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Investigating Horticultural Applications of Liquid Digestate

By

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in collaboration with

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Abstract

Background. Sixty percent of global crop production depends on inorganic fertiliser, supporting nearly half of the world's population. However, this production accounts for 1.6% of global CO_2 emissions and is based on increasingly unreliable, finite resources. Thus, there is an urgent need to find sustainable alternatives. Digestate, a by-product of anaerobic digestion, can be used whole as a fertiliser, or separated into solid and liquid fractions. Liquid digestate is far more voluminous than solid digestate, making it difficult to store. While digestate has been used in broadacre agriculture for decades, its use as in horticulture is not widely studied. 1Up Natural, a British biogas company, produce separated digestate fertilisers from a plant-based feedstock, for retail in domestic horticulture. On account of liquid digestate's high volume, it is pertinent to evaluate it as a replacement to synthetic fertilisers in horticulture.

Methods & Results. To assess liquid digestate's application as a fertiliser, a series of progressive pot-based trials were designed. Firstly, liquid digestate was compared to industry-leading synthetic fertilisers at half and full manufacturer's recommended concentration. Synthetic fertiliser outperformed liquid digestate at the recommended concentration, which was attributed to differing nutrient levels. Secondly, liquid digestate was matched for nitrogen against a synthetic fertiliser. Again, synthetic fertiliser increased plant growth relative to liquid digestate, likely due to limiting influence of non-matched nutrients. Finally, liquid digestate was applied at different concentrations in a sand-culture experiment, to establish its suitability as a sole nutrient source. Liquid digestate induced NH_4^+ toxicity and deficiency in other nutrients.

Conclusions. Based on these experiments, it is apparent that liquid digestate must be altered to be suitable as a sole nutrient source or alternative to synthetic fertilisers in low-nutrient mediums. However, liquid digestate may be suitable for application in high-phosphorus soils to prevent excess phosphorus application.

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Contents

List of Figures

List of Tables

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Author's Declaration

I, Thomas Escott, declare that this thesis is my own work, in my own words, and has not been submitted for the award of a higher degree elsewhere. Many of the ideas in this thesis were the product of discussion with my supervisors Professor Ian Dodd and Professor Phil Haygarth.

Chapter 1

Introduction

1.1 Food Supply and the Green Revolution

Throughout human history, adequate nourishment through a balanced diet has remained a significant challenge for most civilisations and populations (Wiener, 2018). Food supply is one of the most important limiting factors of population and can be subdivided into three major sources: cropland, grassland, and fisheries (Gilland, 1983). Of this, cropland provides 80% of humanity's primary food needs (Gilland, 1983), and thus the productivity of cropland is intrinsically linked to the size of a population. The control food supply exerts on population size has been causally demonstrated through the development and uptake of new technologies, which have facilitated greater yields and increased nutrient content (Smil, 1999*b*).

The Green Revolution, first termed by William Gaud of the United States of America International Development (USAID) programme, spanned the 20^{th} century, and facilitated a rapid increase in agricultural production (Jain, 2010). These changes were driven by the development of new crop varieties, extensive application of nitrogen (N) fertilisers, and irrigation infrastructure (Jain, 2010; Zeng et al., 2014). A seminal example of the paradigm shift in commercial agricultural practices can be found in the breeding programmes of the Office of Special Studies (OSS), a collaboration between the Mexican government and the Rockefeller Foundation, under the supervision of William Borlaug in the 1940s (Perkins, 1998). Through the application of Mendel's Laws of Inheritance, which marked an enhanced understanding of plant genealogy borne out of hybridisation experiments on pea plants, researchers created high-yielding semi-dwarf varieties of maize and wheat to improve disease tolerance and response to fertilisers (Naithani, 2021).

By combining this knowledge with another tenet of 19^{th} century agricultural science, Liebig's Law of the Minimum - that plant growth is limited by the scarcest nutrient, rather than total resources - researchers on the OSS programme were able to increase yield eight-fold by applying synthetic fertilisers (Naithani, 2021). This research coincided with rapid growth in the availability of synthetic fertiliser - largely through the industrial synthesis of urea through the Haber process - and discovery of large phosphate rock deposits in Morocco (Haygarth et al., 2013; Smil, 2000). Borlaug, the architect of the OSS project, was also a proponent of newly-discovered pesticides to reduce disease prevalence, such as Paul Müller's dichlorodiphenyltrichloroethane (DDT), which was used extensively until its worldwide ban in 2004, due to its probable carcinogenic properties and effects on non-target organisms (Borlaug, 1972; SSC, 2019).

Through the application of these scientific principles, the OSS developed a package of improved seeds, crop nutrition, and pest and weed control which was applied to many other developing countries and crop varieties (Naithani, 2021). This laid the groundwork for the so-called "Green Revolution", contributing to a doubling of global population, and tripling in crop yield between 1961 and 2010 (Figure 1.1b).

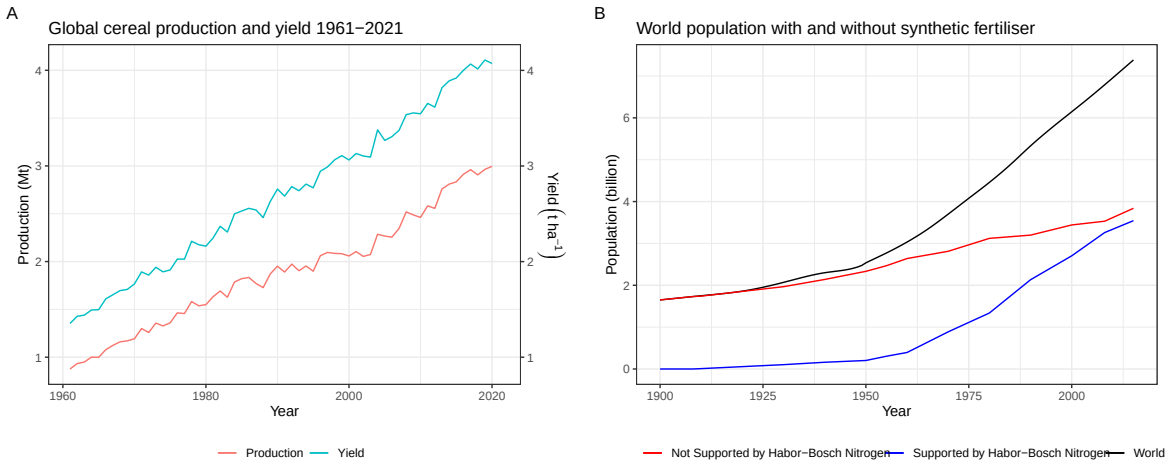

Figure 1.1: (A) Global cereal production and yield from 1961 to 2021 in mega-tonnes (Mt) from Our World In Data (2022) and (B) World population since 1900, and number of people supported and unsupported by nitrogen derived from the Haber-Bosch process (Source: Erisman et al., 2008).

1.2 Fertiliser Production and Use

This enhancement of yield has come at a cost, however, and agricultural systems have become increasingly dependent on supplementation by synthetic fertilisers and pesticides to maintain production. As much as 60% of global food production is now

reliant on inorganic fertilisers (Roberts et al., 2009). Fertilisers typically provide one or more of three elements essential for plant growth: nitrogen (N), phosphorus (P) and/or potassium (K).

