average treatment comparison metric
Bergstrand et al., (2020)	Organic household waste (37%), manure (31%), slaughter residues (19%), other organic food waste (13%), iron chloride (0.03%) based biogas	na	Aerobic fermentation	Pak Choi (<i>Brassica campestris</i> v. <i>chinensis</i>)	113.6 g (fresh weight)	60.43 g (fresh weight)
Churilova et al., (2019)	Industrial kitchen waste	na	Vermiculture	Pak Choi (<i>Brassica chinensis</i> L.)	9394 g/m ² (fresh shoot weight)	9611 g/m ² (fresh shoot weight)

				cv. Shanghai)		
Faruq et al., (2021)	Vegetables	1:1	Anaerobic fermentation	Mustard greens (<i>Brassica juncea</i> L.)	29.24 g (fresh weight)	41.79 g (fresh weight)
Gimenez et al., (2020)	Tomato (71%), Onion (17%), Vineyard residue (12%)	na	Compost	Lettuce (<i>Lactuca sativa</i>)	2.22±0.05 g (fresh biomass)	2.00±0 g (fresh biomass)
Kawamura et al., (2014)	Fish (33%), vegetables (33%), café food waste (33%)	na	Powdered	Lettuce (<i>Lactuca sativa</i> var. <i>capitata</i>)	16.7±6.1 g (fresh shoot weight)	15.4±4.3 g (fresh shoot weight)
Loera-Muro et al., (2021)	Kitchen waste (50%), animal manure (50%)	na	Vermiculture	Mint (<i>Mentha spicata</i>)	1.6±0.5 g	3±0.5 g
Loera-Muro et al., (2021)	Kitchen waste (50%), animal manure (50%)	na	Vermiculture	Rosemary (<i>Rosmarinus officinalis</i>)	4.5±1 g (fresh shoot weight)	5.2±4.9 g (fresh shoot weight)
Yusuf et al., (2021)	Seaweed (<i>Ulva Lactua</i>)	na	Boiled	Lettuce (<i>Lactuca sativa</i>)	8.39 g (fresh weight)	2.23 g (fresh weight)

Reflection on feedstock

As the feedstock source nears the consumer, variability in inputs and methods become increasingly exaggerated, highlighting a fundamental consideration for future research. Consideration for the importance of scalability is critical to the economic viability of developing hydroponic solutions, but the exploration of novel methods can be beneficial to develop niche solutions adapted to more specific conditions set by input material or environmental constraints. For example, *Kawamura et al. (2014)* utilised a method where the final product was a powdered product. While the method required additional steps of dry-freezing and milling, the extra energy costs produced a powdered fertiliser that provides opportunities aqueous solutions may have difficulty overcoming. These benefits may include shelf-stability, transportability, and replicability. This lowers logistical costs required to

ensure solution stability and allows for an inert base that is receptive to modification without additional biological activity.

It is clear that methodology is a key player in developing a successful fertiliser, however it should be emphasised that feedstock plays a similarly important role on the outcome of a solution. This can be seen by comparing the nutrient profiles derived from two similar vermicompost based solutions. Despite the similarities in method, the solutions produced by *Churilova et al.*, (2019) and *Loera-Muro et al.*, (2021) differ greatly. *Table 1.10* shows differences in the “EC : Available nitrogen” ratios, of which *Loera-Muro’s* study had a more optimal concentration. The relevant factors which may have influenced outcomes include differences in time before flushing, pre-composting, and feedstock.

Table 1.10: A comparison between two vermiculture based solutions.

	Churilova (2019)	Loera-Muro (2021)
EC : Available N (ds/cm : mg/L)	1 : 88	1 : 526
Time in vermiculture (days)	70	84
Inputs	Cow paunch material	Kitchen waste (50%), animal manure (50%)

Without additional research, it is unclear which of these factors is plays a predominant role in improving available nitrogen relative to EC. With considerations for other known actors in nitrification, it may be sensible to suggest that the determining factor in the disparity between the two solutions can be attributed to the presence of cow manure. Compared to cow paunch material, manure tends to have a naturally higher nitrogen content. However, it is difficult to entirely attribute the elevated N in *Loera-Muro’s* solution to the manure itself entirely, as it only composes 50% of the feedstock. The other 50%, identified as “kitchen waste” highlights one of the obstacles facing research on consumer-level waste-based solutions. The variety of food preferences around the world causes broad terms such as “kitchen waste” to be vague in identifying the constituent feedstocks of a solution – although at larger scales this sort of sorting may be impossible to achieve.

The large spread of inputs and methodologies provides two approaches to exploring existing literature. It was determined that grouping by methodology was a more effective way to examine trends in the research area. Ultimately, the infancy of the research limits the scope of this review to understanding the effect certain methodologies have across a spectrum of inputs, as opposed to the understanding of the reactions of specific inputs to niche methods.

1.3.3 Current state of fertiliser development methodologies

Methodology is the primary factor researchers influence within FWBHF research. This review defines methodology as the application of processes which prepare, facilitate, or alter the bioconversion of food-waste into FWBHF’s. Current knowledge attributes the underlying success of a methodology to biological activity, highlighted by *Fig 1.2*, which identifies aerobic and anaerobic fermentation as the most popular method trialled. Fermentation, or digestion, is the breakdown of organic matter into inorganic compounds using microorganisms – a process which is essential to nutrient cycling in natural systems (*Garcia et al.*, 2018). While variable, bioconversion methods can be guided with a range of tools to enable the replication of results when utilizing a fixed feedstock. Confirming the theory that

identical methods will produce the same results is an essential element in the continued development of FWBHF's. Adhering to this assumption, this section covers the distribution of methodology across the literature included in this review and attempts to identify the associated strengths and weaknesses attributed to each method of FWBHF production.

By far, the most frequently used methodology was digestion, with 26 out of the 37 reviewed papers utilizing some form of fermentation. Fermentation was distinguished into aerobic, anaerobic, and novel forms due to the distinct differences in their mechanisms and outputs. A brief comparison between aerobic and anaerobic processes reveals that the primary difference is the presence of oxygen in the solution during the process. This absence of oxygen limits the respiratory activity from microbial populations, creating two drastically different microbiomes. The microbiomes deviate in process after initial glycolysis, where oxygen availability dictates the continuation of metabolic processes such as NAD⁺ generation and nitrification. In anaerobic settings, these do not occur and are replaced with less energetic interactions which produce ethanol or lactic acids (Buckel, 2021).

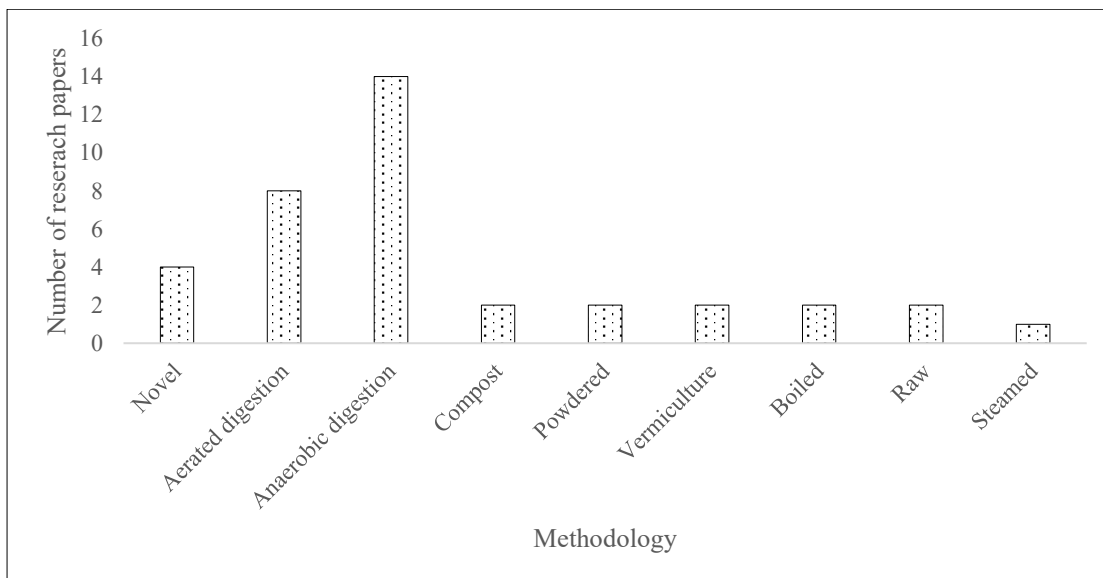


Figure 1.2: The distribution of methodologies utilized by the selected research papers ($n = 37$)

Thus, when considering the impacts aerobic and anaerobic digestion have on organic matter, the processes of carbon breakdown and nitrogen mineralization are the most impacted by the availability of oxygen. Carbon compound breakdown deviates after initial glycolysis, where pyruvate acids are either aerobically metabolized into CO₂ and H₂O, while anaerobically fermented pyruvate compounds produce either ethanol or lactic acids (Melkonian, 2021). Whether ethanol or lactic acids are produced is dependent on the resident microbial community – inoculations of fungal yeasts or bacterial *Lactobacillus*, respectively, can be used to dictate the output (Tristezza et al., 2016). Nitrogen mineralization, similarly, requires oxygen as an electron acceptor after anaerobic processes break organic nitrogen into ammonium (NH₄⁺). Without oxygen, nitrates (NO₃⁻) cannot be synthesized, and the resulting solution could be unviable due to risks of ammonium toxicity (Buckel, 2021).

Other elements, such as potassium and phosphorus do not directly utilize oxygen to become plant available. Phosphorus is often more available in flooded conditions, as the hydrolysis of phosphate compounds solubilizes inorganic phosphates – a process not limited by oxygen availability. Potassium availability is minimally influenced by anoxic environments as K exists predominately in inorganic form. Potassium ion availability is still primarily determined by the rate of organic breakdown (Fageria et al., 2011). Instead, the pH of the solution is important in determining the availability of plant macro- and micro-nutrients. Generally, a pH between 6.5 and 7 is preferred for majority of horticultural crops in hydroponic systems. While there are exceptions, this range provides the greatest variety of plant available nutrients. A slightly acidic solution solubilizes iron, zinc, boron, manganese, and copper which are unavailable in alkaline conditions.

Both aerobic and anaerobic methods of digestion create solutions that tend toward a lower pH. This is the result of either lactic acid production or through the formation of carbonic acids from dissolved carbon dioxide found within the solution. Generally, anaerobic solutions tend to have a more acidic base compared to aerobic pathways. This is attributed to the increased availability of H⁺ ions produced by lactic acid pathways (Abedi et al., 2020) as well as the tendency for aeration to displace dissolved CO₂ (Colt et al., 2013). The absence of oxygen tends to limit energetic respiration – preventing excessive volatilization and preserving a greater density of nutrients within the solution.

Despite the clear functional similarities between aerobic and anaerobic systems, the difference in outputs distinguishes the two as entirely separate methodologies. This has led to exploration into methodologies which exploit the benefits of both processes. These novel methods are either semi-aerated or multi-step. These systems are capable of altering their microbiota through populations of “facultative species” which can tolerate limited oxygen environments and exist on a spectrum between strictly aerobic and anaerobic species. They are ubiquitous across all but the strictest fermentation methods. Thus, with the understanding that there is no such thing as an entirely aerobic or anaerobic population, this paper differentiates between aerobic and anaerobic fermentation based on i) deliberate and stated intentions, ii) actions that result in a certain population being supported, and iii) final nutrient compositions.

The remaining methods, lack the interdisciplinary interest of traditional fermentation techniques. As a result, the specific microbial, chemical, and physical mechanisms of organic matter breakdown represent a significant gap in knowledge. Instead, based on provided nutritional analysis and diagnostic information, the next sections of this review will describe the limitations which face existing FWBHF research.

1.3.4 Nutrient deficiency and solutions

Plant available nutrients are first and foremost, dictated by the concentration of a given element within a hydroponic solution. Whether an element is present or absent can be attributed to an array of factors. This review has identified commonalities across studies that may have led to an impact on the availability of micro- and macro-nutrients. This section will explore the challenges and solutions that affect organic solutions.

As stated before, the unavailability of oxygen in anaerobic systems limits the mineralization of ammonia. This can lead to an imbalance between nitrates and ammonia – causing a deficiency in plant available nitrogen while risking ammonia toxicity. This behaviour is evidenced across several studies, with *Tikasz et al.*, (2019) finding that an aerated turkey manure produced a superior fresh harvest weight compared to a synthetic control. The methodology involved the aeration of various manures for 1.5 days. While the turkey solution

was the only treatment to surpass the control solution, this was unlikely the result of a superior feedstock. The success of this solution could be attributed to the collection method of the turkey waste – which left it resting for six months prior to intentional fermentation. This resting period is likely to have allowed for a more complete mineralization compared to the 1.5-day aeration period all other treatments were given. Investigation of the nutrient analysis shows that the successful turkey treatment had an $\text{NO}_3^-:\text{NH}_4^+$ ratio of approximately 10:9, while the best performing chicken treatments had a ratio of 3:350.

This prompts questions into the ideal time required for complete mineralization of organic nitrogen into inorganic forms. While prolonged periods of fermentation ensure total mineralization of organic nitrogen, the quandary in this practice is that there is evidence that would suggest prolonged fermentation of any sort can lead to the eventual loss of other key nutrients. This behaviour is observed across several studies. *Riera-Vila et al.*, (2019) compared raw and anaerobically digested brewery wastewater in a growth trial. A comparison of the two solutions shows an N:P content of the raw solution contained 7 : 80 (mg/L), while the digested solution contained 170 : 22 (mg/L). The increase in nitrogen is the result of supplementary ammonium nitrate, however the phosphorus content has decreased to only 25% of its original content. This is consistent with existing research, which shows that prolonged aeration of a solution increases alkalinity, which reduces soluble P by up to 75% within the first 24 hours (Zhu et al., 2001). This sort of degradation is tolerable for phosphorus, as the ratio of phosphorus required relative to nitrogen is significantly less demanding than the ratio of nitrogen to potassium.

Potassium degradation in extended fermentation periods was not observed in any study. Phibbunwatthanawong (2019) performed nutrient analysis at multiple stages of fermentation, between day 15 and 30 potassium availability was stable. Although, literature suggests that continued fermentation after ammonium has been converted to nitrates may cause a reduction in available potassium. This can be attributed to the conversion of positively charged ammonium molecules into negatively charged nitrates - shifting the microbial community into favouring denitrifying micro-organisms such as *Bacillus* and *Pseudomonas* which require an electron donor to reduce nitrates into nitrites (Shukla et al., 2021). Studies into the optimisation of *Bacillus* shows that potassium improves the rate of exopolysaccharide production – a proxy for the rate of metabolism (Lee et al., 1997). This suggests that there is an optimal timeframe for anaerobic fermentation. The premature usage of a solution risks nitrogen deficiency or ammonia toxicity, while an excessive fermentation period may lead to deficiencies in other key macronutrients. Further research may be necessary into the exploration of an ideal timeframe for the efficacy of nitrogen mineralisation in relation to the plant availability of phosphorus and potassium.

In order to compensate for nutrient deficiencies, there are a range of solutions that have been explored. Outside of altering the direct inputs, alternative methods of nitrification, as well as the use of organic and synthetic supplements have been trialled. A potential solution to balancing potassium degradation alongside nitrification is explored in *Pelayo-Lind et al.*'s (2019) trial. This method utilises an integrated nitrification reactor, which actively mineralises raw biogas digestate during the growth period of hydroponic crops. Using this method of “active nitrification,” an appropriate NPK ratio of 18:4:25 was able to be established at the beginning of the growth trial. With active nitrification and specific pH management, the treatment was able to meet the yield outcomes of the synthetic control when given an additional week of growth. This delayed vegetative growth may be symptomatic of a nitrate bottleneck caused by the limitations of an active conversion process. Maintaining a more neutral environment is essential, as an acidic environment generally inhibits the

nitrification process (Tarre et al., 2004). This suggests the active conversion process may be limiting the available nitrogen content. It may be worth further study to into a two-part system, which uses a pre-fermented solution in conjunction with an active fermentation system to optimise macro-nutrient release and availability.

Alternative methods of “active nitrification” have also been explored by *Kawamura et al.*, (2014) and *Tong et al.*, (2021). While neither utilise fermentation methods, both strive to supply the necessary nutrients through the use of microbial activity in slower ways that accommodate for perennial crop species. *Tong* (2021) explores the potential of utilising a bokashi compost substrate as a method for introducing nutrients into a hydroponic system. This method relies on the eventual conversion of nutrients from within a bio-active compost to provide the necessary resources required for efficient plant growth over time. The compost was made from a combination of wheat bran, oil cake, and chicken manure with an intentional inoculation of a microbial starter. This method proved to return superior yields relative to the direct application of the compost leachate. This could be due to the benefits of having a delayed release of phosphorus and potassium – which encourage flowering, while early on availability of N would increase vegetative growth prior to the reproductive stages of the crop. By emulating a rich soil, the substrate is able to release nutrients in a controlled fashion while maintaining a stable microbiome for the crop rhizosphere. This biome could manage nitrifying bacteria which prevent diseases as well as optimise nutrient uptake relative to the exposed roots of traditional hydroponic systems. The use of microbial actors to promote plant growth will be further discussed in *3.6 Microbial Activity*. The only compost treatment tested was a 40-40-10 combination of perlite, coco coir, and the bokashi compost. Future studies should alter ratios and accompanying substrates to optimise the release of nutrients over time and in appropriate quantities.

Kawamura et al., (2014) adopts a “slow release” philosophy like *Tong et al.*’s (2021) bokashi compost. By drying and powdering a combination of meat, vegetables, and café food-waste, the treatment involves a daily dose of powder each day to produce the necessary nitrogen required for plant growth. The nitrifying population resides in a bag of tree bark, and other macro- and micro-nutrients are synthetically supplied. A key difference between the two studies is the system type used. *Tong* utilises drip irrigation, while *Kawamura* adopts a floating system. Drip irrigation benefits from relying on the retentive capabilities of its substrate to maintain an appropriate environment for plant roots, conversely floating systems involve the complete submersion of a plants roots into the growing solution. *Kawamura* found that without active water movement, yield outcomes between the treatment and the control were significantly different. The addition of water movement may encourage nitrification through aeration or prevent pest and diseases from establishing. The nature of these slow-release technique is ideal for perennial, fruiting crops such as tomatoes. The slow release of nitrates over time does not lead to excess vegetative growth and thus inhibiting flowering or fruit development.

As observed in *Kawamura*’s (2014) study, the development of organic solutions is not constrained to the function of being an “all-in-one” solution. Many papers seek to determine if additions or substitutions can assist growers in achieving yields similar to commercial fertilizers. The ability for a solution to function independently of supplementation varies from study to study – although feedstock is generally the primary determinant of which supplements a solution will ultimately need. For example, potassium deficiency is a frequent occurrence in aquaponic systems, as marine species primarily produce nitrogenous based waste, and as such synthetic additions of potassium are often added to compensate for the

missing element (Yep et al., 2019). This deficiency was observed across all 5 papers that utilized fish based aquaponic sludges as a primary feedstock.

Table 1.11: The NPK ratios from 7 FWBHF's which utilized the fermentation of aquacultural waste.

Author/s	Species	Method	NPK ratio
<i>Canna (2022)</i>	Control	Synthetic (Aqua Vega A/B)	6 – 1 – 6
<i>Ezzidine et al. (2020)</i>	Salmon	Aerobic Fermentation	9 – 1 – 1
<i>Ezzidine et al. (2020)</i>	Salmon	Aerobic Fermentation	90 – 16 – 13
<i>Ezzidine et al. (2021)</i>	Brown Trout	Aerobic Fermentation	62 – 18 – 11
<i>Panana et al. (2021)</i>	Pikeperch	Aerobic Fermentation	12 – 19 – 2
<i>Delaide et al. (2021)</i>	Pikeperch	Aerobic Fermentation	11 – 0.1 – 0.3
<i>Goddek et al. (2016)</i>	Nile Tilapia	Aerobic Fermentation	53 – 3 – 17
<i>Goddek et al. (2016)</i>	Nile Tilapia	Anaerobic Fermentation	56 – 3 – 16

Table 1.11 shows an imbalanced NPK ratio observed across all papers that utilized fish-based aquaponic sludges as a primary feedstock. Future research in this area can explore methods to compensate for the low inherent potassium content. Another concern surrounding aquaponic based fertilisers is the presence of heavy metals, such as cadmium, lead, and mercury.

Ezzidine utilizes chitosan, a metal chelator derived from the carapaces of ocean crustaceans as an organic method of managing heavy metals without reducing concentrations of metals necessary for plant growth. Chitosan was also utilized by *Sawain et al. (2018)*. *Sawain* explored the calcium rich carapace of crab shell as a supplement to an aquaponics system. When crushed and mixed in with manure, the resulting sludge significantly improved watercress wet weight without affecting the survival rate of catfish over a 30-day period. The direct effects of chitosan have not been isolated in hydroponic or aquaponic systems. As such, the nutritional contribution of chitosan requires additional investigation, as it is largely insoluble at neutral and higher pH levels.

To achieve optimal levels of nutrients, both organic and synthetic supplementation can be utilized. *Sunarya et al., (2020)* anaerobically fermented mushroom production casings, known as baglog, to produce a solution which, while sufficient for growth, did not have the required level of nitrogen for an optimal yield. The study supplemented the baglog with cow manure and banana peels, improving the NPK ratio from 10:4:13 to 14:7:23. Similarly *Arshad et al., (2018)* utilizes banana peels to supplement K in a goat manure-based solution. Neither solution was trialled in growth systems, however, nutritional analysis expresses the potential to supplement a fertiliser with other products to optimise its composition.

Synthetic remediation presents itself as a more reliable and convenient option. *Liedl et al., (2004)* found that ammonia toxicity caused by a poultry-based treatment resulted in tomatoes with fewer and smaller fruits. This was remedied by the supplementation of calcium nitrate, improving the nitrate-ammonia balance – leading to the recovery and improved performance

of the crop. Other nutrient deficiencies, such as magnesium deficiencies were also discovered, and were remedied by a foliar treatment of magnesium sulphate – boosting yields to meet that of the control treatment. An extension of this remediation would be to simply substitute portions of commercial solutions.

While not a complete replacement, the reduction of use in mineral fertilisers can still create significant impact. Of the 11 papers which trialled substitution, 10 were able to substitute a portion of the control solution to the point where there was no significant loss in yield performance. The range of trialled substitute tends to range between 20% up towards 80%. The highest rate of substitution without significant loss in yield was observed by *Anggraini et al.*, (2020) at 66%, utilising an anaerobically digested tofu wastewater. In particular, *Kawamura et al.*, (2014), *Abd-Elmoniem et al.*, (2001), and *Al-Mehadee et al.*, (2020), found that supplementing the nitrogen provided by organic sources with synthetic P, K, and micronutrients was an effective strategy in maintaining yield performance relative to the control.

The benefit of substitution alleviates dependency on mineral solutions, reutilises food-waste, and improves producer resilience to fluctuations in the quality and availability of organic nutrient. *Siddiqui et al.*, (2021) found that seasons affected the availability of certain nutrients found at the fruit market. In the winter season, phosphorus deficiency was noticed, with the produced solution having an NPK of 12 – 1 – 35, compared to spring solution ratio of 2 – 1 – 8. This seasonal difference introduces another dimension of consideration when designing food-waste based hydroponic solutions. There will inevitably be seasonal preferences for certain food-products based on price, consumer demands, or supply availability – highlighting the importance of resilient production techniques. The aforementioned substitution allows producers to incorporate organic sources of nutrients into their solutions, providing a more stable source of nutrients for their crops while contributing towards a more environmentally friendly production method.

Substitution, however, presents its own set of challenges. Economically, adoption of substitutive solutions may have limited cost efficiency, as the organic component of a conventional-organic hybrid solution may be more expensive. Furthermore, partial substitution of a solution may inhibit the availability value-adding labels such as “organic”. Scientifically, it may be difficult to produce a solution with bioactive components to interact with raw, soluble nutrients. There is a risk that the sudden introduction of nutrients from synthetic solutions may encourage eutrophication or the propagation of pests and disease (Schwarz et al., 2004).

1.3.5 Salinity and Sodicity

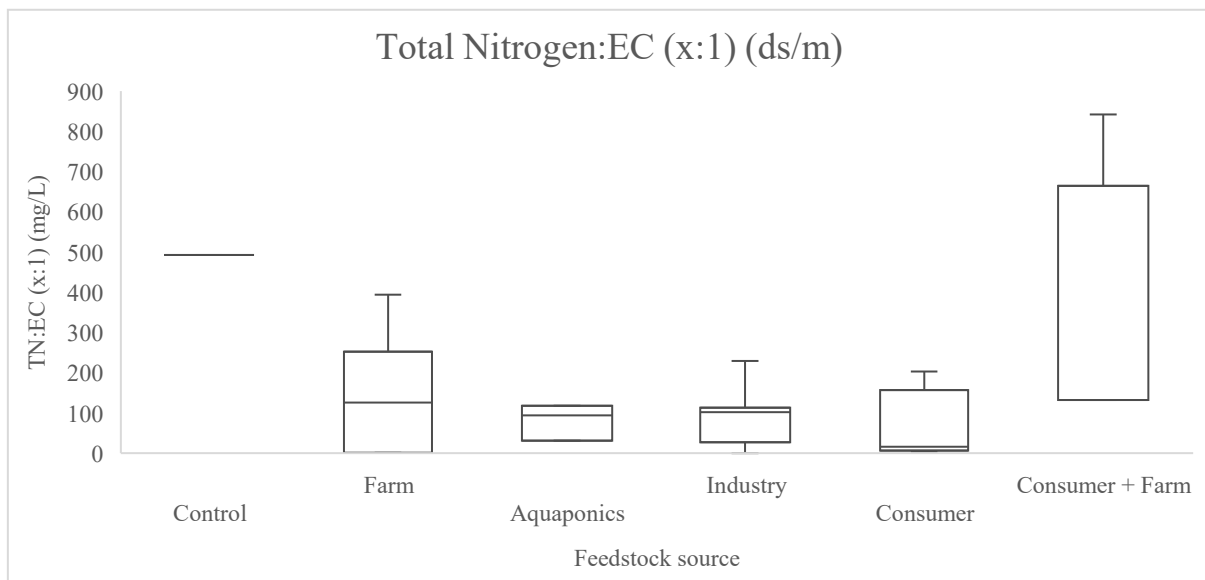
A dense pool of accessible nutrients is key for a successful yield, however there must be considerations towards the salinity of a solution. The balance between nutrient availability and salinity is critical in a viable nutrient solution. Salinity is measured by electrical conductivity (EC) and suggested optimal levels are contentious. Generally, however, tolerable levels of EC range from 0.9 to 2.5 dS/m in hydroponic solutions for leafy greens (Ding et al., 2018). If the salinity exceeds a plants tolerance level, osmotic adsorption of minerals through the roots of a plant may be inhibited (Hosseini et al., 2021). As such, the three key parameters that must be observed are nutrient levels, pH and EC. While the three share many interactions, none are strictly dependent on one another – with means of independent adjustments for all three factors.

Managing salinity has been identified as a particularly difficult challenge in the development of food-waste based solutions. This is attributed to the addition of salts in cooking, as well as

the high levels of naturally occurring sodium in protein rich waste, such as meat. This section explores sources of salinity as well as strategies to manage and maintain efficacy in the face of excessive salinity. Of the 37 papers reviewed, 19 provided undiluted EC values for their trialled solutions – allowing for the development of figure 3, which shows the distribution of total nitrogen relative to EC (TN:EC) separated by feedstock type. The importance of this relationship is predicated upon the osmotic capacity of nutrient adsorption based on the salinity of a solution. As EC increases, the osmotic potential of a plant decreases, causing nutrient deficiencies in a crop – regardless of the nutrient content within the solution.

Each solution has been standardized to represent a nitrogen content when EC is set to 1, and benchmarked against a standard Hoagland’s solution, which when at an EC of 1.6 provides approximately 210 mg/L of inorganic nitrogen.

Excess salinity is found across a range of processing methods, from digestion, to boiling, and steaming. Although some crops are more salt tolerant than others, common hydroponic species, such as leafy greens, have lower salinity tolerances compared to other species (Albornoz et al., 2015). Seemingly, the predominant factor in solution salinity is the input, as shown in figure 1.3.



When allotted into “Farm”, “Industry”, and “Consumer” categories, clear distinctions between groups can be made. Referring to table 12, there is a marked difference in between feedstock sources. The average TN:EC ratio drops is similar across all but consumer level inputs. While the standard deviation is high, relative to the mean, the average TN:EC ratio is distinctly different in consumer-based solutions. This trend could be explained by the nature of feedstock, as farm and industrial level inputs tend to be unrefined and unreduced byproducts. An acceptable ratio of TN:EC is achieved when a crops nutrient needs are met without surpassing its inherent tolerance of salinity.

A closer examination at consumer-based solutions finds two studies which provide insight into the skewed balance. 2 papers specify the usage of restaurant or café waste. *Siddiqui et al.*, (2021) utilises waste from a service club, and a fruit and vegetable market across several seasons. This research isolates the relationship between feedstock and salinity. All feedstock types were steam heated, ground, minced, and strained to produce a liquid fertiliser. While the solution was not utilised in any growth trials, nutrient analysis found differences between

the N – P – K – Na ratios of the treatments. The spring fruit market produced a ratio of 2 – 1 – 8 – 1, whilst the service club treatment had a ratio of 4 – 1 – 12 – 17. This is likely due to the “pub style” foods, with steak, hot potato chips, chicken schnitzel, gravy, and an assortment of seasoned vegetables. This reflects the importance of constituent materials in managing salinity.

In terms of methodology, boiling distinguished itself as a particularly inefficient method of developing an effective solution. *Arshad et al.*, (2018) boiled goat manure mixed in with banana peels until a 20L vat of liquid was reduced to 5L. The nutrient profile derived from this produced a solution with an TN:EC ratio of 1:3. A study by *Yusuf et al.*, (2021) explored the potential of sea lettuce (*sp. ulva lactuca*), after it had been heated up to 70°C and strained. While there was no available nutrient profile, the solution was dosed to reach a TDS of 560, 800 and 900ppm based on the growth stage of the lettuce crop. None of the treatments could meet the efficacy of the control. It is likely that simply boiling organic material does not extract adequate available nutrients. Future research should continue exploration into the effects boiling has on nutrient extraction and if there is potential to avoid the excess salinity produced by liquid reduction.

Table 1.12: The Total Nitrogen:electrical conductivity ratios categorised by feedstock source.

	TN:EC (x :1.6) (mg/L: ds/m)				
	Farm (n = 17)	Aquaponics (n = 3)	Industry (n = 7)	Consumer (n = 4)	Consumer + Farm (n = 4)
Mean	140	81	96	60	310
SD	119	36	67	83	307
Median	126	94	102	16	132

Just as important as the ratio between nutrients and salinity, is the total saturation of an element. *Bergstrand et al.*, (2021) aerobically fermented a combination of manure, slaughter residues, and “other organic food-waste,” which was diluted and dosed at rates where EC (ds/m) was equal to 1, 2, and 4. Notably, the solution with an EC of 1 was the closest to emulating the yield efficacy of the control. The control had an EC of 2, and unusually, the organic trial with the equivalent EC did not perform as well. While all 3 trials have a linearly increasing NO₃⁻, there is also a linear increase in NH₄⁺. Ideally, a 3:1 ratio of NO₃⁻ to NH₄⁺ is preferred, with low levels of ammonium toxicity being alleviated by the presence of nitrates (*Du et al.*, 2021; *Zhu et al.*, 2021). The control solution contained a ratio of NO₃⁻: NH₄⁺ of 21:6, while the organic solution, at all application rates, was closer to 6:5. It is possible that the increase in total available nutrients may have led to a concentration of ammonium that inhibited plant growth.

A final consideration is that solutions with excessive EC still have potential functionality as supplements. For example, *Li et al.*, (2021) produced condensed molasses soluble (CMS), a solution derived from anaerobically fermented molasses wastewater with an undiluted EC of 23ds/m. Even when diluted, the ratio of NPK to EC cannot accommodate plant production. Despite this, a minor supplement of 100mg/L of condensed molasses soluble (CMS) at the seedling stage was found to enhance the growth of canola to a significant level when used in conjunction with inorganic fertilisers. This improvement could be due to the deposition of early nutrients, although it may also be attributed to microbial activity in the rhizosphere and solution. Alternatively, the density of nutrients provides a promising research area for desalination projects to improve the viability of wastewater reutilisation. Although technology has improved over time, current commercial technologies are limited to the

removal of all mineral elements – including nutrients – from a solution using reverse osmosis technology. Even so, the operation of such machinery at an industrial scale would require excessive amounts of capital and ongoing funding (Ang et al., 2022). Current research into selective reverse osmosis techniques show that there is potential in the future for such technology to be available (Han et al., 2022).

Sub-system types and growing techniques

Food-waste based solutions have different demands relative to conventional solutions, evidenced by the unanimous success of the control solutions in the studies included in this review. Sub-system and nutrient application techniques have an unmeasured impact on the success of a given organic solution. As such, there are no links between subsystem types, nutrient application methods, and the yield outcome of any organic fertilisers.

In total, 7 different sub-systems were identified i) Ebb and Flow (EF), ii) Nutrient Filter Technique (NFT), iii) Deep Water System (DW), iv) Wick System, v) Floating System, vi) Drip irrigation System (DI). NFT systems were the most used systems ($n = 13$, Figure 1.4), with other systems having a rate of usage between $n = 6$ and $n = 1$.

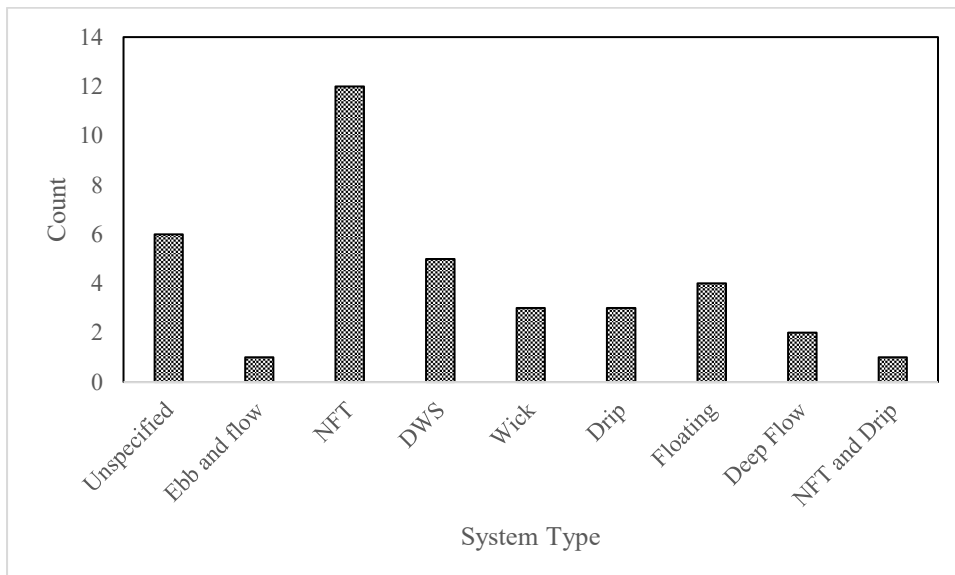


Figure 1.4: Distribution of hydroponic system types across reviewed literature ($n = 37$)

The low number of replications across the range of system types limits the comparability between literature. Hence, statistical analysis correlating system types with yield outcomes based on the identified literature in this review is unreliable. External literature comparing the effects of different system types is limited. Within this review, 35 of the 37 papers reviewed utilized one form of hydroponics system. The outliers, Churilova et al., (2019) and Gimenez et al., (2020), compared the performance of conventional and organic solutions across two system types: DWS and DIS.

Gimenez et al., (2020) utilised a compost-based solution on the growth of baby lettuce in DW and DI systems. Both systems produced statistically similar fresh weight yields to the control. The DW system averaged a slightly higher yield, with an approximate 10% greater fresh weight (DW = 2.22g, DI = 2.00g). This difference, however, was exacerbated when both treatments were inoculated with the root disease *pythium*. It was found that the DW system had suffered less yield loss, increasing the gap to around 20%. Conversely, however, the DI

system produced lettuce with lower nitrates, a known carcinogen (Chazelas et al., 2022), as well as a greater density of phenolics and flavonoids, which are associated with nutritional value and flavour (Yao et al., 2007). Further research should seek to identify the impacts system types have upon the quantity and quality of yields. Additionally, optimization of crop/sub-system combinations in conventional systems should extend towards identifying optimal crop/sub-system/organic fertilizer combinations.

The initial consideration when applying a nutrient solution is the rate of application. Ultimately, this determines both the concentration of the solution, as well as manages fluctuations in salinity, pH, and nutrient availability for the plant throughout its growth stages. The method for application can be broken down into *Temporal* and *Conditional* factors. *Temporal* considerations include the “when” of application: *i*) Once at the beginning of the growth period, *ii*) at x time interval, *iii*) at x EC, *iv*) at x nutritional density. *Conditional* considerations ask “how much” solution is required, including: *i*) To x EC, *ii*) to x nutritional density, *iii*) to $x\%$ of total volume, *iv*) x volume.

There is no strong link between temporal factors and the success of trials. Trials which dosed to the required nitrogen had a higher rate of success relative to other methods (Kechasov et al., 2021; Tikasz et al., 2019). Papers which dosed based “to x EC” generally selected to meet the recommended EC of conventional solutions (Wang et al., 2018; Sunarayo et al., 2018; Abd-Elmoniem et al., 2001). The most representative technique, however, was to dose at fixed dilution rates without further explanation in methodology (Nanik et al., 2021; Liedl et al., 2006; Guntara et al., 2021). Future research should strive to specify both temporal and conditional factors when noting methodology.

1.3.6 Microbial Activity

This review explores two aspects of microbial activity, *i*) the role of microbes as a tool for the production of organic hydroponic solutions, and *ii*) the role of microbes during cultivation. The perspectives adopted by this review challenge conventional expectations of sterile hydroponics. Traditionally, hydroponic systems strived to minimise microbial activity to serve its original function as a research tool for growing in a *ceteris paribus* scenario, with the goal of eliminated unpredictable or pathogenic microorganism behaviour. As a result, conventional growth solutions are synthetic, minerally sourced nutrients – sterile by nature. Today, industrial applications of hydroponic systems retain this culture of sterilisation (Garland, J.L, 1994). Extensive research has been undertaken to understand, and subsequently minimise the presence of microbial communities within systems. However, there is potential to apply existing understanding of micro-community interactions to develop a balanced ecosystem within growing systems. While no paper compared an inoculated with a non-inoculated organic solution, papers such as Kechasov et al., (2021) created synthetic treatments with an identical nutritional composition of the organic solution. A comparison of the treatment and its synthetic clone found that there were less trusses, but greater fruit weight in the organic solution. This could be attributed to the availability of available nitrogen in early stages of plant growth but may also indicate another factor augmenting the performance of the solution.