Nitrogen is available to plants in two main mineral forms: nitrate (NO_3^-) and ammonium (NH_4^+) (Bhattacharyya et al., 2020). It is the most important element for plant growth, as it is a major component of DNA, chlorophyll, and amino acids. Without N, plants would not have structure or a means of energy transfer. The Haber-Bosch process,

$$N_2(\mathrm{g}) + 3H_2(\mathrm{g}) <=> 2MH_3(\mathrm{g}) \qquad \Delta H^\theta = -92\mathrm{kJ\ mol}^{-1}, \tag{1.1}$$

produces the overwhelming majority of N fertiliser and is responsible for 80% of global ammonia production (Qin, 2019). As shown in Figure 1.1a, 40% of the world's population is dependent on the process for survival (Smil, 1999*b*). The artificial N-fixing process involves heating air to react atmospheric N with hydrogen, which produces ammonia (Figure 1.2). Despite being exothermic in the forward reaction (1.1), increased rates of reaction at higher temperatures mean that heating to 450 °C is industry standard, under a pressure of around 10-15 MPa (Chen et al., 2019). To enable equilibrium to be reached more quickly, iron(II, III) oxide is traditionally added as a catalyst, however more effective catalysts, such as hydride, are currently being investigated to reduce operating temperatures and pressures (Humphreys et al., 2021). While N can be sourced from the air, 99.5% of the hydrogen produced for the process derives from fossil fuels, typically methane (Fasihi et al., 2021). Despite significant improvements in efficiency since its inception, the Haber-Bosch process - largely on account of its scale - is the most energy-demanding chemical process in existence and consumes around 2% of the global energy supply, contributing 400 Mt of carbon dioxide annually – 1.6 % of total global emissions (Liu, 2014). The accelerated speed and scale of N fixation from the Haber-Bosch process also contribute to a significant imbalance in the N cycle. If not correctly managed, overapplication or incorrect timing of fixed N can have severe ecological consequences, particularly within waterbodies (Carpenter et al., 1998).

Phosphorus is only available to plants as phosphate (PO_4^{3-}) ions. It plays a crucial role in fundamental biochemical reactions, and an adequate supply is needed to increase response to applications of N and K (Smit et al., 2009). While phosphorus is typically quite abundant in soils, 95-99% is found in insoluble, unavailable forms (Bhattacharyya et al., 2020). Plants can access these forms through the production of organic acid root exudates, such as acetate and citrate, which convert P into plant-available forms: either by forming soluble P complexes, or by acidifying the root zone and releasing ions

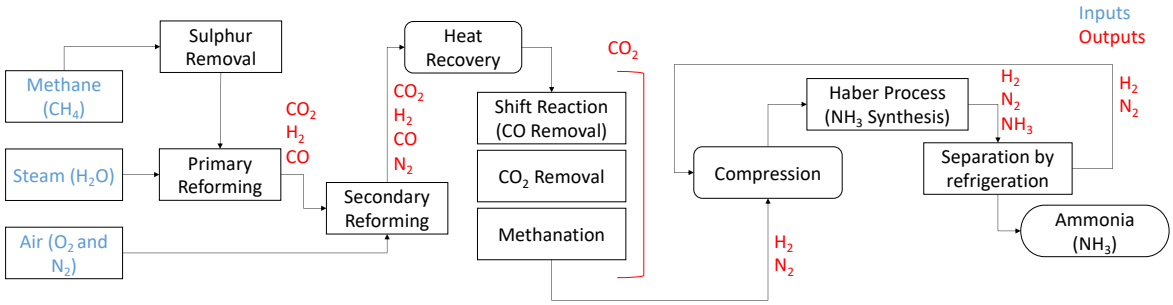

Figure 1.2: Flow diagram of the Haber-Bosch process, including inputs and outputs. Modified from Lichtarowicz (2016).

into solution (Hees et al., 2002). Some organic acid exudates, such as citrate, may also enhance P uptake by chelating with other ions, such as aluminium, to prevent toxicity (Ma et al., 2001). Phosphate rock is the sole economic source of phosphorus for fertiliser production, and derives almost exclusively from sedimentary marine origin (Oelkers and Valsami-Jones, 2008). Apatite is the dominant mineral in phosphate rock, which makes up 0.35 % of the Earth's crust (Manning, 2008). Apatite generally associates with fluoride, and can also associate with other metals, including barium, cadmium, and uranium posing a potential environmental contamination risk in extraction and application (Smit et al., 2009). Phosphates are generally found in agricultural fertilisers as acid-treated soluble PO_4^{3-} salts, typically as superphosphate or triple superphosphate (Manning, 2008). Superphosphate is produced through the reaction of phosphate rock with sulphuric acid, while triple superphosphate reacts phosphate rock with phosphoric acid, leaving a much purer product (Smit et al., 2009).

Reliance on rock phosphate for meeting the phosphorus demands of plants has problems both in supply and use. Firstly, reserves of readily available PO_4^{3-} are finite, and increases in demand resulting from economic development and population growth pose a risk of depletion (Smit et al., 2009). Economically viable phosphate rock reserves may be exhausted by 2080 if current trends are continued (Smil, 2000). These reserves are also heavily concentrated in certain regions: over 70% of the world's phosphate rock reserves are in Morocco and its disputed territory of Western Sahara, posing geopolitical and monopolistic risks (USGS, 2022). Historically, this asymmetric distribution has led to exploitation of coastal communities: Clark and Szerszynski (2020) talk about the joint history of colonialism and phosphate mining in the destruction of Nauru and Banaba, two PO_4-rich Pacific islands rendered "perfectly uninhabitable for men" through the removal of vast quantities of minerals for fertiliser by colonialists. Scarcity, distribution and dependence on external energy source can also cause major fluctuations

in synthetic fertiliser price: rising oil prices caused a seven-fold increase in PO_4^{3-} fertiliser price in 2007-2008, triggering violent riots in India (Cordell et al., 2009).

Potassium is another element essential for plant growth and is associated with the movement of nutrients and water through plant tissues, as well as enzymatic and regulatory processes such as adenosine triphosphate (ATP) production and stomatal response. Similarly to PO_4^{3-}, while potassium is often abundant in soils, typically only 1-2% of it is available to plants (Bhattacharyya et al., 2020). The fraction of potassium readily available to plants is known as solution potassium, which is water-soluble or adsorbed to the cation exchange sites of clay particles. This plant-available fraction is also referred to as potash, of which 95% is derived from potassium chloride, known as muriate of potash (MOP) (Stewart, 1985). Potash is obtained using either solution mining or conventional underground mining methods. These processes can lead to surface subsidence and water quality issues, both in extraction and waste management (UNEP and IFA, 2001). While potash reserves remain relatively secure in physical terms, they are concentrated within the borders of a few countries: Canada, Russia, Belarus, and China currently account for over 75% of the world's known potash reserves (USGS, 2022). Following sanctions against Russia and Belarus for their involvement in the invasion of Ukraine, potash price has become increasingly volatile (AHDB, 2022); a sustainable and secure domestic supply would help buffer against international price fluctuations.

1.3 Anaerobic Digestion and its Role in Horticulture

Anaerobic digestion (AD) is a method of recovering biogas and nutrients from organic residues in the absence of oxygen and has been adopted at many different scales since its discovery in the mid-19^{th} century (Liedl et al., 2004; Pullen, 2015). Biogas generation capacity through anaerobic digestion has consistently increased in both the UK and globally (IRENA, 2021; IEA, 2022), and bioenergy generation now accounts for about 61% of the UK's renewable energy generation (BEIS, 2021). China is the largest user of AD, with over 8 million installations, while Germany has the highest uptake in Europe, with over 6,800 large-scale digester plants (Pullen, 2015).