The role of microbes in the development of hydroponic solutions

Microbial activity played a role in developing FWBHF’s in 32 of 37 reviewed papers. The most replicated method was fermentation or digestion ($n = 22$). This process relies on microbial actors to remineralise organic material into plant-available inorganic nutrients. An unmanaged fermentation will naturally develop, and host microbial communities based on the environment and available sources of nutrients. This spontaneous community will break

down organic matter into a variety of forms over time. While spontaneous, the microbial communities which establish and populate a fermentation can be influenced by manipulating environmental factors such as temperature, aeration, and inputs. Using these tools, researchers can optimise the repeatability, nutritional content, and quality of a solution through the cultivation of ideal microbial populations. Temperature can be adjusted to favour certain species; particularly as different stages of breakdown occur. Alterations in temperature can affect the speed of digestion, as warmer environments increase microbial activity, and thus accelerate stages which rely upon microbial agents (Youcai et al., 2021). Additionally, certain temperature ranges can favour particular microbial biomes – such as increasing temperatures up to 60°C to kill parasite eggs effectively in anaerobic fermentations (Youcai et al., 2021). Similarly, the rate of aeration influences the speed of nutrient breakdown, such as increasing rates of mineralisation of ammonia. In addition, aeration can also be used to increase pH through the removal of CO₂, or prevent the development of unwanted anaerobic species (Colt et al., 2013; Thauer R., 1998). An example of application could be to lower temperatures and begin aeration of an anaerobic solution as it begins methanogenesis. The combination of increasing available oxygen and cooling the solution prevents the development of methanogens, and pivots NH₃ breakdown away from CH₄ and towards solubilised NO₃ – a plant available nutrient.

Alteration of a FWBHF through inputs can be distinguished into “primary feedstock” and “additives”. Primary feedstock includes the bulk of the nutrient source, while additives cause change through chemical, physical, or biological influences. Primary materials include the bulk of organic matter which is to be broken down into the organic solution. Ideal primary inputs will also include a balanced composition of NPK rich matter coupled with carbon-based nutrients to supplement microbial populations. Additionally, primary inputs may be pre-processed – converted from food-waste into a primary input by a number of physical alterations, including heating, mashing, chopping, crushing, or otherwise alter its physical form to improve surface area and microbial accessibility. Chemical additives can include pH manipulation, which alter nutrient availability and microbial communities, or include enzymatic agents such as proteases which may accelerate the breakdown of feedstock. Physical additives include increasing available surface areas for microbial colonisation. This generally consists of inert, porous material such as volcanic rocks, filter media, or sponges. By improving the population and stability of the microbial environment, the rate of fermentation increases. Biological additives are generally the direct inoculation of preferred microbial species. By exploiting the closed system of fertiliser development, a microbiome can be inoculated and managed to cultivate a preferred microbial species - leading to potential improvements in replicability, nutrient availability, and/or time of production.

The role of microbes during cultivation of plants

In growing systems, tradition dictates a sterile environment. However, upon reviewing historical literature, this philosophy was known to be misguided as far back as 1994. Still, the development into the eradication of microbial communities continued, with multiple commercially available of chemical additives designed to purge hydroponic systems of microbial communities. An example of a beneficial community is the naturally occurring population around crop rhizospheres. These populations are dedicated to the decomposition of dead tissue and root secretions, improving resilience to disease and improving nutrient uptake capacities (Porter, G., 1994). Recent research has provided a more intentional and active role for microbes, with *Pelayo Lind et al.*, (2021) utilising an aerated nitrification reactor which actively converted ammonia into nitrates during the growing cycle.

Such beneficial microbes found within hydroponic systems have been identified as “plant growth promoting microorganisms” (PGPMs) (Sheridan et al., 2017). Such organisms have been greatly studied in recent years, with variants of traditional soil-based PGPMs, such as *Arbuscular mycorrhiza* fungi and *Rhizobium* species (Dhawi F., 2023). The identification of beneficial strains can enable growers to adjust environmental conditions to cultivate these species to improve yield outcomes and reduce use of pesticides, algacides, and synthetic fertilisers.

A final consideration is how biologically active a FWBHF is when it is ready for use in a hydroponic system. A sterilised solution presents itself as a vulnerable environment for any microbial population to exploit – wanted or not. Options to continually sterilise a solution without disturbing root biomes include UV sterilisation. However, a study into the efficacy of UV sterilisation as a preventative to *Pythium* found that while there were short-term benefits, eventually a population would establish itself in the rhizosphere of plant roots anyway – especially as non-target bacterial species start to decline as a result of irradiation (Zhang et al., 2000). An alternative, that has been, and is continued to be explored is inoculation of beneficial or benign bacteria that can prevent plant diseases from establishing impactful populations. The introduction of *Bacillus cereus* into hydroponic systems was shown to lower the rate of *Pythium* root rot occurring by up to 20% (Lee et al., 2015). Additional research utilised *Streptomyces griseoviridis* as an alternative to physical and chemical methods to successfully control *fusarium* wilt (Lee et al., 2015).

Inoculation and cultivation of microorganisms

This review has found no strong evidence for inoculation or supplementation of soluble carbohydrates as determining factors for the success of a food-waste based solution. The direct inoculation of microbial strains was only mentioned in 10 of the 37 research papers. Four of these papers were digestate based, one was powder based and the last was raw. None of the strains were specified by species or name. It is difficult to prove a correlation between the inoculation of microbes and an altered outcome in the resulting yield of a trial. In future studies it may be worth comparing the effects of deliberate inoculation with otherwise identical inputs, systems, and crop species.

Similarly, the effects of simple carbohydrate additives were not convincingly successful or unsuccessful. The encouragement and facilitation of microbes did not play a pivotal role in the performance of a solution. Across the solutions, nine utilised simplified carbohydrates, most frequently sugars of white sugar, brown sugar, and molasses.

Inoculation

Inoculation involves the deliberate introduction of a specific or group of micro-organisms into a solution. Through inoculation the micro-community of a solution can be customised to perform specific functions. These functions can range from establishing a community that can optimise breakdown, prevent disease, and enhance rhizosphere activity in plant root systems. For example, when digesting meat, the protected environment of a digester limits processing agents to microbial activity. Without naturally occurring arthropods and vertebrates, microbes are limited in their ability to penetrate tissue material. While physical barriers can be broken with physical processing techniques, microscopic elements such as cellulose, lignin, and proteins still require chemical or biological breakdown. A potential solution may be the introduction of specialised microbes, such as proteolytic bacteria can be introduced to produce protease enzymes and accelerate the breakdown process of the inputs. This can be further optimised to include multi-stage inoculations depending on the

breakdown stage of the input. *Xu et al., (2022)* performed a multi-stage inoculation utilising different bacteria species on composted food-waste and found that this process expedited the rise in temperature and extended the thermophilic period from four to seven days – expressing a prolonged period of elevated microbial activity. Additionally, the resulting digestate had a more complete mineralisation, with only 10% of the initial NH_4^+ content remaining after the trial period – with neither the control nor one-stage inoculation being able to reduce the NH_4^+ content beneath 33% in the same time period.

Methodology with details of inoculation were limited, although several stand out as promising avenues for future research. *Figueroa et al., (2015)* exposes an anchovy wastewater-based solution to an autoclaving process prior to fermentation. Sterilisation of a treatment solution prior to fermentation is a unique method in this review. Researchers can inoculate their nutrient rich solution with specific strains of bacteria, such as *Bacillus* that are optimal for the breakdown of their specific inputs. Whether or not this additional step impacts the outputs is unknown, as a non-sterilised treatment group was not present. Future research exploring the impacts of sterilisation may enable more reliable inoculation stages – particularly exploring whether sterilisation pre- or post- fermentation is more effective in managing a productive growing system.

Within the scope of this report, no paper reported specific inoculant species. 10 papers reported deliberate inoculation during the development of their solutions. Eight of these were digestates. This area of research is generally underreported. Future research should strive to identify, as best as possible, the selected strains of inoculants, as well as time of inoculation and conditions of the system.

Cultivation of bacteria

Whether inoculated or not, a micro-biome can be encouraged to grow through the addition of soluble carbohydrates such as sugar or molasses. This provides, in theory, several benefits regarding the longevity, expansion, and efficacy of microbial colonies. The presence of sugar provides a fast source of energy for colonies to expand and establish quickly in a solution (*Lievens et al., 2015*), it also allows microbes in solutions with primary inputs composed of fibrous vegetation with complex polysaccharides – such as bamboo or sugar cane stalks– to have a source of nutrients while the material is slowly broken down. Only 8 papers reported additives directed for microbial consumption. *Phibbunwatthanawong et al., (2018)* measured the effects of a fermentation with and without sugar. Utilising ethanol slop, sugarcane leaves, and molasses in differing ratios, fermentations were anaerobically fermented for 30 days.

Table 1.13: Ratio of ethanol slop, sugarcane leaves, molasses, and filtered water (volume, weight, volume, volume) utilised in Phibbunwatthanawong et al., (2018).

Treatment	Ethanol slop	Sugarcane leaves	Molasses	Filtered water
F1	1	0	0	0.25
F2	1	0	0.1	0.25
F3	1	0.1	0	0.25
F4	1	0.1	0.1	0.25
F5	1	0.25	0	0.25

Nutrient analysis was performed once at 15 days, and again at 30 days. Day 15 found that the F4 solution had the highest nitrogen and potassium content. However, by day 30, F6 had a superior nitrogen and potassium content. Despite this, in the ensuing growth trial, the lettuce

(*Lactuca sativa*) yielded the greatest fresh weight was F3, matching the synthetic control in terms of fresh and dry weight. A comparison between F3 and F2 shows that the source of carbohydrate may play a role in the conversion of food-waste into a functioning nutrient solution. Molasses represents a relatively simple carbohydrate which does not require a diverse microbial community to break down, while sugarcane leaves require more specialised microbes which are capable of processing cellulose, lignin, and other structural materials. F3 may cultivate a more diverse community, providing greater opportunities for optimal spontaneous fermentation to occur. This highlights a gap in knowledge regarding the selection of supplements for microbes. Further research into the efficacy of long- and short-term sources of carbohydrates, as well as the resulting microbial communities may be key in identifying microbial species worth inoculating in future methods.

Current literature does not explicitly explore the role of sugars in food-waste derived hydroponic solutions. However, literature from fields in microbiology specialising in waste breakdown have identified the potential drawbacks of soluble carbohydrate additives. As shown in F2 of *Phibbunwatthanawong's* research, additions of sugar may hinder the development of a balanced biome. Additions of sugar should be carefully considered – especially in solutions where the primary inputs are rich in proteins. In the decomposition of meat, proteolytic bacteria play an essential role in the breakdown of protein. However, proteolytic bacteria have a relatively slower growth rate compared to bacteria that rely solely on carbohydrates to grow (Kieliszek et al., 2021). Thus, when adding sugar, the amount added should be calculated such that it provides adequate energy to the relevant microbial communities without excess sugars. Excessive sugar can lead to the growth of unwanted microbes and impede the development of microbes better optimised for the breakdown of proteins and other more resilient carbohydrate sources (Horvath et al., 2020).

Remaining literature probes at the potential effects of carbohydrate supplements in hydroponic systems. *Kano et al.*, (2021) uses corn steep liquor (CSL) as an additive to develop a rhizosphere community. With the goal of cultivating bacteria capable of mineralising organic nitrogen, it was found that when used alongside a commercial organic solution, the fresh weight of lettuce was able to meet the efficacy of the synthetic control if given an extra week prior to harvest. While there was no specific study into the impacts of the CSL itself, this research continues exploration into the idea of a biologically active hydroponic system. Future studies should explore the dynamics of supplements and microbial communities in hydroponic systems during cultivation.

1.4 Gaps in Knowledge and future recommendations

This section is organised in order of the discussion, identifying key gaps, and recommending potential avenues in feedstock, methodology, and microbial activity, for future research. Additionally, recommendations in reporting standards are also included.

Feedstock

Feedstock in the context of this review has a strong focus on the development of a functioning solution, however consideration towards differences in feedstocks based on consumer behaviour may also play a key role in organising future research. While largely auxiliary to the primary focus of this report, regional variations in food waste arise from socio-economic, cultural, and geographic differences. For example, households with increased income tend to consume – and waste – proportionally greater amounts of fresh produce and proteins compared to carbohydrates (Ishangulyyev et al., 2019; Barrera et al., 2021). An example of cultural preference influencing food waste is a vegetarian population

producing food-waste with a lower average protein content compared to a more carnivorous population (Vatika et al., 2018). Finally, the geography of a region influences what is readily available, such as rice in warm, wet climates compared to wheat in drier, cooler climates (Sharma et al., 2022). All these factors may influence the composition of a feedstock, which in turn may impact the effectiveness of particular methods and the nutrient composition of a final product. This review identified nine papers which utilised waste generated from consumer or retail levels, only two papers provided complete profiles of the feedstock (Gimenez et al., 2020; Yusuf et al., 2021). The remaining seven papers identified food waste as “café/kitchen/industrial kitchen-waste”. Improved reporting and research exploring regional variations in food-waste clarifies directions for future research by organising processing methods based on feedstock similarity – ultimately optimising processing methods for local food-waste sources. Finally, the functional requirements of a FWBHF vary regionally. Consumer preferences determine whether a FWBHF is of sufficient efficacy. Simply, if a population demands fresh strawberries, the FWBHF must satisfy the nutritional requirements to yield profitable amounts of strawberries. Understanding regional food-waste availability and produce preferences familiarises researchers with their feedstocks and the desired nutritional outcomes they must meet.

Quantifying how feedstock interacts with processing methods is the backbone of refining FWBHF research. Interactions can be primarily quantified by yield outcomes, although assessing nutrient content, food-safety risks, and disease resilience may also prove useful. Along with this, unique interactions with food-waste should also be explored – some waste may have properties which can alter the solubility or microbial composition of a solution, such as *Sawain et al.*, (2020) using of powdered crab shell as a natural flocculant, or *Kano et al.*, (2021) utilising corn steep liquor to enhance existing synthetic or FWBHF solutions.

Overall, this review has identified the importance of adjusting methodology to accommodate for available food waste. Future research should focus on managing existing sources of urban, consumer level food waste. This can be achieved through both specialist and broad approaches. A specialist approach should strive to assess the nutritional, microbial, and chemical changes in specific foods under specific conditions. A broad approach better captures the inevitable variance of food-waste, including shifts in competing microbial populations, changes in pH, or nutritional fluctuations. An example of a specialist approach is the process of milling and powdering fish as a source of hydroponic nitrogen. *Kawamura et al.*, (2014) implemented this specific methodology while measuring for a specific outcome (Available nitrogen) – providing an intimate understanding of how fish acts as a FWBHF after it has undergone a powdering process. Conversely, *Siddiqui et al.*, (2021) utilises digestion with a large range of foods sourced from the restaurant industry. By providing a pilot study on the interaction between digestion and real food-waste, limitations such as excess sodium were able to be identified. Hence, both approaches are relevant in the study of FWBHF development. With the current level of research, both hold great potential in furthering development of FWBHF's.

Methodology

Processing methodologies are focus of research in FWBHF development. Existing research covers range of trialled methodologies under a myriad of conditions, producing a largely fragmented patchwork of knowledge. The currently most understood processing method is fermentation, due to both its prominence in FWBHF as well as its relevance in other disciplines. Despite this, there is little evidence in the reviewed literature to suggest that fermentation, either aerobic or anaerobic, is better than other methods. The lack of consistent feedstocks, system types, substrates, lighting conditions, and crops across papers limits the identification of common trends of success.

Fermentation, however, highlights the importance of effective and clear reporting. Existing research of fermentation techniques included examining yield efficacy, nutritional content, and length of fermentation (Siddiqui et al., 2021; *Phibbunwatthanawong* et al., 2019). The chemical changes because of both anaerobic and aerobic fermentation are well documented – methanogenesis, nitrogen mineralisation, and acidification are well studied pathways endemic to fermentation. This provides a clearer research direction for other methodologies – to develop a similar level of chemical, mechanical and functional understanding of the changes food-waste experiences under their respective conditions.

Many of the methodologies in this review can be considered novel and/or unique. After individual assessments of each method, the overall gaps in knowledge can be summarized as follows:

- 1) How processing methods impact organic matter breakdown chemically, microbially, and physically.
- 2) How microbial populations interact with particular processing methods with respect to processing time, nutrient availability, and safety of usage.
- 3) How different environmental and physical factors affect processing method efficacy.
- 4) How energy-efficient, scalable, and flexible a processing method is.

These conditions may also include alterations in substrates, optimal lengths of processing, microbial inoculations/facilitation, subsystem-specific interactions, active integration into subsystems, and the impacts made by feedstock content. Alongside existing studies, future research recommendations include exploring heavy metal analysis, food-safety studies, plant disease impacts, microbial content analysis, and optimal system types. An example of future research could explore boiling and steaming as a method for FWBHF production. While the effects of boiling and steaming on nutrients for human health are well understood, there is limited information regarding plant-relevant nutrients as a result of boiling or steaming food-waste (Lee et al., 2018; Yong et al., 2019). Future studies into these processes could explore plant-nutrient availability differences between a 15-minute blanch and a 3-day hard boil of vegetative waste. Although research into these two methods should also consider energy costs, and its scalability to industrial levels.

Similar to fermentation, research into vermiliquer benefits from a multidisciplinary presence. While this review was not able to claim that an unmodified vermiliquer is an adequate FWBHF, on-field trials have provided evidence for improved long-term yields, increased

microbial activity, and potential to remove heavy metals and pathogens from substrates (Churilova et al., 2019; Singh et al., 2011). Within this review, only the effects of raw vermiliquer have been documented, future research should explore whether post-processing techniques such as fermentation may impact the efficacy of a vermiliquer FWBHF.

A fermented vermiliquer posits the potential of synergy between processing methods in FWBHF development. The impacts of synergistic, or multi-stage processing on FWBHF's are largely understudied. Examples of multi-stage processing can be observed in papers which utilise biogas digestate. Generally, additional processing includes additional anaerobic fermentation of biogas followed by supernatant extraction (Ronga et al., 2018; Lind et al., 2019; Ntinis et al., 2021). A deviating methodology included adding biogas digestate into an operating hydroponic system with active aeration to facilitate the mineralization of ammonia into plant available nitrates. Another example is the drying, and powdering of feedstock (Kawamura et al., 2014; Sawain et al., 2018). Further research could explore the benefits of each method, such as utilising vermiculture to homogenise food-waste into a liquid prior to bioconversion through fermentation processes. Alternatively, autoclaving may improve inoculation and cultivation of ideal microbial communities in a solution. The primary limitation of utilising multi-stage processing are the costs associated with additional processing stages, energy, extending processing times, required space, and labour. Hence, cost-benefit should be an eventual consideration when exploring multi-stage processing techniques.

A largely unmentioned synergistic process is the intentional, mechanical breakdown of feedstock, which is only mentioned in three of the nine consumer level research papers (Faruq et al., 2021; Kawamura et al., 2014; Siddiqui et al., 2021). These processes can include grinding, milling, chopping, crushing, and mincing. The effects of prior mechanical processing have an unmeasured impact on the quality of FWBHF's, with none of the literature in this review performing a comparative process between mechanically processed feedstock and whole feedstock. There is a strong case for the exploration of utilising artificial mastication, which include improvements in microbial diversity, rate of breakdown, and plant-pathogen reduction (Mishra et al., 2018; Liu et al., 2022). Uniquely, it is the only process in this review that is exclusively used in conjunction with another method, likely due to its inability to incite large chemical changes in feedstock. The benefits largely derive from the breakdown of particles, mixing of materials, and homogenising of feedstock. For example, *Liu et al.*, (2022) found that using fine bonemeal instead of whole bones in a cellulose rich compost reduced the presence of pathogens such as *Phaeoacremonium*, *Acremonium*, and *Geosmithia* as well as increasing the final TN content. Hence, future research may choose to explore the effects pre-processing feedstock by masticating and mixing feedstock have on the outputs of other methods.

Future research could focus on optimising hydroponic systems by adapting to the unique properties of FWBHF. This optimisation may include adjustments in solution temperature, system type, lighting, and irrigation scheduling. Practically, it is preferable to modify fertiliser development to fit established hydroponic systems, such as NFT or floating raft systems, incorporating small modifications such as mesh traps, porous substrates, and

bioreactors as methods to manage debris, support microbial communities, and improve nutrient conversion during the growth period. A notable innovation could be the integration of active nitrifiers, similar to those used in aquaponics. This approach replaces fish waste with processed food-waste as a source of nitrogen, potentially improving the longevity of existing plant-available sources of nitrogen for FWBHF use.

Salinity and sodicity are a major obstacle in the development of effective FWBHF's. The imbalance between EC (electrical conductivity) and plant available nutrients is observed across majority of papers. Current research into the management of salinity and sodicity in FWBHF's is limited. Existing desalination projects include the conversion of from sea- or saline groundwater into potable water, as well as the neutralisation of sewerage waste (Khawaji et al., 2008). These systems tend to be industrially sized, and have high initial and ongoing costs associated with their operation – requiring industry level effort of food-waste management. New research into ion sieves for desalination may prove to be a future solution, however this solution is far from commercial application. Whether any processing methodology impacts salinity is largely unknown. Future research could entail methods of lowering EC relative to other nutrient levels, although cost effective methods to do so are somewhat limited.

Microbial Activity

Applications of microbial actors in FWBHF's is an understudied area of research. While new papers are common, the range of available future research far exceeds existing literature. Beginning with managing existing microbial populations, considerations for future research include optimal temperatures, aeration, substrate selection, and chemical additives to optimise cultures for plant production. The extension of this research is to explore potential species for inoculation – these populations may have the potential to improve yield, prevent disease, as well as stabilise nutrients (Dhawi, F., 2023). Continued optimisation can include identifying optimal inoculation timing, interactions with different food groups, as well as whether if inoculated species should be removed prior to use in an operating hydroponics system.

Standardized reporting

While in its infancy, the development of food-waste into organic hydroponic systems is quickly gaining momentum as a topic of research. The opportunity to re-utilise food waste as a method of securing local fresh produce in a sustainable manner has greatly increased interest in this research area. Currently, independent investigations are probing potential techniques for processing a plethora of food-waste sources. In lieu of this, it would be prudent to begin developing a standardised reporting system. *Table 1.14* lists a range of elements found to be relevant that should be included across FWBHF research. These factors are chosen and ranked into three priorities: *high*, *medium*, and *low*.

- *High* priority reporting elements have been selected as essential components of research which are relevant across all disciplines within FWBHF research. They have been selected due to relevance as well as being easy to measure and report.
- *Medium* priority reporting elements may have more specific areas of research or are limited by costly or time intensive methods of data collection.

- *Low* priority reporting elements tend to be niche and difficult to collect. This information is largely delegated to the study of specific interactions and should be included if available and relevant.

Table 1.14: This table can be used as a reference guide for key variables for future research in FWBHF development.

Stage	Priority	Reporting element	Description	Example
Inputs	High	Primary inputs	A % mass/volume breakdown or total mass/volume measurement of the feedstock.	Household waste (20% meat and bones, 40% veg scraps, 40% eggshells)
	High	Physical additives	Any materials which are added to alter the physical environment to prevent settling, host microbes, or manage pH	Lava rock, bio-balls, stirrers, etc.
	High	Chemical additives	Any supplements which affect the chemical attributes of a solution	pH buffers, antiseptics, nutrient supplements
	High	Biological supplements	Any inoculations or intentional introduction of materials which aim to alter microorganism activity.	Microbial strains, sugars
Techniques	High	Pre-processing	Methods which do not cause significant chemical change to the feedstock.	Mash, chop, crush
	High	Processing method	The primary processing method/s utilised	Fermentation, vermiculifer
	High	Processing time	A time measurement of any processes that feedstock has undergone.	30-day fermentation, 21 day steam
	High	Solution temperature	The temperature of the solution during processing	Fermentation managed at 30°C
	High	Aeration	Include specific details such as method, flow rate, and operational hours	Stirred for 30 seconds twice a day, aerated at 320L/hr

Analysis	High	EC	A measurement of the undiluted solution	EC = 4.5 dS/cm
	High	pH	A measurement of the undiluted solution	pH = 4.5
	High/medium	NPK	An analysis of key macronutrients	N = 1090 µg/L P = 230 µg/L K = 1560 µg/L
	Medium	S, Ca, Mg	An analysis of key macronutrients	As above
	Medium	Micronutrients	Nutrient analysis of assorted micronutrients	As above
	Low	Microbial analysis	Methodologies used which identify microbial populations	S16 extraction
During Operation	High	System type	The type of subsystem used	NFT, DWS, DS
	High	Frequency of irrigation	If NFT system, include flow rate and volume of reservoir. For DWS include frequency of flooding and duration.	(NFT) Flow rate of 200L/hr w/ 400L reservoir. (DWS) Flooded every 6 hours for 2 minutes.
	High	Crop species	The crop species, including scientific name and variety	Lettuce (<i>Lactuca sativa</i>)
	High	Substrate used	The type of substrate the crop is planted in	Rockwool, Cocopeat
	High	Frequency of FWBHF application	Application rate of fertiliser, including dilution rates, timings, or goal pH/EC/NPK levels	Dosed to EC of 1.6 dS/cm Dosed to TN = 200ug/ml
	High	Ambient conditions	Hours of lighting, air temperature, humidity and the presence of ventilation or CO2 supplementation	16 hours of light a day at ~30°C with 65% humidity.

1.5 Conclusion

Continued development into FWBHF will benefit both food-waste management and food production systems in urban environments. This development assists in the transition of a linear flow of nutrients towards a cyclical structure, reframing “disposal” of food-waste as “leakage,” ultimately normalizing notions of recycling food-waste. By introducing a stigma

against disposing of food-waste, future infrastructure can accordingly accommodate to sort, process, and utilize the waste generated by urban populations.

The existing landscape of FWBHF research presents a variety of novel approaches with varying degrees of success. Reviewed literature is largely exploratory, trialling different methodologies with a range of feedstocks. Future research should strive to isolate individual agents which facilitate the greatest change. Whether it be processing methods, feedstock additives, growth system modifications, or microbial inoculations, the continued improvement of existing methods, or the discovery of new methods is critical to building towards developing effective food-waste management techniques as well as a sustainable foundation for urban horticulture.

Chapter 2. A comparative nutrient analysis of food-waste based hydroponic fertilisers derived from vermiquer and individually fermented food items.

Abstract

Developing food-waste based hydroponic fertilisers (FWBHF) addresses growing urban sprawl and the growing adoption of urban horticulture, improving nutrient cycling and sustainability in our food production systems. This study profiles plant-available nutrients across a range of food products, divided into three main groups (protein-rich, carbohydrate-rich, and fibre-rich) after exposure to anaerobic and aerobic fermentation and vermicompost techniques. Leachates were analysed for inorganic nutrients and heavy metals to compare with a conventional synthetic hydroponic fertiliser. Key findings identified deficiencies in nitrogen and calcium across all treatments. Nutrient availability in the protein-rich food group (beef, chicken) was consistent across both aerobic and anaerobic treatments, with the exception of aerobic fermentation producing a significantly higher level of phosphorus and potassium compared to the control. This was contradicted by the high variation between treatments in the carbohydrate-rich group (pasta, bread), which often produced contradicting nutrient outcomes in the same treatment group, this was attributed to differences in wheat variety and value-adding processes affecting the inorganic nutrient availability in this group. The vermicompost leachate, vermiquer, contained statistically similar levels of potassium, phosphorus, magnesium, and zinc when compared to the control, but was significantly deficient in nitrogen, calcium, and sulphur. Despite this, it was identified as a potential “all-in-one” solution, being low in heavy metals, elemental toxicity, and odourless when compared to fermentation treatments. These results aim to assist future developments in FWBHF by profiling inorganic nutrient availability across food groups when exposed to vermicomposting and aerobic/anaerobic fermentation techniques.

2.1 Introduction

Urban horticulture has gained tremendous momentum in recent decades, leading to improved efforts to optimise the sustainability of the industry. One topic of interest is the development of food-waste based hydroponic fertilisers (FWBHF) as an alternative to the existing industry standard synthetic fertilisers (Wang et al., 2024). Synthetic fertilisers generally rely on energy intensive and non-renewable sources of nutrients such as industrial ammonia production and phosphate rock mining. In addition to the environmental degradation these methods cause, increasing scarcity of minerals and cost of energy inhibits the economic viability of future urban horticulture industries (Cordell et al., 2011; Liu et al., 2020). As such, the re-utilisation of food-waste as a FWBHF attempts to address concerns surrounding nutrient leakage as well as a sustainable future for urban horticulture.

Studies into FWBHF development explore the potential of utilising organic waste sourced from farms, industry, and consumers (Wang et al., 2024). Existing literature provides a patchwork of results, which utilise a variety of primary feedstocks, inoculations, additives, hydroponic system types, substrates, methods of processing, and so forth (Wang et al., 2024).

The output of these studies generally include yield outcomes relative to a synthetic control solution. However, select studies also include additional analysis such as microbial load measurements (Kano et al., 2021), yield quality (Bergstrand et al., 2020), and nutrient analysis of the FWBHF (Wang et al., 2024). From the outputs, it was identified that yield performance was best predicted by the similarity of nutritional contents between FWBHF and their respective synthetic control solution. *Kechasov et al. (2021)* found that mineral replication of a pig-manure and household-waste based FWBHF was unable to meet the yield efficacy of the treatment – suggesting a biological mechanism, such as beneficial micro-organisms, may have enhanced the efficacy of the solution.

Another identified trend was the tendency for consumer level waste to have a greater variability in feedstock relative to “Farm” and “Industry” level producers. Unlike in farm or industrial settings, large volumes of homogenous inputs are not available at consumer levels. This introduces another confounding factor in the production of consistent, replicable hydroponic solutions. These changes are further amplified when considering how socio-economic, cultural, and geographical factors influence the type of food waste an urban population generates. Hence, the primary challenge of FWBHF development is the transformation of variable non-homogenous feedstocks into effective hydroponic solutions capable of supporting crop growth.

A large range of novel methods have been identified across existing literature. While the primary factor in a successful FWBHF is its efficacy in emulating the yield outcomes of a synthetic solution, considerations must be made towards the efficacy of the processing method. Considerations for the energy efficiency, time of processing, effective volume, and replicability must all be evaluated when selecting a method. *Yusuf et al., (2021)* utilised steaming and boiling, however, this may require more energy relative to methods which utilise bioconversion, such as digestion or vermiculture. The latter methods exploit self-propagating microbes and invertebrates to break down organic matter into plant-available form. This enables scalability for widespread applications without exponentially increasing energy costs (Arancon et al., 2019).

The three described trends i) Nutrient similarity, ii) Variability of inputs, and iii) Variety of novel processing methods, has created a need for a quantitative research project which aims to begin the documentation of plant-nutritional outcomes from novel processing methods. This paper identifies and compares trends between, and within, major food groups, such as vegetative matter, carbohydrates, and proteins. The selected methodologies include the fermentation of individual food groups, as well as two controls – a conventional synthetic hydroponic solution, and household-waste based vermiquer. In addition, this paper also measures the impact aeration plays in the final nutrient composition of both fermentation and vermiculture treatments. It is hypothesised that aeration will improve the availability of nutrient species, such as nitrate, which rely on aerobic microbiological activity (Sparks et al., 2022). The goal of this research is to begin developing a database of expected outcomes for protein-rich, carbohydrate-rich, and fibre-rich food groups. The application of this study aims to assist future research when seeking food-waste based nutrient supplements for specific minerals, better understand the behaviour of inorganic nutrient composition of specific food groups when exposed to novel treatments, and to publish the range of mineral deficiencies found in FWBHF's.

2.2 Materials and Methods

Treatment preparation

The control solution in this study utilises a two-part Hoagland's variation "*Aqua Vega AB* solution" (Canna), dosed at the recommended value into tap water (Sydney, Australia), which contains a host of trace elements to ensure preservation of water drinking quality.

The study was conducted in a darkened fume hood over 90 days in a completed randomized design with 2 different treatments applied to 5 different foods. The tested foods were chicken, beef, bread, pasta, and assorted vegetables. 500 grams of each treatment was conventionally prepared, without addition of salts or seasoning. The chicken breast and scotch fillet were cooked in a convection oven at 180°C for 25 minutes. Pasta was boiled at 105°C for 11 minutes. By weight, the salad consisted of carrot (11%), seedless green capsicum (22%), truss tomato (4%), pink lady apple (18%), naval orange (18%), oak leaf lettuce (21%), and Lebanese cucumber (10%).

Individual foods were then blended into a fine paste and placed in jars of 1.25L of deionised water. Each individual food was replicated 4 times. The 2 treatment groups included an aerobic and anaerobic trial. Air temperature was kept at 24°C. Additionally, 8 bio-balls (AquaOne) were provided to increase colonisable surface area for bacterial growth by providing increased surface area. Aerobic trials utilised air pumps with a flow rate of 60 L/hr. Anaerobic trials were fitted with one-way air valves to facilitate gas release. The digestates were left in a covered fume hood for 90 days.

Fresh vermiliquer was collected from a single worm farm which was fed, by volume, eggshell (25%), coffee grounds (15%), green vegetable waste (30%), carbohydrates (including rice and pasta) (10%), garden waste (10%), and fruit peels (10%). The solution was left to sit outdoors in 20L buckets, in full sunlight, with loose fitting lids for a 90-day period. Prior to analysis, the vermiliquer was treated either aerobically or anaerobically. Aerobic treatment consisted of 72 hours of aeration at a rate of 60 L/hr. Anaerobic treatments were sealed and left undisturbed for 72 hours in the dark.

Sample analysis

Treatments were filtered (Whatman no.4) before being put into cold storage at 4°C. Aliquots were analysed for pH, EC, and essential plant nutrients. pH and EC were taken using a hand-held reader (HI9813-61, HANNA). A flow injector analyser was used to take total nitrogen, nitrates and ammonia readings. Preparation of the samples included only a 20-times dilution.

The remaining elements (*P, K, S, Ca, Mg, Zn, Cu, Mn, Fe, Pb, and Cd*) were all measured using an ICP-MS. Samples were prepared with an acid digestion of 0.125ml of HNO₃ and 0.375ml of HCl to 0.5ml of the solution. They were heated to 60°C in a water bath for two hours to ensure total digestion. Samples were frozen immediately after and thawed immediately prior to 10-times dilution for ICP-MS analysis.

Data analysis

Data analysis was carried out using Rstudio (2024.04.2 Build 764). Normality for measured parameters (pH, EC, nutrient analysis) was assessed using Shapiro-Wilks test, qq-normal plots and histograms, all response variables were determined to be normal. Equal variance between treatments was tested with a Levene's test and were analysed with ANOVA. A Dunnett's test was performed to quantify the difference between each treatment and the

control mean. In addition, nutrient concentration comparisons are mathematically diluted/concentrated into a standardised form using the equation:

$$\text{Adjusted nutrient conc.} = \left(\frac{\text{Raw EC}}{1.6} \right) * \text{Raw nutrient conc.}$$

This standardised form uses an EC of 1.6 dS/m as a baseline due to its ubiquitous tolerance by hydroponic crop species. This is required as EC variation impacts the rate of nutrient availability to crop species and is further explored in *Results and Discussion*. Plots were visualised using the “ggplot” package.

2.3 Results and discussion

2.3.1 Indicators and roles of pH

Depending on the intended crop, conventional hydroponic solutions tend between a pH of 5 and 7 (Velazquez-Gonzalez, et al, 2022). A pH within these bounds optimises plant nutrient availability while precipitating potentially toxic elements, such as aluminium (Sparks et al., 2022). In our experiment, the control in this trial contained an average pH of ~5.3. Majority of treatment solutions exceeded the upper bounds for an “ideal” pH range. The exceptions were for the anaerobic carbohydrate-rich treatments, bread and pasta. Both treatments averaged a pH of 4.5 - acidic enough to begin the solubilisation of aluminium, a phytotoxic element (Sparks et al., 2022). Aeration treatments caused significant levels of variance in vegetable, pasta, vermicuquer, and bread treatments, while protein-rich beef and chicken were statistically similar ($p < 0.05$). This difference may be caused by the specialisation of similar microbial communities required to break down dense, protein-rich waste compared to the variety of microbial communities competing for readily available carbohydrate energy (Burcham, et al 2024). Similarly, the vermicuquer benefits from an established community of invertebrates and micro-organisms to transform varied food-waste into a relatively homogenous solution (Loera-Muro et al., 2021).

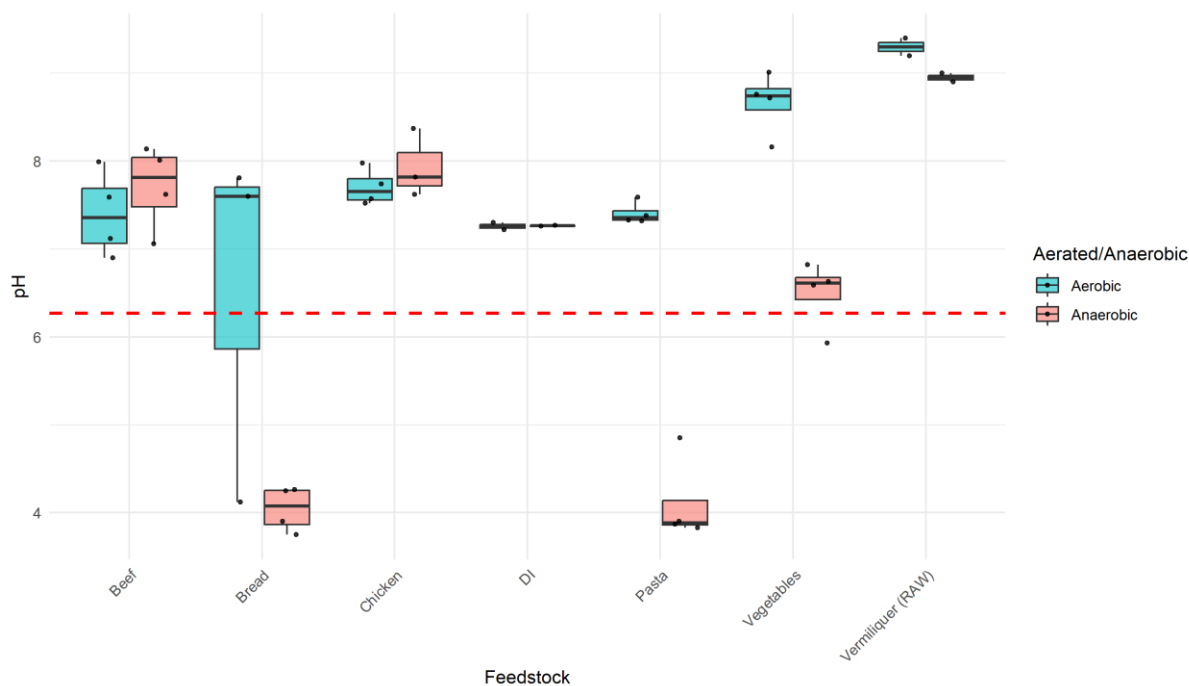


Figure 2.1: pH across undiluted food groups after 90 days of aerobic/anaerobic fermentation. DI = Deionised water. Horizontal line represents the mean pH of the control solution.

While pH is primarily determined by the presence of H^+ , the overall cation/anion ratio can influence the proportion of acidic cations (such as Al and Fe), over a period of time (Sparks et al., 2022). This is caused by nutrient uptake mechanisms in plants by either absorbing an inverse proton or hydroxyl, or by exchanging a proton or hydroxyl for the required cation or anion. This is relevant as the availability of certain species, such as nitrogen, appear in both anionic (NO_3^-) and cationic forms (NH_4^+). Studies in soil systems have observed both alkalisation and acidification in rhizosphere pH as a result of the uptake of each nutrient,

respectively (Custos et al., 2020). However, in hydroponic systems, this is largely understudied. It is unknown if changes in rhizospheric pH impact the rest of the hydroponic solution supply.

This behaviour of having both anionic and cationic availability is unique to nitrogen. Most crop species share similar molecular preferences, with macro-nutrients having either cationic or anionic uptake pathways (Table 2.1). As a result, future iterations of experimental hydroponic solutions should consider how ionic availability of plant-preferred nutrients can impact pH in a solution.

Table 2.1: Ionic preference of plant available macro-nutrients (Sparks et al., 2022)

Element	Anionic Form	Cationic form
Nitrogen	Nitrate (NO_3^-)	Ammonium (NH_4^+)
Phosphorus	Phosphate (H_2PO_4^-)	-
Potassium	-	Potassium (K^+)
Calcium	-	Calcium (Ca^{2+})
Sulphur	Sulphate (SO_4^{2-})	-
Magnesium	-	Magnesium (Mg^{2+})

Adjustments to pH are possible without altering macro-nutrient ratios through the addition of chemical pH buffers. Hydroponic pH buffers generally favour citrates and carbonates due to affordability, non-phytotoxic properties, and compatibility with other macro-nutrients (avoiding precipitation, or toxic outcomes) (Zhang et al., 2024).

2.3.2 EC, salinity, and sodium

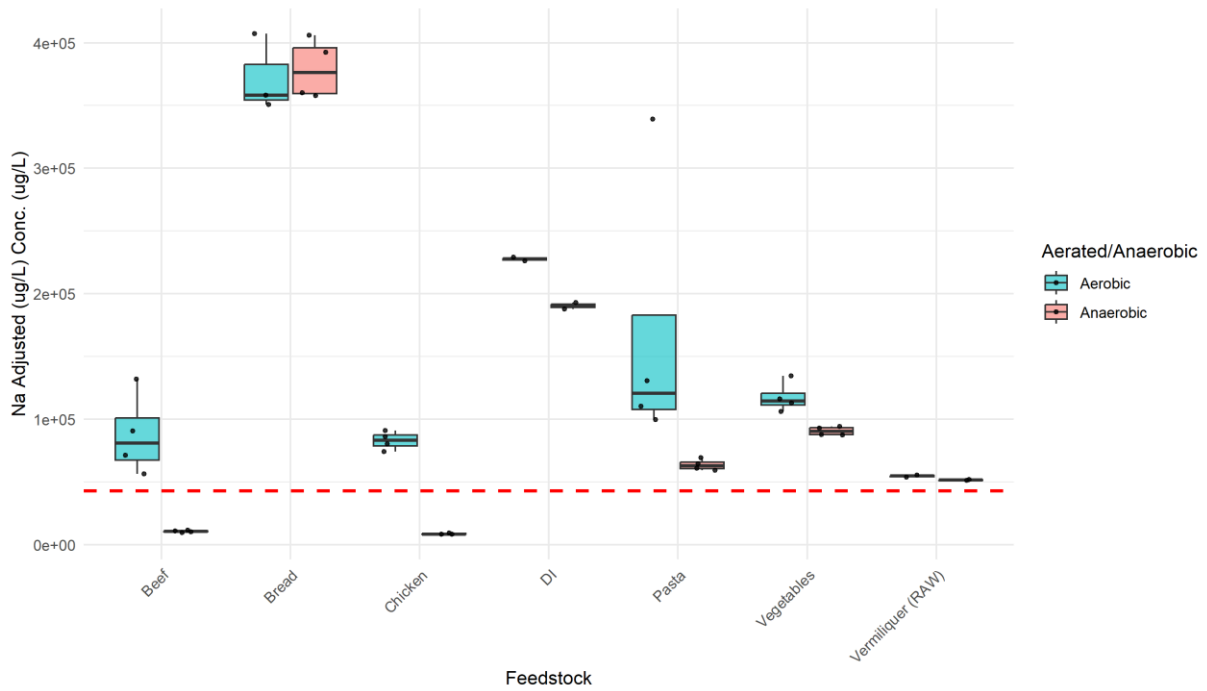


Figure 2.2: Sodium content in aerobic/anaerobic food groups and vermiliqueur after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

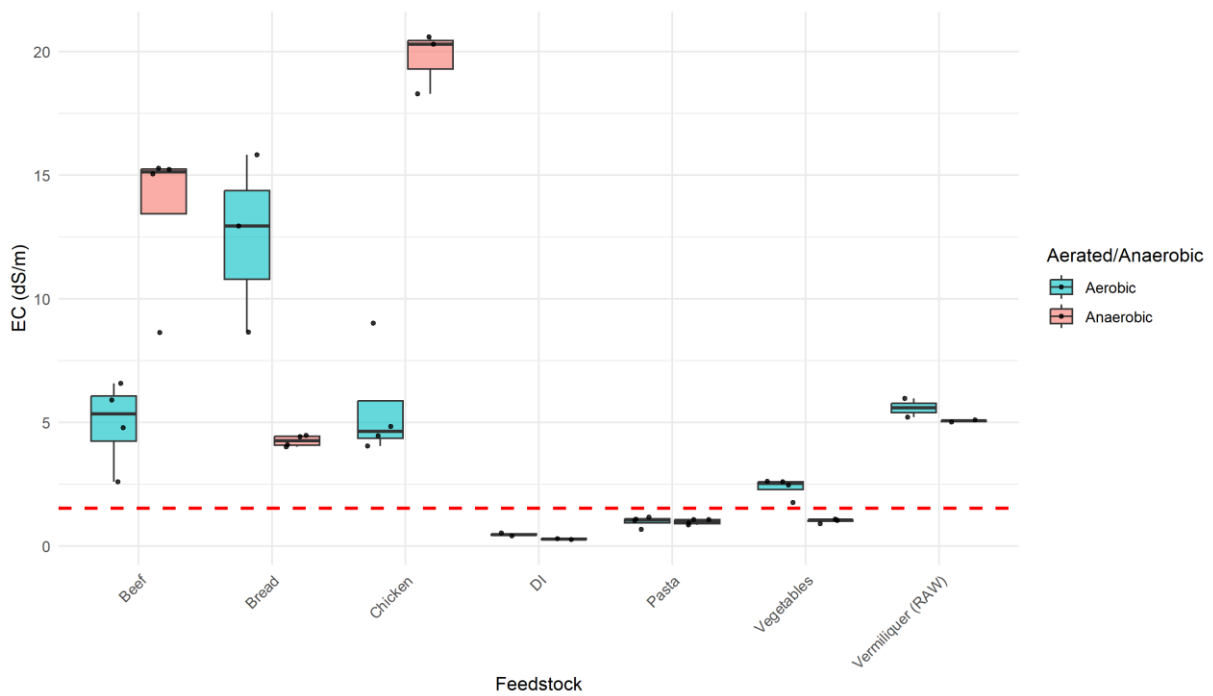


Figure 2.3: EC (dS/m) across undiluted food groups after 90 days of aerobic/anaerobic fermentation. DI = Deionised water.

While not an essential nutrient for C3 plant species, sodium (Na^+) is the final cation alongside potassium (K^+), calcium (Ca^{2+}), and manganese (Mg^{2+}) which contribute to salinity in a solution (Brownell P.F, 1980; Sparks et al., 2024). These cations are essential for plant function and are dosed to a salinity EC of ~ 1.6 dS/m. Multiple studies have found that the optimal EC range changes depending on crop species, but usually includes a range from 1.2 to 2.4 EC (Wortman, S.E., 2015, Dunn et al., 2016, Shareef et al., 2024). This range is optimal for plant absorption by optimising osmotic potential, while maintaining a nutrient dense solution. In scenarios with excess salinity, plants can suffer from ion toxicity, and osmotic stress, while the solution may see symptoms of precipitation between nutrients, such as potassium and calcium salts, inhibiting nutrient availability (Sparks et al., 2022). From a producer perspective, excess salinity will lead to reduced, or total yield failure. Hence, a successful FWBHF should have an adequate supply of all plant nutrients in relation to its EC level.

The sodium content across treatments tends to be similar or significantly greater than that of the control. The vegetable, pasta, and bread treatments all show significantly greater sodium levels relative to the control. This excess sodium is non-essential in C3 plant species, and thus occupies potential room for other salt species which are essential for plant growth. This sort of salinity has been a major obstacle in the development of FWBHF's. The source of sodium tends to be from table salt (NaCl) which is a common ingredient in both industrial (bread and pasta), consumer, and retail food processes. Practical and cost-effective methods of removing sodium from treatment solutions are limited, with options such as osmotic sieves, being prohibitively expensive for crop production uses (Han et al., 2022).

2.3.3 Essential plant macro- and micro- nutrients.

While pH plays a critical role in the availability of plant nutrients the molecular presence of plant essential macro- and micro-nutrients is foundational for a viable hydroponic solution. Current understanding of nutrient requirements for hydroponic crops are largely dependent on crop species requirements. No existing scientific evidence suggests there is a universally optimal composition of minerals. This can be attributed to different nutrient requirements for crop species, system types, and growing conditions. The following analysis studies the concentrations, functions, and synthesis of each element across treatments. In addition, the impacts of aerated/anaerobic fermentation will be examined and discussed.

Macro-nutrient analysis

Plant macro-nutrients include nitrogen, phosphorus, potassium, calcium, sulphur, and magnesium. Of the six essential plant macro-nutrients, no treatment was similar to either nitrogen or calcium within one confidence interval. Conversely, most treatments exceeded nutrient requirements for phosphorus, potassium, sulphur, and magnesium. Insufficient levels of macro-nutrients stunt the development of crops, whilst excessive levels lead to elemental toxicity.

Nitrogen

Nitrogen is usually the macro-nutrient required in the greatest abundance of vegetative crop species. The element is essential in the production of amino acids, chlorophyll, and in the regulation and uptake of nutrients and water. Nitrogen deficiency can be observed as chlorosis, stunted vegetative growth, and, in extreme cases, plant death (Sparks et al., 2022).

Nitrogen content in treatment solutions all did not meet the control (Figure 2.4). Vermiliquer was the treatment with the highest total nitrogen content, with both aerobic and anaerobic treatments having $\sim 50\%$ of the nitrogen content found in the control. No other treatment was

considered significantly similar to these two. The impacts of aeration on nitrogen content only significantly impacted the bread treatment, which increased as a result. This is potentially due to aeration improving oxygen availability for nitrifying species. It is unknown why the other carbohydrate group (pasta) did not share this behaviour – although it may be attributed to differences in processing and original wheat species.

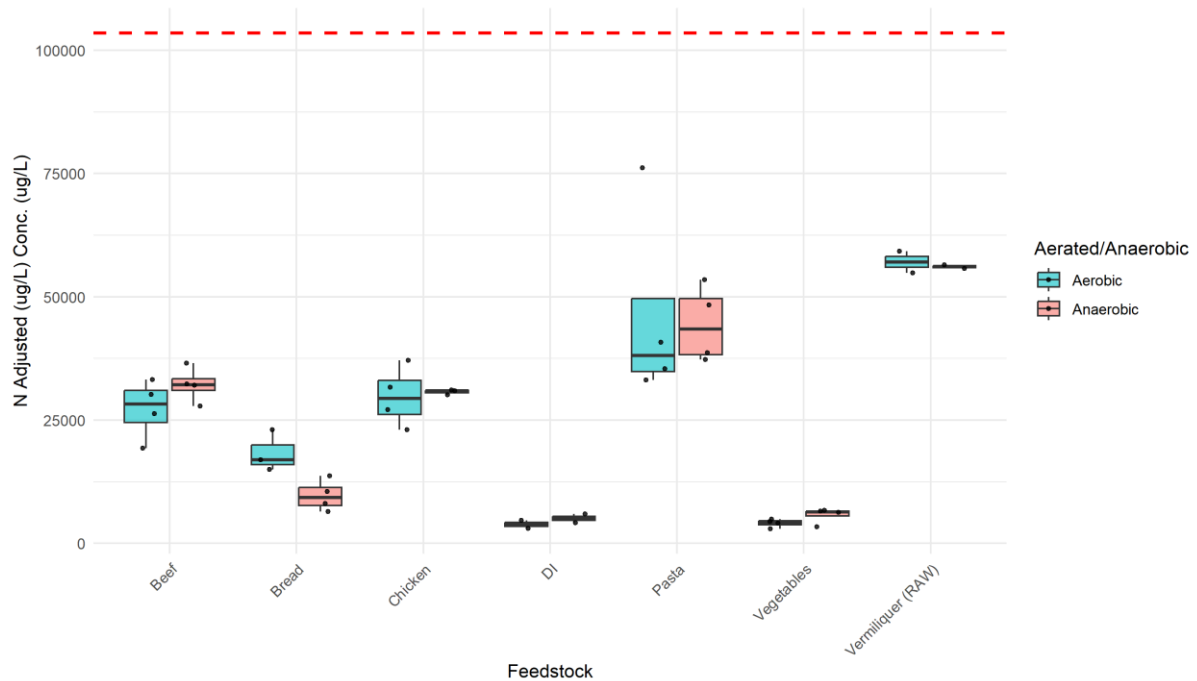


Figure 2.4: Nitrogen content in aerobic/anaerobic food groups and vermiliquer after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

While plant uptake of ammonium (NH_4^+) is possible, there is a preference for nitrates (NO_3^-) depending on species. Common hydroponic species, such as lettuce, strawberry, and tomatoes tend to suffer negative yield outcomes when the ratio of $\text{NH}_4^+ : \text{NO}_3^-$ exceeds 75:25 (Demsar et al., 2004; Tabatabaei et al., 2008; Nawarathna et al., 2021). At this ratio, ammonium toxicity begins to inhibit the uptake of cations K^+ , Mg^{2+} , and Ca^{2+} which inhibit normal plant development. In addition, recent studies have also identified upregulation of stress-related enzymes which can further hinder development. As such, it is essential that candidate FWBHF's have $\text{NH}_4^+ : \text{NO}_3^-$ ratios which provide chemical stability and remain non-toxic to crop species. Chemical stability aims to minimise fluctuation in pH as a result of NH_4^+ and NO_3^- uptake and conversion. In hydroponic systems, it has been suggested that an optimal ratio of $\text{NH}_4^+ : \text{NO}_3^-$ is 25:75, as it has been observed to self-regulate pH by offsetting acidification caused by nitrate uptake through the exchange of H^+ ions during ammonia cation uptake (Dickson & Fisher., 2019). However, a true optimal ratio is subject to case-by-case conditions, as factors such as nitrogen concentration, crop species and growth stage, pH levels, and growing medium can influence nitrogen uptake in crop species (Chen et al., 2024).

The control solution contained a $\text{NH}_4^+ : \text{NO}_3^-$ ratio of approximately 15:85, which only the vermiliquer treatment group was able to surpass (Figure 2.5). The vegetable treatment group had a positive ratio, with more than 50% of available nutrients being in NO_3^- form. Unexpectedly, the vegetable, bread, and pasta treatments contradicted the hypothesised impacts of aeration. The predicted impact of aeration was that there would be a greater

increase in nitrification processes. This is due to the presence of oxygen supplying aerobic species such as *Rhizobiales* and *Nitrobacter* with oxygen for the conversion of NH_4^+ to NO_3^- (Delgado et al., 2007; Nicholls and Ferguson, 2013; Hu et al., 2023). A potential explanation for this reversal may be due to the availability of accessible carbohydrates in the carbohydrate, and vegetable treatments. Ammonium oxidisers have demonstrated the ability to assimilate organic carbon while oxidising NH_4^+ to NO_2^- for energy production, however current literature does not suggest this provides any significant growth advantage (Prosser, J.I., 2007). Other explanations include excessive acidity and heat inhibiting nitrification to occur (Hu et al., 2023). The absence of an elevated NH_4^+ in vermiliquer treatments suggests that either isolated fermentation, or the absence of macropod bioturbation/processing may also impact the process of nitrification and nitrate retention.

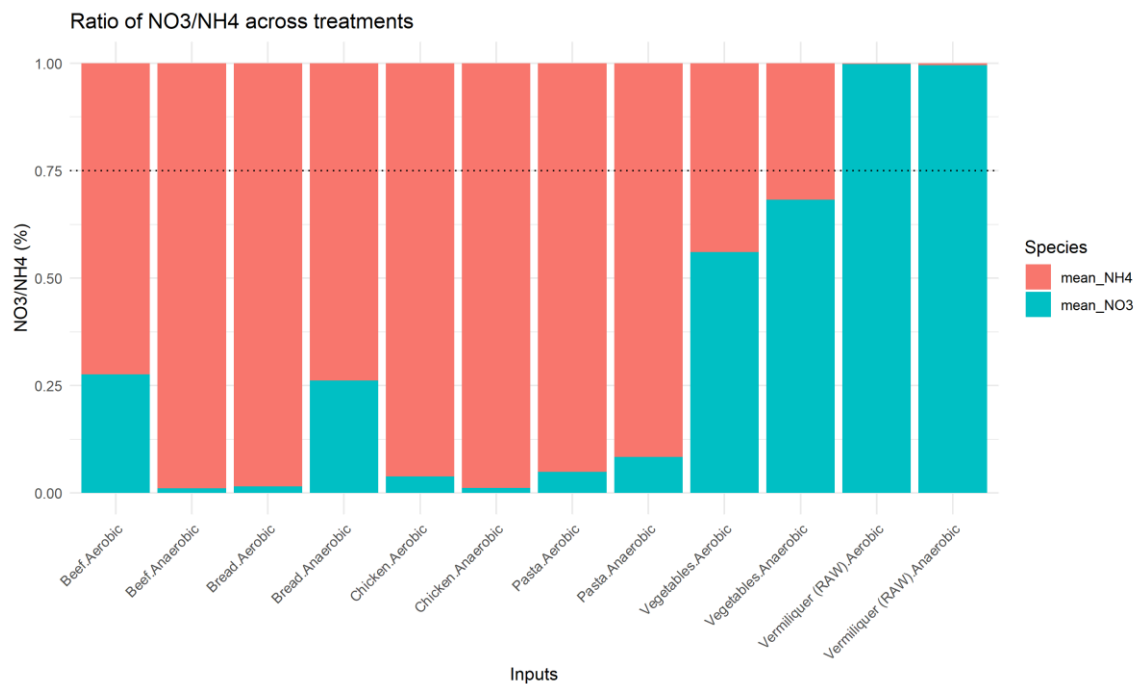


Figure 2.5: Distribution of nitrogen species, nitrate and ammonia, across treatments. Dotted line represents the ideal 25:75 ratio of $\text{NH}_4^+ : \text{NO}_3^-$ (Dickson & Fisher., 2019)

The rest of the treatment groups are consistent with the hypothesis that aerobic environments promote nitrification processes. The beef treatment saw a significant improvement in NO_3^- content as a result of aeration ($p < 0.05$), while the increase in chicken was not considered significant. Conversely, carbohydrate groups bread and pasta both showed elevated concentrations of nitrates in anaerobic treatments – this may be due to excessive fermentation times causing denitrification. Vegetable treatments saw a significantly lower $\text{NH}_4^+ : \text{NO}_3^-$ ratio compared to other fermentation treatments. This is most likely due to the composition of raw vegetable products being composed of primarily nitrates, as opposed to proteins found in carbohydrates and proteins which deaminate into ammonia species (Knez et al., 2022; Păucean et al., 2024). This highlights the importance of feedstock as an underlying determinant in the composition of a hydroponics solution. Like the only vegetable treatment, the vermiliquer treatment is mostly composed of vegetative waste, and the improved NO_3^- ratio is reflected by this.

A pilot trial found that a 48-hour aeration of vermiliquer did not significantly change the levels of total organic nitrogen, nitrates, or ammonia to a significant level. It may be possible that the conversion from ammonia to nitrates occurs prior to leachate extraction, or

that prolonged storage may enable the anaerobic nitrification to occur. Additional studies into the nitrate/ammonium balance of freshly harvested vermiliquer may be beneficial to understand this interaction.

Phosphorus

Phosphorus is essential for normal plant growth, forming genetic material as well as energy carrier ATP, and reductase NADP which facilitates nutrient transport, photosynthesis, and formation of genes (Khan et al., 2023). Limited phosphorus limits the utilisation of photosynthesised carbohydrates. This bottleneck results in dark, green colouration in foliage, as well as a reduction in vegetative and fruit quality and quantity (Khan et al., 2023).

Phosphorus content varied across treatments, generally trends saw aerobic treatments having greater concentrations relative to anaerobic treatments (Figure 2.6). All treatments except for vermiliquer had at least one aerobic or anaerobic form statistically similar to, or greater than the control ($p < 0.05$). Notably, vermiliquer is the only treatment without significant difference when aerated.

Both aerobic protein treatments were abundant in phosphorus. This is likely due to the greater availability of phosphorus in protein-based material due to its role in muscle synthesis and energy creation as creatine phosphate (Kohlmeier, 2015). Aeration of the treatment likely enabled aerobic microbial species such as *Pseudomonas putida* to solubilise phosphorus in the plant available form $H_2PO_4^-$ (Singh et al., 2024).

Conversely, there is a reduction in available phosphorus after aeration in bread and vegetable treatments. This is most likely due to the greater availability of phosphorus in protein-based treatments, as well as the tendency for phosphorus to be found primarily as phytate in the bran of wheat grain (Shabnam et al., 2018). The inclusion of bran in wholemeal bread may assist in the fortification of available phosphorus in the bread treatment. The differences between carbohydrate treatments can be hypothesised as either differences in utilised wheat varieties (durum for pasta, white for bread) or as differences in post-harvest processing prior

to final sale.

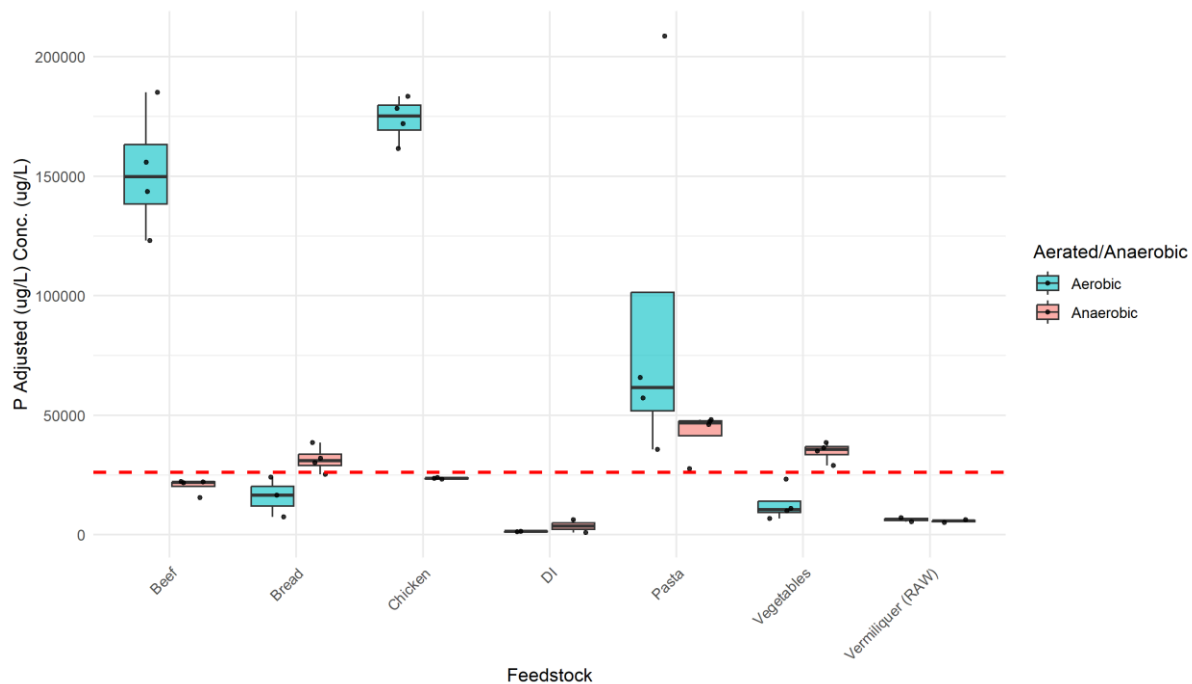


Figure 2.6: Phosphorus content in aerobic/anaerobic food groups and vermiliquer after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Generally, phosphorus is more available in slightly acidic conditions (pH 6.0-7.0), where a larger range of phosphorus-rich minerals, such as calcium phosphate, solubilise into inorganic form for plant uptake (Sparks et al., 2022). Majority of treatments show a slight tendency towards alkalinity, and as such there is potential for acidic amendment for the remediation of phosphorus deficiency. This may not be necessary for food-waste rich in protein which have been aerobically fermented, nor would it be necessary for anaerobically fermented carbohydrates and vegetables. The vermiliquer treatment would benefit the most from this remediation, due to high alkalinity (pH = 9) and low phosphorus availability

The high levels of phosphorus alone make either the fermentation or vermiliquer FWBHF treatments potentially viable for commercial application. This is due to predicted shortages in phosphorus fertilisers within the next century. This scarcity is attributed to the over-extraction of available phosphate rock – the primary source of phosphate fertilisers (Jupp et al., 2021). As a result, methods to recapture and reutilise phosphorus should be explored further, especially for hydroponic systems, which heavily rely on mineral sources of the element.

Potassium

Unlike nitrogen and phosphorus, potassium is not a molecular component in any plant structures. This can make it difficult to directly associate potassium to a specific metabolic function within a plant. However, the importance of the mineral should not be understated, potassium facilitates enzyme regulation, stomatal behaviour, gas exchange, and an assortment of other osmotic functions throughout the roots, shoots, and fruit (Sardans et al., 2021). The capacity to efficiently regulate osmotic behaviour in the root system is particularly useful in FWBHF systems, which can suffer from excessive sodium levels (Figure 2.2). Excess sodium can limit plant nutrient availability by interfering with nutrient exchange in the root system. Soil-based trials have shown that both potassium (K^+) and calcium (Ca^{2+}) improve sodium

exclusion mechanisms in tomato and olive plants (Capula-Rodriguez et al., 2016; Larbi et al., 2020).

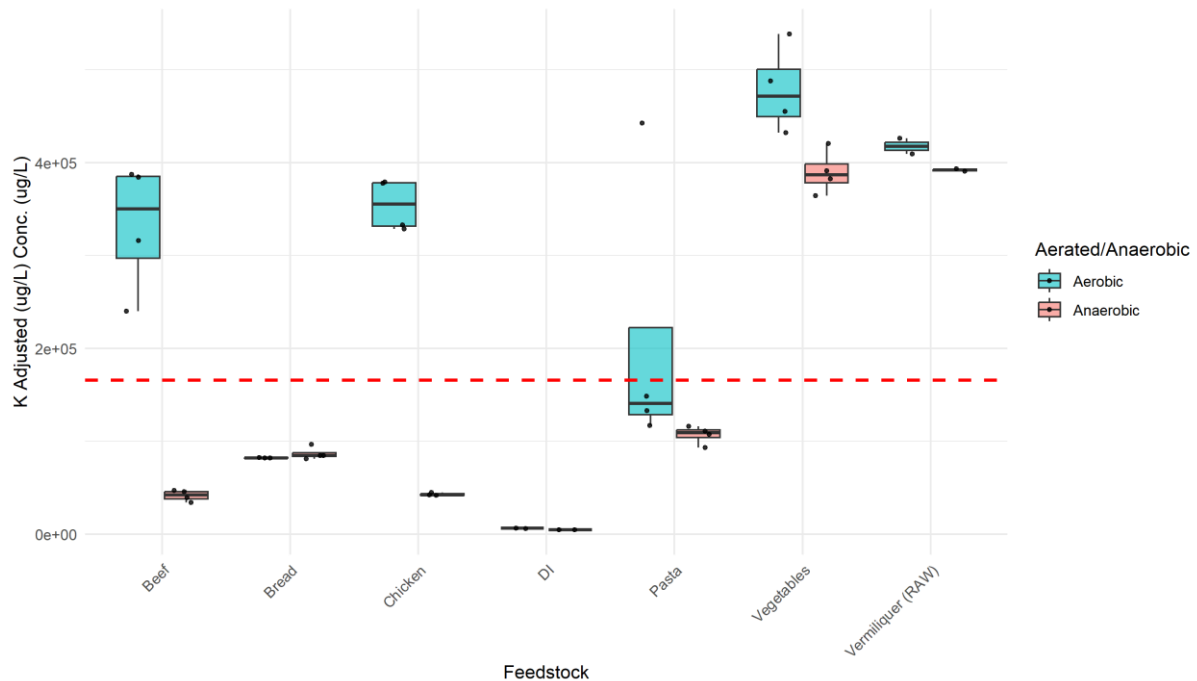


Figure 2.7: Potassium content in aerobic/anaerobic food groups and vermiliqueur after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Potassium availability across treatments generally favoured aerobic environments, with all but one treatment having no significant difference because of aeration (Figure 2.7). This adheres to current understanding of microbial K fixation. Microbe groups such as bacterial *Bacillus sp.*, and *Pseudomonas spp.*, or fungal *Aspergillus sp.* rely on available oxygen to complete metabolic solubilisation of K^+ . In the bread treatment, it is possible that the elevated EC (Figure 2) relative to other treatments may have inhibited the development of K fixing populations. Another potential theory may be that high availability of carbohydrates creates microbial populations which outcompete K fixing microbes, although there is no existing literature which quantifies either hypothesis. Future studies should include microbial analysis to confirm the presence of K fixing microbes and clearly discern whether aeration triggers a biological or chemical change in K^+ availability.

Once K^+ solubilisation occurs, however, treatments show that there is an abundance of the element. While potassium toxicity is largely asymptomatic. There is evidence to suggest that if potassium exceeds an optimal dosage, there is significant loss in both root and shoot development (Xu et al., 2020). Research into optimal potassium levels in hydroponic systems is largely unexplored, and would likely vary across different crop species, nutritional compositions, and system types.

Calcium

Calcium plays a role in both structuring plant cells, facilitating nutrient transport between cell membranes, and signalling. Due to its major role in signalling, Ca^{2+} must utilise the xylem for transport, as such the primary driver of calcium uptake and distribution is transpiration. (Thor K., 2019). In hydroponic systems, the risk of insufficient transpiration is unlikely due to the

artificially managed light, humidity, temperature, and aqueous nutrient solution. Ca^{2+} presence may be particularly useful in FWBHF solutions, as the element (alongside K^+) demonstrates the capacity to improve salt tolerance when in higher concentrations (Hadi and Karimi, 2012). It should be noted that while Ca^{2+} can improve intracellular salt tolerance, there is little evidence to suggest it can significantly alter the osmotic deficits caused by a highly saline nutrient solution (Reid and Smith, 2000).

Calcium was the other element besides nitrogen that was identified as deficient across all treatment solutions (Figure 2.8). This may be caused by excessive acidity or alkalinity causing precipitation into carbonates and other salts (Sparks et al., 2022). The treatment with the highest calcium content (other than De-ionised water (DI)) was pasta. Another potential cause may be caused by the lack of calcium-rich feedstocks such as bones, eggshells, and dairy products. In either case, these deficiencies can be amended by either adjusting pH to an acceptable range (pH 6.0 – 7.5), or by supplementing a solution with more calcium, ameliorating detrimental effects of the sodium (Shabala et al., 2006).

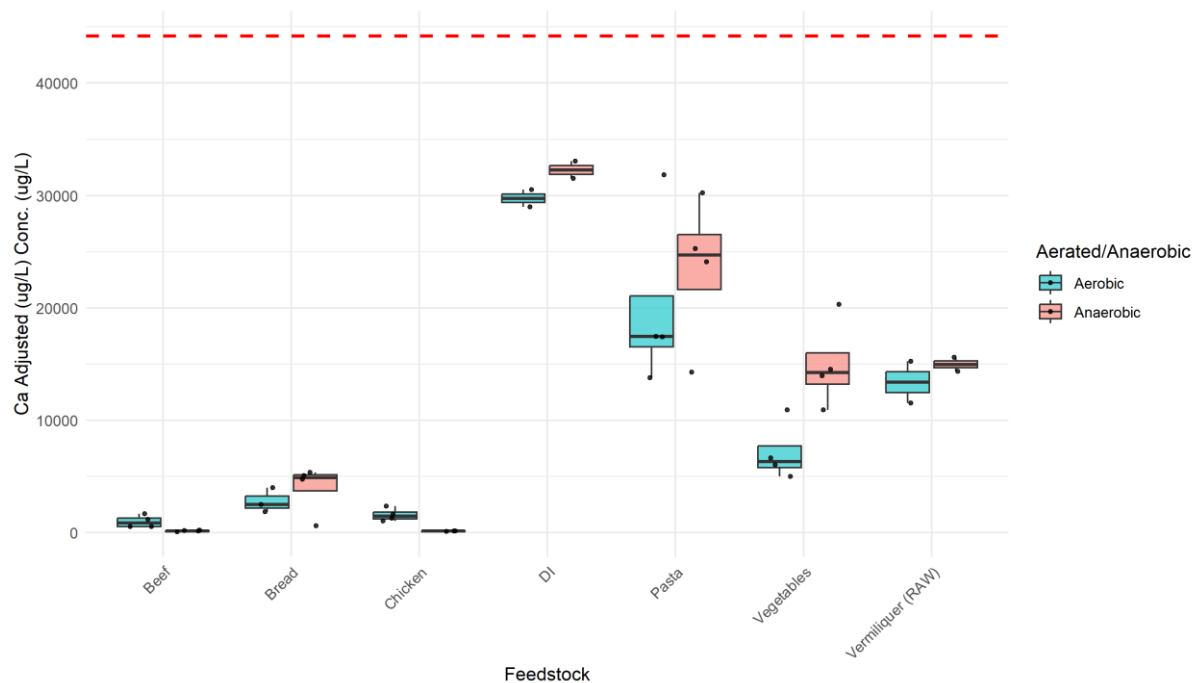


Figure 2.8: Calcium content in aerobic/anaerobic food groups and vermiliqueur after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Another potential explanation for calcium deficiency is the uptake caused by aerobic microbial species such as soil-based *Pseudomonas sp.* and *Bacillus sp.*, utilise the element in signalling, metabolic, and reproductive processes (Kolodkin-Gal et al., 2023). This is supported by the significantly greater (although still deficient) levels of calcium in anaerobic treatments in carbohydrate, vegetable, and vermiliqueur treatments. Notably, the DI treatment was shown to have the greatest level of calcium. This may be due to the trace calcium carbonates found in Sydney’s drinking water (NHMRC, 2024). The absence of excessive nutrients in the DI treatment may have limited the development of populations which utilise calcium. Unsurprisingly, both protein groups had the lowest amount of calcium, which is expected due to the low inherent calcium level in lean meat (Mortenson et al., 2024). Efforts should be made into the exploration of the biological and chemical relationship of calcium in

FWBHF development, including if supplementation of the mineral improves nutrient availability in final FWBHF treatments.

Sulphur

The primary purpose of sulphur is as a constituent of essential amino acids for protein synthesis. Sulphur is mainly absorbed as sulphate (SO_4^-) (Narayan et al., 2023). The distribution of sulphur in treatments varies greatly (Figure 2.9). Protein treatment groups tended to have adequate amounts in aerated conditions but were significantly lower than the control in anaerobic conditions ($p < 0.05$). Conversely, carbohydrate and vegetable treatments shared higher levels of sulphur in anaerobic conditions. Notably, the carbohydrate group can be observed to have a large disparity in overall sulphur concentration ($p < 0.05$).