Anaerobic digestion produces three broad fractions of product: gas, liquid and solid. The inputs to a digester, known as feedstock, dictate the ratio of these products and their potential energy yield (Pullen, 2015). Biogas is typically the primary economic product of AD and is a mixture of methane, hydrogen, carbon dioxide and other trace

gases. Biogas can be used for many purposes: it can be combusted onsite in a cogeneration engine, compressed and bottled, or refined and injected directly into the gas grid. Cogeneration is the most common use of AD biogas, and modern gas boilers have an efficiency of greater than 90% (Pullen, 2015). This option requires only minimal refinement: hydrogen sulphide and water must be removed to prevent the generation of sulphuric acid in the generator. Methane, which constitutes 50-70% of biogas, has 21 times the warming potential of carbon dioxide (Pullen, 2015). Through the capture of this during anaerobic digestion, methane emissions to the atmosphere can be effectively eliminated, reducing the warming effect versus uncontained organic decomposition.

The remaining solid (known as "fibre") and liquid (known as "liquor") fractions are collectively known as digestate and are considered byproducts of the method, formed through acidogenesis and methanogenesis, respectively. Acidogenic digestate consists of fibrous plant matter and possesses high moisture retention, amino acid, and mineral content, whilst methanogenetic digestate consists of a high-nutrient sludge (Pullen, 2015). Both forms of digestate are overwhelmingly utilised in agriculture, with over 97% being applied to arable lands and forests in Germany (BMUV, 2018).

Digestate can be applied whole, or separated into solid and liquid fractions. Applying whole digestate can potentially supply a greater range of nutrients over different timescales, with fibre acting as a high-P, slow-release fertiliser and liquour as fast-release source of N and potassium (Szymanska et al., 2022). This could offer year-round benefits to soil nutrition and structure into the long term. Alternatively, through separation - usually with a screw-press (Guilayn et al., 2019) - two distinct products can be generated. This can give greater control over application, depending on criteria (Al Seadi and Lukehurst, 2012). For instance, fibre may be preferred in soils with low organic matter (OM) and phosphorus, but that are high in N; the reverse may be true for liquor (Al Seadi and Lukehurst, 2012). Furthermore, solid and whole digestate may improve soil physical properties, by reducing bulk density and moisture retention of soils, which could prove particularly useful in compacted or OM-depleted soils, especially in water-stressed areas (Möller, 2015).

Liquor is far more abundant than fibre and has a higher volume and mass than the feedstock influent, on account of water addition during AD (Pullen, 2015). A 1.5 MW AD plant typically produces over 45,000 t liquor annually, which can be reapplied to agricultural lands (Mason, 2017). However, this can typically only be done in spring and summer in many areas, as precipitation events can cause runoff into local catchments (Pullen, 2015). Thus, liquor must either be stored onsite or transported if the plant is operational over winter; the transport of liquor can be expensive: upwards of

€20 per tonne (Pullen, 2015). Liquor is currently restricted to agricultural applications, due to uncertainty of its suitability for other uses (Pullen, 2015). In the United Kingdom, digestate produced from waste products must adhere to the Publicly Available Specification (PAS) 110, which sets limits on contaminants and pathogen content to be marketable. However, digestate produced from non-waste products (e.g., energy crops grown for AD) is not subject to this legislation (EA, 2014).

Attitudes and recommendations surrounding digestate application are also important in governing its effective utilisation. Based on its primary uses as a means of waste management and energy generation, digestate may be viewed by agricultural communities as a waste product, rather than a valuable source of crop nutrition (Dahlin et al., 2015). This sentiment may be enhanced by lack of subsidies to digestate fertiliser markets, and the waste-like characteristics of digestate, notably its offensive aroma (Dahlin et al., 2015; Case et al., 2017). Consequently, emphasis must be placed on integrating digestate application into fertiliser management plans (Kovacic et al., 2022; Al Seadi and Lukehurst, 2012), to avoid overenrichment of soils.

The domestic horticulture market has experienced exponential growth following the outbreak of COVID-19, with garden centres reporting up to a 4.5-fold increase in sales, out-competing production of ornamentals (Klinkenberg, 2020; Cruz et al., 2022). Of this growth, a large proportion of sales represents new gardeners, typically millennials (Mullins et al., 2021). Mullins et al. (2021) found that over half of those surveyed grew at least one type of fruit or vegetable at home, with over 17% beginning during the pandemic. Tomatoes (*Solanum lycopersicum L.*) are the most cultivated outdoor vegetable in home horticulture, with 86% of residential food gardens in the United States growing them, followed by cucumbers (*Cucumis sativus*) at 46% (NGA, 2014). Furthermore, while salad greens and herbs remain the most popular crop for indoor cultivation, there is a growing interest in growing tomatoes indoors (Cruz et al., 2022). This represents a phase shift in the home horticulture market, toward a more nutrient-demanding crop, with a longer growth cycle (Cruz et al., 2022). It is important for producers to meet increasing fertiliser demand sustainably, as over half of new gardeners are motivated to grow food at home to reduce their environmental footprint (Mullins et al., 2021).

To maximise sustainability, digestate products must be marketed responsibly. For instance, where the nutritional content of digestate is unclear or variable – even if it is not immediately bioavailable – there is a potential for over-application by horticulturalists not aware of digestate's inherent nutrients, or who do not have access to proper soil testing facilities. Over-application of soil amendments, often through excess applica-

tion of organic matter, is commonplace in domestic horticulture, even amongst growers with an education in soil health and fertility (Nelson et al., 2022). As a result, domestic cultivated soils regularly exceed double the recommended organic matter content (Nelson et al., 2022). While effects of excess organic matter are variable and poorly understood (Nelson et al., 2022), this highlights a broader 'more is better' philosophy (Moebius-Clune, 2016). Therefore, responsible manufacturer dosage recommendations will account for a propensity for growers to over-apply.

Anaerobic digestion is predominantly a means of waste management and maximisation currently, and thus the feedstock is frequently variable or sub-optimal for plant growth (Dahlin et al., 2015). Theoretically, through controlling feedstock inputs, biogas production can be made more reliable, and a more homogenous digestate will be produced, although this is only recently being evaluated (Häfner et al., 2022). 1Up Natural Ltd is a firm based in Cumbria, United Kingdom which owns and operates an AD plant. The biogas produced is converted to electricity using a dual CHP generator, which generates 1.1 MW/h electricity at 97% efficiency. The feedstock is controlled to consist of 70% grass and 30% energy crops (e.g., maize): co-digestion of readily degraded grass and more cellulose-rich energy crops improves the speed and efficiency of the process. As the digestate consists of virgin crops and not waste, the product is not subject to PAS 110 requirements and is saleable without further refinement.

While digestate has been used extensively in broadacre agriculture as a means of nutrient recycling and waste management, very little is understood about its potential in different markets, especially liquor application in home horticulture (Weimers et al., 2022). To be viable in competitive domestic and industrial marketplaces, digestate must provide similar or superior benefits for plant growth and health, at a similar or cheaper economic cost to synthetic fertilisers. Therefore, this thesis seeks to evaluate the value of the liquid fraction of digestate as a fertiliser for use in domestic horticulture, through a series of commercially-realistic experiments.