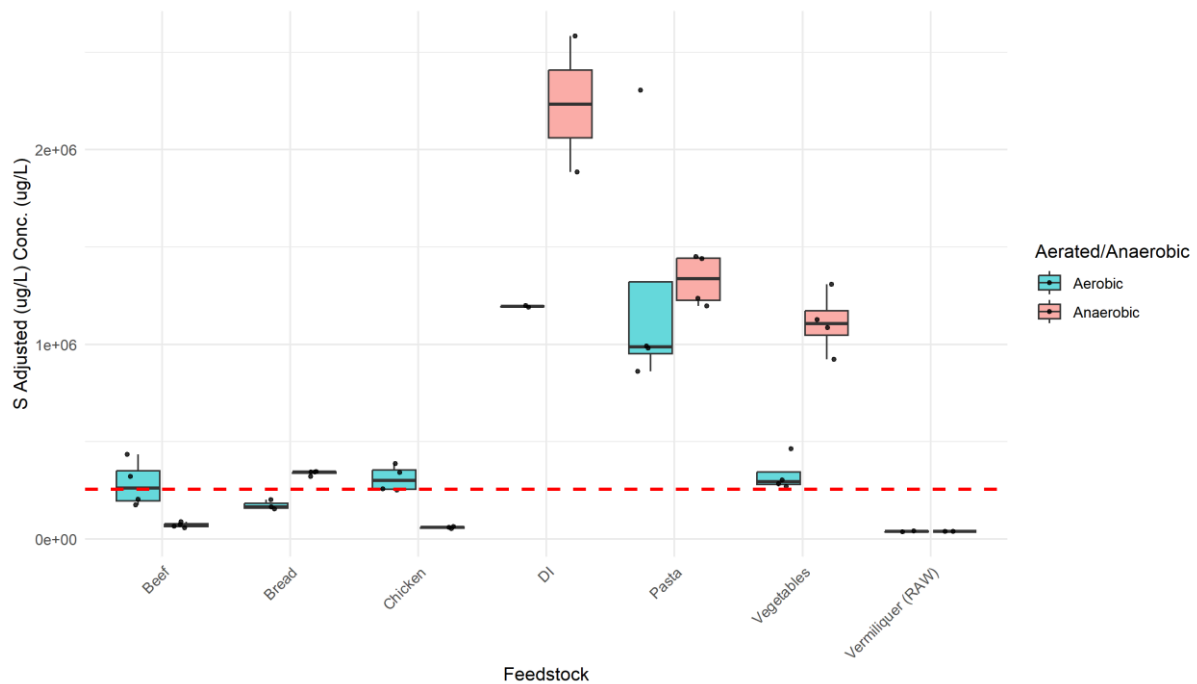


Figure 2.9: Sulphur content in aerobic/anaerobic food groups and vermiquier after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

A possible explanation for such an abundance of sulphur may be attributed to anaerobic treatments limited capacity for desulphurisation, which relies on excess H^+ ions and aeration (Izadi et al., 2023). In addition, bacterial biomass may also represent a significant proportion of sulphur (Heinze et al., 2021). As such, it is possible that treatments which facilitate perennial bacterial populations (anaerobic and carbohydrate rich) result in greater overall sulphur levels. While sulphur toxicity in plants is rare, the effects of excess sulphur in soil systems are well documented. In soil systems, excess sulphates cause acidification, which can cause the precipitation of key nutrients such as Ca_2^+ and increase risk of Al and Fe toxicity. Whether this effect is replicated in hydroponic systems is not well studied (Narayan et al., 2023).

Another consideration of sulphur is that excess amounts can form odorous compounds (Hoffmann et al., 2009). All treatments, other than vermiquier, expressed a highly repugnant olfactory experience. The impact of elevated sulphur on the strength of this odour was not quantified. While this does not directly affect plant development, this should be a

consideration for future research, as application of FWBHF's should not come at the detriment of human health.

Magnesium

The function of magnesium is largely found in chlorophyll pigments and enzymatic reactions throughout a plant. In the case of deficiency, a plant will show stunted root growth, interveinal chlorosis of mature leaves, and a reduction in total biomass (Ishfaq et al., 2022). Protein based treatments tended to have a lower level of magnesium relative to the control (Figure 2.10). While pasta and vegetable treatments were sufficient regardless of aeration, aerobic treatment on bread caused a significant reduction in magnesium compared to anaerobic treatment. The availability of magnesium in soil systems is limited by acidity, with a soil solution of pH<6 causing a sharp drop of availability. This is not observed in the FWBHF treatments. Both anaerobic bread and pasta treatments had observed pH of ~4.5. In both cases, magnesium was significantly superior in anaerobic bread, and statistically similar in anaerobic pasta treatments. More studies into the solubility and availability of magnesium in aqueous solutions are required to understand the mechanisms behind its plant availability.

Deficiency of this element is exacerbated by acidic conditions, with a sharp drop of availability below pH 6.0 (Ishfaq et al., 2022).

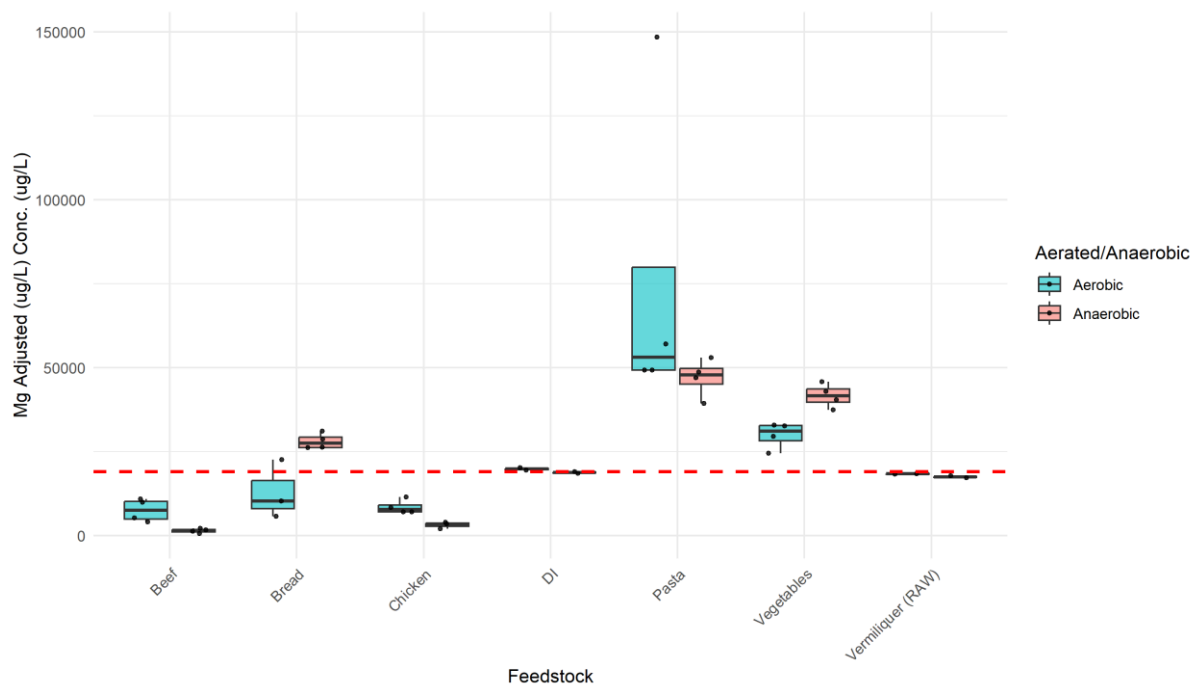


Figure 2.10: Magnesium content in aerobic/anaerobic food groups and vermiliqueur after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Micro-nutrient and heavy metal analysis

Micro-nutrients are essential for plant growth but are required in smaller amounts relative to macronutrients. Analysis of 4 micro-nutrients, zinc, copper, manganese, and iron were performed using ICPMS. In addition, analysis of heavy metals including lead and cadmium were also performed, as excess heavy metals can lead to detrimental effects in human health.

Zinc

All treatments have similar or significantly greater levels of zinc compared to the control (Figure 2.11). Notably pasta, bread, and vegetable treatments show a concentration of zinc high enough to cause zinc toxicity in crop species. The threshold for zinc toxicity varies by plant, but generally irrigation water is recommended to have a maximum zinc concentration of 2000 ug/L (Noulas et al., 2018) before symptoms of toxicity begin to impact yield. At the current concentration of each treatment (EC = 1.6 dS/m), the anaerobic pasta and vegetable treatments are at risk of yield reduction in crop species. Symptoms of toxicity can be ameliorated by raising solution pH above 6.0, which remains an acceptable threshold for majority of hydroponic species. This partially explains why anaerobic carbohydrate and vegetable groups have such high concentrations of the element, they all share a relatively low pH compared to their aerobic counterparts (Noulas et al., 2018). Protein groups show increased concentrations in aerobic treatment compared to anaerobic treatments, again this may be caused by the slight difference in pH causing heavy metal precipitates to form.

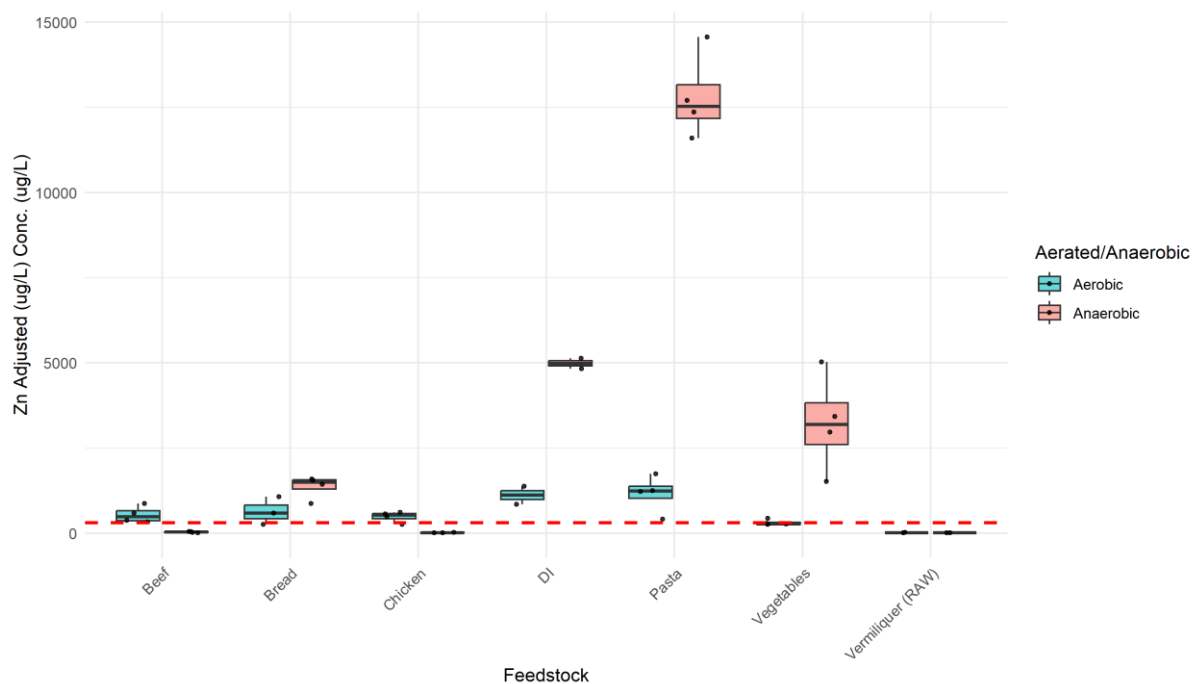


Figure 2.11: Zinc content in aerobic/anaerobic food groups and vermiliqueur after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Copper

Copper has the unique trait of being both essential and toxic for plant and human development (Kumar et al., 2021). Like zinc, copper is a metal cation ($\text{Cu}^+/\text{Cu}^{2+}$) and is most available in acidic conditions. All treatments have significantly lower copper contents relative to the control (Figure 2.12). Protein groups share similar behaviour, with aerated treatments yielding a higher, more varied copper content relative to anaerobic counterparts. Notably, pasta and vegetables share a similar reaction to aeration, while bread and vermiliqueur both show little variation and are statistically similar. This may be due to the insolubility of copper

in totally aqueous solutions, or due to the absence of copper in feedstocks.

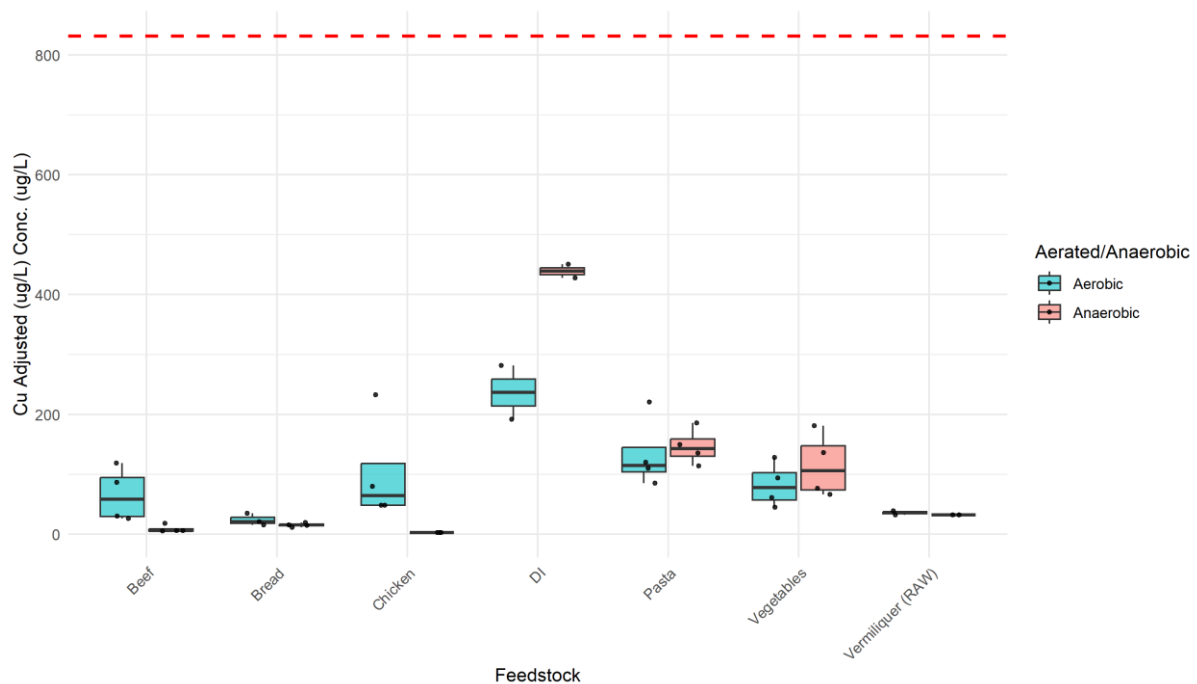


Figure 2.12: Copper content in aerobic/anaerobic food groups and vermiliquer after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Manganese

Manganese is an essential cofactor in photosynthesis, catalysing the process of splitting water in photosystem 2 reactions (PSII). Anaerobic bread and pasta treatments were significantly greater than control solution (Figure 2.13). Their aerobic counterparts were the second closest to meeting significant similarity to the control. This is likely due to the manganese content found in wheat seed, an essential nutrient in ensuring germination and early growth in plants (Marcar and Graham, 1986). In FWBHF application, the inclusion of grain-based inputs may be useful in fortification of manganese. In plant available form, the cationic Mn^{2+} shares a similar uptake path to calcium (Ca^{2+}) and is most readily available in slightly acidic solutions (Alejandro et al., 2020).

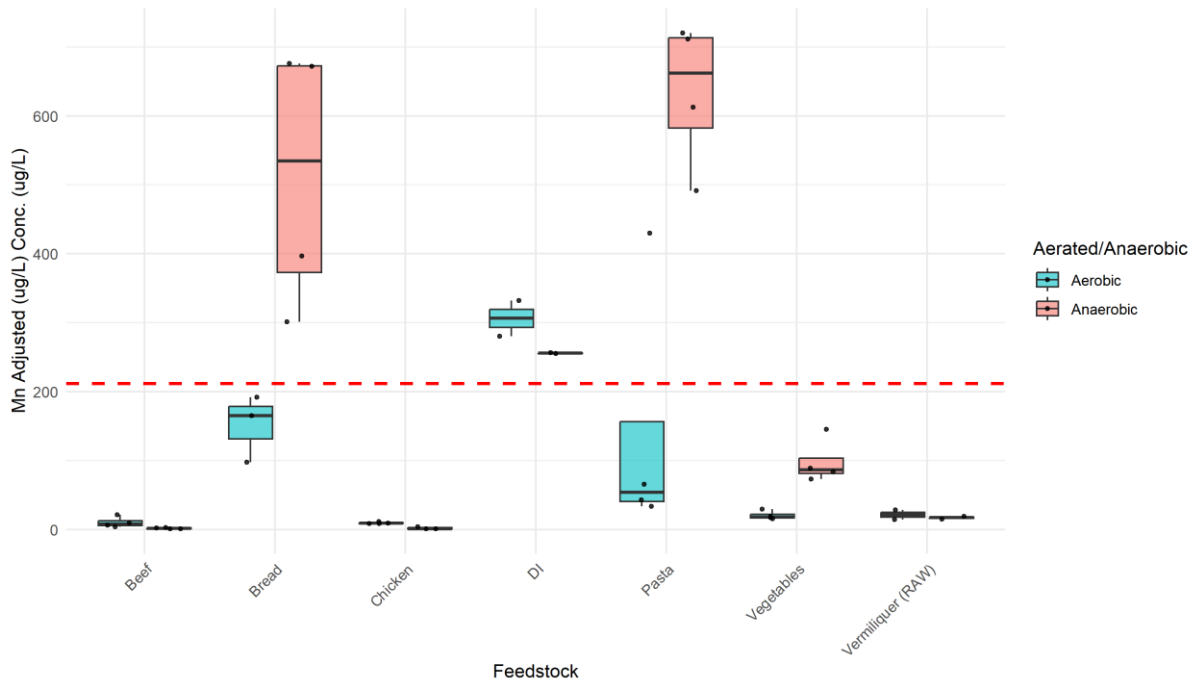


Figure 2.13: Manganese content in aerobic/anaerobic food groups and vermiliquer after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Iron

Iron is essential for electron transport in both photosynthesis and respiration in plants. Across feedstocks, all had at least one aerated/anaerobic treatment which was considered significantly similar or greater than the control (2.14). Iron is primarily absorbed in cationic Fe^{2+} and Fe^{3+} and solubilises readily in the presence of oxygen (Ning et al., 2023). The outlier to this hypothesis is in the vegetable treatment, the mechanisms of this behaviour are unknown. For other treatments, excess iron can cause yield reduction through interruptions of metabolic and photosynthetic processes (Ning et al., 2023).

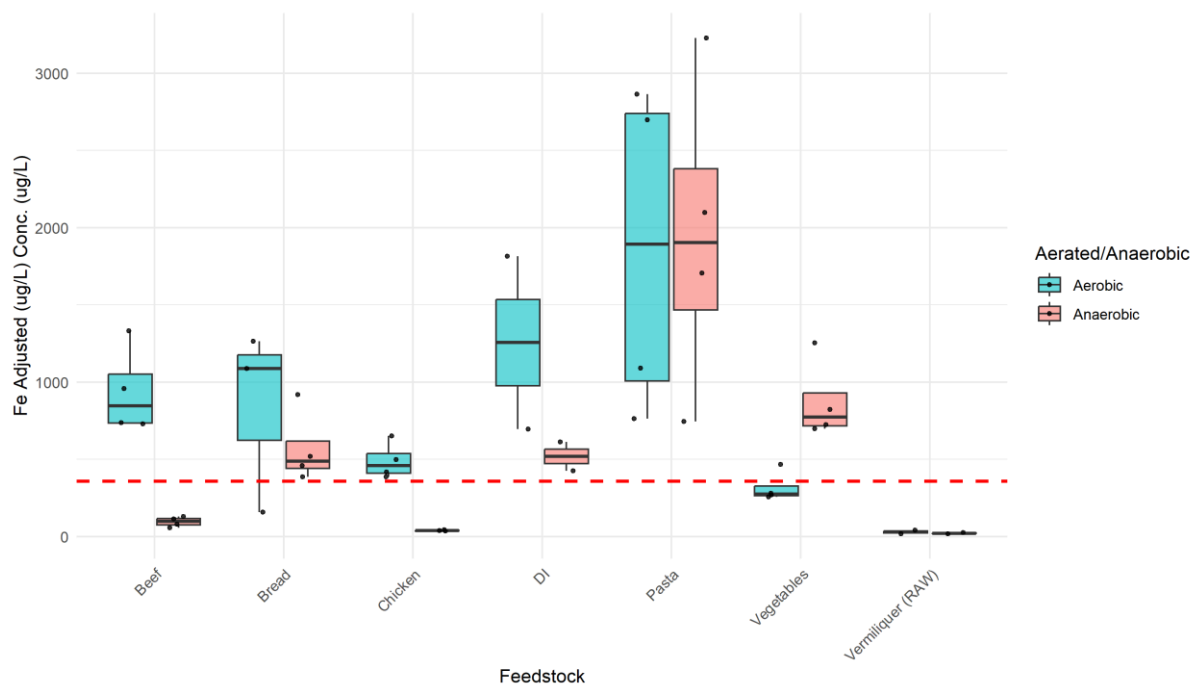


Figure 2.14: Iron content in aerobic/anaerobic food groups and vermiquor after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Heavy Metals (Lead and Cadmium)

Heavy metal contamination in agricultural fertilisers can adversely impact both plant and human health. As plants absorb heavy metals from soil solutions, these metals are transported to vegetative material, roots, and carbohydrate sinks in crops – causing bioaccumulation when consumed by humans. The level of tolerance for heavy metals is a well-studied field, with guidelines establishing a wide array of conditions to assist in determining acceptable limits. In Australia, fertiliser regulations are mandated by state governments, in New South Wales the maximum accepted levels of cadmium and lead are 10000ug/kg, and 100000 ug/kg, in non-phosphatic fertilisers (<2% phosphorus) (NSW Government., 2017). No treatment here exceeds this threshold (Figures 2.15, 2.16). This is congruent with existing knowledge of heavy metal contamination in conventional hydroponic systems.

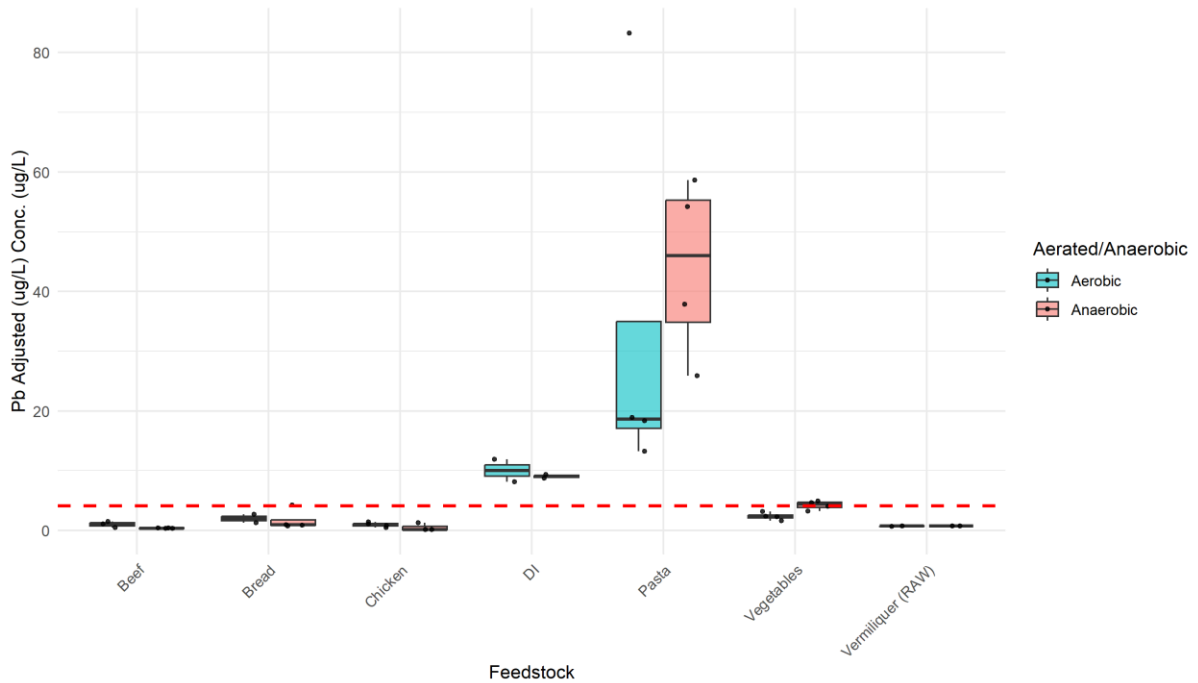


Figure 2.15: Lead content in aerobic/anaerobic food groups and vermiliquer after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

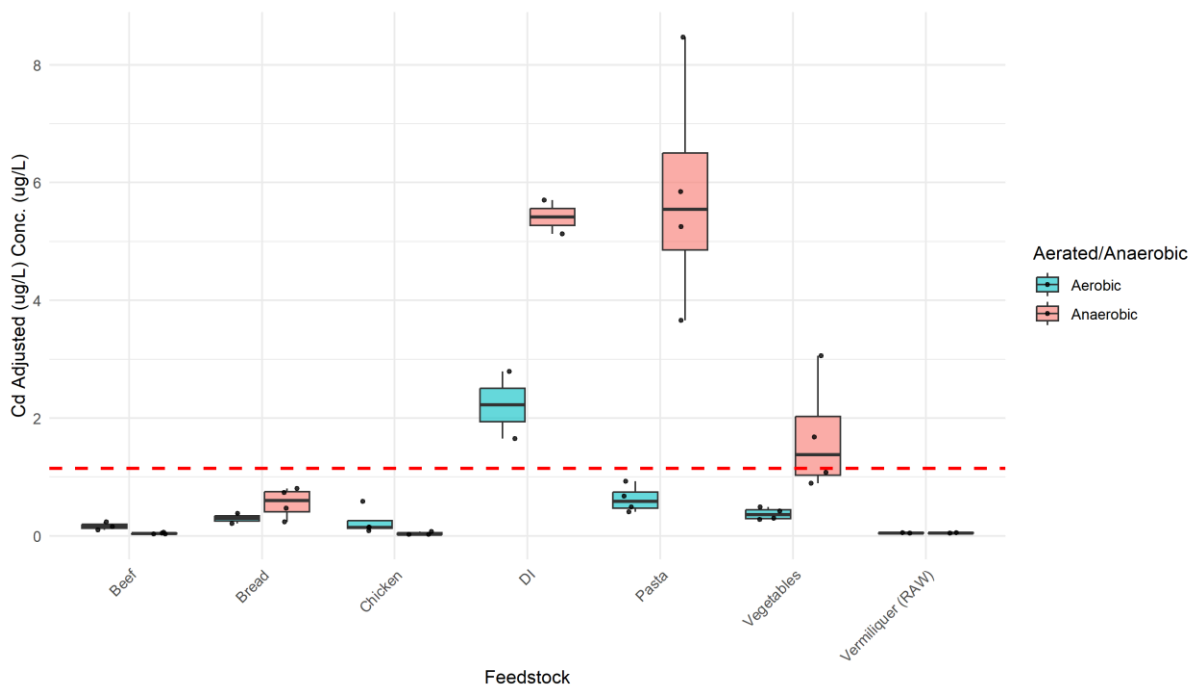


Figure 2.16: Cadmium content in aerobic/anaerobic food groups and vermiliquer after diluting to EC of 1.6 dS/m. DI = Deionised water. Horizontal line represents mean control sodium content.

Unlike in soil systems, conventional hydroponic systems are infrequently contaminated with heavy metals, largely due to the precise formulation of their synthetic solutions. However, the risk of heavy metal contamination increases when utilising FWBHF's. Trace heavy metals

from food products, such as in the pasta, bread, and chicken are solubilised during fermentation for plant uptake (Ghanati et al., 2019; Aljohani et al., 2023). In addition, plants grown in hydroponic systems have greater phytoremediation properties compared to soil systems – meaning they absorb heavy metal pollutants at a greater rate than soil-based counterparts (Sumalan et al., 2023). While this benefits the capacity for hydroponic systems to remove heavy metals from contaminated bodies, it increases the potential risk for heavy metal toxicity in both plant and human subjects (Sumalan et al., 2023). As such, considerations and checks for heavy metal toxicity should not be disregarded in future studies in this area.

2.4 Conclusion

This study suggests that individual food groups are not capable of forming an “all-in-one” solution and suggests future studies to introduce composite FWBHF solutions using the same techniques and comparing results with these findings. It does find similarities within food groups of similar composition. Protein-rich food groups shared similar reactions in mineral synthesis when exposed to aerobic and anaerobic fermentation treatments. Conversely, carbohydrate-based treatments occasionally differed in reaction to treatments. This may be due to post-processing methods altering composition of nutrients through baking, moisturising, supplementing with other ingredients, or even the fortification of foods with human essential nutrients. All fermentation treatments shared the same odorous trait, which may be difficult to overlook in commercial or retail application.

While the composite vermiliquer treatment was deficient in nitrogen, calcium, copper, and manganese nutrient groups, it was odourless and demonstrated a capacity for nitrification. In addition, it contained adequate potassium, sulphur, magnesium, zinc, and iron. Current applications of this treatment may be as supplements to existing synthetic fertilisers, or as a standalone fertiliser when used in conjunction with nutrient supplements.

Future directions should include identification of potential microbial fixers of nutrients. Identification and cultivation of microbes capable of partaking in the breakdown of organic material into inorganic nutrients may be the key to optimising nutrient cycling in FWBHF development. The extension of cultivating communities during FWBHF production is to maintain similar communities in active growth systems. Exploring the potential of creating a stable micro-biome in operation hydroponic systems provides potential to explore continual supplementation of organic nutrients during growth stages and minimising chemical and physical methods of pest and disease control.

This paper has identified that current iterations of FWBHF development methods are incapable of meeting the nutrient levels of synthetic solutions. The primary challenge appears to be excessive salinity limiting available nutrients. High salinity environments place stress on plant nutrient uptake, and thus solutions must be dosed at levels which may not provide optimal nutrition to plants. Developments in the isolation, precipitation, or removal of excess salts should be explored going forward in FWBHF development. Using FWBHF to achieve optimal yields will likely require the use of synthesised solutions, although efforts to explore the effects of substitution should be made. A partial replacement of synthetic solutions still works towards the goal of improving nutrient cycling in our food production systems and bringing urban horticulture into the growing field of sustainable agriculture.

Chapter 3. Evaluating the efficacy of a vermiliquer based food-waste based hydroponic fertiliser derived from household waste.

Abstract

Developing methods of re-utilising food-waste in hydroponic systems aims to improve nutrient cycling in urban environments and provide alternative sustainable methods for urban horticulture. Current research in food-waste based hydroponic fertiliser development (FWBHF) centres around multi-step processing – usually a combination of mechanical, biological, and chemical methods which aim to efficiently convert organic food-waste into plant-available inorganic form. FWBHF preparation utilised a four-step process: 1) mastication, 2) vermiculture, 3) anaerobic fermentation, 4) aerobic fermentation. Nutrient analysis using ICP-MS and FIA found significantly lower concentrations of nitrogen, calcium, and phosphates in the FWBHF compared to the commercially available synthetic solution. However, the concentration of sulphur and potassium were both significantly greater. Growth trials with lettuce (*Lactuca sativa*) found a reduction in fresh weight, SPAD, and root length with the FWBHF. It is likely that normal growth was inhibited due to excessive sodium ($\text{Na} = 46.3 \text{ mg/L}$) and a high nitrate:ammonia ratio (300:1), creating a saline-alkali solution. Causal factors for this may be a saline feedstock, excessive aeration, or extensive anaerobic fermentation. This research details the potential changes, and/or additions, in methodology which may remediate these imbalances and subsequently improve the viability of future FWBHF's.

3.1 Introduction

Efficient methods for recapture and reutilization of the nutrients found in consumer-level food-waste could extend concepts of circular economy of nutrients to the urban food production industry. The development of food-waste based hydroponic fertilisers (FWBHF) aims to address the nutrient leakage created by urban food-waste by recycling organic matter into plant-available inorganic form for use in urban hydroponic settings. This approach aims to provide a cost-effective, sustainable source of plant nutrients for urban food-production, with the aim of improving food security by increasing the availability of locally sourced fresh produce while reducing import reliance.

Experimental methods for processing food-waste into experimental FWBHF usually involve some sort of physical preparation (e.g. mastication, heating, boiling) followed by a period of biological breakdown (e.g. fermentation) (Wang et al., 2024). This two-stage approach emulates natural breakdown processes, with human intervention substituting the role of scavenger and detritivore behaviour. The physical breakdown of food-waste tissues increases the available surface area that microorganism communities can access and develop in. These communities ultimately facilitate the breakdown of organic material into plant available inorganic form.

Current challenges facing FWBHF production include variability of feedstock, excess salinity, and shortages in plant available macro- and micro- nutrients. Compared to primary

and industrial producers, predicting the volume and composition of food-waste at consumer levels can be difficult. This is largely caused by seasonal variations in food availability, unexpected shortages, cultural/palette preferences, and the ubiquitous nature of global cuisines in major urban areas. These factors contribute to increasing the variability of feedstock composition, requiring processing methods to be resilient and elastic to an excessive range of inputs.

The food waste produced by end-consumers are commonly saturated with oils, and salts. These contribute towards salinity issues common in trial FWBHF's (Wang et al., 2024). Salinity in hydroponics must be managed carefully, as the optimal range of uptake for many species is an electrical conductivity (EC) of between 1.6 and 2.2 dS/cm (Dunn & Singh, 2016). Exceeding these bounds generally results in stunted yield, or crop failure either due to malnutrition or excessive osmotic stress on the root system. As a result, excessive salinity in FWBHF is often the limiting factor in nutrient solution application, causing a shortage of essential nutrients.

These factors have shaped the processing methods adopted in this study. The methods of this study can be broken into four distinct steps: i) mastication, ii) vermiculture bioconversion, iii) anaerobic fermentation, and iv) aerobic fermentation. The adoption of vermiculture bioconversion is predicated upon the proven benefits red earthworms (*E. fetida*) provide to soil-based systems. Earthworms facilitate organic breakdown of large organic particles into plant-available inorganic nutrients found in their waste, known as casts. Casts are rich in nitrogen, and accelerate nitrogen mineralisation by hosting diazotrophic microbial species which have been found to provide between 10-38% of the required nitrogen for a variety of grain-crops in broad-acre settings (James S.W., 1991; Parmelee & Crossley, 1988; Parmelee & Whalen, 2000; Pathma & Sakthivel., 2012; Blouin et al., 2013). Diazotroph species can proliferate in a range of environmental conditions, with dryland, root-bound species (*sp. Rhizobium*), to aqueous cyanobacteria (*sp. Anabaena, Nostoc*) and free-living bacterial species (*sp. Azospirillum, Clostridium*) (Rodrigues et al., 2018).

This report aims to detail whether the integration of aeration and vermicompost techniques can improve the nutrient availability of

The first step, mastication, aims to homogenise food-waste to increase surface area for microbial and invertebrate access, aerating the feedstock to avoid putrefaction, and prevent the formation of food-specific fungi. Vermiculture bioconversion utilizes red earthworms (*E. fetida*), bacteria, and fungi to convert organic matter into inorganic nutrients. The outcome from this process is multi-faceted, in addition to bioconversion earthworms also play a role in aerating feedstock, and inoculating feedstock with nutrient cycling microbes (Raimi et al., 2022). The anaerobic fermentation of vermiliquer aims to homogenise of feedstock and provides organic matter an opportunity to break down in the absence of solids. The final step, aeration, aims to facilitate aerobic biochemical and chemical reactions to begin fixing nutrients into plant available forms. While the primary intention is to facilitate free-floating diazotrophic species, oxygenation can also influence the solubilisation of other elements into available forms (Caceres et al., 2006).

In addition to nitrogen, earthworms have been observed to increase phosphorus by 50%, potassium, calcium, and magnesium (James, S.W., 1991; Suarez et al., 2004; Adejuyigbe et al., 2006). A side-effect of this improvement is an increased rate of nitrogen and phosphorus leaching. This can be considered beneficial in FWBHF production, as the flushed leachate, known as vermiliquer, is the primary component used in hydroponic application. Conversely, the high calcium content of casts facilitates the exchange of soil sodium, increasing salinity in

the vermiliquer (Blouin et al., 2013; Li et al., 2021). It is largely unknown whether this behaviour exists in a soil-less vermiculture system. However, symptoms of sodium-induced stress may be mitigated by the high level of calcium found in earthworm casts (Tammam et al., 2021).

The addition of anaerobic and aerobic fermentation provides time for leachate homogenisation, fixation of nitrogen species, and acclimation of the microbial community in an aqueous environment. This additional step is a modification of a previous study, which demonstrated that an aerated vermiliquer solution produced superior crop yields to an unaerated, recirculated vermiculture-hydroponics system (Churilova & Midmore, 2019).

The primary objectives of this study are to determine the initial nutritional content of the resulting FWBHF in relation to these processing methods, as well as the changes macro-nutrients, and nitrogen species, undergo throughout a 25-day growth cycle with Lettuce (*Lactuca sativa*). In addition, observing the yield outcomes from their application. By utilising an array of energy/cost efficient techniques, this paper hopes to begin developing methods to begin recirculating food-waste in urban horticulture settings.

3.2 Materials and Methods

FWBHF preparation

A FWBHF was prepared through fermented vermiliquer methods. Vermiliquer was harvested from an established worm farm which was fed household waste over the course of one month. The household waste constituted of a fluctuating volume of raw and cooked food-waste products, including vegetables, eggshells, coffee grounds, pasta, rice, bread, and raw/cooked bones. The worm farm was turned, and the contents masticated with a trowel each week. In addition, 1 litre of water was flushed through the system and the resulting leachate/vermiliquer was collected and left to sit in a 22°C storeroom with a loose lid for 60 days and then placed in a cold room set at 4°C. 48 hours prior to application, the FWBHF was aerated for 48 hours with an airstone with an air-flow rate of 60L/hr. The control treatment was a commercially sourced “leafy green” synthetic hydroponic solution (Aqua Vega, CANNA).

Chemical water nutrient analysis

Nutrient solutions taken from the beginning and ends of the trial were prepared for flow injection analysis with a 20-times dilution. Flow injection analysis was used for taking total Kjeldahl nitrogen, nitrates, and ammonia readings (FIAlyzer-1000, FIA Labs). ICP-MS analysis was also performed on nutrient solutions collected at the beginning and ends of the trial, measuring total nutrient availability as well as presence of heavy metals (P, K, S, Ca, Mg, Zn, Cu, Mn, Fe, Pb, and Cd). Samples were prepared with an acid digestion of 0.125ml of HNO₃ and 0.375ml of HCl to 0.5ml of the solution. They were heated to 60°C in a water bath for two hours to ensure total digestion. Samples were frozen immediately after and thawed immediately prior to 10-times dilution for ICP-MS analysis (Nexion 1000 ICPMS, PerkinElmer) at 4.5L/min helium kinetic energy discrimination mode.

Growth trial and post-harvest yield analysis

Lettuce (*Lactuca Sativa*) was germinated over a 14-day period in one of two commercially available, organic substrates. The first substrate (S1) is composed from peat, bark and biodegradable binder (Root!t!, Natural Rooting Sponges), and the second (S2) is primarily composed from coco coir, peat, and a biodegradable binder (Xtract Plugs, Quickplugs). The control was dosed in all treatments at 50% strength (0.8 dS/m) after majority of seedlings developed true leaves (8th day). After seedlings had developed a second set of true leaves (14th day) they were transplanted into nutrient flow technique (NFT) growth systems (Figure 3.1).

Four heads of lettuce were planted in each of the 18 separate NFT systems. Each system hosted a 20-litre capacity, and a flow rate of 200L/hr. Air temperature was maintained at 24°C, with a room humidity of 70%. Lighting included two 36W fluorescent tube lights of “Cool White” per system, which produced approximately 4000 kLux at substrate level. Nine vermiliquer systems were trialled against nine control systems, with the treatment layout utilising a completely randomised design.

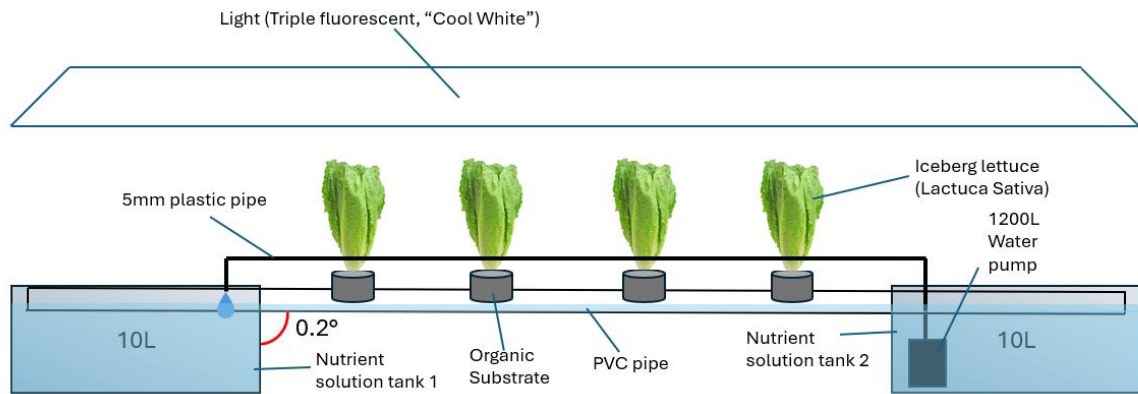


Figure 3.1: Diagram of nutrient film technique setup utilised in this trial.

Initial introduction of seedlings to the NFT system utilised a tap water and 50% strength concentration (20ml A/B or 500ml vermiquer) of either control or treatment solutions. The corresponding treatment was added every 72 hours in ~ 0.4 dS/m increments (10ml A/B; 250ml vermiquer) until an EC of 1.6 dS/m was achieved. In addition, tap water was added again at the end of each growing period. To maintain an appropriate EC of 1.6 dS/m, a handheld EC/pH reader (Hi9813-61, HANNA) recorded EC and pH, and solutions were variably re-dosed back to the expected level. pH was not adjusted, as this trial aims to observe and measure the behaviour of the vermiquer treatment in the absence of chemical intervention.

This method was replicated in a pilot study, with the only difference being an elevated air temperature of 28°C.

After 21 days in the growth system, lettuce was harvested by cutting at the base of the stem. Just prior to harvest, a chlorophyll content reading was taken with a hand-held SPAD reader (SPAD 502 Chlorophyll meter, Spectrum Technology). To qualify for post-harvest analysis, a plant had to have at least two pairs (4) true leaves. Fresh weight and root length were then measured followed by a 72-hour air-dry oven treatment for dry weight measurement.

Data analysis

Data analysis was carried out using Rstudio (2024.04.2 Build 764). Normality for measured parameters (yield outcomes, nutrient analysis) was assessed using Shapiro-Wilks test, qq-normal plots and histograms. Both nutritional analysis and yield outcomes were compared against the control. Chemical analysis found that all treatments were normal, and utilised parametric one-way ANOVA.

Significant changes in height, root length, and average SPAD were determined using ANOVA. Shapiro-Wilk's found that fresh weight distribution was not normal, and Kruskal-Wallis was used to determine significant differences between groups. Root-shoot ratio was calculated with plant height and root length. This unconventional method is due to the difficulty in separating the substrate blocks from the fine root systems of the samples.

3.3 Results

3.3.1 Chemical properties of the Vermiliquer

The initial EC of the undiluted vermiliquer was 6.6 dS/m and had an alkaline pH of 9.2. Once dosed into growth systems to an EC of 1.6 dS/m, the vermiliquer showed a gradual increase of mean pH of 7 to 8.5. The pH of the control solution reduced from an average of 7.4 to 5.8.

Table 3.1: ANOVA significance test results to determine significant differences between raw vermiliquer and the control once diluted to EC = 1.6 dS/m. Significance () denotes the difference between the control and vermiliquer.*

Element	Control \pm standard deviation (mg/L)	Vermiliquer \pm standard deviation (mg/L)	p-value (<0.05)
Nitrogen	140.2 \pm 45.0	55.9 \pm 7.7	0.0003*
Phosphorus	19.8 \pm 6.1	7.1 \pm 1.1	0.005*
Potassium	96.0 \pm 31.7	248.5 \pm 16.4	0.0003*
Calcium	52.9 \pm 15.8	20.0 \pm 3.1	0.005*
Magnesium	21.4 \pm 6.3	10.1 \pm 1.2	0.005*
Sulphur	84.4 \pm 25.3	94.5 \pm 9.5	0.975
Sodium	16.9 \pm 4.1	41.1 \pm 3.2	0.005*

There were significant differences between the control and treatment in all macro-nutrients (Table 3.1). The control solution contained significantly greater nutrient levels in all elements except for potassium. Particularly, nitrogen and phosphorus were only 1/3rd of the control. Sodium was significantly higher in the vermiliquer. Finally, heavy metals lead and cadmium were significantly lower in the treatment. In addition, it should be noted that there is an increase in variation of each macro-nutrient in the control, compared to the vermiliquer treatment.

Changes in vermiliquer nutrient composition over time

Nitrogen species ammonia and nitrates were both measured before and after the growth trial. It was found that nitrates reduce over time, and ammonia rises. For both elements, the changes are considered significant ($p < 0.05$).

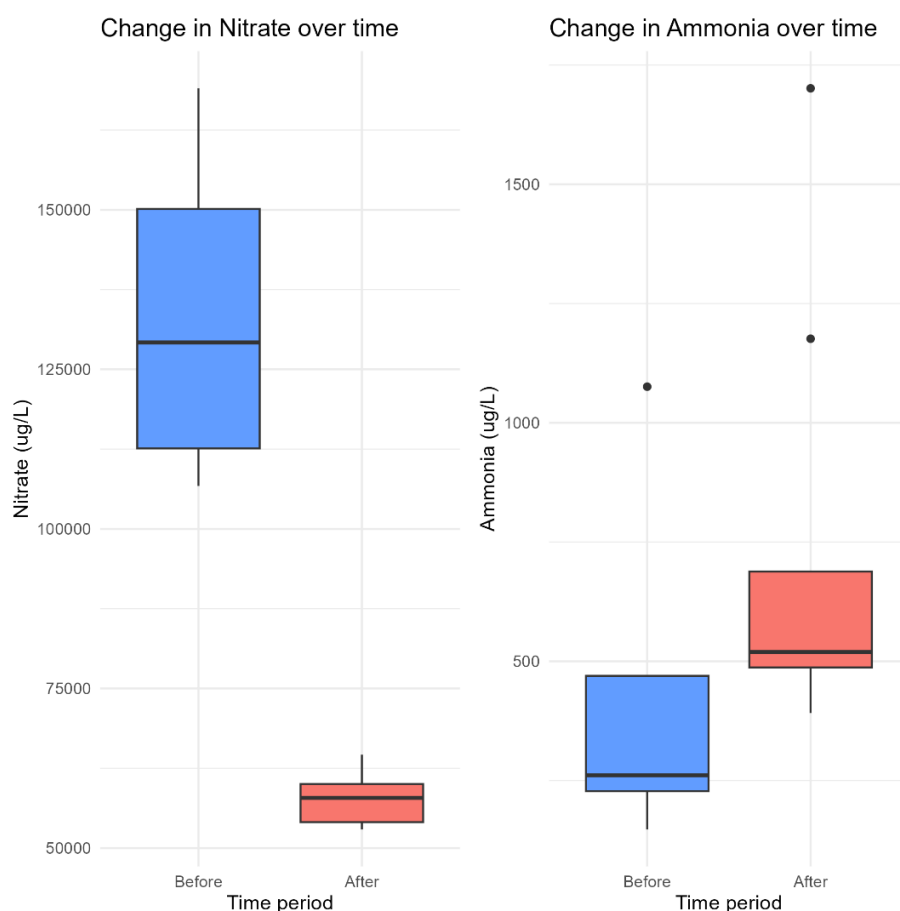


Figure 3.2: Comparison of nitrate and ammonia over time in the vermiliquer treatment. Before treatment measures the raw solution adjusted to an EC of 1.6, and the after solution measures the vermiliquer after a 25-day growth trial, adjusted to an EC of 1.6.

Notably, there was an observed inconsistency in nutrient concentration behaviour between the pilot study and the current growth trial. After adjusting for EC (1.6 dS/m), a comparison of the changes between day 1 and day 25 in nutrient composition found that nitrogen, phosphorus, and magnesium showed an opposite behaviour across growth trials. In addition, increases in calcium were significantly greater ($p < 0.05$) in the pilot study compared to the current study. This may be due to the elevated temperature conditions in the pilot study growth trial (28°C) and requires further investigation.

Table 3.2: The percentage change in macro-nutrient content between the adjusted undiluted raw vermiliquer and the vermiliquer after 25 days of plant growth. Includes both the pilot study and the current trial (GT3). Significance (*) denotes differences in solution nutrient composition between day 1 and day 25 of the growth trial.

Element	Pilot study change (%) ($p < 0.05$)	Current change (%) ($p < 0.05$)
Nitrogen	-38%*	-1%
Phosphorus	-25%*	+18%
Potassium	-50% *	-38% *
Calcium	+229% *	+40%*
Magnesium	+70% *	-43%*
Sulphur	+181% *	+144%*

3.3.2 Yield outcomes

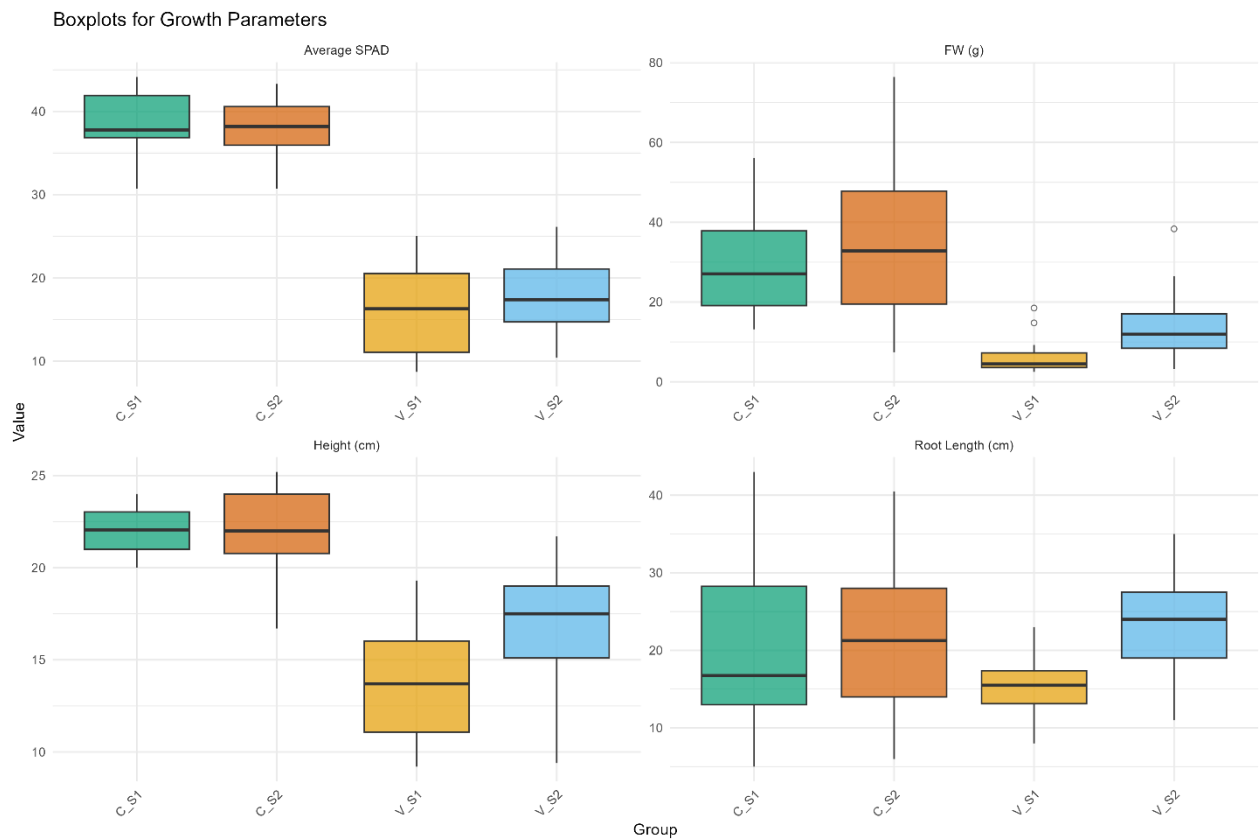


Figure 3.3: Boxplot figures of: a) average SPAD b) fresh weight (g), c) plant height (cm), and d) root length (cm) across 4 treatment groups: “C_S1” = Control substrate 1, “C_S2” = Control substrate 2, “V_S1” = Vermiliquer substrate 1, “V_S2” = Vermiliquer substrate 2.

Average SPAD acted as a proxy for chlorophyll content and was significantly lower in vermiliquer treatments compared to the control ($p < 0.05$). Similarly, the fresh weight of the vermiliquer treatment was considered significantly lower than the control ($p < 0.05$). In addition, V_S2 yielded a significantly greater fresh weight compared to V_S1 ($p < 0.05$). Vermiliquer treatment also saw a significant decrease in height ($p < 0.05$), but there was no significant difference caused by substrate. Overall, vermiliquer treatments were similar to the control in root length. S2, however, had a significantly higher root length compared to S1 in the vermiliquer treatments. Generally, the vermiliquer had a greater root-shoot ratio relative to the control ($V = 1.3$, $C = 0.75$), this is likely to be due to the lower average fresh weight of the treatment.

3.4 Discussion

3.4.1 Chemical Properties of the Vermiliquer and yield outcomes

Excess sodium and deficiencies in macro-nutrient species nitrogen and calcium are likely the primary causes of the significantly lowered fresh weight observed in the vermiliquer treatment (Figure 3.3, Table 3.1). The excess sodium and alkalinity is most likely attributed to the level of base sodium species and an imbalanced nitrate: ammonia ratio (Beltrao Valenca et al., 2021). Generally, there is a degree of tolerance from crop species to mildly saline (<3%) or mildly alkaline (pH: 7.1 – 8.5) conditions, where growth remains unaffected through osmotic regulation, expression of acidic exudates in root systems, or shifts in ion uptake/outflow behaviour (Hasanuzzaman and Fujita, 2022; Yang et al., 2024). In tandem, however, even mild saline-alkali conditions (salt: <3%, pH: 7.1-8.5) compound the individual pressures of salinity and alkalinity, and inhibit regulatory functions which mitigate their effects on yield outcomes. (Oster et al., 1999). As a result, plants facing even mild saline-alkali conditions can experience inhibited root development, photosynthetic function, antioxidant systems, and osmotic regulation capacity. Without intervention, this often results in serious yield loss or plant death.

Salinity, measured as electrical conductivity (EC) in this study, is essential to plant growth as it represents essential plant nutrients in the form of solubilised salts. However, excessive accumulation of non-essential salts (such as sodium) can disrupt normal plant growth by interfering with plant uptake mechanisms. Salinity represents a primary challenge in FWBHF development, as hydroponic crops are more vulnerable to excess salinity, compared to in-soil systems (Tavakkoli et al., 2010). Our experimental vermiliquer contained proportionally greater sodium levels compared to other nutrients. This trend is pervasive throughout FWBHF research, which commonly report high levels of sodium (Wang et al., 2024).

When our vermiliquer is dosed at a level which does not induce osmotic stress (EC = 1.6 dS/m), sodium induced nutrient imbalances interfere with the selective uptake of ions, with over-represented elements (sodium) displacing less abundant cations (nitrates, calcium and magnesium) from plant uptake. The current vermiliquer iteration would need to be dosed at three times the strength in order to meet the nitrogen of the control. If this were the case, the solution would have an EC of 4.5 dS/cm – far exceeding the osmotic tolerance of lettuce causing disruptions in intra-cellular osmotic regulation, protein transport chains, and nutrient uptake/outflow behaviours.

A potential avenue for improving salinity tolerance is to improve maintenance of an ideal pH of between 5.5 and 6.5. This study found that the vermiliquer treatment had a mildly alkaline composition compared to the control (V_ mean = 8.3, C_ mean = 5.3). Generally, mild alkalinity is manageable by most crops – with many species having genetic responses to maintain stable intracellular pH levels. However, the addition of saline stress begins to hinder the efficacy, and even deployment, of these mechanisms. The stress of regulating intracellular pH in a plant is exacerbated by efforts to manage cell structures, ionic toxicity, and physiological drought as a result of salinity stress (and vice versa) (Fang et al., 2021).

In addition, improving pH regulation leads to improvements in overall nutrient availability within a solution (Yang et al., 2024). At the current level of alkalinity, there begins to be a deficiency in iron, phosphorus, boron, manganese, copper, and zinc (Clark and Baligar et al., 2000).

3.4.2 Changes in nutrient composition over time

An unexpected finding of this paper was the inconsistency of change in macro-nutrient levels of the vermiliquer solution between the pilot and current study. *Table 2* shows the differences between two growth trials utilising the same vermiliquer solution – with the only difference being the temperature of the growth trial room (Pilot study = 28°C, Current study = 24°C). Generally, changes in FWBHF composition during the growth trial are not documented (Wang et al., 2024), however, understanding the chemical changes which occur throughout a growth period may provide insight into optimising fertiliser application, minimising risk of pest and disease, and cutting down operational costs.

The most likely causes for inconsistencies in chemical concentration over time are i) acceleration of biological activity within the solution, and ii) accelerated growth of lettuce in response to increased temperatures. A trial conducted by *Yang et al.*, (2024) documented the changes in both plant growth and nutrient composition of a hydroponic solution over two seasons (summer and winter). The study found that total nitrogen, magnesium (Mg^{2+}), and sulfate (SO_4^-) behaved significantly differently between the warmer (27.2°C) and cooler periods (23.7°C). As expected, elevated temperatures produced significantly higher yield outcomes in the summer growth period, as such the summer nutrient solution saw a reduction in total nitrogen, phosphorus, and potassium, and an increase in magnesium and sulphur. Conversely, the lower yielding fall trial did not see a significant reduction in total nitrogen or an increase in magnesium. In this study, the observed findings between the pilot trial and the current trial correspond with *Yang's* findings. A greater yield was observed in the pilot study (28°C), as well as the significant reduction in the vermiliquer nitrogen, phosphorus and potassium content, and increases in magnesium and sulphur. Meanwhile the current study (24°C) saw significantly lower yields compared to the pilot ($p < 0.05$) and not see the same uptake of nitrogen and shared a reduction in magnesium. The similarity of these findings suggests that increased rates of growth lead to a greater demand of nutrients, leading to a greater rate of plant-solution exchange. This hypothesis is reinforced by the observed decrease in EC and increase in pH, which are characteristic of nutrient uptake in plant-solution interactions.

Despite the similarities, there were some differences observed between this study and *Yang's* findings. Firstly, this study found a significant increase of calcium in both growth trials, which is absent in *Yang's* observations. The increase in calcium is unusual, as the alkaline pH in both trials (pilot: 8.9, current: 8.4) would usually cause calcium to begin precipitation. However, this may be attributed to upstream municipal supplements in the irrigation water during the pilot study growth period. Secondly, the gravity of fluctuations in macro-nutrient content were much more volatile. The proportional increase and decrease of elemental abundance were much greater compared to *Yang's* findings. It is possible this is due to the bio-active nature of FWBHF. It may also be attributed to the due to lower overall abundance of nutrients, which causes regular levels of nutrient uptake to create greater proportional changes in nutrient content.

The observed changes in the nitrate: ammonia balance in the current study was also conducive with existing research. An ideal nitrate: ammonia ratio in hydroponic operation is considered to be ~75:25 (Chen et al., 2024). Maintaining this ratio provides three benefits: i) Ensures nitrogen species are optimized for plant uptake through improvements in N storage and root development (Cesco et al., 2012); ii) improves intracellular exchange and pH balance (Vidal and Gutierrez, 2008); iii) Increases the development of plant growth, and N metabolising enzymes (Hachiya and Sakakibara, 2017; Wang et al., 2019). The initial nitrate: ammonia ratio in the vermiliquer treatment was considered high (300:1), as expected, it

gradually reduced (80:1) over the growth period. This shift is likely due to plant uptake favouring nitrates and denitrification processes. The high nitrate concentration produced an environment conducive with microbial denitrification, largely due to hydroxyl exchange from nitrate uptake providing ionic materials and alkaline conditions (Albina et al., 2024). While this process is “self-correcting” towards a more ideal nitrate: ammonia ratio, the side-effects of excessive microbial denitrification can lead to a continued increase in alkalisation due to hydroxyl production (Albina et al., 2024). Therefore, future FWBHF development should aim to provide an appropriate nitrate: ammonia ratio of approximately 8:2. Alternatively, future research may explore the effects of pH buffers on the alkalisation caused by high nitrate concentrations and microbial denitrification.

3.4.3 Approaches to FWBHF development – managing nutrients.

The goal of FWBHF development research is to develop a method which can process organic matter into a solution of inorganic nutrients optimized for crop production. Similar to most existing literature, the results from the growth trial found that the trial methodology was not capable of replicating the yield outcomes of the control ($p > 0.05$) (Wang et al., 2024). The inhibition of lettuce development is most likely attributed to a nutrient imbalance and the subsequent saline-alkali conditions it produces. The observed deficiency in nutrient content is a persistent issue in FWBHF development and can be attributed to the inherent discrepancy between what is collected as food-waste and what is ideal for crop production. Usually, the greatest limitation is an excess of sodium, which regularly fluctuates in feedstocks due to shifts in seasonal supply and consumer demands (Wang et al., 2024). This creates the need for a method which is both capable of optimizing essential nutrients and filtering excessive salts, while being robust enough to tolerate variations in feedstock.

Efforts to remediate nutrient deficiency in FWBHF systems can take place either during FWBHF development or during growth periods. A literature review found that a majority of existing research focusing on the development of FWBHF utilised some form of microbiological activity to break organic matter down into inorganic forms (Wang et al., 2024). The ensuing inorganic elements are then further processed chemically or biochemically into plant-accessible nutrients. Furthermore, supplementary literature exploring modifications in management and/or the implementation of hardware intended to improve plant-essential nutrients in a solution draw from research in aquaculture, soil science, wastewater management, and biogas effluent management. This section seeks to highlight methods which take place during FWBHF development or growth periods to potentially improve the speed, quality, and consistency of future FWBHF's. The intended outcomes of the following methods range from optimisation of organic matter breakdown, inhibition of disease, or to guide biochemical and chemical pathways in preferred directions.

While the methods employed in this paper were aimed at optimising nutrient solution outcomes, these methods were not able to produce a FWBHF as effective as the synthetic control. Using the control as a nutritional baseline, the experimental FWBHF was deficient in nitrogen, phosphorus, and calcium. Conversely there were excess levels of potassium, and sodium, which may contribute to elemental toxicity – further inhibiting yield. A notable feature of the methods included are the multifaceted outcomes they produce. This is likely a common outcome in a range of potential methods due to the interrelated nature of biological, chemical and physical properties within a FWBHF. Such effects should be noted for each

applied method, and future studies should explore reactions of each property both individually and in relation to each other.

As discussed, plant responses and measured nutrients lead to the conclusion that the observed difference in yield is most likely due to nitrogen deficiency. Both the lowered fresh and dry weight, alongside the significantly lower measured chlorophyll are symptomatic of nitrogen deficiency (Broadley et al., 2000). Such a deficiency is particularly prominent in vegetative species, such as lettuce. With the FWBHF containing only $\sim 1/3^{\text{rd}}$ of the nitrogen provided by the synthetic control, there is a strong need to amend this missing element. Within FWBHF research, the contributing factors towards having an adequate supply of nitrogen (without synthetic supplementation) had highly vegetative feedstocks or utilised some form of bio-active nitrification mechanism during growth periods (Wang et al., 2024).

Phibunwatthanawong and Riddech (2019) developed a FWBHF with similar levels of nitrogen when compared to a conventional synthetic solution using an aerated fermentation technique. The high level of nitrogen could be attributed to the feedstock, sugar cane stems, which are both densely ligneous and rich in simple carbohydrates for microbial species to feed on while break down of harder plant matter occurs. The caveat to this approach is the inability of researchers to control food-waste generation – that is, at the consumer level it is not guaranteed to receive ideal feedstock for FWBHF applications. Active nitrification has been explored in food-waste, biogas, and wastewater based hydroponic fertiliser development trials. This method aims to overcome initial nitrate deficiencies by installing an aerated, porous surface alongside some form of organic carbon into an active hydroponic system (Kawamura-Aoyama et al., 2014; Pelayo Lind et al., 2020). This provides a constant supply of nitrates as ammonia is gradually converted throughout the growing period. In addition, this assists in maintaining a 75:25 ratio of nitrates to ammonia, which provides a range of previously discussed benefits to nutrient availability and yield outcomes.

Utilising an active nitrification system also enables a potential form of nutrient management in an operational hydroponic system. While untested, the gradual supplementation of ammonia species into an active nitrification hydroponic system aims to provide a non-intrusive method of pH management. By capitalising upon the exchange of cationic ammonia and anionic nitrates with plant uptake systems, periodical supplementation of ammonia mitigates the alkalisng effects of nitrate uptake, which is the dominant nitrogen species preferred by plant species. This is achieved by both microbial oxidation of ammonia, as well as the release of organic acids in response to ammonia uptake by the plant. (Frijlink et al., 1992; Zhu et al., 2021; Chen et al., 2024). Future studies could measure the efficacy of this method compared to conventional techniques, especially in relation to yield outcomes.

In terms of directly improving the nutrient balance of FWBHF without altering feedstock, optimising fermentation conditions may assist in future FWBHF research. The optimisation of fermentation conditions could help create a superior FWBHF. A previous trial found that food waste comprised of proteins, vegetables, and carbohydrates showed elevated sodium level in response to aerobic techniques, an anaerobic phase may help precipitate excess sodium prior to application. Similarly, improving time under aeration may prevent over-nitrification, which has been proven to produce a lower yield weight compared to an optimal 75:25 nitrate: ammonia ratio (Chen et al., 2024).

Future technologies may also play a role in FWBHF. Currently, the application of plasma-fixation and selective reverse osmosis in FWBHF are not considered cost efficient and are thus unlikely to be utilised in the near future. Plasma-fixation of nitrogen aims to provide a sustainable alternative to the existing Haber-Bosch systems which have supplied majority of the worlds nitrogen fertiliser over the last century (Zhao and Tian, 2023). This new method

provides a greater energy efficiency and lower emissions. Selective reverse osmosis could be applied to filter out excess nutrients and salts from a FWBHF, correcting any nutrient imbalances in a solution. However, much like plasma-fixation, the cost of current infrastructure and ongoing costs prevents its application in current studies (Ang et al., 2019).

Remediation of nitrogen deficiency is a key step in improving the efficacy of FWBHF, however, research in the availability of other macro-nutrients should also be considered. In particular, the balance of nitrogen, phosphorus, and potassium (NPK) must be suitable for hydroponic application. While the exact composition of NPK varies by crop, there should be a priority in developing a greater understanding of processes which can accurately balance the required levels of these three key macro-nutrients.

3.5 Conclusion

This study confirms the inherent distribution and total availability of plant nutrients in food-waste as a major barrier for FWBHF development. Saline-alkaline conditions and the ensuing nutrient deficiencies in nitrogen seem to be the primary inhibitor of lettuce development. This trial found that multi-step treatments can produce a solution capable of sustaining crop growth, although not to the current yield standards of conventional solutions. The findings from this study have reinforced that future FWBHF development should pursue the improve the ratio of essential nutrients, particularly nitrogen, and reduce buildup of sodium-based salinity. As discussed, efforts for future development should focus on the development of robust, elastic techniques which can be applied to a range of changing food-waste sources.

Chapter 4. Evaluating the role and composition of lettuce (*Lactuca sativa*) rhizobiome assemblages in a food-waste based vermiliquer hydroponic system.

Abstract

The biologically active nature of food-waste hydroponic fertilisers (FWBHF) may lead to changes in the microbiome of the substrate and rhizosphere in an active hydroponic system. This study performs a metagenomic analysis of the rhizobiome in hydroponic lettuce (*Lactuca sativa*) grown using an experimental vermiliquer hydroponic FWBHF. Substrate samples were taken after a 25-day growth period, and next generation sequencing of the 16S (V3-V4) and ITS1 regions (Illumina MiSeq v3, 2 X 300 bp) found significant increases in both the abundance and diversity of identifiable genera. In addition, it was found that changes in bacterial taxa were more influenced by fertiliser type, while fungal taxa were more influenced by substrate type. Initial SIMPER analysis of fertilisers prior to application found significant differences in both taxonomy and functional group population distribution. By the end of the growth period, it was found that while taxonomy was still considered significantly different, functional groups (e.g. diazotrophs, plant pathogens, etc) were considered similar. This finding suggests that the hydroponic conditions, or rhizosphere activity from lettuce was able to exert a greater level of influence in substrate material than previously thought. These findings improve understanding of hydroponic substrate microbiome dynamics and aim to support future research exploring microbially beneficial inoculants in organic hydroponic systems.

4.1 Introduction

Current iterations of FWBHF's are unable to meet the yield outcomes of conventional, synthetic hydroponic solutions. This is largely attributed to nutrient imbalances, such as excess sodium and deficiencies in nitrogen. However, we expect that the biologically active experimental FWBHF has a greater microbial diversity compared to the conventional solution. Existing research in soil, and hydroponic systems have associated a diverse microbiology with improvements in yield quality, quantity, and disease resilience (Gravel et al., 2006; Khalil et al., 2009; Spaepen et al., 2014; Thomas et al., 2023). We hope that understanding the impacts FWBHF has on the metagenome of hydroponic growth media may provide unique use-cases for its application.

Existing research efforts into the microbial aspects of hydroponic systems have historically focused on the control of plant pathogen species (Schnitzler W.H., 2004). This is a component of the overall sterile culture of conventional hydroponics which utilise integrated pest management systems which aim to prevent, control, and/or eradicate the presence of microbial activity within protected cropping systems. This includes sterilising substrates, structures, and workers to prevent pathogenic actors from accessing the freely available nutrients in irrigation water. In addition, the homogeneity of crop species, and absence of perennial substrate ecology contribute to the vulnerability of these systems. Existing control systems are largely physical and chemical – with UV lighting, heat-treatments, and anti-microbial chemicals significantly reducing the impact of pest and disease affecting yield

outcomes (Ehret et al., 2001; Van Bruggen et al., 2006; Sheridan et al., 2017). Despite this, total sterility in hydroponics is virtually non-existent, as microbial communities have still been found across growth systems with stringent sanitation policies (Sheridan et al., 2017).

The inevitability of microbial colonization leads us to believe there is merit in manipulating and monitoring these populations as a method of biological pest management in hydroponic systems. While inoculation of specific biological control agents (BCA's) has been associated with the control of common root-based diseases such as *pythium* spp. and *fusarium* spp., the improved accessibility of metagenomic sequencing enables a more holistic view of the population dynamics of bacterial and fungal genera (Gravel et al., 2006; Khalil et al., 2009; Spaepen et al., 2014; Thomas et al., 2023). This approach aims to observe changes in response to the upstream inoculation of an ecologically diverse solution.

An additional biological actor observed in this paper is the interaction between the lettuce (*Lactuca sativa*) rhizosphere and introduction of a FWBHF. The degree of influence crops have on their immediate rhizobiome in soilless systems are contentious. *Lobanov et al.*, (2022) argues that crop species have enough control over their rhizobiome that upstream sources may not have any impact on composition, while *Meeboon et al.*, (2022) suggests that hydroponic microbiomes are not resilient enough to resist upstream inoculation. Despite this, it is unanimously agreed that the existence of a complex, ecologically redundant rhizobiome is essential for a range of direct and indirect crop benefits, including nutrient mobilisation, phytohormone production, and the management of pathogenic populations through competition or predation (Prashar et al., 2014; Fend et al., 2024).

This paper utilises next-generation sequencing to compare the metagenomes of a synthetic solution and an experimental vermiliquer FWBHF. The aims of this research seek to observe the impacts of biologically active upstream inoculant sources, on both substrate ecology and yield outcomes. This research hopes to provide insight into the role of substrates, fertilisers, and plant-substrate interactions when considering microorganisms as a biological method of integrated pest management systems in hydroponic systems.

4.2 Materials and Methods

Treatment preparation

A FWBHF was prepared through fermented vermiliquer methods. Vermiliquer was harvested from an established worm farm which was fed household waste over the course of one month. The household waste constituted of a fluctuating volume of raw and cooked food-waste products, including vegetables, eggshells, coffee grounds, pasta, rice, bread, and raw/cooked bones. The worm farm was turned, and the contents masticated with a trowel each week. In addition, 1 litre of water was flushed through the system and the resulting leachate/vermiliquer was collected and left to sit in a 22°C storeroom with a loose lid for 60 days, and then placed in a cold room set at 4°C. The FWBHF was aerated for 48 hours prior to application with an airstone with an air-flow rate of 60L/hr. The control treatment was a commercially sourced “leafy green” synthetic hydroponic solution (Aqua Vega, CANNA).

Growth trial and post-harvest analysis

Lettuce (*Lactuca Sativa*) was germinated over a 14-day period in one of two commercially available, organic substrates. The first substrate (S1) is composed from peat, bark and biodegradable binder (Root!t!, Natural Rooting Sponges), and the second (S2) is primarily composed from coco coir, peat, and a biodegradable binder (Xtract Plugs, Quickplugs). The control was dosed at 50% strength (EC = 0.8 dS/m) after majority of seedlings developed true

leaves (8th day). After seedlings had developed a second set of true leaves (14th day) they were transplanted into nutrient flow technique (NFT) growth systems.

Each NFT system contained a 20-litre capacity and a flow rate of 200L/hr (Figure 4.1). Ambient air temperature and humidity were maintained at 28°C, and 70%. Each system was lit with two 36W fluorescent tube lights of “Cool White” per system, which produced approximately 4000 kLux at substrate level. Of the 18 systems, nine contained the vermiliquer treatment, and the other nine contained the control. Each system held 4 heads of lettuce, two using substrate 1 (S1) and the remaining two using substrate 2 (S2). Thus, there are four distinct treatment groups in this study, i) Vermiliquer + substrate 1 (VS1), ii) Vermiliquer + substrate 2 (VS2), iii) Control + substrate 1 (CS1), and iv) Control + substrate 2 (CS2).

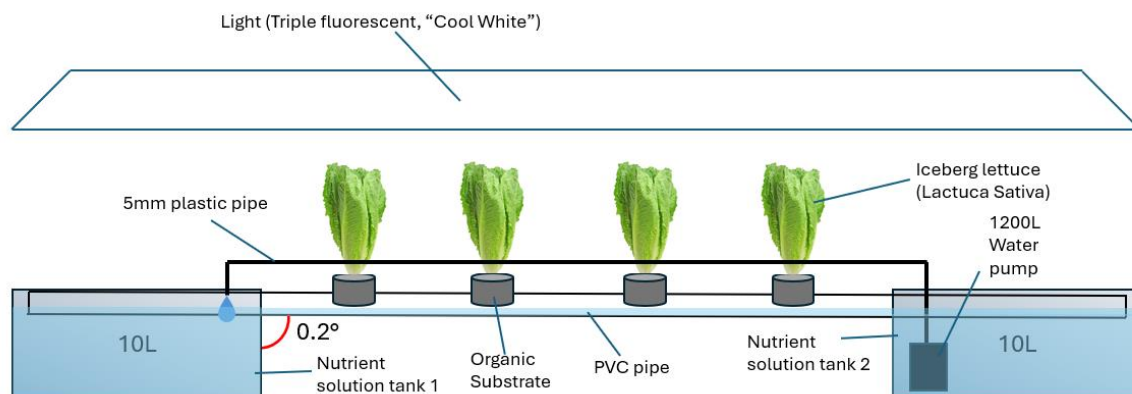


Figure 4.1: Diagram of the nutrient film technique setup utilised in this trial

Initial introduction of seedlings to the NFT system utilised a tap water and 50% strength concentration (EC = 0.8dS/m; 20ml A/B or 500ml vermiliquer) of either control or treatment solutions. The corresponding treatment was added every 72 hours in ~0.4 dS/m increments (10ml A/B; 250ml vermiliquer) until an EC of 1.6 dS/m was achieved. In addition, tap water was added again every 7 days to maintain 20L of volume. To maintain an appropriate EC of 1.6 dS/m, a handheld EC/pH reader (Hi9813-61, HANNA) recorded EC and pH, and solutions were variably re-dosed back to the expected level. pH was not adjusted, as this trial aims to observe and measure the behaviour of the vermiliquer treatment in the absence of chemical intervention.

After 21 days in the growth system, lettuce was harvested by cutting at the base of the stem. Just prior to harvest, a chlorophyll content reading was taken with a hand-held SPAD reader (SPAD 502 Chlorophyll meter, Spectrum Technology). To qualify for post-harvest analysis, a plant had to have at least two pairs (4) true leaves. Fresh weight and root length were then measured followed by a 72-hour air-dry oven treatment for dry weight measurement.

Sequencing the metagenome.

Rhizospheric DNA samples were prepared by shaving excess substrate from around the root system, dicing the remaining root ball, flash freezing with liquid nitrogen, and grinding to a powder. From this, ~0.1g was taken for extraction using a SPINeasy soil DNA extraction kit (MPBio). Amplification was performed on 16S V3-4 regions using the primers: 16S_341f (5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3') (Klindworth et al., 2013) and 16S_805r (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3') (Klindworth et al., 2013). ITS1 regions were amplified with ITS1f (5'-

TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGAGGAAGTAA-3') (White et al., 1990) and ITS2 (5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGCTGCGTTCTTCATCGATGC-3') (White et al., 1990) primers. Sequencing was performed using the Illumina MiSeq v3 in a 2 X 300bp paired end run.