As such, the following hypotheses are presented:

I Plant-based liquid digestate offers comparable performance at the manufacturer's recommended concentration for tomatoes compared to synthetic fertilisers.

II When matched for nitrogen, plant-based liquid digestate offers comparable performance to synthetic fertiliser.

III Plant-based liquid digestate is suitable as a sole nutrient source for tomatoes under protected horticulture.

Chapter 2

Literature Review

While digestate has been widely used in agriculture for decades, this has traditionally been a function of availability, and as such use has typically been a function of vicinity to biogas reactors (Feiz et al., 2022). However, a growing sustainability market has created commercial opportunities for biofertilisers, and subsequently digestate application has broadened to different sectors, including domestic horticulture and hydroponics (Network, 2019; Rossdeutsch et al., 2022). To be successful in these markets, digestate must be refined to produce a more reliable, homogenous product with predictable outcomes for plant growth. One method of achieving this, and which also aids storability, is to separate whole digestate into solid and liquid fractions (Möller and Müller, 2012). As the liquid fraction comprises 75-80% of digestate fresh matter (Möller and Müller, 2012), it could represent a major additional income source to plant operators if valuable as a fertiliser. To evaluate liquid digestate in this context, a comprehensive evaluation of typical compositions of liquid digestates, and the implications that this may have on plant growth and yields - particularly of important horticultural crops, such as tomato - is essential.

2.1 Characterising Liquid Digestate and its Properties

The composition of the liquid fraction of digestate displays high variability depending on feedstock, reactor set-up and method of separation (Akhiar et al., 2017). As Table 2.1 shows, both solid and liquid digestate have a high pH, while liquid digestate is typically high in nitrogen (N) and potassium (K), and the solid fraction higher in phosphorus (P) (Hjorth et al., 2010). The concentration and form of nutrients in

digestate is dependent on both the feedstock and digestion process, and there can be high variability (European Commission, 1996; Table 2.1). The dominant form of N in both fractions of digestate is ammonium (NH_4^+), due to anaerobic conditions in the reactor, which restrict nitrate (NO_3^-) formation. Based on Table 2.1, liquid digestate appears to be higher in calcium (Ca) but lower in heavy metals than the solid fraction. In contrast, other studies demonstrated that copper (Cu) and zinc (Zn) partitioned onto the fine particles found in the liquid fraction of digestates derived from animal slurry, food and/or industrial waste (Popovic et al., 2012; Kupper et al., 2014). Therefore, while a general overview can be obtained from examining general characteristics of liquid digestate, the exact composition is source-dependent, and depends highly on feedstock.

According to Möller and Müller (2012), the ratio of NH_4^+ to total-N depends on the feedstock: a highly degradable feedstocks (e.g., cereal grains and grass silage) will produce a higher NH_4^+-N/total-N ratio, and a lower Carbon (C):N ratio than more fibrous feedstocks (e.g. silage maize). Degradability is related to the C:N ratio Möller and Müller (2012), and thus more fibrous, cellulose-rich feedstocks will remain in organic form for longer. The digestate produced by 1Up Natural Ltd derives from grass, maize and whole crop silage, with relative ratios depending on seasonality (W Tuer 2022, personal communication, 23 September). Phosphate in digestate is mostly found as struvite ($MgNH_4PO_4 \cdot 6H_2O$) and hydroxyapatite (HAp, $Ca_5(PO_4)_3OH$), both largely insoluble minerals, with Güngör et al. (2007) finding they comprised 78.2% and 21.8% of total PO_4^{3-}, respectively, in a dairy manure-based digestate. These compounds are formed when metal salts (iron (Fe^-), Ca^-, Aluminium (Al^-)), added to digestates for desulphurisation, cause P to precipitate out of the liquid fraction, entering the solid fraction (Möller and Müller, 2012; Möller et al., 2018). As a result, solid digestate is typically high in P, with a large portion - at least 88% (Regelink et al., 2021)- in inorganic form, although it is largely insoluble. However, the high organic matter content of digestate may counteract this unavailability, through competitive adsorption to soil and metal ion surfaces by organic molecules, releasing P into the soil solution (Yang et al., 2019). The magnitude and nature of this effect is site-specific and affected by numerous factors, including soil pH, with contrasting results also being reported (Hiradate and Uchida, 2004). Furthermore, the organic P contained within both the liquid and solid fraction of digestate - approximately 10% of total P (Tuszynska et al., 2021) - may also enhance P reserves in soil, and become available through solubilisation by phosphobacteria or lowering of soil pH through organic acid root exudation (Deng, 2021). Therefore, while P contained in digestate may not be immediately plant-

available, it may act as a longer-term P source, particularly in acidic soils (Regelink et al., 2021), providing nutrition to plants and reducing vulnerability to nutrient run-off into waterbodies.

As Table 2.1 shows, K^+ is unaffected by anaerobic digestion, and thus mostly remains dissolved in the liquid fraction (Möller and Müller, 2012). Micronutrient concentrations are variable within and across digestate (Table 2.1), particularly for Fe - likely a function of addition volumes in the biogas reactor.

The conditions and duration of liquid digestate storage prior to application can significantly affect composition (Plana and Noche, 2016). Due to its high volume and seasonal restrictions on fertiliser application to fields, liquid digestate is typically stored in large ponds, known as lagoons: these may be covered or uncovered (Plana and Noche, 2016). Installing air-tight covers to these lagoons can reduce greenhouse gas emissions by 65% (Luostarinen et al., 2011) compared to open storage, and retain 55-100% of the NH_4^+ in the liquid digestate (Rehl and Mueller, 2011). Additionally, liquid digestate stored in uncovered lagoons is subject to photodegradation by sunlight, dilution by precipitation and nutrient leakage through adverse weather conditions (Al Seadi and Lukehurst, 2012).

Liquid digestate is biologically-active, and microbial communities are largely a function of feedstock. While some have identified pathogenic bacteria, particularly in manure-based digestates, with Furukawa and Hasegawa (2006) measuring 10 times more *E. coli* and faecal *Streptococci* in manure-based digestate than one derived from food-waste. However, there appears to be high variability even within feedstock groups (e.g *E. coli* in Table 2.1 and 2.2) with standard deviations often as high as the mean. Thus the size and composition of microbial communities are likely a function of individual biogas reactors. Bacteria/fungi ratios are variable in liquid digestate, but bacteria dominates when applied to soils - Walsh et al. (2012) found a 1.8-fold greater increase in soil bacteria when liquid digestate was applied relative to undigested solids.

2.2 Evaluating Suitability of Liquid Digestate as a Biofertiliser

The high mineral-N: total-N of liquid digestate (Table 2.1) represents a promising source of plant-available N (NH_4^+), which could reduce dependency on artificial sources. However, while NH_4^+ assimilation is energetically favourable for plant uptake compared to the other major form of mineral N, nitrate (NO_3^-; Salsac et al., 1987), the former can

Table 2.1: Nutrient analysis of liquid digestates and solid fraction depending on feedstock, compiled from different sources by Reuland et al. (2021*a*). Values are a mean average with ± Standard Deviation.