Data analysis

MiSeq outputs were extracted, and primers were removed. They were then filtered, trimmed, and merged through RStudio package "DADA2". Read length (truncLen) was set to 260 for forward reads, and 220 for reverse reads, maximum expected errors (maxEE) was set to 2, and Phred quality truncation (truncQ) was set to 2. Merged reads were analysed with the NCBI BLAST+ software (v2.16.0, NCBI) against the NCBI ribosomal_RNA database (11 Nov 2024), or the NCBI ITS_RefSeq_Fungi database (15 Nov 2024). The resulting accession codes were assigned full taxonomy with the RStudio package taxonomizr (0.10.6) using the NCBI nucleotide database. Functional groups were assigned to 16S V3-4 outputs with FAPROTAX (Ver 1.2.9) (Louca et al., 2016). Guilds were assigned to ITS1 outputs with FunGuild (Ver. 1.1) (Nguyen et al., 2016). In addition, genera found in prominent, published literature was manually added to supplement identification.

Distribution of microbial taxa and functional groups were plotted with Principal component analysis (PCoA) in RStudio. PCoA was performed with Bray-Curtis distances, and clusters were identified and plotted against treatment variables. Cluster similarity and quantification were assessed with the use of pair-wise comparisons with adonis2 in R-Studio (Table 2), and p-values were adjusted using the Bonferroni correction. Similarity percentages (SIMPER) were calculated using "Spearman" distances. Visualisation of data was performed with ggplot and ggraph packages in RStudio. Significance tests were determined with a combination of Permutational analysis of variance, similarity percentage tests ("Spearman" method), Kruskal-Wallis (for non-normal data), and linear modelling, with outputs being corrected with Bonferonni corrections and confirmed with post-hoc Tukey's tests. Faprotax databases

4.3 Results

4.3.1 Microbial analysis of rhizobiome abundance and composition across treatments and substrates

Across all treatment groups, a total of 19810 unique bacterial species were identified, and 13093 unique eukaryotic species were identified using NCBI databases.

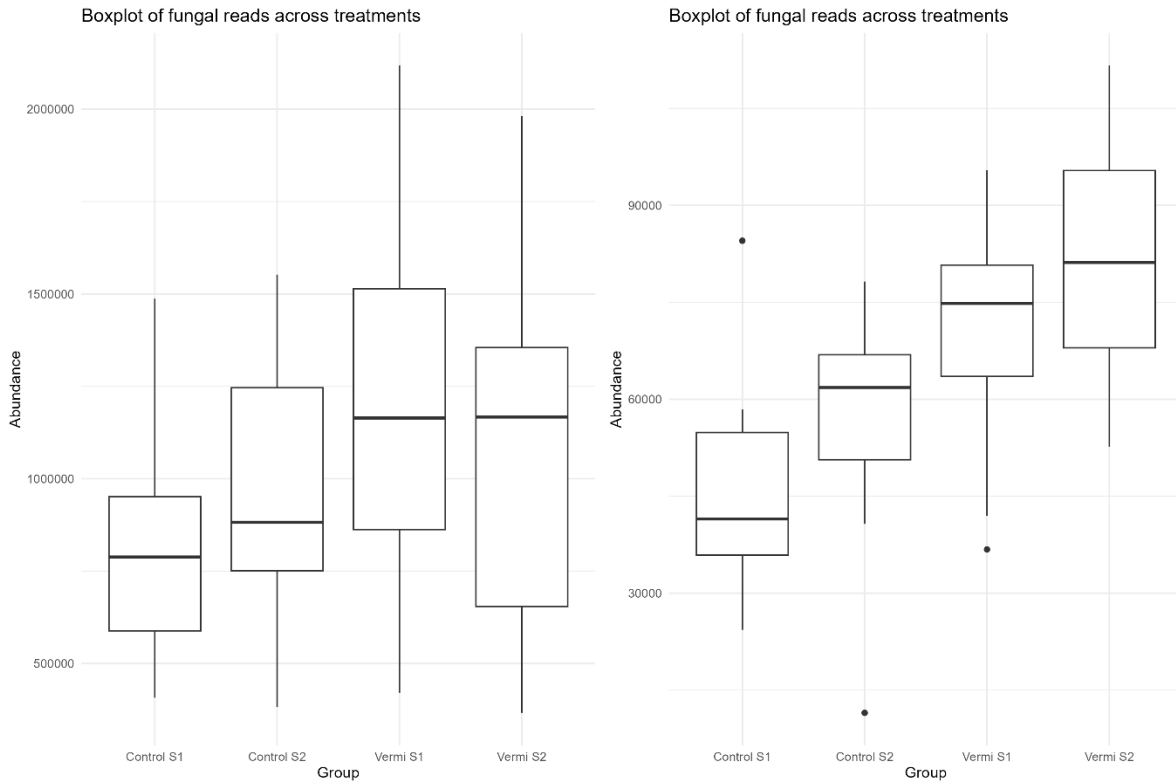


Figure 4.2: Boxplots of bacterial and fungal reads across treatments. Abundance represents the total reads within each replicate. Treatment groups: i) Control + substrate 1 (CS1), ii) Control + substrate 2 (CS2), iii) Vermiliquer + substrate 1 (VS1), iv) Vermiliquer + substrate 2 (VS2).

Both solution and substrate played a role in affecting identifiable fungal abundances ($p < 0.05$). Bacterial abundances, however, were not significantly affected by solution-substrate combinations ($p > 0.05$). However, when differentiated by only solution type, both bacterial and fungal abundances were greater in the vermiliquer treatments ($p < 0.05$). Substrate type did not significantly affect the abundance of bacteria; however, it had a significant effect on the abundance of fungal species ($p > 0.05$).

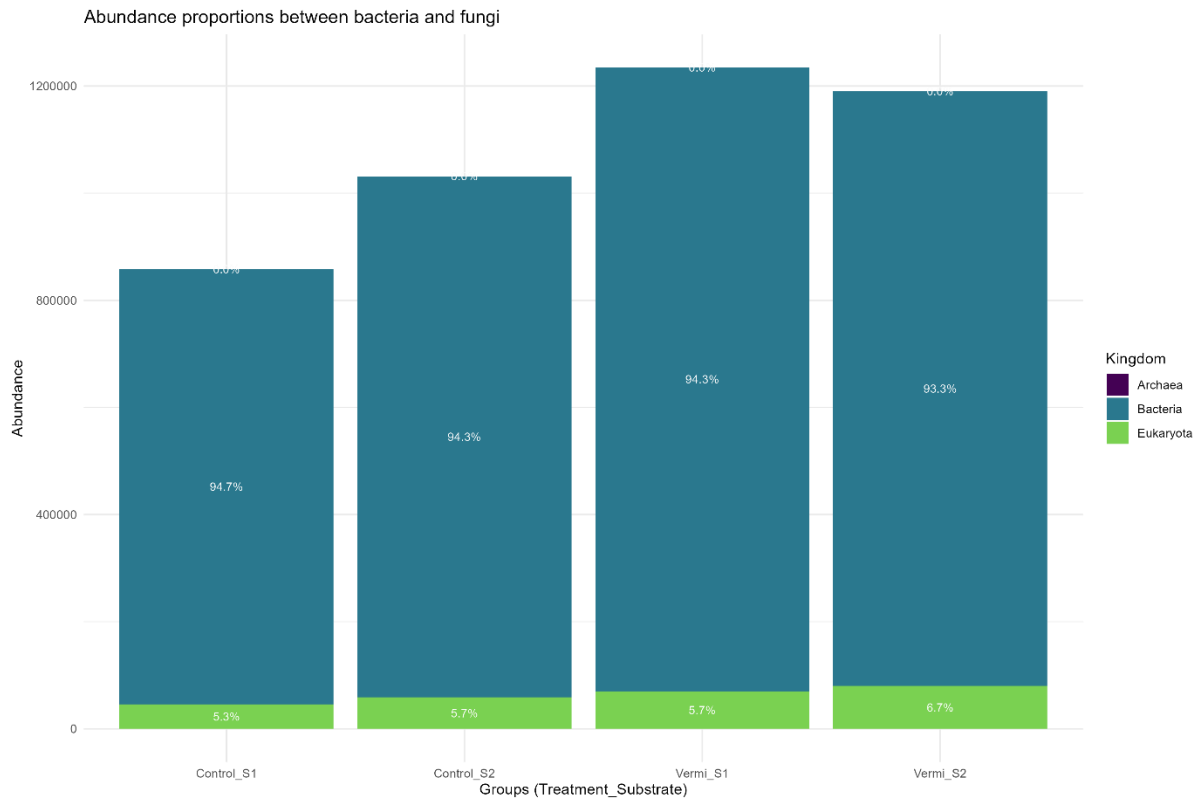


Figure 4.3: Abundance of identifiable microbiota represented as kingdoms. Archaea (0.0%) were not identified using the NCBI database in this analysis. Treatment groups: i) Control + substrate 1 (CS1), ii) Control + substrate 2 (CS2), iii) Vermiliquer + substrate 1 (VS1), iv) Vermiliquer + substrate 2 (VS2).

Both vermiculture treatments had a significantly greater overall abundance of bacterial and eukaryote species ($p < 0.05$). Across all treatments, there was no significant difference in the ratio of eukaryote and bacterial species. Differences in bacterial distribution, however, began at the phylum level. A pairwise PERMANOVA, with Bonferroni corrections, found that the proportional distribution of all bacterial phyla was significantly different across all combinations of treatments and substrates ($p < 0.05$).

ITS1 taxa was divided. Pairwise comparisons between CS1 x CS2, CS1 x VS1, and CS1 x VS2, showed significant differences at phyla level (Figure 4.4). A SIMPER analysis found that these differences mainly stemmed from different proportions of the fungal phyla *Ascomycota*, *Basidiomycota*, and *Chytridiomycota*. The remaining pairs, CS2 x VS1, CS2 x VS2, and VS1 x VS2, were similar in proportional distribution of eukaryotic phyla (Figure 4.5) – with differences occurring between these pairs at the Class level.

The common denominator causing significant differences at the phyla level is the CS1 treatment. The dissimilarity with CS2 implies that changes in substrate material led to changes in rhizobiome eukaryotic species composition. Conversely, the similarities in phyla composition between VS1 and VS2 challenges the influence of substrate in eukaryotic composition.

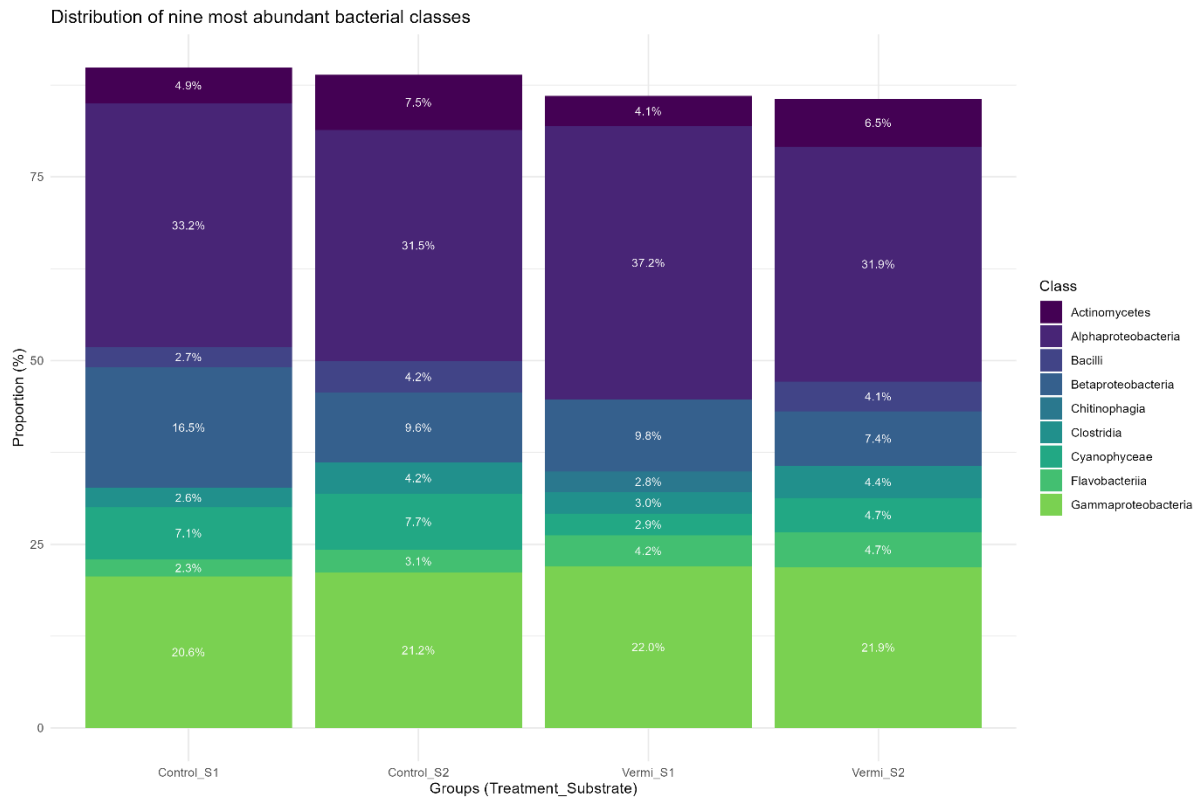


Figure 4.4: Proportional abundance and distribution of identifiable bacterial classes in each treatment group. Treatment groups: i) Control + substrate 1 (CS1), ii) Control + substrate 2 (CS2), iii) Vermiliquer + substrate 1 (VS1), iv) Vermiliquer + substrate 2 (VS2).

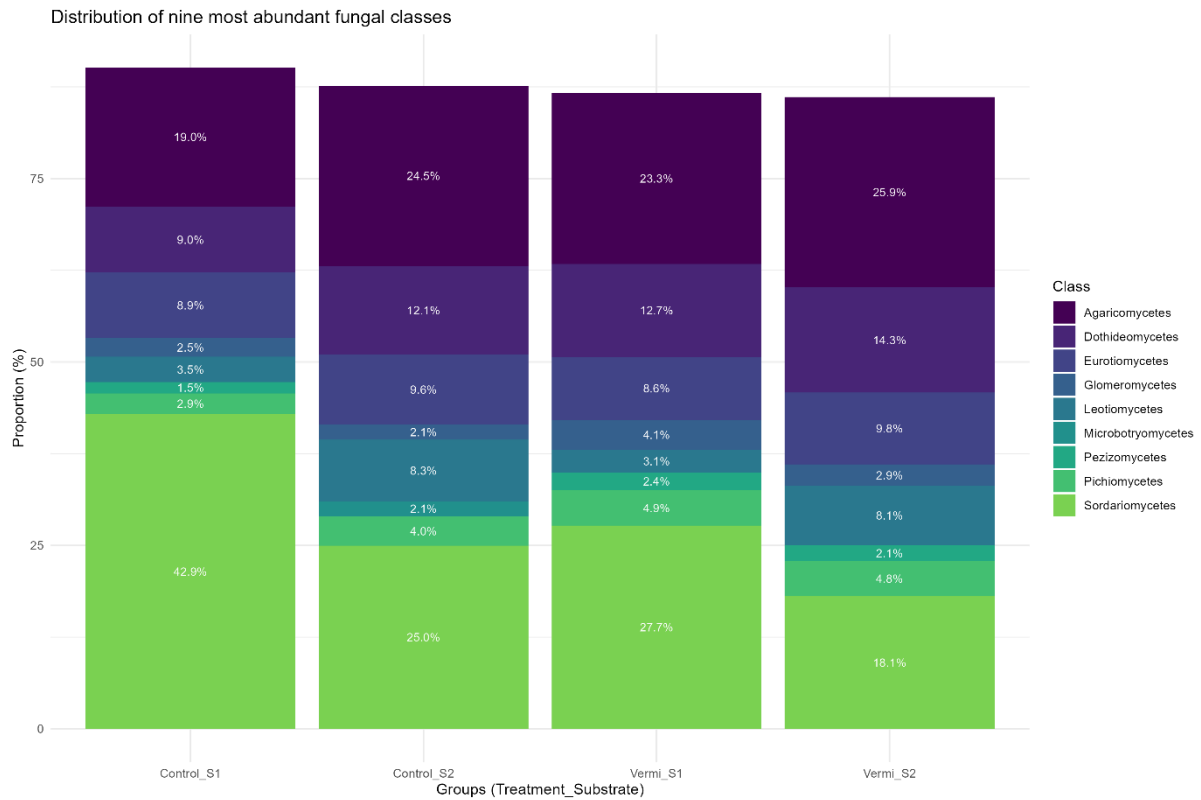


Figure 4.5: Proportional abundance and distribution of identifiable eukaryotic classes in each treatment group. Treatment groups: i) Control + substrate 1 (CS1), ii) Control + substrate 2 (CS2), iii) Vermiliquer + substrate 1 (VS1), iv) Vermiliquer + substrate 2 (VS2).

4.3.2 Impacts of treatments and substrates on rhizobiome composition

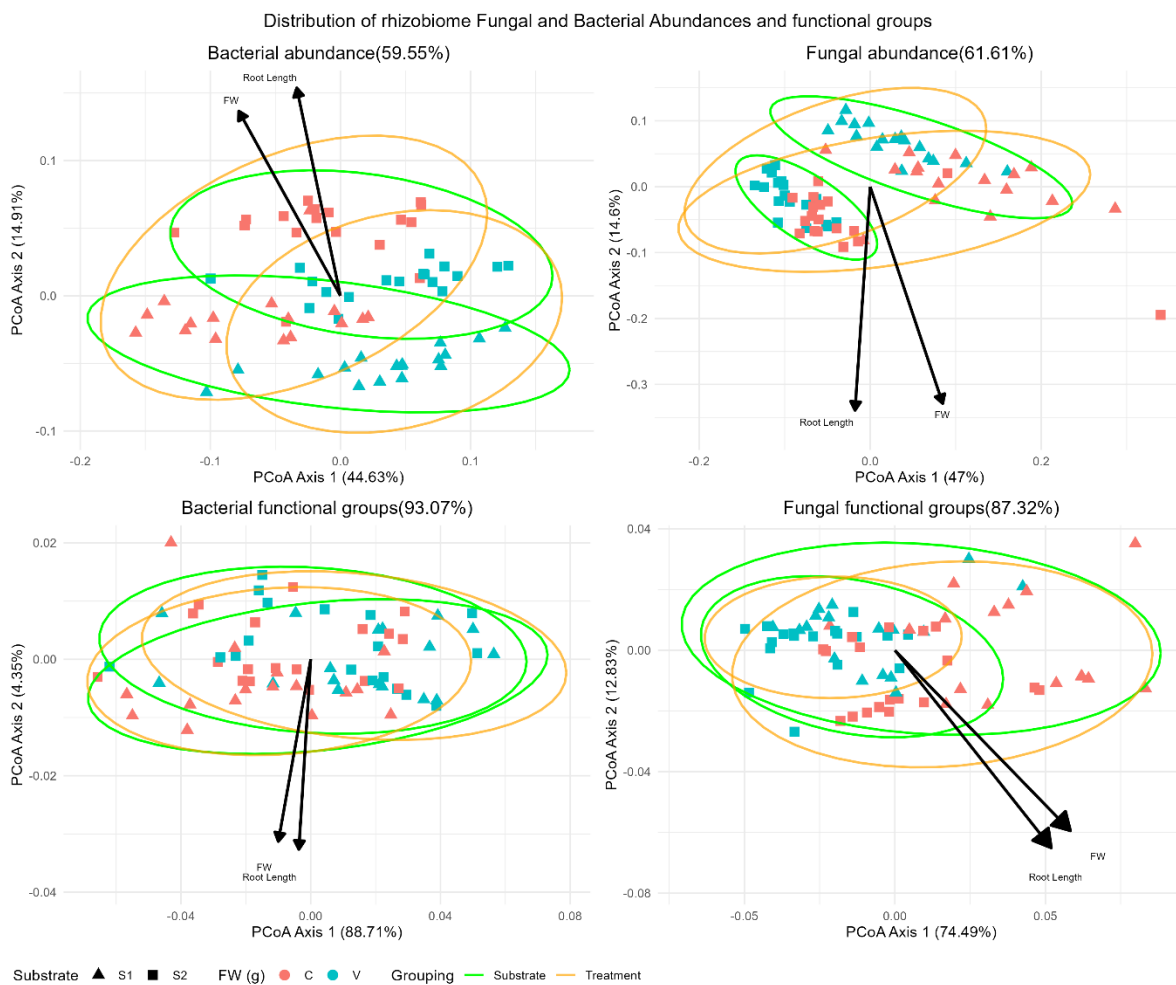


Figure 4.6: PCoA's of: 6a) bacterial taxonomic abundance distribution, 6b) fungal taxonomic abundance distribution, 6c) bacterial functional group abundance distribution, 6d) fungal functional group abundance distribution. Shapes represent substrate types (triangle = S1, square = S2), colour denotes treatment (red = (C) control, blue = (V) vermiliquer), and ellipses capture groups with 95% confidence (green = substrate, yellow = treatment).

Figure 4.6a shows marginal clustering across each unique solution/substrate combination across bacterial species. Fungal species show a distinct clustering behaviour across substrates and show a degree of separation between control and vermiliquer treatments (Figure 4.6b). Contrastingly, both PCoA plots showing differences in functional group abundances show significantly less clustering across both solution/substrate combinations (Figures 4.6a & 4.6b). These functional groups classify species into groups based on their functional processes (e.g. nitrogen fixing, plant pathogenic, human pathogenic etc.). Further proportional pair-wise analysis confirms significant differences in taxonomy between solution/substrate combinations ($p < 0.05$) and finds no significant differences between functional groups ($p > 0.05$). Hence, it can be stated that despite having a high level of taxonomic diversity across treatment combinations, there is a high level of similarity in the functionality of these populations.

R^2 interpretation from a PERMANOVA analysis found that both solution and substrates had reproducible effects on bacterial taxa, fungal taxa, and fungal functional groups (Table 4.1). The exception to this is the degree of influence substrate played in determining bacterial

functional group composition, which was mainly impacted by changes in solution type. While majority of variation is unexplained by the R^2 value, Table 4.1 captures the relative impacts of substrate and treatment on taxa and functional groups. From this we can determine that bacterial taxonomic composition and functional groups were more influenced by solution type, whilst fungal taxonomic composition was more influenced by substrates.

*Table 4.1: R^2 values of substrate and treatment impacts on PCoA distribution across bacterial and fungal abundances and functional groups. Significance code * = $p < 0.05$*

PCoA Plot	Substrate R^2	Treatment R^2	Substrate + Treatment R^2
Bacterial Taxa	0.12*	0.19*	0.31*
Fungal Taxa	0.24*	0.11*	0.36*
Bacterial Functions	0.01	0.13*	0.14*
Fungal Functions	0.17*	0.27*	0.45*

The impacts made on bacterial and fungal populations as a result of changes in either solution or substrate are highlighted in Figures 4.7 and 4.8. Bacterial compositions showed distinct clustering across all treatment/substrate combinations, suggesting that both treatment and substrate play a role in the taxonomic makeup of the bacterial rhizobiome population (Figure 4.7). Fungal composition was more mixed in response to treatment/substrate combinations. This can be seen in the difference in clustering behaviour in Figure 4.8. While substrate groups maintain distinct clusters (8a & 8b), treatment groups can be seen overlapping (8c & 8d) and are considered significantly similar after Bonferroni corrections ($p = 0.12$). This observation further suggests that the fungal rhizobiome population is more dependent on substrate compared to the treatment. It should be noted that PERMANOVA outputs do not directly align with PCoA plots. This is likely due to the PCoA1 axis only accounting for between 44% and 68% of the total variance across treatments.

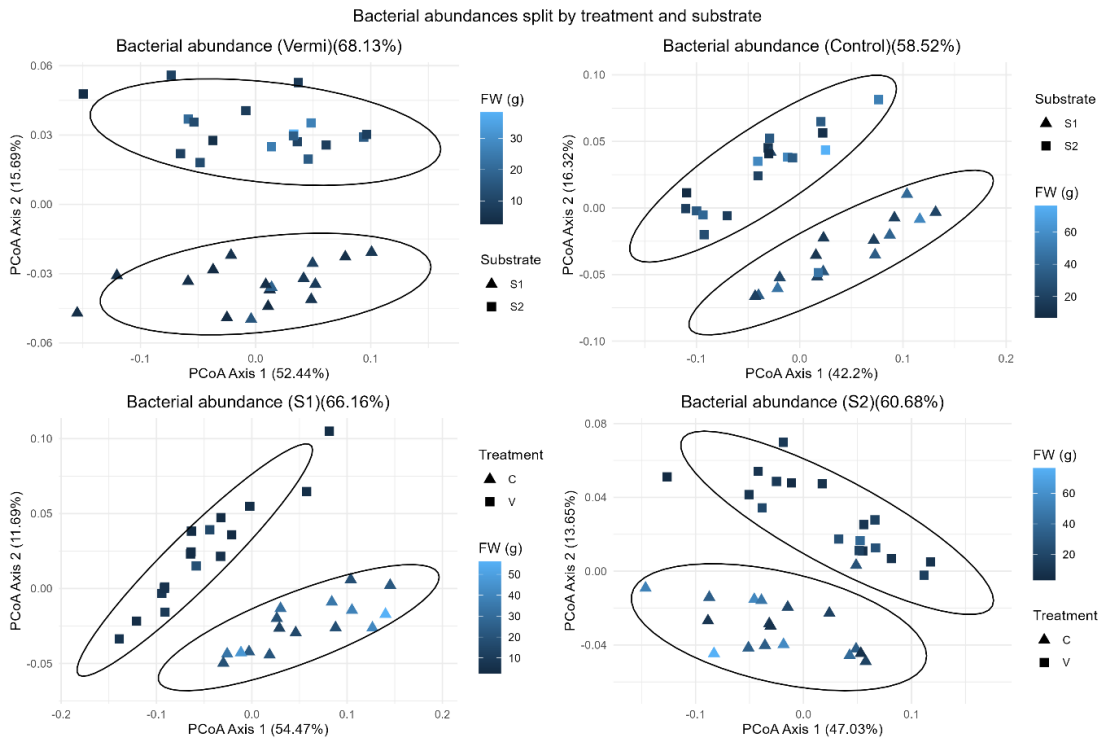


Figure 4.7: PCoA of bacterial abundances, split by treatment and substrate. From left to right, top to bottom: 4.7a) vermiliquer solution, 4.7b) control solution, and bacterial abundance in 4.7c) Substrate 1, and 4.7d) Substrate 2. C = Control, V = Vermi, S1 = Substrate 1, S2 = Substrate 2.

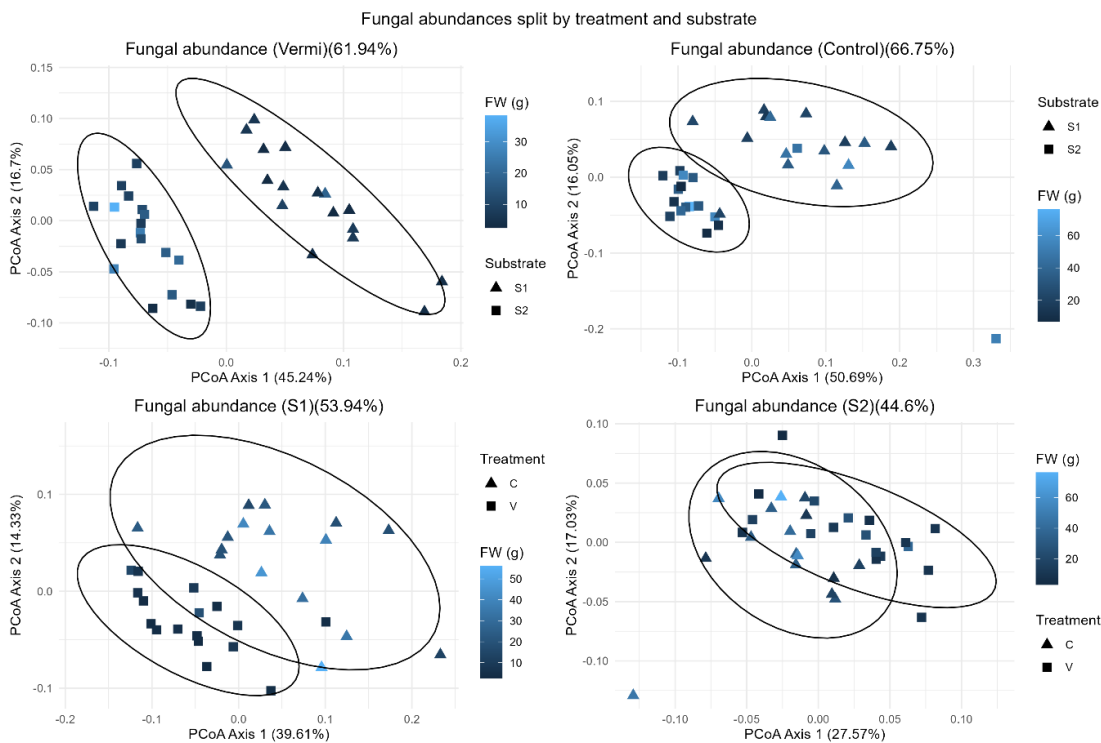


Figure 4.8: PCoA of fungal abundances, split by substrate. From left to right, top to bottom: 4.8a) vermiliquer solution, 4.8b) control solution, and bacterial abundance in 4.8c) Substrate 1, and 4.8d) Substrate 2. C = Control, V = Vermi, S1 = Substrate 1, S2 = Substrate 2.

Figure 4.9 shows the distribution of taxa in both bacterial and fungal populations relative to the raw vermiliquer (VRAW). Once introduced into the growth system, the behaviour of both bacterial and fungal taxa is to split two clusters based on substrates. This is more pronounced in fungal taxa. In addition, the population composition tends to shift towards the population found in the control solution treatments.

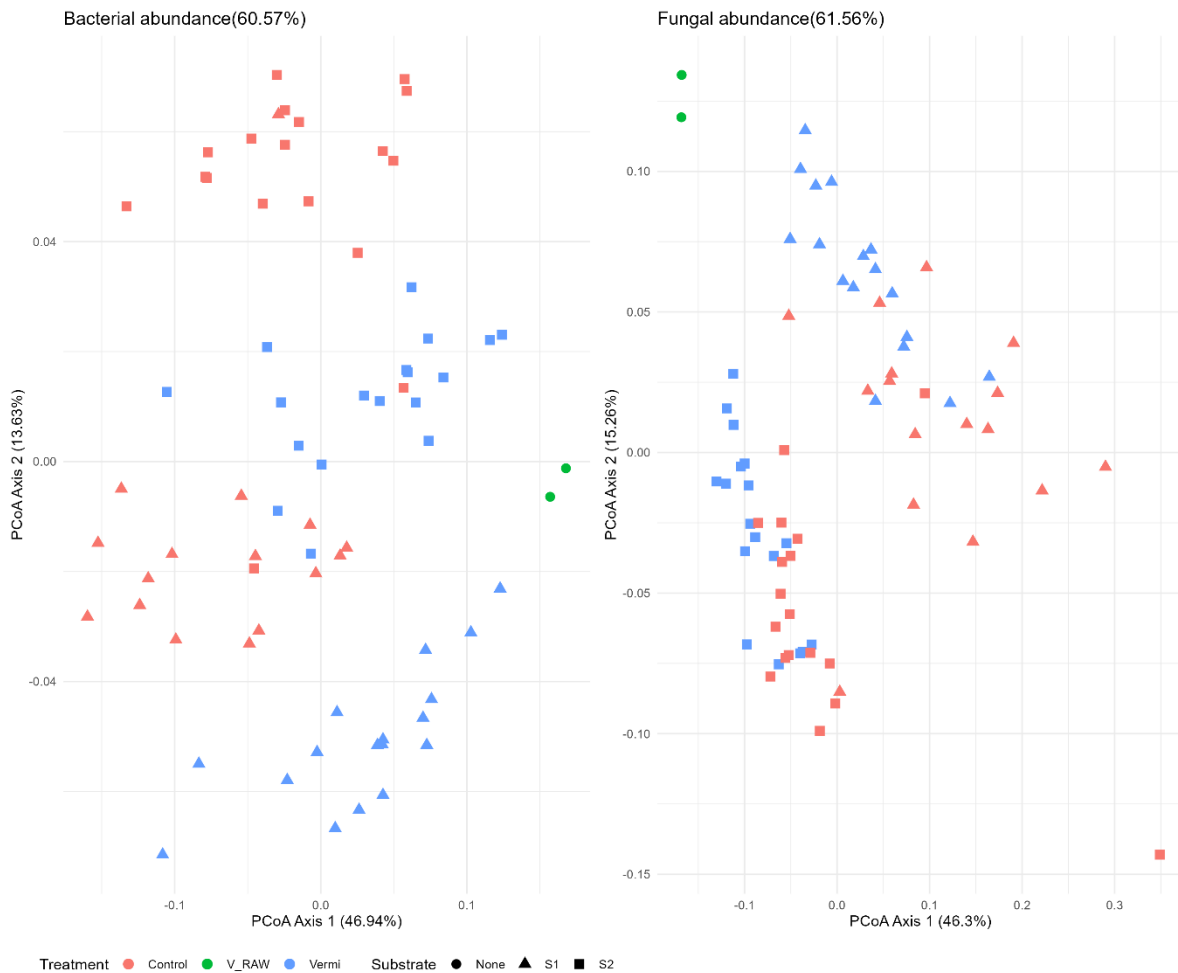


Figure 4.9: PCoA plot of: 4.9a) bacterial (left), and 4.9b) fungal (right) rhizobiome composition. The composition of raw vermiliquer (VRAW) is also included. S1 = Substrate 1, S2 = Substrate 2.

4.3.3 Taxonomic differences and functional similarities

A particularly interesting finding were the similarities in the proportional distribution of functional groups across all treatment groups - despite significant differences in taxonomic compositions. Figures 4.10 and 4.11 contrast the distinct clustering in taxonomic analysis with overlapping functional groups and fungal guilds across treatments and substrates.

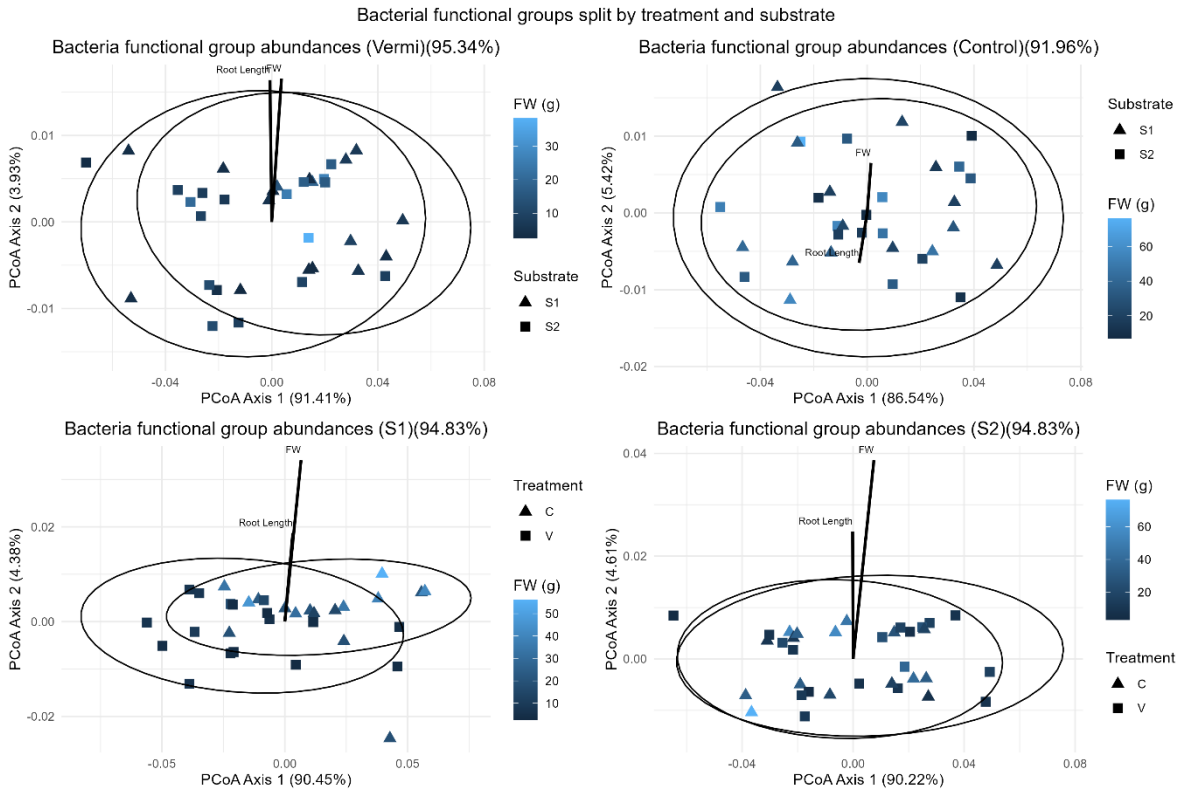


Figure 4.10: PCoA of bacterial functional groups, split by treatment (4.10a, 4.10b) and substrate (4.10c, 4.10d). C = Control, V = Vermi, S1 = Substrate 1, S2 = Substrate 2.