Parameter	**Unit**	**Liquid digestate from manure and energy crops (n=95)**	**Liquid digestate from biowaste (n=41)**	**Liquid digestate from biowaste, manure and/or energy crops (n=86)**	**Solid digestate from mixed source (n=6)**
pH		7.9 ± 0.3	8.0 ± 0.2	8.0 ± 0.4	7.8 ± 0.5
Dry Matter (DM)	Fresh Weight g kg^{-1}	63.7±28.1	51.8 ±30.5	43.1±19.7	129.3 ±32.0
Organic Matter (OM)	Dry Weight g kg^{-1}	702.1 ±80.1	614.8 ±82.5	626.5 ±97.1	604.4 ±133.8
Total Organic Carbon (TOC)		390.0 ±44.5	341.5 ±45.8	340.9 ±61.4	335.8 ±74.3
Total Nitrogen (TN)		93.2 ±33.8	111.9 ± 59.0	128.5 ±77.2	43.6 ±23.3
Ammonium (NH_4^+-N)		51.5 ±25.0	75.3 ±45.5	75.8 ±51.5	8.6 ±5.8
Potassium Oxide (K_2O)		80.7 ±26.7	62.0 ±27.6	81.3 ±40.4	30.9 ±11.0
Phosphorus Pentoxide (P_2O_5)		28.6 ±11.1	11.8 ±5.4	34.5 ±23.3	34.8 ±24.8
Calcium Oxide (CaO)		44.4 ±17.1	74.0 ±11.5	37.7 ±22.5	10.3 ±1.5
Magnesium (Mg)	Dry Weight mg kg^{-1}	9.6 ±3.1	8.7 ±2.9	7.6 ±5.5	9.0 ±4.0
Chromium (Cr)		8.5 ±7.1	31.9 ±8.4	8.2 ±3.3	41.0 ±3.1
Cadmium (Cd)		0.4 ±0.2	0.8 ±0.2	0.3 ±0.2	0.8*
Lead (Pb)		3.7 ±2.4	16.0 ±1.3	4.2 ±2.7	
Nickel (Ni)		8.6 ±3.6	19.1 ±4.5	9.4 ±5.4	31.9 ±5.9*
Zinc (Zn)		395.4 ±388.2	304.7 ±41.5	334.9 ±207.8	1144.0 ±1.4*
Copper (Cu)		97.3 ±110.9	90.2 ±11.7	83.3 ±78.6	476.0 ±120.2*
Mercury (Hg)		0.1 ±0.0	0.2 ±0.1	0.0 ±0.0	
Iron (Fe)		3193.3 ±988.6		3795.3 ±247.1	
Manganese (Mn)		264.3 ±73.7		314.6 ±308.6	
TOC:TN	%	4.7 ±1.5	3.9 ±2.2	3.8 ±2.5	5.5 ±0.8
NH_4^+:TN		54.4 ±12.8	68.8 ±19.9	61.5 ±16.8	24.0 ±15.2
E. coli		47.3±62.5		161.2±327.0	

Table 2.2: Average pathogenic microbial communities contained in different digestates in Log colony forming unit (CFU) g^{-1} fresh matter, from an experiment by Furukawa and Hasegawa (2006). Values are a mean average, and ± denotes standard deviations.

Digestate	**Coliform Group**	***Escherichia coli***	***Faecal streptococci***	***Vibrio parahaemolyticus***
Kitchen waste effluent	3.3 ±3.4	1.9 ±2.1	1.9 ±2.2	3.1 ±3.1
Cattle manure effluent	2.7 ±2.7	2.7 ±2.7	3.0 ±3.0	2.8 ±1.6

cause toxicity in low doses, particularly in tomatoes (Britto and Kronzucker, 2002). To prevent interference with energetically-similar K^+ (which affects pH regulation of the cell membrane), NH_4^+ must be immediately converted to amino acids, primarily glutamine and glutamate (Schulze et al., 2019*b*). These amino acids donate the NH_2 groups used to make protein; however, this process releases protons, which are excreted through the root (Schulze et al., 2019*b*). The resulting acidification of the rhizosphere can lead to a release of heavy metals and basic cations contained in the soil, which are toxic to roots (Schulze et al., 2019*a*). Under NH_4^+ toxicity plants tend to grow slower, while developing interveinal chlorosis and eventually necrosis of leaves (Britto and Kronzucker, 2002). NH_4^+ toxicity has been observed in numerous plant growth trials using liquid digestate (Table 2.4). A common method of ameliorating this issue is to increase the relative proportion of NO_3^-, through supplementation (e.g. Liedl et al.,

2004), and/or nitrification: either through sparging and/or a biofilm (e.g.Liedl et al., 2006; Weimers et al., 2022).

Furthermore, NH_4^+ toxicity can induce deficiency and toxicity in other nutrients. Firstly, the protein complexes that must be produced to assimilate NH_4^+ safely require sucrose for the C-skeleton: toxicity both consumes and restricts this supply by limiting the rate of photosynthesis by decreasing CO_2 fixation, due to changes in the chloroplast structure, and uncoupling of plasmatic energy gradients (Britto and Kronzucker, 2002). While not directly reported as C deficiency, the stunted growth observed under liquid digestate trials encountering NH_4^+ toxicity (e.g. Liedl et al., 2004) were likely due to, among other things, C deficiency. Furthermore, NH_4^+ uptake can competitively inhibit magnesium (Mg) uptake (Schulze et al., 2019*b*). Combined with already low amounts of Mg in liquid digestate, this can lead to deficiency in plants treated with liquid digestate, including tomatoes (Liedl et al., 2004).

The low concentrations of P in liquid digestate resulted in deficiency in several studies (Table 2.4), and supplementation was often required (e.g., Stoknes et al., 2016; Weimers et al., 2022). Weimers et al. (2022) also reported boron (B) and sulphur (S) deficiency in pak choi (*Brassica rapa, ssp. chinensis*) as unamended digestate had insufficient concentrations - this was successfully ameliorated through mineral supplementation.

Studies on the effectiveness of the liquid fraction of digestate as a fertiliser have predominantly focussed on either hydroponic or field-based applications of manure or waste-based liquid digestates (Table 2.4). While these have yielded results applicable to industrial systems, they may not relate well to traditional domestic horticulture practices. Furthermore, there is high heterogeneity in methodology, with different criteria for comparing treatments (e.g. concentration vs total amount, matching for one nutrient vs EC etc.), and the use of different plant species, which likely influence outcomes. However, studies appear to concur that – albeit often through amendment - liquid digestate can be a suitable nutrient source for plant growth, and so there is merit in evaluating the applications of a plant-based digestate within domestic horticulture.

Studies evaluating the microbial effects of liquid digestion as a separate fraction have only begun recently (Möller and Müller, 2012). As a result, the mechanisms and outcomes of liquid digestate application to soils, and implications on plant growth, remain mixed and poorly understood. While several pathogens were identified in the digestates applied by Furukawa and Hasegawa (2006), these were not detected in the soil at harvest. Panuccio et al. (2021) observed accelerated mineralisation of organic matter and enhanced microbial activity following liquid digestate application, although this

study did not study impact on plant growth. However, Barduca et al. (2021) observed a decrease in soil ΣCO_2 efflux following liquid digestate application, which they attributed to high NH_4^+ concentration and negative competitive effects: digestate is bacteria-rich and, when applied to soil, may overwhelm autochthonous fungal communities. Following soil analysis, they found a fungal/bacterial ratio of 0.25 (Barduca et al., 2021). Previous studies of the effect of liquid digestate application on soil microbes and plants have largely been conducted in isolation from one another. Whilst valuable for a detailed mechanistic understanding, separate evaluation of liquid digestate's effects on microbes and plants may not be practical: for instance, if liquid digestate must be applied in large quantities to stimulate microbial growth, high nutrient levels (in particular NH_4^+) may surpass the threshold for toxicity.

2.3 Suitability of Liquid Digestate for Growing Tomatoes

Tomatoes (*Solanum lycopersicum L.*) are the most popular outdoor crop both commercially and domestically, and are grown in 86% of residential food gardens in the US (Schwarz et al., 2014; NGA, 2014). Since they readily grow in a variety of systems, they are one of the most studied plants (Schwarz et al., 2014). Under hydroponic systems, one of the most widely used tomato-specific nutrient solution is that of De Kreij et al. (1997), outlined in Table 2.3 (Schwarz et al., 2014). At different stages of development, tomato plants have different nutrient requirements - for instance during the vegetative stage, calcium (Ca), which is makes up 2-5% of leaf dry mass, is required in higher quantities than when producing fruit, which contain 0.15% Ca by dry mass (Schwarz et al., 2014). Therefore, Table 2.3 serves as a useful indicator of optimal proportions of nutrients, from which the utility of a fertiliser can be estimated. By normalising other nutrient concentrations against NH_4^+, the averages for liquid digestate derived from manure and/or energy crops in Table 2.1 were compared to Table 2.3 (Table 6.2 in Appendix B). Based on these ratios, deficiency may occur when a typical liquid digestate is applied at the recommended NH_4^+ concentration, particularly for K, Mg, Ca and Fe in the root zone. Indeed, Mg deficiency has been identified in previous hydroponic tomato growth trials using liquid digestate (Liedl et al., 2004). Phosphorus deficiency has also been identified in these trials (Stoknes et al., 2016), and can induce stomatal closure and root elongation in tomato (Clarkson and Scattergood, 1982).

Tomatoes have high nutrient demands, making them a suitable crop for plant nu-

trition studies. Some experiments applying liquid digestate have used tomatoes (Table 2.4), with variable results.

As reflected by its application quantity in Table 2.3, N, as in most plants, is probably the most important nutrient for growth (Benton-Jones Jr, 2007). The form of N is important: as is widely established in literature, and reflected in the NO_3^-:NH_4^+ ratio (Table 2.3), tomatoes prefer N as NO_3^-, and are sensitive to NH_4^+ (Benton-Jones Jr, 2007). Ammonium toxicity has been reported as an issue previously when using liquid digestate hydroponically to grow tomatoes (Liedl et al., 2004; Stoknes et al., 2016). While conventional effects of NH_4^+ toxicity (e.g. stunted growth, chlorosis and abscission) are also observed in tomato, mild NH_4^+ stress can increase chlorophyll concentration of tomato (Fernández-Crespo et al., 2015).

Thus far, liquid digestate applications to tomatoes as part of a hydroponic system have been limited to a small pool of authors, who used manure and waste-based digestates in pH-reduced hydroponic systems (Table 2.4). As digestate composition is highly dependent on feedstock, the application of an entirely-plant based liquid digestate must first be assessed as received, and then any potential alterations made.

Table 2.3: Recommended optimal nutrient solution concentrations for hydroponic tomato cultivation (De Kreij et al., 1997)

Nutrient/element	**Unit**	**Starter solution**	**Root environment (range)**	**Refilling solution**
EC	dS m^{-1}	3.7	2.5-5.5	1.5
pH		5.6	5.6	5.6
NO_3^-	mmol l^{-1}	23	15-31	10.75
NH_4^+		0.1	0.1-0.5	1
K		8	5.3-10.6	6.5
Ca		10	6.6-13.3	2.75
Mg		4.5	3-6	1
SO_4		6.8	4.5-9.0	1.5
P		1	0.7-1.3	1.25
HCO_3		<1	0-1.0	
Na		<12	0.1-12.0	0
Cl		<15	0.1-15.0	0
Fe	μmol l^{-1}	25	13-38	15
Mn		5	2-7.5	10
Zn		7	3.5-10.5	4
B		50	25-75	20
Cu		0.75	0.4—1.1	0.75
Mo		0.5	0.3-0.8	0.5

Table 2.4: Summaries of methodologies and outcomes of previous studies evaluating liquid digestate as a fertiliser.

Study	**Liquid Digestate Feedstock**	**Experiment Design**			**Results**
		System	**Treatments**	**Crop**	
Stoknes et al. (2016)	Source-separated food waste	Closed loop, container experiment using recirculated nutrient solutions in peat-based substrate	Nitrified liquid digestate at different ECs and pHs, mineral fertiliser control	Herbs (Lemon balm, coriander, parsley, basil, dill and thyme)	At low concentrations liquid digestate offered comparable performance to mineral fertiliser, while reducing pH improved N effect. Optimal concentration varied with the species.
		Closed loop, crate-based substitution experiment using recirculated nutrient solutions	Nitrified liquid digestate and mineral fertiliser control applied to peat-based substrate and spent mushroom compost	Cape gooseberry (*Physalis peruviana*)	Mineral control outperformed liquid digestate on account of NH_4^+ toxicity and excessively high nutrient content in the latter.
		Crate-based soilless system	Diluted liquid digestate, diluted solid digestate applied directly to peat-based or completely hydroponic media	Tomato and lettuce	Applying dilute liquid digestate directly caused crop failure. Reasons not addressed in paper.
		Closed loop, crate-based soilless system using recirculated nutrient solutions	Nitrified liquid digestate with mineral fertiliser controls grown in peat-based or waste-based substrates matched at an EC of 2.3	Tomato	
Weimers et al. (2022)	37% organic household, 29% pig and cattle manure, 21% slaughter waste, 5% grease fat, 8% food waste, <0.3% iron chloride and iron sludge	Soilless pot-based experiment, matched for mineral N (650 mg plant^{-1}) and watered according to need	Nitrified liquid digestate, supplemented nitrified digestate, mineral fertiliser	Pak choi (*Brassica rapa, ssp. chinensis*)	Unamended liquid digestate showed signs of sulphur (S), boron (B) and P deficiency; amended digestate performed comparably to mineral fertiliser.

Liedl et al. (2006)	Digested poultry litter liquid (DPL)	Two-stage raised bed trial	Between 1999-2003 digested poultry solids (DPS) at two concentrations against a commercial natural fertiliser and pelletised municipal sludge, matched at the lower concentration of DPS. In 2004, switched DPS to DPL at two concentrations, natural fertiliser to a chemical, and municipal sludge to organic fertiliser	**1999-2003**: Potato (*Solanum tuberosum*), sweet corn (*Zea mays*) and tomato (*Solanum lycopersicum*) **2004**: Potato, broccoli (*Brassica oleraceae*) and tomato.	DPL, applied at 2× the N recommended in the synthetic fertiliser, out-performed other treatments for tomato and potato, while broccoli performed the same as chemical fertiliser when N was matched across treatments.
		Field trial	DPL, commercial granular fertilisers, and urea at different rates based on N application	Tall fescue, orchard grass (*Dactylis glomerata*), and red clover (*Trifolium pratense*)	DPL outperformed commercial fertilisers, and performed the same as urea.
		Hydroponic trial	Three concentrations of DPL against a commercial nutrient solution	Tomato (*Solanum lycopersicum*)	Increasing concentration of DPL reduced lettuce quality, but at lower concentrations was comparable. DPL stunted growth in tomato due to NH_4^+ and required sparging and amendment with Mg and a chelator for comparable performance with the commercial solution.