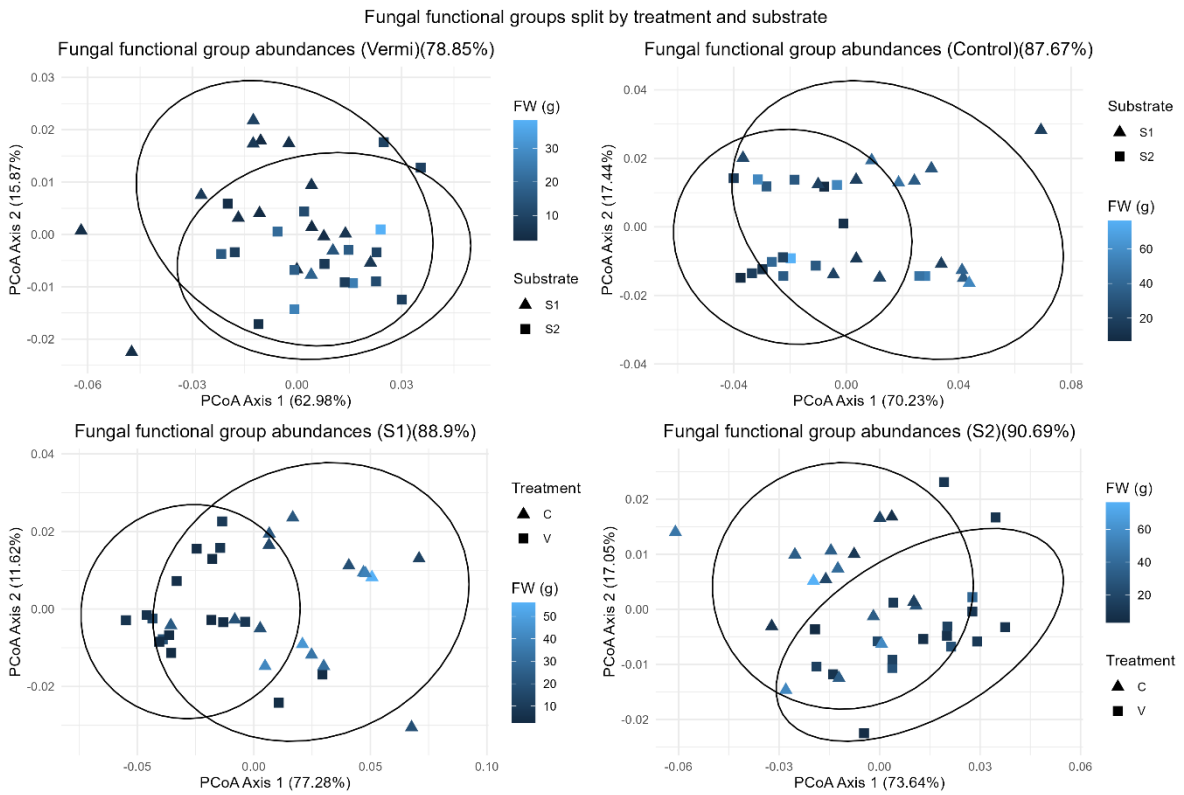


Figure 4.11: PCoA of bacterial functional groups, split by treatment (11a, 11b) and substrate (11c, 11d). C = Control, V = Vermi, S1 = Substrate 1, S2 = Substrate 2.

In addition, Figures 4.10 and 4.11 differentiate the levels of impact each treatment had on bacterial and fungal composition. The high PCo1 in across Figure 4.10 suggests that contributing factors to the variation are limited or highly correlated. This provides insight into the populations of bacterial group composition, which may extend into potential biotic or abiotic systems which regulate its formation and maintenance.

4.3.4 Relationship between microbial community and yield outcomes

Across all treatment and substrate combinations, there was no significant relationship between yield and the microbial composition of the rhizosphere. Despite this, visual observation of Figures 4.16 and 4.17 shows contrasting dynamics in fresh weight outcomes between the control and vermiquer treatments as a result of microbial abundances. In control treatments, there is a negative relationship between yield and microbial abundances, while vermiquer treatments show a relative improvement in yield when microbial abundances are higher.

Analysis of root length in relation to microbial abundances was more unpredictable, with limited consistent behaviour across PCoA visualisations. A linear model found that there was no significance in the role of root length in the microbial assemblage ($p = 0.08$), although its closeness to significance may warrant further investigation.

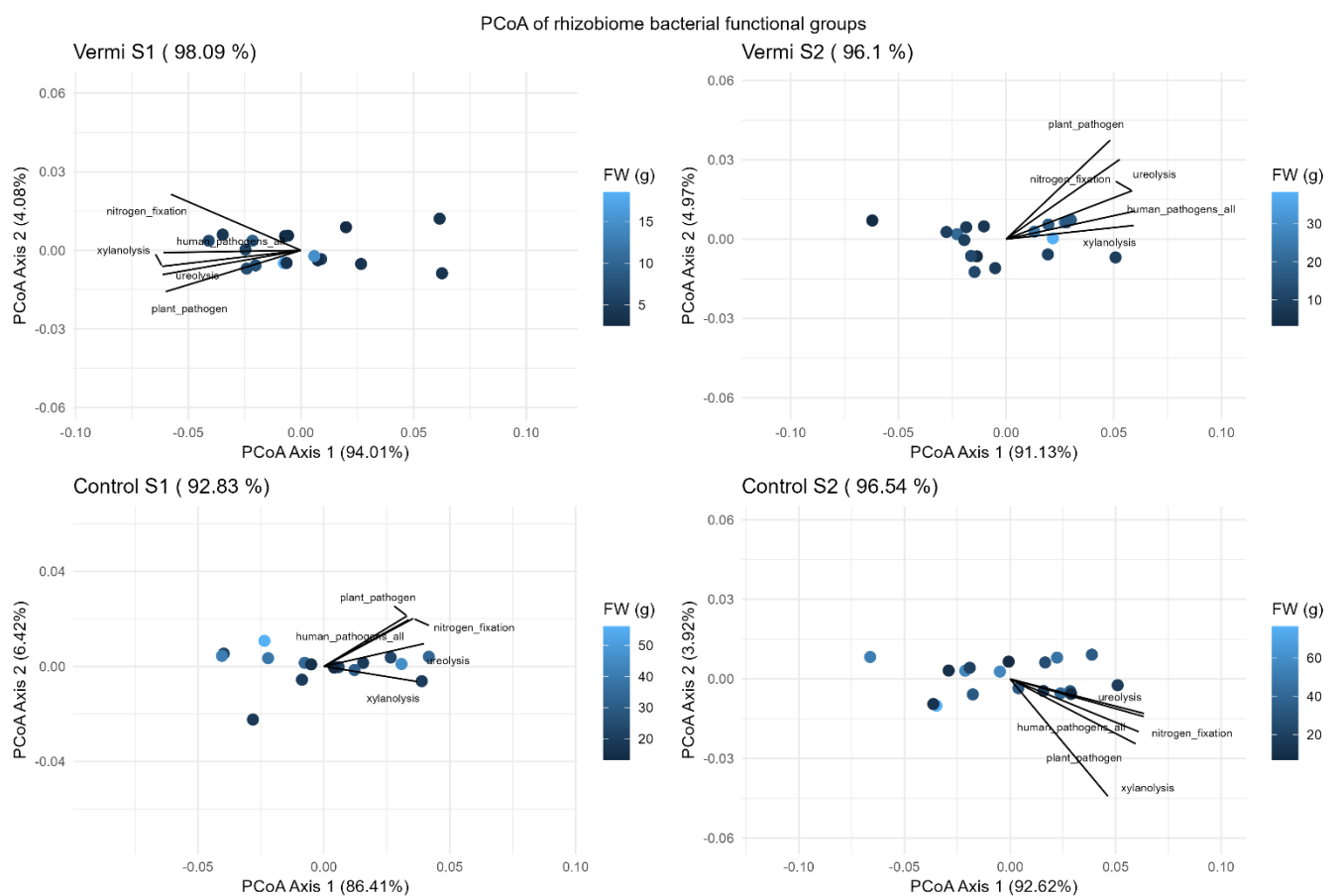


Figure 4.12: PCoA of bacterial functional group distribution for: 4.16a) Vermi_S1, 4.16b) Vermi_S2, 4.16c) Control_S1, and 4.16d) Control_S2. FW (g) represents fresh weight, and vectors show relative abundance of functional groups.

PCoA of rhizobiome fungal functional groups

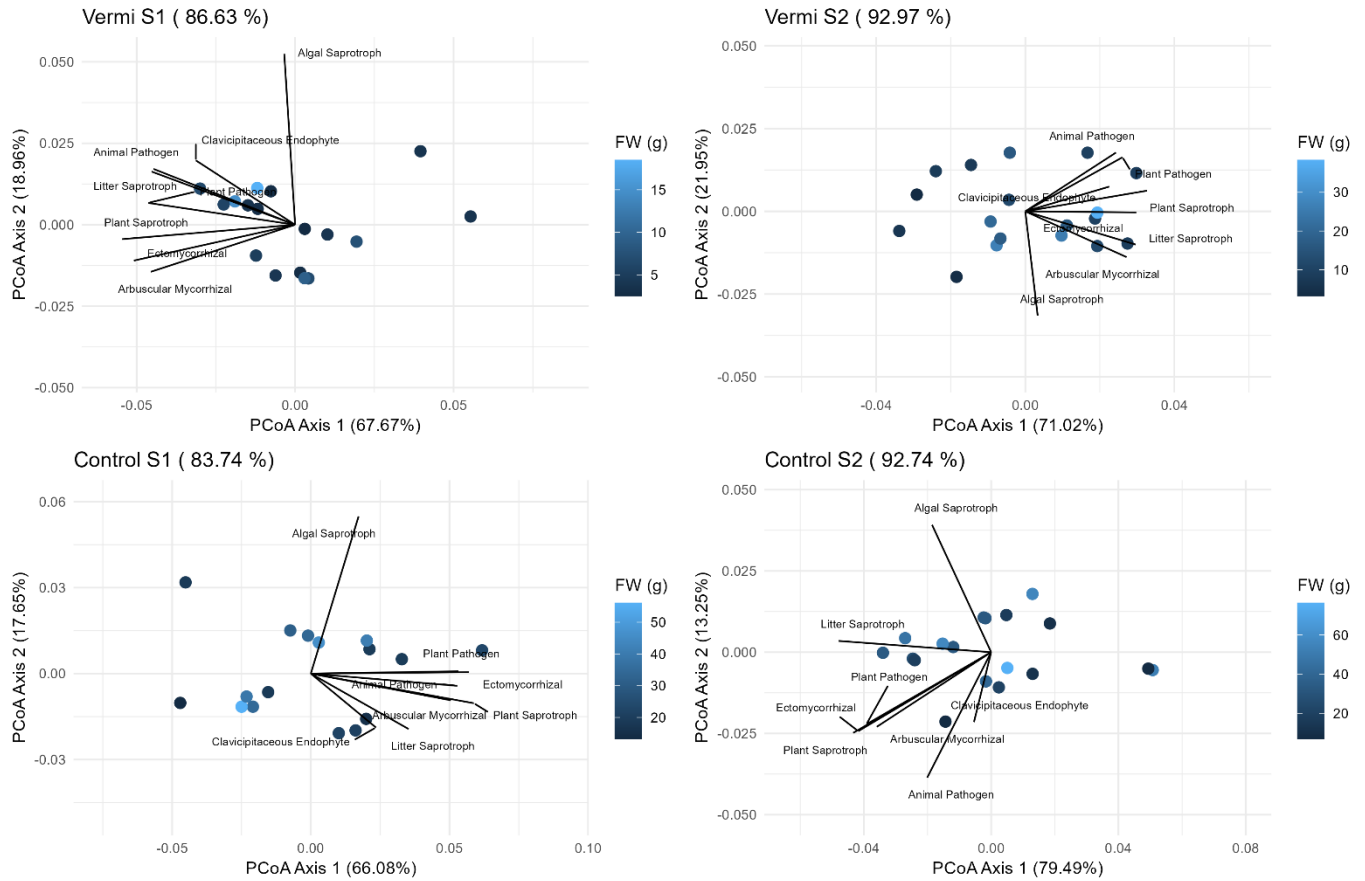


Figure 4.13: PCoA of fungal functional group distribution of: 4.17a) Vermis_S1, 4.17b) Vermis_S2, 4.17c) Control_S1, and 4.17d) Control_S2. FW (g) represents fresh weight, and vectors show relative abundance of functional groups.

4.4 Discussion

4.4.1 Impacts of substrate and treatment on the microbiome

The impacts of the vermiliquer based FWBHF was found to cause significant increases in microbial abundance and diversity in both substrate groups. This is likely due to the bioactive vermiculture processing method and the increased availability of complex organic matter compared to a synthetic hydroponic solution. Non-homogenous organic material provides greater colonisable surface area, variety of complex nutrients, and ecological niches for microorganism species. This presents as an increase in complexity of the trophic web, leading to the observed increase in both microbial abundance and diversity. Furthermore, a reduced nitrogen and phosphorus content in the FWBHF may have improved the gradient of bioconversion, as high levels of inorganic nitrates (NO_3^+) and phosphates (P_2O_5) inhibit microbial populations which break organic matter into these elements (Chen et al., 2022). As such

Substrate research in soilless production systems usually focus on the physio-chemical properties of substrates, and the effect they have on yield outcomes across crop species (Dannehl et al., 2014; Rahman et al., 2019). Existing literature into the microbiome of soilless substrates comprehensively documents the substrate-rhizosphere interactions which occur in the commercially popular rockwool. However, this study focuses primarily on biota which occurs as a result of conventional fertiliser application (Thomas et al., 2023). As such, literature comparing rhizobiomes between differing solutions and organic substrates are limited (Wang et al., 2024).

The role of substrate in this study was identified to have more impact on eukaryotic species compared to bacterial species. This is largely in-line with soil-based research, which found that soil texture played a greater role in predicting fungal abundances compared to bacterial abundances (Xia et al., 2020). The substrates in this trial had similar compositions of peat and “biological binder,” however they demonstrated different levels of influence in the taxonomic makeup of the crop rhizobiome. Notably, S2 exerted such an influence on the fungal taxonomy that vermiliquer and control treatment solutions (VS2 and CS2) were considered to have similar eukaryotic taxonomic makeups. The mechanism for this degree of influence may be from i) physio-chemical properties of the substrate providing conditions suitable for specific taxa, and/or ii) the substrate initially contained a population which survived initial sterilisation.

The similarities in microbial population dynamics between soil-based research and our findings suggests there is merit in the (tentative) application of existing soil-based research to guide future hydroponic microbiome research. These include investigating the effects of introducing keystone microbial species/communities and measuring the effects of an increased alpha diversity in substrate ecology.

The concept of a keystone microbial community can be referred to as a “microbial hub.” Agler et al., (2016) posits that in soil-based systems there are a group of highly interconnected taxa which exercise a disproportionately large influence on the surrounding microbial communities of plant hosts and often act as important intermediates between abiotic and host factors. Usually, these hubs are formed from the rhizospheric activity of a host species and have been observed to have consistent and predictable taxonomic outcomes across phylogenetically similar species (such as maize and sugarcane) (Castellano-Hinojosa & Strauss, 2021). These observations in soil-based research could be applied to the findings

of this study. Identifying and transplanting highly interconnected species found across treatments into a sterile system could enable a replicable inoculation of microbial communities. This research may be even more applicable in large-scale perennial systems which may benefit more from the stability that are provided by known “microbial hub” communities.

Another approach drawing from soil-based research is to explore the effects of a complex and diverse microbiome in hydroponic growing media. Improvements in soil alpha-diversity have been found to be associated with greater levels of functional redundancy (taxa that support a common function), greater levels of biota which support multiple functions, and improved complexity in taxa which support plant-relevant functional groups (Escalas et al., 2019; Compant et al., 2019). The importance of complex and biodiverse microbiomes in soil systems have been shown to provide adaptability in response to short- and long-term changes in environment, resistance to introduced pathogenic species, and improve yield outcomes. Such benefits may be imparted by the FWBHF vermiliquer treatment, which was shown to host a greater diversity of plant antagonist and diazotroph species compared to the control. Research in this area should test the effectiveness of vermiliquer as a biological integrated pest management (IPM) method in hydroponic systems. In addition, the effects of increased plant pathogen diversity on the expression of disease symptoms are not well understood and require further investigation.

4.4.2 Tendencies towards functional similarity in the rhizobiome

Exposure to vermiliquer-based FWBHF and different organic substrates caused significant changes in the taxonomic composition of the rhizosphere in hydroponic lettuce. The contribution of the vermiliquer FWBHF treatment to the rhizobiome was distinct from the control. While this change in biota was not associated with increased yield outcomes, it provided valuable insight into the population dynamics of hydroponic rhizobiomes. A key finding of this study is the tendency for the composition of rhizobiome taxa to tend towards a similar functional composition, regardless of inoculation source. This is observed in both bacterial and fungal species across all substrate types.

The rhizobiome of an individual hydroponic lettuce crop can be characterised as an “island”, which resides in a single treatment group, or an “island chain”. Island chains represent taxonomic compositions formed by primary differences created by unique combinations of solutions and substrates. Each island within a chain represents a secondary level of differentiation – where samples within the same treatment group, are similar but not totally homogenous in taxonomic composition. Hence, the uniquely composed rhizobiome of an individual lettuce, which, while similar to neighbouring islands, is usually considered reasonably distinct. Despite the taxonomic differences across island chains and the islands within, this study has found that the ecological functions on each “island” are similar in purpose and proportion. That is, regardless of actual taxa, the rhizobiome of each plant, across all treatments, is comprised of species which carry out the same functions in similar proportions.

There are three possible explanations for this behaviour, i) natural population dynamics formed by hydroponic conditions, ii) the control and vermiliquer provide similar microbiomes, or iii) the lettuce has a degree of autonomy over its own rhizobiome. This paper endorses the possibility that the environment formed in hydroponic substrates provide the conditions for particular functional compositions to form. On the other hand, it is highly unlikely that the control and vermiliquer hosted identical proportions of functionally similar microbes – especially after dilution into an operating hydroponic system. However, the

results of this trial and existing literature mostly suggest that lettuce (*Lactuca sativa*) may host a mechanism for functional selectivity in the rhizosphere. This hypothesis is largely supported by existing literature in soil-based research, which has identified functionally similar rhizobiome assemblages across multiple crop species across multiple field trials (Castellano-Hinojosa & Strauss, 2021). Despite this, discussions surrounding crop-rhizobiome community dynamics in soil-less systems are contentious. While some research suggests that crops have a capacity to resist inoculation from upstream sources (Lobanov et al., 2022), others argue that upstream sources can easily infest a hydroponic system due to the absence of a robust microbial ecosystem (Meeboon et al., 2022). This research has observed behaviour which sits between either argument – that upstream inoculant sources can colonise the growth media within a hydroponic system with a complex ecosystem; however, this does not necessarily result in an unstructured ecology. Instead, crop species seem to be able to exercise a selective mechanism which composes a complex and interconnected rhizobiome with specific proportions of functional groups using the available taxa.

Future research in this area should aim to identify i) the role of natural population dynamics as a result of hydroponic conditions in rhizobiome composition, ii) whether inorganic substrates affect this behaviour, and iii) if there are yield benefits to changes in taxa in the same functional group.

4.4.3 Yield outcomes and potential commercial applications

The yield outcomes of the growth trial found that the control solution had a significantly greater fresh and dry weight compared to the vermiliquer solution (Chapter 3). However, overall root length was considered similar across all treatments. As noted in the results, control and vermiliquer treatments showed opposite reactions in response to microbial abundances; with the control showing non-significant yield reductions with increases in microbial abundance, and the vermiliquer showing non-significant yield increases with increases in microbial abundance. The cause of this behaviour is unknown, and its implications for FWBHF should be further explored.

The priority for a commercially ready product in horticulture is the preservation of the existing yield and quality outcomes that current management practices maintain. This stipulation requires that the inclusion of FWBHF in commercial applications does not reduce the fresh harvest weight of lettuce, visually damage aesthetic qualities, or produce lettuce containing human pathogens. The integration of biological actors in hydroponics systems is a double-edged sword. On one hand, the development of FWBHF as a microbial inoculation may offer a more robust, cost-effective, and sustainable approach to disease management in hydroponic cultivation. On the other hand, the deviation from the sterile culture of conventional hydroponic systems limits the applicability of established and effective physical and chemical pathogen control methods. Currently, the effects of FWBHF microbiology in hydroponic systems are understudied. Key areas of interest include the conductivity of human pathogens in FWBHF into produce, the effects of asymptomatic populations of plant pathogen species, and the efficacy of FWBHF as a biological IPM method for annual and perennial cropping systems.

The vermiliquer FWBHF had a greater diversity and abundance of human and plant pathogen species compared to the control. In Australia, current legislation does not specify any critical limits of human pathogens in irrigation water (FPSC, 2022). While this enables the application of FWBHF in commercial hydroponic systems, there are regulations for microbial limits of food-borne pathogens in fresh produce such as lettuce. Pathogens of note are usually waterborne and require a lower microbial load to cause symptomatic reactions compared to

other human pathogen species identified in this study (Riggio et al., 2019). The presence of human pathogens in the rhizosphere do not necessarily equate to the presence of pathogens in food products (Saldinger et al., 2022). Generally, there are two mechanisms for human pathogens to enter produce. The first is when contact is made between contaminated irrigation water and consumable components of a product, and the second is internalisation of pathogens through root uptake systems (FPSC, 2022; Saldinger et al., 2022). While the first is generally remediated through management practices such as utilising drip irrigation, the likelihood of the second in hydroponic systems is up for debate.

A literature review studying the rate of human pathogen internalisation from contaminated irrigation water in hydroponic systems found that the rate of human pathogen infiltration was greater in hydroponic systems compared to soil systems (Saldinger et al., 2022). This was attributed to the accessibility of roots directly to contaminated vectors, and the absence of a microbiome to compete against the pathogen species (Warriner et al., 2003). Within the review, only synthetic solutions were included, and it found that internalisation was largely unpredictable – with a variety of leafy greens showing total internalisation of human pathogens through the entire crop, to only having pathogens detected in the growth media (Wang et al., 2019; Ilic et al., 2022). Despite the unpredictability across healthy crops, *Saldinger* highlights the correlation between plant disease and physical damage to vegetative and root systems with an increase in internalised human pathogens.

Minimisation of symptomatic plant pathogen populations preserves yield quality and quantity. While there is a greater diversity of plant pathogens in the vermiliquer FWBHF, there was no visual evidence of foliage or root damage due to disease. FWBHF as a biological IPM technique aims to establish an ecologically complex and robust microbiome in hydroponic growing media. If the behaviour of such a microbiome adheres to existing soil-based research, this ecology could suppress symptomatic populations of human and plant pathogens through competition of resources, predation, and fortification of crop health (Ali et al., 2022, Thambugala et al., 2020). Existing research on hydroponic pathogens and their impact on microbial composition have found that the initial invasion of pathogenic species causes a sharp increase in microbial diversity and followed by a decrease in diversity below the original baseline (Wei et al., 2018, Vargas et al., 2022). This is hypothesised to be a result of infected crops releasing exudates as a “cry-for-help” mechanism to attract a wider range of microorganisms to assist in suppressing, competing and preying upon pathogenic species (Rolfe et al., 2019). Following the infection, a plant weakened by disease may be unable to exude effective rhizosphere interactants to re-establish its original rhizobiome. The application of an ecologically diverse inoculant aims to normalise and fortify the available biota in response to the behaviour of the “cry-for-help” defence mechanism. The efficacy of this approach may also be improved through management strategies which promote environments which are porous, easily aerated, and stable.

Whether FWBHF inoculants benefit from organic substrate groups should be investigated in future research. This study identified S2 as a substrate with a resilient fungal population which resisted upstream inoculation. It is unknown whether this is due to previously established populations, or if the environment is suitable for a particular composition of fungal species. The S2 substrate showed a lower abundance of bacterial and fungal crop diseases compared to S1. This may be associated with anti-fungal fungi species such as *Trametes versicolor* (Parrani et al., 2019), which was found across S2 samples in relatively greater abundance compared to S1. This finding hints towards the role of substrates in biological IPM strategies, as a hub for key microbial species to establish and develop a

symbiotic relationship with crop species. Studies into the exact physio-chemical properties of substrates, and ideal methods of inoculation with FWBHF should be explored.

The risk of large-scale changes in existing management practices demands that research into the viability of FWBHF should be comprehensive. As such, there are a litany of research questions which must be answered prior to the commercialization of FWBHF as a replacement or supplement to commercial hydroponic systems. Beginning with the scope of application, the establishment of inoculated microbiomes may not be as effective for crops with shorter growing periods, such as in lettuce. There may be greater benefit in inoculating and cultivating the substrate microbiome in perennial species, where an established rhizobiome can maintain a stable ecology for a longer period of time. Additionally, the integration of existing physical and chemical methods of pest and disease control still pose a challenge, as these may nullify the benefits provided by FWBHF application. Hence, it is imperative that comparisons between methods, as well as trials combining methods are performed.

4.5 Conclusion

Vermilique derived FWBHF has a significant impact on the fungal and bacterial populations in the rhizosphere of hydroponically grown lettuce. The changes in composition are largely taxonomic, and do not result in significant changes in the functional capacity of the rhizosphere. In this respect, there is potential to explore and identify the role of specific taxa in filling ecological niches within the growth media of hydroponic systems. Studies should identify the resulting changes in microbial composition as a result of changes in processing methods, as well as feedstock. The continued development of how urban food-waste can translate into nutrients suitable for urban food production is essential to the development of nutrient cycling in our food production systems.

The increased rhizobiome diversity and complexity provided by the FWBHF begins to mimic the interactions observed between crops and soil-based systems. As such, it is tempting to apply soil-based research in hydroponic rhizobiomes. However, as this study found, the behaviour of hydroponic microbiomes are not always in line with the observations of soil-based research. Hence, when applying theory from on-field trials, caution should be given to avoid applying assumptions of soil systems. The infancy of hydroponics rhizobiome research should prioritise how microbial populations respond to environmental changes and eventually use this knowledge to study how microbial populations can effect change in their environment. This ensures future research into hydroponic rhizobiomes is established upon structured, and reviewed, knowledge – as opposed to operating on assumptions that soil-based research is immediately applicable to hydroponic systems.

Chapter 5. High temperature sterilisation of vermiliquer food-waste based hydroponic fertiliser and its effects on solution composition and lettuce (*Lactuca sativa*) yield.

Abstract

Chapter 3 found that the nutritional content of the solution was inadequate as a complete replacement of synthetic solutions. In response, this study aims to identify i) if FWBHF can be used to partially substitute applications of synthetic hydroponic fertilisers, and ii) if high temperature sterilisation of FWBHF impacts nutritional composition and yield outcomes. Sterilisation of the FWBHF was carried out in an autoclave at 121°C for 15 minutes. The effects of high-temperature steam sterilisation caused significant changes in nutrient composition of FWBHF, significantly elevating phosphorus, potassium, magnesium, and sodium ($p < 0.05$). This study performs a growth trial of lettuce (*Lactuca sativa*), comparing two treatments (V1 = 66% control/33% sterilised FWBHF; V2 = 66% control/ 33% non-sterilised FWBHF) against a synthetic A/B control solution (Hoaglands Aqua Vega, Canna). 6 replicates of each treatment and control found that substitution did not cause any significant yield loss and reduced the variation in fresh weight (g) outcomes, though not to a significant level ($p = 0.08$). In addition, sterilisation did not cause significant changes in yield performance (fresh weight, disease resistance, growth rate).

5.1 Introduction

A frequent finding of growth trials utilising a food-waste based hydroponic fertiliser (FWBHF) is its inability to meet the yield standards of a conventional synthetic fertiliser (Wang et al., 2024). This is largely attributed to the imbalanced nutritional content found in FWBHF's, namely an excess of sodium, and a deficiency of nitrogen and calcium hinder yield outcomes (Wang et al., 2024). Despite this, there may be potential in exploring potential application of FWBHF's as a partial substitute or supplement to a conventional system. This alleviates reliance on synthetic fertilisers, encourages nutrient cycling, and encourages sustainable urban horticulture. Use-cases for the experimental vermiliquer FWBHF may be as a partial substitution for an array of nutrients, a supplement for a specific nutrient (e.g. phosphorus or sulphur) (Chapter 3), or as a source of microbial inoculation.

A handful of past studies explore partial substitution and/or supplementation of conventional solutions with FWBHF's. This is usually done as a remediation step to bolster the nutritional content of the FWBHF, as opposed to the substitution of an all-in-one nutrient solution. Regardless, the supplementation of single nutrient species (e.g. nitrogen only) or substitution of a portion of an all-in-one solution consistently leads to significant increases in yield outcomes (Abd-Elmoniem et al., 2001; Kawamura-Aoyama et al., 2014; Li et al., 2020; Chaorlina et al., 2021). Due to the variety of hydroponic systems, crop species, and FWBHF

nutritional compositions it is difficult to ascertain an optimal rate of substitution to minimise the require synthetic solution without causing yield losses.

In addition to essential plant nutrients, FWBHF substitutions also provide an inoculation of diverse microbiota and organic matter, which can establish within the growth media and improve the ecological complexity of the rhizobiome; potentially leading to improvements in disease resilience, crop development, and crop quality (Chapter 4; de la Feunte Cantó et al., 2020; Dhawi et al., 2023). Determining whether the presence of this ecology benefits overall yield outcomes may lead to improvements in the commercial viability of FWBHF's.

Utilising uniform high-temperature sterilisation aims to discern whether the micro-ecology cultivated during FWBHF development plays a positive, or negative, role in yield outcomes. In addition, the auto-claving process addresses other factors such as i) facilitating complete breakdown of organic matter through high temperatures, and ii) ensuring control of human/plant pathogens.

Sterilisation of final product may have some effect on remaining organic matter, as a systematic review (Wang et al., 2024) identified four research papers which utilised some form of heat treatment in the development of a FWBHF. Three of the papers utilised steaming and boiling on raw organic matter prior to further processing (Arshad et al., 2018; Siddiqui et al., 2021; Yusuf et al., 2021). One paper, Kechasov et al., (2021) identified an increase in pH as a result of autoclaving a finalised solution (7.5 to 9.7), with no reported change in EC. Thus, the exact effects of high-temperature sterilisation on the nutritional composition of are undocumented. It is known that high-temperatures can interact with organic compounds in food-products, such as the simplification of starches, breakdown of cellulose and formation of covalent bonds between protein components (Chang et al., 2018; Yu et al., 2023), which may influence chemical composition or how microbiota interact with the remaining organic matter in the FWBHF. This may improve the availability of carbon and other nutrients, as well as further prevent the buildup of detritus in the operating hydroponic system.

Similarly, the potentially pathogenic populations observed found in the substrate of lettuce grown in Chapter 4 (genus *Clostridium*, *Salmonella*, and *Escherichia*) present themselves as a potential risk to food safety. The presence of these genera in the solution pose a risk as surface contact with contaminated water, and occasional internalisation of pathogenic species through water uptake has been observed in conventional hydroponic systems. (Saldinger et al., 2016).

Additionally, Yu et al., found that autoclaving was more effective at halting microbial activity over the 180-day trial period compared to pasteurisation and acidification– potentially earmarking it as an option for long-term storage of a FWBHF solution. This study will perform nutrient analysis on the sterilised solution identify changes in nutrient composition and help assess whether high temperatures can assist in improving nutrient availability.

As such, this paper aims to determine if an experimental vermiliquer FWBHF can partially substitute a synthetic A/B hydroponic solution. In addition, it seeks to determine if the removal of an established, complex ecology is detrimental to the function of this vermiliquer FWBHF. It also discusses the benefits, drawbacks, and potential opportunities which arise from the use of sterilisation in FWBHF development.

5.2 Materials and Methods

FWBHF preparation and sterilisation

Both vermiliquer FWBHF were prepared identically, with four main steps. Household waste (vegetables, eggshells, coffee grounds, pasta, rice, bread, and raw/cooked bones) was collected and added to an established red earthworm (*Eisenia fetida*) vermicompost system. Each week, waste would be masticated with a trowel, and the system would be flushed with 1 litre of water. The runoff leachate, vermiliquer, was collected and stored in a 22C storeroom with a loose lid for 60 days. After, they were placed in a 4C cold room. 72 hours prior to application, the FWBHF was aerated for 48 hours. Vermiliquer was then stirred and portioned into two litre reagent bottles. Three of the bottles were placed back into the cold room as the unsterilised treatment (V1). The remaining three sterilised treatments (V2) were autoclaved at 121.1°C for 20 minutes and then placed back in the cold room. After 24 hours at 4C, both samples were ready for application.

The control used was a commercially sourced “leafy green” two-part A/B synthetic hydroponic solution (Aqua Vega, CANNA).

Chemical water nutrient analysis

Aside from analysis of the raw V1 and V2 solutions, samples for nutrient analysis were collected on day 14, 48 hours after the final dosage of treatment solutions and EC had peaked at 1.6 dS/m. Nitrates and ammonia were measured with a flow injector analyser (FIAlyzer-1000, FIALab). Samples were diluted with DI water at rates of x25 for expended nutrient solutions and 50x for raw nutrient solutions. Elemental phosphorus, potassium, calcium, magnesium, sulphur, and sodium were measured using ICP-MS (Nexion 1000 ICPMS, PerkinElmer). Samples were prepared by combining 0.5ml of treatment, 0.375ml HCl, and 0.125 HNO₃ in a 1.5ml Eppendorf tube. Samples were inverted and placed in a water bath at 60C for 2 hours. Finally, samples were centrifuged at 2400rpm for 30 seconds to separate solids, the remaining aliquot was filtered (Whatman 451, Whatman) and 1ml of the aliquot was transferred to a clean Eppendorf tube. Samples were frozen immediately and thawed immediately prior to 10-times dilution analysis at 4.5L/min on helium kinetic energy discrimination mode.

Growth trial and post-harvest yield analysis

A processed mushroom baglog waste substrate was auto-claved at 121C for 15 minutes 24 hours prior sowing. Lettuce seeds (*Lactuca sativa*) were soaked in DI water for 30 minutes prior to sowing into hydroponic growth media composed of Seedlings were transferred to a germination chamber, and warmed with a heating mat to 24C, receiving 12 hours of light per 24-hour cycle. After 9 days, a diluted A/B solution of 0.6 dS/cm was added to the germination chamber. At 14 days plants which had begun developing a second set of true leaves and were transplanted into a continuous NFT system. Each system contained 4 heads of lettuce, across 18 separate NFT systems, 6 were entirely synthetic (Control, C), 6 were non-sterile vermiliquer (V1), and 6 were sterile vermiliquer (V2). Treatments were assigned in three separate blocks with identical conditions to avoid cross contamination. Room temperature was maintained at 24°C, and humidity at 70%. Lighting included a 16-hour light cycle from two 36W fluorescent tube lights of “Cool White” per system, which produced approximately 4000 kLux at substrate level.

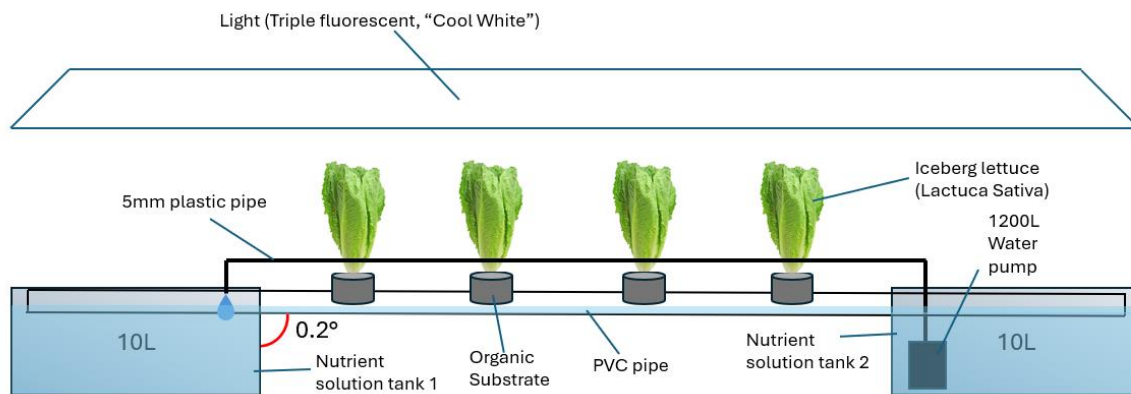


Figure 5.1: Diagram of nutrient film technique setup utilised in this trial

The dosing regimens for C, V1, and V2 are shown in Table 1. 45 ml of AB were applied to the control in total, and 30 ml was applied to V1 and V2 – with 400ml of V1/V2 making up the missing third of A/B in the FWBHF treatments.

Table 5.1: The dosing regimens for C, V1, and V2.

Treatment	Day 1	Day 4	Day 8	Day 12
Control	10ml A/B	5ml A/B	15ml A/B	15ml A/B
V1	10ml A/B + 100ml V1	100ml V1	10ml A/B + 100ml V1	10ml A/B + 100ml V1
V2	10ml A/B + 100ml V2	100ml V2	10ml A/B + 100ml V2	10ml A/B + 100ml V2

After 25 days, samples were harvested and measured for fresh weight (FW), chlorophyll content (SPAD), height, and leaf count. An average SPAD reading was taken after sampling the longest 3 leaves along their lengths at intervals of approximately 3cm. Height was determined by measurement of the longest leaf, and leaf count included all lettuce leaves greater than 2cm in length. Samples were then dried for 72 hours in a plant oven set to 60C, and dry weight was measured. Root samples were measured as distance from the base of the substrate to the tip of the root. Root weight was unable to be measured due to challenges with separation from growth substrate. Root-shoot ratio was calculated with plant height and root length.

Data analysis

Data analysis was performed in R-Studio (2024.04.2 Build 764). Normality for all measured parameters (nutrient analysis, fresh weight, dry weight, leaf count, plant height, root length) was assessed using Shapiro-Wilks test, qq-normal plots and histograms, all response variables were determined to be normal. Equal variance between treatments was tested with a Levene’s test, and variables with equal variance were analysed with ANOVA followed by a post-hoc Tukey’s to identify significant group pairings. Variables with unequal variance were analysed with a Welch’s ANOVA and significant pairs were identified with post-hoc Games-Howell tests. Plots were visualised using the “ggplot” package. In addition, manual pairwise Levene’s tests were performed post-hoc to identify significant pairs and corrected with Bonferroni corrections – although these should not be considered strict determinants of significance. This was used as an indicator for potential differences between individual treatments that global differences were unable to identify.

Data for nutrient analysis were transformed by an EC factor, which was applied to the raw measurements of each sample. EC factor was calculated by $ObservedEC * ECFactor = 1.6$ which aims to mitigate disproportion in nutrient levels caused by differences in EC across treatments.