Stoknes et al. (2018)	80% food waste, 20% animal manure	Closed loop, container-based soilless experiment, recirculated nutrient solutions at a target EC	Vermicomposted solid digestate and aerated liquid digestate versus a peat and mineral control	Cherry tomatoes (*Solanum lycopersicum*, *Favorita F1*)	Comparable yields between digestate treatment and conventional, but digestate treatment was considerably higher monitoring to maintain pH, EC, DO chloride, nitrate and phosphate levels in the solution.
Liedl et al. (2004)	Digested poultry litter liquid	Automated soilless fertigation	Unamended liquid digestate, commercial fertiliser; then switched liquid digestate to either commercial fertiliser or air-sparged, calcium nitrate-amended liquid digestate, matched for N at 150 ppm	Tomato (*Lycopersicon esculentum Mill. 'Trust'*)	Unamended liquid digestate caused NH_4^+ toxicity and Mg deficiency. Once sparged to remove 75% of NH_4^+ and supplemented with $Ca(NO_3)_2$ and $MgSO_4$ offered comparable performance to synthetic alternatives. Authors reported using large amounts of H_3PO_4 to lower pH, resulting in high levels of P in solution.
Ronga et al. (2019)	43% maize silage, 22% triticale silage, 27% cow slurry, 8% grape stalks	Dripper-fertigated soilless recirculating system	Liquid digestate or a standard nutrient solution were applied to peat moss, clay-loam soil, agriperlite, solid digestate or pelleted digestate. Treatments matched within a N, EC and pH range.	Baby leaf lettuce (*Lactuca sativa L. cv. 'Chiari'*)	Liquid digestate worked well in combination with agriperlite, while solid digestate and pelleted digestate performed well as substrates with standard nutrient solution.
Liu et al. (2011)	Biogas slurry, origin not stated	Hydroponic pot-based experiment	Biogas slurry diluted 5.22 times supplemented with different macro and micro-nutrients, including 0.1 mmol EDTA-Fe and 0.33 mmol KPO_4 with the former applied either within the solution or as a foliar spray	Lettuce (*Lactuca sativa*)	Supplementation with Fe and KPO_4 together significantly improved yields, but when applied individually there was no significant effect. Direct application to the liquid was required, foliar spray was not effective.

Walsh et al. (2018)	Lactating cow slurry	Field study on a clay-loam cambisol grassland previously subject to sheep grazing	No fertiliser, undigested cattle slurry, liquid digestate from cow slurry, solid digestate from cow slurry, inorganic N fertiliser, inorganic NPK fertiliser. Matched for total N with an application of 100 kg N/ha in mid-April for first harvest, then 50 kg N/ha post-harvest	Perennial ryegrass (*Lolium perenne L.*) and white clover (*Trifolium repens, L.*)	Liquid digestate gave comparable yields to solid digestate application, undigested slurry and the inorganic NPK, and outperformed the inorganic N fertiliser. Plant N content and digestibility did not differ across any treatment including control.
Furukawa and Hasegawa (2006)	(a) Source-separated kitchen waste (b) cattle manure	Field experiment on a Typic Hapludands	Chemical NPK fertiliser, cattle manure liquid digestate and kitchen waste liquid digestate applied at 22 g N^{-2}. All treatments were supplemented with 10 g m^{-2} calcium superphosphate due to low P in the soil and digestate, while KCl was supplied to match K rate across treatments	Spinach (*Spinacia oleracea cv. 'Sanpia')* and komatasuna (*Brassica rapa var. perviridis, cv. 'Kokattana'*)	Liquid digestate from both sources was comparable with the synthetic fertiliser. Volatilisation of NH_3 did not appear to be an issue. A comment was made on the poor odour of the digestate treatments.
Sigurnjak et al. (2017)	30% energy maize, 30% pig manure, 40% food waste	Three-year field experiment investigating partial substitution of mineral fertiliser with liquid digestate	Liquid digestate with either animal manure or digestate was compared against synthetic fertiliser applied with raw animal manure.	Silage maize (*Zea mays L*)	Liquid digestate provided a suitable N-K source for maize cultivation, without loss of yield versus traditional methods.

Panuccio et al. (2021)	(a) poultry, cow and sheep manure, (b) olive waste and citrus pulp, maize silage and animal manure	Pot-based greenhouse experiment using 3.5 kg alkaline sandy-loam soil	Two liquid digestates (from feedstock a and b) applied at 10, 20 30% v/w compared to two solid digestates (a and b) with a synthetic N:P:K fertiliser applied at 1.2 g/pot was the control	Tomato (*Solanum lycopersicum L.*)	Liquid digestate at all concentrations significantly increased leaf number and area versus the untreated control, particularly the more plant-based digestate (b). A significant dose-response was observed, which was more influential than feedstock. High salinity (EC) attributed to decreased fruit production versus synthetic control, but did not affect quality - increasing phenols and flavonoids versus control.

2.4 Literature Gap and Focus of Study

Liquid digestate is a highly heterogenous product, and its composition is heavily influenced by feedstock, reactor set-up and separation method (Möller and Müller, 2012). However, its abundance and generally high N & K content suggest that it could be used effectively as a partial or complete replacement to synthetic fertiliser (Sigurnjak et al., 2017). While widely applied in broadacre agriculture, usually as whole digestate, there are emerging commercial opportunities in the retail market, which have yet to be comprehensively evaluated. Thus far, literature has almost exclusively focussed on liquid digestate as a byproduct of waste management: utilising manure and waste-based digestate under industrial applications. Thus, very little is known empirically about performance of liquid digestate within domestic horticultural applications, particularly those derived from an entirely plant-based feedstock. Consequently, there is a need to establish an initial performance baseline against retail synthetic fertiliser and the potential mechanisms explaining any disparity in plant growth or physiology.

The overarching aim of this thesis is to answer whether liquid digestate is effective as a replacement to synthetic fertilisers for tomatoes under realistic domestic horticulture conditions. Within this scope, the thesis aims to evaluate whether liquid digestate is suitable as a sole nutrient source for tomatoes, particularly within soilless systems. This will provide mechanistic and commercially-relevant information on the current status of liquid digestate-based fertilisers, and help inform their future role in meeting nutritional requirements in the horticultural industry.

Chapter 3

Comparing Commercial Liquid Digestate and Mineral Fertilisers

3.1 Introduction and Background

Assuming all other plant needs are met (i.e. water, light etc.), nutrient status affects plant physiology and growth significantly (Schulze et al., 2019*b*). Nutrient status can be inferred in-situ through stomatal conductance (g_s), which is affected by hydraulic conductivity (Bartletta et al., 2016), and chlorophyll concentration, a measure of photosynthetic rate (Ma et al., 1995). Under nutrient deficiency, stomata generally close due to reduced hydraulic conductivity, restricting photosynthesis (Pirasteh-Anosheh et al., 2016). Similarly, lack of nutrients - especially nitrogen (N) - cause degradation of chlorophyll due to remobilisation to new growth or seeds (Schulze et al., 2019*b*). Both stomatal closure and reduced chlorophyll concentration decrease photosynthetic rate, restricting plant and leaf growth (Pirasteh-Anosheh et al., 2016). A similar response is generally observed in response to excess nutrient supply, particularly N (Schulze et al., 2019*b*). It is important to note that nuances exist, for instance mild NH_4^+ toxicity may induce an increase in chlorophyll concentration (Fernández-Crespo et al., 2015). Thus, a holistic assessment which considers both plant physiological and growth measurements is important is essential to evaluating whether nutritional needs have been met by a fertiliser.