5.3 Results

5.3.1 Impact of high-temperature sterilisation on nutrient solution composition

The effects of high-temperature sterilisation were largely uniform across replicates, implying a consistent response of FWBHF to the treatment. However, due to the sample size ($n = 3$ per treatment) significance cannot be attributed to the outcomes of this section.

pH and EC remained largely unchanged as a result of the autoclaving process, with a slight increase in both, without any deviation. The concentration of available nutrients, however, saw an increase in all elements besides nitrogen (Table 5.1). Nitrogen was observed to have a 0.7% decrease in concentration as a result of the treatment. Similarly, potassium and sulphur also saw proportionally small increases of approximately 5%. Calcium, magnesium, and sodium experienced greater levels of change, with the increases ranging between 10% and 15% greater than the initial concentration. Finally, phosphorus saw the greatest increase at a 26%, although this is likely attributed to the initially low levels of the element causing a disproportionately greater increase in percentage concentration compared to other, more abundant, elements.

Table 5.2: Table of mean nutrient content between undiluted non-sterile (RAW V1) and undiluted sterile (RAW V2) vermiliquer FWBHF ($n = 3$). Nutrient content has been transformed with the EC factor as described in the Methodology section.

Nutrient	Mean RAW V1(\pm SD) (mg/L)	Mean RAW V2(\pm SD) (mg/L)	Percent Change (%)
1 pH	7.9(\pm 0.0)	8.2(\pm 0.0)	3.2
2 EC (dS/m)	7.9(\pm 0.0)	8.0(\pm 0.0)	0.3
3 N Adjusted	209.4(\pm 4.8)	207.9(\pm 2.9)	-0.7
4 P Adjusted	18.1(\pm 1.1)	22.8(\pm 1.0)	26.2
5 K Adjusted	1298.1(\pm 55.6)	1364.6(\pm 27.1)	5.1
6 Ca Adjusted	847.5(\pm 56.4)	931.3(\pm 31.3)	9.9
7 Mg Adjusted	88.9(\pm 3.7)	101.8(\pm 1.3)	14.5
8 S Adjusted	287.1(\pm 0.7)	303.1(\pm 1.3)	5.6
9 Na Adjusted	206.6(\pm 8.5)	232.9(\pm 2.4)	12.8

5.3.2 Changes in nutrient composition throughout the growth period

Nutrient sampling after the final round of treatment applications (day 14) found a relatively wide spread of chemical responses across all treatment groups. Significant decreases in pH were observed in V1 treatment groups, this was not observed in V2, which instead showed a non-significant tendency towards alkalinity (2a). EC was found to be similar across all

treatments, although V1 skewed EC compared to the control, while the V2 was prone to significant outliers both above and below the mean(2b).

The distribution of macronutrient content across treatments were semi-consistent with the nutrient levels observed in the raw treatment solutions (Figure 5.2, Table 5.1). It is highly unlikely this consistency is due to plant nutrient uptake, as yield quantity and quality outcomes are considered similar across all groups (Figure 5.3) Calcium and sulphur were the only two elements considered statistically similar to the control for both V1 and V2 treatments.

Nitrogen and phosphorus were both significantly lower in V1 and V2 compared to the control. While nitrogen was considered similar between V1 and V2, phosphorus was considered significantly higher in the V2 compared to V1.

Both potassium and sodium were significantly higher in V1 and V2 compared to the control. Notably, V2 contained a significantly greater level of potassium compared to both V1 and C. In addition, visual observation shows a slight increase of sodium in the V2 treatment.

Uniquely, magnesium in the V2 treatment was considered similar to the control, while the V1 contained a significantly lower concentration of the element.

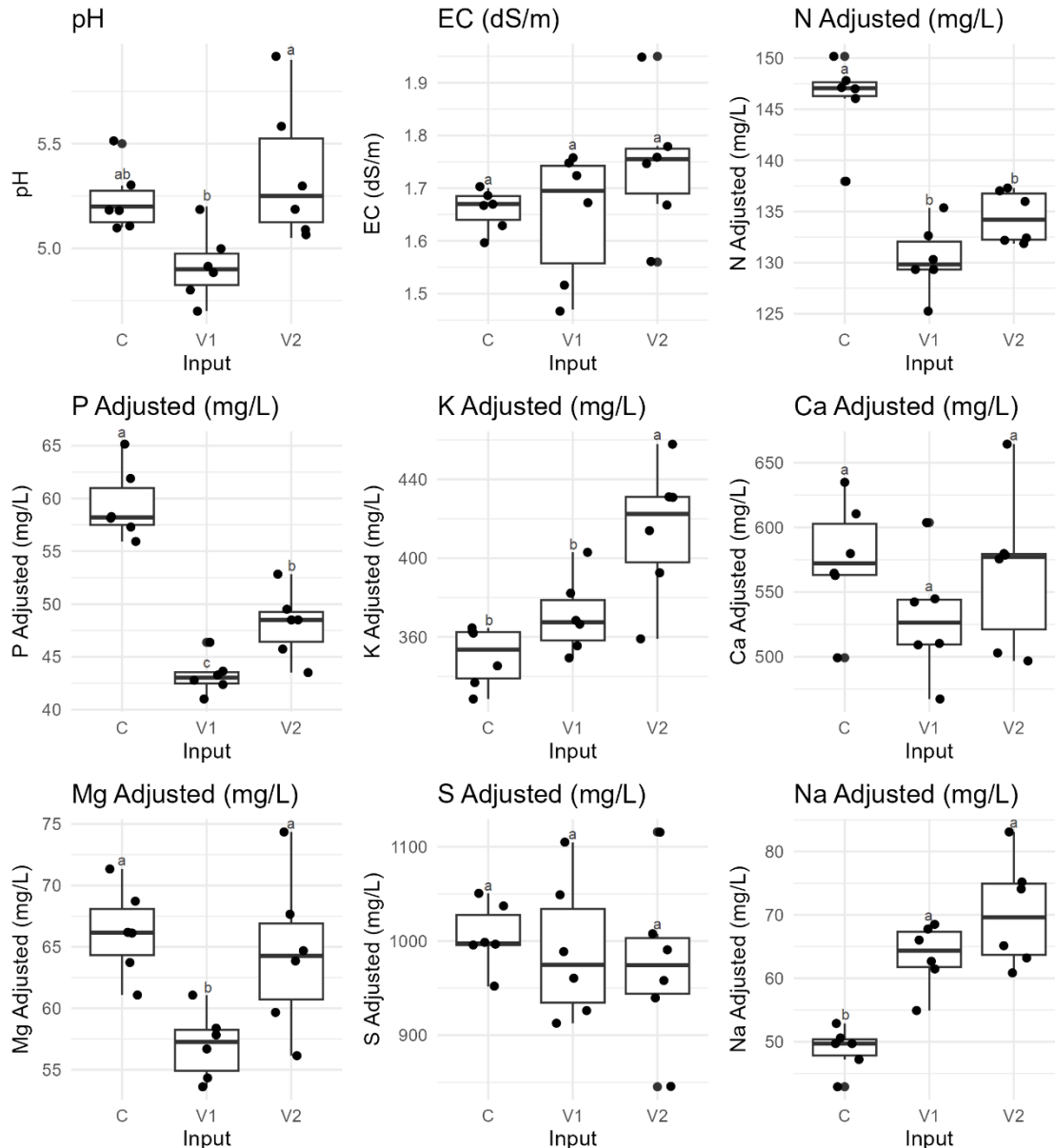


Figure 5.2: Jittered boxplots of differences in nutrient content between control (C), non-sterile (V1), and sterile (V2) treatments 48 hours after the final fertiliser application (day 14). From top left to bottom right: a) pH, b) EC, c) total nitrogen (mg/L), d) phosphorus (mg/L), e) potassium (mg/L), f) calcium (mg/L), g) magnesium (mg/L), h) sulphur (mg/L), and i) sodium (mg/L).

5.3.3 Yield outcomes

Yield outcomes measured in fresh weight (g), dry weight (g), leaf count, and height did not find significant differences across all treatments (Figure 3a, b, c, e). Similarly, SPAD readings and root length were also considered similar (Figure 3d, f). That is, there is no significant loss in yield, root development, or chlorophyll content as a result of the FWBHF substitution.

Notably, some measured variables show a change in variance across treatments. A global Levene's test found that FW, DW, SPAD, and root length are all similar in variance, while leaf count and height are unequal. However, visual observation of DW and FW (figures 3a, b)

show a reduction in range and interquartile range. Pair-wise Levene's with Bonferroni corrections were performed as a post-hoc analysis of the global Levene's test to identify pairs of interest. These found that variance was significantly different in FW and DW between the V1 and C treatments. Meanwhile, V2 shows some reduction in variance, although pair-wise comparisons found V2 to have equal variance with both C and V1 treatments. It should be noted that post-hoc Levene's are not definitive indicators of significant change and instead indicate behaviours which may of interest in future research projects.

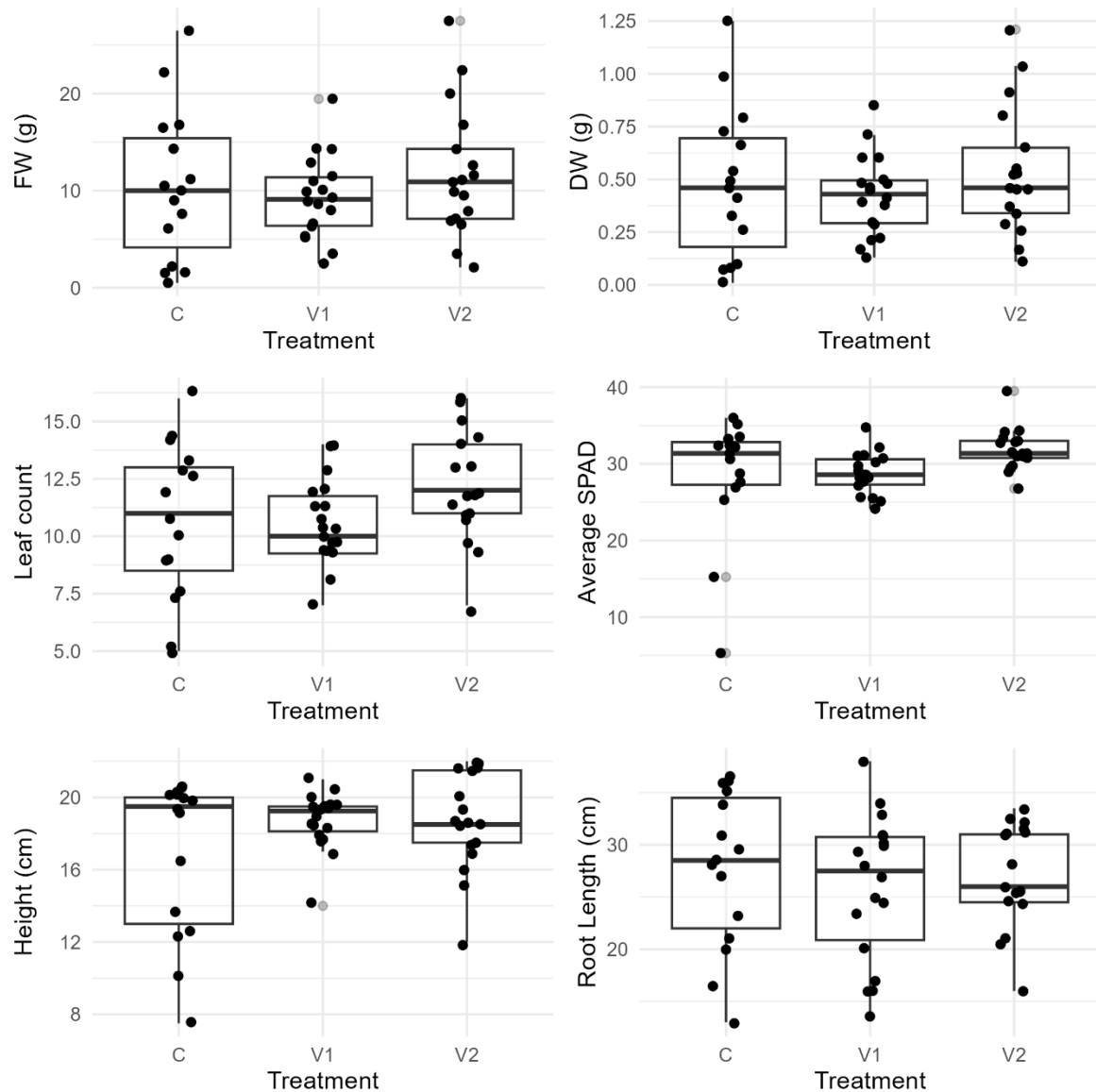


Figure 5.3: Jittered boxplots of yield outcomes. Top left to bottom right: a) Fresh weight (g), b) dry weight (g), c) leaf count, d) average SPAD, e) height (cm), f) root length (cm). C = Control, V1 = non-sterile vermiliquer FWBHF, V2 = Sterile vermiliquer FWBHF.

5.4 Discussion

5.4 Impact of high-temperature sterilisation on chemical, biological, and yield outcomes

High temperature sterilisation did not cause any significant differences in yield outcomes (FW, DW, height, root length) between the non-sterile and sterile treatments in this trial. In addition, both treatments were able to meet the yield outcomes of the control. Future tests should include a negative control (66% concentration) to better quantify the effects of partial FWBHF substitution. This observation may be due to plant-related rhizospheric activity limiting the impact upstream inoculants have on the functional composition (e.g. proportional distribution of diazotrophs, pathogens, PGPM's etc.) of its rhizosphere (Chapter 4).

The two use-cases for autoclaving protocols outlined in this paper are i) sterilisation to eliminate potential human and plant pathogens, ii) induce chemically beneficial changes which improve nutrient availability. While the effectiveness of autoclaving protocols for producing a sterile output are well known, incorporation of high-temperature sterilisation requires careful consideration of its multi-faceted impacts on both microbiological and nutritional aspects of a FWBHF (Chang et al., 2018; Yu et al., 2023).

The removal of pathogenic species can be considered objectively beneficial, however, the collateral sterilisation of the entire microbiome limits potential use cases. While this study did not observe a significant difference between the sterile and non-sterile treatments, there is an observable non-significant reduction in variance for height and fresh weight in the non-sterile treatment group (Figure 5.3). This reduction in variance could be attributed to a redundant ecology providing stability or plant growth promoting microbes (PGPM's) which homogenise yield outcomes across replications. The benefits of an ecologically redundant rhizosphere in hydroponic crop systems are two-fold (F. Dhawi., 2023). First, from PGPM's, which directly provide services such as improving nutrient uptake, and plant-hormone expression (Compant et al., 2019). Second, the presence of a rich, redundant rhizosphere creates an environment which can improve resilience of unfavourable abiotic conditions, as well as resistance to root-borne diseases through both predation of pathogenic species and competition for resources (Li et al., 2021; Ali et al., 2022; Carmen Orozco-Mosqueda et al., 2022). Together, these factors may simultaneously improve crop resilience and reduce the viral load of pathogenic species in the rhizosphere. However, to our knowledge, there is no literature which explores the effects of FWBHF on yield reduction. Similar literature in soil-based research attributes the causal factor of yield variation to be the capacity for inoculants to establish and perform microbiological fixation of nitrogen (i.e. uniformity in available nitrates creates a more uniform yield) (Jáuregui et al., 2019). Hence, the cause of this effect is not likely due to differences in nutrient availability, as all three treatments shared similar levels of variance across all macro-nutrient groups. Future research should explore this crop response to FWBHF and ascertain the exact mechanism causing this reduced variation.

Conversely, sterilisation produces a microbiological vacuum which provides opportunities for biological intervention or improvements in logistics. If a sterilised solution was left unaltered and applied in a hydroponic system, it would certainly become recolonised by microbial life. These populations may come from the environment or from surviving "commercially sterile" thermophilic populations of bacteria and fungi (T. Deak., 2014). Biological intervention, such as the inoculation of conventional hydroponic systems have shown significant increases in fresh weight yield and overall root-shoot development across a range of common hydroponic crop species (Oliveira et al., 2022; Stegelmeier et al., 2022; Wang et al., 2023). Introduction of PGPM's such as *Azospirillum brasilense*, *Bacillus subtilis* and *Pseudomonas fluorescens*

were found to increase the vigour of arugula (*Erica sativa*) (Oliveira et al., 2022), and subspecies *Arthrobacter pascens* (BUAYN-122) and *Bacillus subtilis* (BUABN-01) were found to improve both biomass and nutritional content of lettuce (*Lactuca sativa*) (Wang et al., 2023). As such, the removal of the existing microbiome may present opportunities for the inoculation of select microbial populations. To our knowledge this research has not been trialled on a sterilised FWBHF, nor has there been a comparative study comparing yield effects between uninoculated and inoculated FWBHF's.

Chemical changes which occurred as a result of high-temperature sterilisation include increases in most plant essential macro-nutrients, excepting nitrogen. There were especially pronounced effects on magnesium, sodium, and phosphorus. The cause of this reaction is likely due to high temperatures inducing the breakdown of organic matter, which has been observed to improve overall nutritional availability (Chang et al., 2018). The optimisation of this process may prove difficult, as Barber (2016) has identified that the temperature, time, and pressure for optimising nutritional outcomes is specific to inputs – that is, there is no existing method which guarantees optimal nutrient extraction from non-homogenous inputs. As such, the primary consideration for future research in this area is to consider the amount of energy expended in relation to nutrients gained. While some of our observations of changes in nutritional content are significant, the overall NPK composition of the nutrients was not greatly improved in the FWBHF. The endemic deficiency in nitrogen remained unchanged, and more importantly the sodium content was found to be elevated as a result – overall this leads to a reduced potential application rate of the solution.

5.5 Conclusion and future recommendations

The absence of yield loss, and non-significant improvement in yield consistency suggests that FWBHF can be utilised as a supplement to a conventional hydroponic solution. In addition, high-temperature sterilisation methods do not affect yield outcomes. Future research surrounding high-temperature sterilisation should retain a strong focus on developing a commercially suitable product. This includes altering the stage sterilisation is implemented, exploring effects it may have on yield quality, as a tool for long-term storage, and a cost/benefit or life-cycle analysis.

Moving autoclaving to an earlier stage of FWBHF development may help alleviate increases in sodium content. The application of high-temperature sterilisation on food-waste prior to bioconversion may provide different benefits compared to its application on a completed FWBHF. These benefits include initial breakdown of organic matter, as well as providing a leaching mechanism for excess sodium. Existing literature has identified that pre-process thermal hydrolysis (autoclaving followed by rapid decompression) improves the methane efficiency and temporal rate of downstream anaerobic digestion (Ismail et al., 2022).

Improving yield quality may be a potential aspect of FWBHF supplementation. Future studies should strive to analyse how FWBHF sterilisation affects antioxidants, flavour, and the nutritional values of crops.

Logistically sterilisation may be a viable method for improving commercial viability by prolonging shelf life. The cessation of biological activity prevents biologically induced changes in nutrient composition. In conjunction with refrigeration, this may enable the homogenisation of FWBHF produced throughout the year – mitigating changes in nutritional compositions which occur as a result of fluxes in feedstock composition.

A final consideration for capital and ongoing operation costs should also be noted. Compared to bioconversion, the energy required for auto-claving is considerable. Ongoing energy costs are mainly attributed to vacuum pumps and heating elements. While the energy requirements of the vacuum pump are fixed, the heating element requires proportionately more energy as the mass of the input material increases (O'Callaghan et al., 2023). Sterilisation may not be necessary if there is no equivalent benefit being gained, either through disease prevention, fresh weight increases, or improved growth rate. As such, the additional step of large-scale high-temperature sterilisation could be considered unnecessary.

Chapter 6. General discussion and conclusion

Maintaining food security in the face of increasing urbanisation and climate instability is a challenge that must be addressed through reforms and improvements in current agricultural practices. This thesis aims to improve the availability of affordable, fresh, horticultural produce in urban areas through the adoption and implementation of hydroponic systems which utilise nutrients derived from food-waste. Besides bolstering urban food security, this approach also aims to improve nutrient cycling and creates opportunities for sustainable urban horticulture practices to develop. This thesis collates the current understanding of FWBHF development and aims to begin addressing knowledge gaps through the quantification of novel processing methods and their effect on food-waste (Chapter 1, Chapter 2). This paper did not find FWBHF to be an adequate substitute for existing commercial hydroponic solutions, instead identifying excess sodium as the primary limitation in its efficacy – much like the findings of other existing literature (Chapter 3). Despite its nutritional limitations, we identified potential use cases of FWBHF as a biological inoculant. Recent advancements in next-generation sequencing have made microbial community analysis in FWBHF systems possible, allowing for a greater understanding of how biotic and abiotic factors function and influence the ecological composition of the hydroponic microbiome (Chapter 4). The development of bio-active FWBHF's also revisits our approach to sterilisation in hydroponic systems, asking how the introduction of a biologically complex, and nutritionally diverse solution affects management practices which have been developed for “sterile” synthetic solutions (Chapter 5).

6.1 Nutrient imbalance is endemic in FWBHF solutions

The success of conventional hydroponic systems is dependent upon the curation of essential plant nutrients in growth solutions, and the inability of FWBHF's to emulate these conditions are a pervasive and central issue surrounding its viability in commercial application. A large contributing factor to the observed nutrient imbalances is due to the dependence of a FWBHF's nutritional potential on its feedstock. Chapter 1 found that distinction between waste-sources was crucial in identifying trends in nutritional imbalances, due to the differences in composition of waste generated at farm, industry, and consumer levels. Farm and industry food-waste was often unprocessed, homogenous organic matter, while consumer waste was highly variable in both composition and over time. As such, this thesis has targeted consumer-level household waste as the primary focus of this research. The findings of our experimental trials identified deficiencies in nitrogen and calcium, and excesses in potassium and sodium. This is in line with observations from other household-waste derived FWBHF's (Chapter 1).

These deficiencies are less common, or absent, in FWBHF's developed from farm or industrial waste, which tend to be composed of unprocessed, homogenous organic waste. It was found that as feedstock collection moves from farm and industry towards consumer levels there is a tendency for waste to increase in variability. Firstly, there is a greater fluctuation of feedstock quality over time. Secondly, and more importantly in the context of nutrient imbalance, household waste has a greater variety of inputs. This can be attributed to differences in levels of processing, with feedstocks including waste which is unmodified from farms (e.g. fruit and vegetable waste, offcuts), while others may have undergone extensive preparation at industry and consumer levels (e.g. lasagna, fried rice, a Reuben

sandwich). These processes can alter the nutritional composition of food-waste by altering physical and chemical attributes, for example: homogenising carbohydrates (milling wheat grain into flour), altering acidity (preservation of vegetables with vinegar), or even just adding sodium for flavour (seasoning with salt). As such, there is a tendency for consumer food-waste to contain a fluctuating level of sodium, regularly exceeding tolerable levels for hydroponic crop production.

To better quantify and compare the nutrient levels across literature and within trials, the equation: “ $AdjustedEC = \frac{ObservedEC}{1.6} dS/m$ ” was developed (Chapter 1). This equation aims to standardize the nutrient levels at a set electrical conductivity of 1.6 dS/m, which is ideal for plant growth in hydroponic systems. While it does not account for chemical changes which may occur as a result of dilution, this provides a standardised frame of reference for the comparison of multiple FWBHF solutions. Using this approach, it was identified that the deficiencies observed in the growth trials (Chapter 3, Chapter 5) are more akin to nutrient imbalances. That is, relative to the level of required macro-nutrients, there is a disproportionately large sodium content. As a result of this, when looking to remediate immediate deficiencies, such as in nitrogen or phosphorus, additional applications of FWBHF are ineffective. This is due to increased salinity, which begins to inhibit root uptake of nutrients, even if they are in a greater concentration.

Hence, the big question in FWBHF development is “How do we address excess sodium?”

This question, to our knowledge, has never been explicitly addressed in FWBHF literature. We have identified across literature and our own trials, a series of processes which have potential to alleviate, but not remediate, the nutrient imbalances observed. Generally, these approaches utilise feedstock supplementations or utilise a novel method to filter out excess sodium. Remediation of feedstock through supplementation of organic matter is an effective method for improving nitrogen content in FWBHF solutions (Chapter 1). The findings of this chapter highlighted that nitrogen deficiency were not as prevalent in FWBHF trials which utilised vegetative farm/industrial waste, and consumer waste trials which utilised supplementations of vegetative organic matter (Phibbunwatthanawong et al., 2018). The primary factor separating the quality of FWBHF derived from farm/industrial waste and consumer level food-waste is the presence of excess sodium.

As such, supplementing feedstock is the earliest level of intervention for the remediation of sodium in FWBHF development. A consistent finding throughout Chapter 1 was that literature which included dense, ligneous vegetative matter (usually from farm/industry) had a greatly improved nitrogen:EC ratio compared to papers which utilised exclusively household/consumer waste. Applying this in an urban environment could be done through the addition of municipal green waste to FWBHF feedstock – diluting the sodium content of food-waste with locally sourced, nitrogenous, non-saline, material.

The next stage of intervention is prior to initial organic breakdown. In literature, this usually involves physical processes, such as mastication, to homogenise material. A potential extension to this process could be the incorporation of high-temperature steam sterilisation. Chapter 5 has already highlighted its microbicidal capabilities, which eliminates pathogenic populations as well as enables the inoculation of ideal microbiota for the breakdown of organic matter. Beyond biological changes, its application to raw food-waste may also create physical and chemical changes. Physically, steam treatment can assist in structural breakdown of feedstock, and improve the porosity of material. Capitalising upon the improved porosity, steam treatment provides liquid which may solubilise and leach sodium from food-waste. Combined with mastication, pressing, and straining, this may be an

effective method to leach out excess sodium prior to bioconversion of organic matter – essentially preserving nutrients in organic matter and removing excess sodium. The caveat of this process is that each step will incur additional labour, capital and ongoing costs.

Commercial adoption of cutting-edge technologies for agricultural application is often met with hesitation. Hence, cutting-edge methods of sodium remediation are unlikely to see significant adoption in commercial settings in the near future. However, as these technologies are developed their associated costs may reduce and their use in FWBHF may become viable. Thankfully (for FWBHF development), there is a high likelihood of continued research into desalination processes on a large scale, as climate change drives urban centres to develop methods for the processing of sea- and wastewater into potable forms. While not all forms of desalination are appropriate for FWBHF development, the use of membrane-based selective reverse osmosis shows promise as a method for filtering out specific minerals, such as sodium. Other novel methods, such as nitrogen supplementation with plasmapheresis may also be potential candidates for improving nutrient balance.

From our findings, it is likely that the application of multiple, novel steps throughout the production chain, is the best approach to improving the nutrient content of FWBHF. Hence, it is essential to continue exploring a range of efficient approaches to manipulate the mineral content of FWBHF.

6.2 Processing methods must be robust and efficient.

Initial exploration in FWBHF sought to determine an optimal combination of processes and food-waste inputs which would be able to produce a FWBHF which was chemically similar to a synthetic solution. However, there was a rapid recognition of the uncontrollability in the food-waste used in FWBHF development. As such, the focus of this thesis shifted away from optimising inputs, and towards post-collection processes which could be used to efficiently convert house-hold waste into a hydroponic solution. The development of FWBHF should consider both spatial and temporal fluxes in food-waste generation, these factors can include geography, culture, and seasonal availabilities which contribute towards variation in feedstock. Hence, the challenges of developing a commercially viable method of nutrient recapture begin to arise – the systems and processes which are implemented may only function in geographically specific zones and must be able to produce a consistent product in the face of fluctuating inputs throughout the year. Current methods to mitigate variance include homogenising FWBHF over a period of time, or supplementing with synthetic fertilisers – although these solutions come with their own caveats and logistical challenges, such as managing solution stability in storage, or potentially disqualifying producers from utilising value-adding certifications. Similar considerations must also be made regarding energy efficiency, where inputs and methods should be considerate of scalability, available infrastructure (for energy, and waste collection), and environmental impacts (i.e. wastewater and emissions).

Most approaches to FWBHF development involve multiple steps, which can be physical, biological, or chemical in nature. However, it is not uncommon for one novel process to influence two or three of these aspects. Physical processes are pertinent through FWBHF development. These processes include initial homogenisation through mastication, chopping, or crushing. Further on, solutions are physically altered by aeration, stirring, or filtering. Changes in biological composition during FWBHF production are often catalysts for bioconversion, which can be defined as biologically driven chemical changes. Bioconversion is the most common method of inducing chemical changes in FWBHF development. Across literature, the most frequently identified method of bioconversion is through

aerobic/anaerobic fermentation techniques. This is likely due to its cost-effective and scalable nature, especially when converting organic material into inorganic forms. In addition, the abundance of literature surrounding fermentation stems from multiple disciplines, which have documented the microbiological and chemical effects of the process across a range of inputs. Other methods such as composting and vermiculture have also been trialled. Chemical intervention is usually reserved for final alterations, with majority of literature omitting its usage, reserving it for end-stage pH manipulation or to supplement deficient nutrient groups (Chapter 1).

This thesis trialled a range of physical and biological methods and did not utilise chemical additives. Physical methods included mastication, and bioconversion was trialled with aerobic fermentation, anaerobic fermentation, and vermicompost. The FWBHF utilised in this thesis' growth trials was a four-step process: 1) physical mastication, 2) vermicomposting, 3) anaerobic fermentation, and 4) aerobic fermentation. This produced a solution which sustained lettuce growth, but not to the standards of the control in both quantity and quality aspects. The deficiency in vigour can be largely attributed to the inherent nutrient imbalances mentioned earlier in this discussion and in Chapter 3. The excess of salt and deficiency of key elements (particularly nitrogen) are still the primary inhibitors of achieving yield parity.

Continued research into how processing methods affect the nutritional makeup of food-waste leachate is critical in developing robust methods for FWBHF production. Strong considerations for documentation are the rates of energy expenditure relative to nutritional yield, as well as the composition of food-waste utilised. As shown in Chapter 2, foods of similar make-up tend to have similar reactions when exposed to novel processing methods. The applications of these studies could allow for modular applications of novel processing methods based on the composition of food-waste in different settings and over time, encouraging an adaptable, holistic approach to FWBHF development.

6.3 Microbial composition in the rhizobiome

The incorporation of FWBHF ecologies into hydroponic systems may be the greatest affront proposed by this thesis towards conventional hydroponic practice. The inoculation and cultivation of a complete microbiome challenges the sterile culture which has historically driven research efforts into the microbiome of hydroponic systems. However, several findings of this thesis suggest that there is merit in reversing the doctrine of sterility – especially with respect to the transition towards an organic source of nutrients. The arguments for the challenge are related to an increased availability of organic matter, a new understanding of plant-substrate rhizosphere interactions, and a more holistic perspective on pathogen control in general. It should be noted that this thesis does not advocate for the complete removal of hygiene and sterile practices, instead it suggests for a revision of current practices to accommodate the integration of a new, biological method of integrated pest and pathogen management.

The most prominent change in the adoption of FWBHF is the compositional material of the solution itself. FWBHF tends to contain large amounts of organic matter which is not fully plant available. On the other hand, synthetic solutions tend to only contain mineralised, optimised nutrients for plant uptake. This creates a new challenge for FWBHF viability, as there is an extra step of bioconversion of organic matter before plant uptake. Compared to inorganic forms, the organic material is structurally complex and requires appropriate microbial actors to facilitate its breakdown. This thesis was unable to fully isolate the effects

of organic matter, although it was found that FWBHF tended to have a greater overall abundance of microbial activity (Chapter 4). The composition of organic matter in substrates was found to create significant differences in fungal species, whereas nutrient solution treatments tended to have greater influences on bacterial composition. However, the current commercial standard is to use synthetically composed rockwool as a substrate. As such, it is highly recommended that future research explores the differences in rhizobiome composition between rockwool and any organic substrate – with a strong focus on the changes in pathogenic species as well as ecological complexity.

The findings of Chapter 4 highlighted the degree of influence plant species had over their immediate rhizosphere. Despite the taxonomic differences between the synthetic and FWBHF treatments, the two both shared a similar distribution of functional groups (E.g. nutrient cycling, heterotroph spp., plant pathogens etc.). It is unlikely that this is a coincidence, as the initial functional distributions of the control and FWBHF were considered significantly different. After the introduction of each treatment, the functional composition of the solutions gravitated towards similarity – suggesting that a common mechanism between the treatments which exercised a degree of control over the rhizobiome composition. It is most likely that this is due to the lettuce crop utilising carbonic exudates to manipulate its immediate rhizosphere. Lettuce has shown in its capacity to mould significantly different upstream ecologies into favourable, functional compositions (Chapter 4). Previous research has tested inoculations of specific species for both pathogen control and plant growth promotion, with varying degrees of success (Gravel et al., 2006; Khalil et al., 2009; Spaepen et al., 2014; Thomas et al., 2023). Our findings show that elasticity of the lettuce rhizosphere suggests that a multi-species inoculation may also be feasible. Future research should explore the inoculation of entire ecosystems, and how they may impact plant growth.

Hence, the inherently biological nature of FWBHF solutions may require a revision of existing sterile culture in hydroponic systems. It has been found in both this thesis and other literature, that non-symptomatic populations of plant pathogenic species are inevitably present in hydroponic systems (Sheridan et al., 2017). As a management method, total elimination of microbial activity through blanket applications of chemical and physical control methods are a common practice, and generally this approach has worked well for commercial solutions. However, the organic components in FWBHF, as well as its bioactive nature, may warrant a new approach to managing pathogenic populations. The presence of stable ecologies in soil systems has long been associated with the fortification of crop health, and the biological regulation of pathogenic populations (van Bruggen et al., 2006). Similar to soil systems, the development of a perennial rhizosphere ecology in hydroponics will require the adoption of holistic IPM techniques, which includes limiting the use of chemical microbicides.

As a caveat, it should be noted that the findings of Chapter 4, Chapter 5 identified no significant benefit in retaining a complete FWBHF derived ecology in lettuce production. It is possible that a stable ecology is simply not necessary in the rapid turnover associated with micro-green or leafy green production in hydroponic systems. Instead, efforts to improve yield in these areas should focus more on rebalancing nutrient species. However, for long-term crop species, particularly those with fruiting bodies, the implementation of a stable ecology may be greatly beneficial. A stable ecology may be more adaptable to the prolonged exposure to different environmental contaminant sources, changing nutrient requirement of fruiting crops, and in FWBHF systems, changes to the microbial/nutritional composition of fertilisers over time.

6.4 Concluding remarks and future directions

The extension of nutrient cycling into urban environments is an important milestone in developing sustainable food production systems. The impacts of modern agriculture on nutrient dynamics have led us to realise the fragility of the land we work, and the scarcity of nutrients we face. In response, we have sought ways to alleviate the pressures of degrading land and growing urban populations by seeking alternative sources of nutrients. The study of FWBHF's joins a wave of research which has identified the value of "waste" generated in urban environments which has been traditionally delegated to landfill. The collective doctrine of these research efforts is to recapture and repurpose the leakage of nutrients from our food production systems. The findings of this thesis have identified distinct obstacles which limit its practical viability. Existing studies frequently share a nutrient imbalance which manifests as an excess of sodium, and deficiency in macro-nutrient species. While this limits the performance of current FWBHF's, there is still research which may improve commercial viability without directly remediating this nutrient deficiency. This includes efforts to utilise FWBHF as a supplement, exploring its effects on yield quality, manage the non-homogenous nature of food-waste, and improve its shelf-stability.

FWBHF did not show improvement in lettuce growth over 25 days, however it may prove useful in a more perennial system as a pre-harvest supplement. Assessments of long-term disease resilience in systems which produce multiple harvests should be performed. We expect that the extended growth period provides time for the microbiome ecology to establish and stabilise. In addition, perennial hydroponic species are usually fruiting crops, which benefit from deficit irrigation and excess potassium when fruit development begins (Liu et al., 2021). The current iteration of FWBHF contains an excess of potassium suitable for fruiting crop production. In addition, its high salinity allows it to simulate the limited water conditions of "deficit irrigation" conditions, potentially leading to improvements in fruit quality parameters such as titratable acids, soluble solids, sweetness, and flavour (Zhang et al., 2016).

Studies which aim to better characterise long-term behaviour of urban food waste can greatly improve understanding of the required processes for optimal FWBHF development. In addition, a stronger documentation of inputs improves the translatability of FWBHF research across urban centres. In turn, novel processes can be applied more intentionally compared to the current state of FWBHF research.

The application rate of FWBHF may also be improved, currently, the density of nutrients provided by 10ml of A/B control solution requires 400ml of undiluted vermiquer to replace. Evaporating or precipitating excess liquid. This may inhibit recolonisation by increasing the concentration of the solution, while addressing the excess liquid in the FWBHF. Future research should test these hypotheses, with special consideration for how dehydration influences the resulting nutrient and microbial composition.

While some FWBHF studies have identified an improvement in yield quality (increases in antioxidants, phenolic compounds, flavonoids etc.), a 2024 meta-analysis of both hydroponic and soil systems was unable to ascertain the definitive improvement in yield quality as a result of "organic" cropping (Ahmed et al., 2021; Rahman et al., 2024). The myriads of mechanisms behind improved crop nutrition are well identified, although the weight of each process is still understudied. As such, future FWBHF research should aim to determine if the

use of vermiliquer FWBHF has any benefits on nutritional quality of produce, and the optimal supplementation rate of FWBHF alongside a synthetic solution to gain these benefits.

A realisation of this project was the scale of food-waste generated by just one household. A rough calculation has found that the household waste generated in Chapter 3 would amount to approximately 0.8L of organic food-waste per day. Over the course of a year, this amounts to ~320L (~160kg). We have estimated that it takes roughly 6L (or 3kg) of food-waste to generate 1L of vermiliquer. This means a single household can generate ~53L of vermiliquer a year. In chapter 5 we found that a 33% vermiliquer + 67% control solution could produce lettuce without yield loss. Hence, at an application rate of 0.075L of vermiliquer per head of lettuce we estimate that from the 320L (160kg) of food-waste generated each year, a single household can provide enough vermiliquer to grow 706 heads of lettuce. This can be expressed as $x = (y/6)/0.075$ – where x represents heads of lettuce, and y is the volume of food-waste (L). Overall, this replaces 2.64L of the commercial synthetic solution, which is a comparably miniscule volume next to the 53L of vermiliquer required. This reframes the priority of this research away from replacing synthetic solutions, and towards managing the overwhelming abundance of food-waste we generate each year.

In addition to managing the growing amount of urban organic waste we generate, climate variability creates an undeniable need for the re-localisation of sustainable food production. Current modes of fresh produce supply have created an overwhelming dependence on regional and international imports. In conjunction with the growing cost of inputs associated with conventional soil and hydroponic systems, there is a growing need for a cost-effective, local, source of fresh produce throughout urban environments. Hence, we hope that this thesis can clarify the current state of FWBHF development, highlight potential avenues for future research, and begin to evaluate how current practices can adapt to integrate FWBHF's.

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