However, the overapplication of fertiliser is a significant problem both commercially (Good and Beatty, 2011) and domestically (Small et al., 2019). As such, it is important that fertiliser be applied at a level which is sufficient to sustain plant growth while not allowing excessive amounts to enter external systems. Consequently, an important

caveat to assessment of fertiliser performance is the utility of its application. For instance, under this remit, a fertiliser applied at half the recommended application should not yield comparable results to the full, as this would indicate unnecessary application surplus to plant requirements.

To be commercially viable as a fertiliser, liquid digestate must offer comparable or superior benefits to plant growth at a similar or reduced cost to synthetic alternatives. While previous studies have evaluated this application and found mixed results (as reviewed in Chapter 2), these have exclusively used manure or waste-based digestates, usually in complex hydroponic systems (Liedl et al., 2004; Stoknes et al., 2018) not commonly practiced in domestic horticulture. Thus, there is a need to scientifically evaluate whether liquid digestate can replace mineral fertiliser under realistic domestic (home growing) conditions. Therefore, I designed a factorial growth trial to closely mimic a typical domestic horticulture set-up, using retail substrates and mineral fertilisers.

For liquid digestate to be critically assessed as a liquid fertiliser, it must first be agronomically and commercially benchmarked against both generally-formulated and crop specific fertilisers – in this case for tomato. As 3.1 shows, liquid digestate was comparable in terms of consumer sentiment and value judgement to the market leaders of the general fertiliser and tomato fertiliser markets, suggesting similar marginal commercial performance.

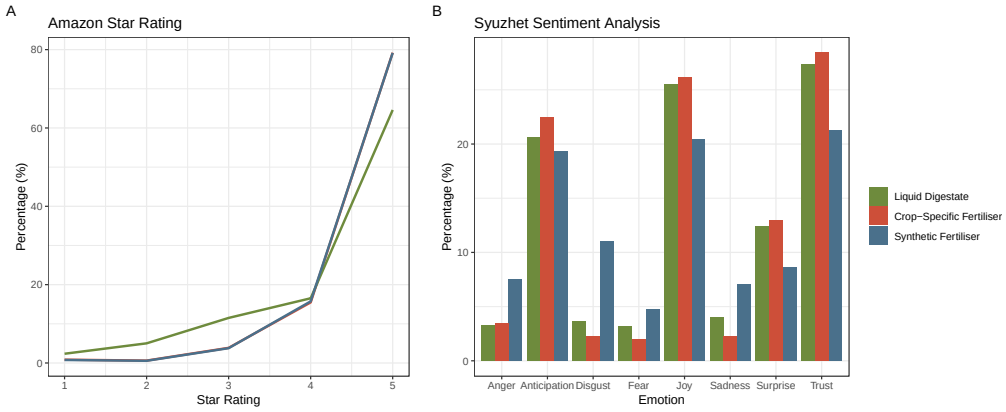

Figure 3.1: (A) Line graph of distribution of Amazon star ratings of the fertiliser products selected for Experiment 1, correct as of 29^{th} September 2022. (B) Clustered bar graph of the percentage of words contained in the 100 most recent reviews for each fertiliser, with words assigned an emotion as per the NRC Emotion Lexicon, as per Mohammad and Turney (2011)

However, the term 'performance' is subjective, even when restricted to effect on plants, depending on the criteria of the consumer. This is particularly true for fruit crops, such as tomato, where growers may deem *"good"* performance as producing

the highest volume, quantity, or quality of fruit; or the most aesthetically pleasing plant/fruit. Based on the short duration of this experiment (sufficient to determine significant differences in plant growth and/or physiology), fruiting was unlikely to occur before harvest, so performance was limited to the following definition: *"the level of physiological activity and biomass production versus other treatments"*.

Thus, to establish a benchmark for liquid digestate as a commercial fertiliser, the following hypothesis was devised:

I Plant-based liquid digestate offers comparable performance at the manufacturer's recommended concentration for tomato compared to synthetic fertilisers.

To further aid evaluation of performance, the following subhypotheses were presented.

(i) Liquid digestate will significantly increase plant growth and physiological response relative to the control and compete with the crop-specific fertiliser and general synthetic fertiliser.

(ii) The manufacturer's recommended concentration significantly increases plant growth and physiological response versus half the recommendation for all treatments.

3.2 Methods and Materials

3.2.1 Experimental Design

The experiment was designed as a factorial experiment, using three different fertiliser treatments at two concentrations (at the manufacturer's recommended concentration and half the manufacturer's recommended concentration), with a negative control of no fertiliser. Ten replicates were grown for each treatment, giving 70 plants in total. The three fertilisers trialled were a plant-based liquid digestate (Will&Al's Natural Plant Food Company, Cumbria, United Kingdom), a crop specific fertiliser (Levington® Tomorite, Cardiff, Wales) and a general synthetic fertiliser (Levington® Miracle-Gro®, Cardiff, Wales). Table 3.1 shows the manufacturer's recommended dilution of each product. Liquid digestate was sampled from the same packaging and fulfilment process as commercially practiced, and the general synthetic fertiliser and crop-specific fertiliser were purchased from a local gardening store to best mimic commercially-realistic storage practices. Table 3.3 shows the results of previous proprietary nutrient analyses conducted on the liquid digestate by the project partner using NRM Laboratories (Coopers Bridge, Bracknell, United Kingdom).

Table 3.1: Retail fertiliser recommended dilutions, and the N-P-K values reported on their packaging

Fertiliser Source	**Manufacturer's Recommended Dilution**	**Stated N-P-K**
Liquid Digestate (LD)	35 mL to 4.5 L	3.9 - 1.23 - 7.48
Crop-specific Fertiliser (CSF)	20 mL to 4.5 L	4 - 3 - 8
General Synthetic Fertiliser (SF)	15 mL to 4.5 L	24 - 8 - 16

The experiment was maintained under glasshouse conditions [22 °C/16 °C day/ night, photoperiod of 14 hours at photosynthetically active radiation (PAR) ∼350 μmol $m^{-2}s^{-1}$]. Environmental conditions for the duration of the experiment can be seen in Figure 3.2 in Appendix A. Tomato seeds (*Solanum lycopersicum*, cv. Ailsa Craig) were sown in cell seed trays in a low-nutrient, commercially available John Innes Seed and Cutting Compost (Westland Horticulture, Tyrone, Northern Ireland) on 2nd November 2021. Trays were irrigated when the substrate surface began to dry. When the majority of seedlings had a second unfolded true leaf, the most representative specimens were transplanted into 2 l square black plastic pots (110 (L) x 110 (W) x 165 (H) mm) filled with John Innes Seed and Cutting Compost on 16th November 2021. An initial 200 mL tap water were given to each seedling to ensure establishment.

Gravimetric water holding capacity (WHC) was determined by saturating three pots of the substrate, covering overnight with a saucer, weighing again and oven drying at 105 °C until a stable mass was reached, as in Robertson and VanderWulp (2019). The average difference in mass between the saturated and dry weights was used to calculate the available water within a pot and a lower threshold of 50% of WHC (1575 g) was established. Randomly selected triplicates of each treatment were weighed daily to determine if WHC had dropped below 50%, in which case all plants were weighed and irrigated using tap water to the target weight, which was originally set at 1700 g (∼ 65% WHC) and increased 24 days after transplanting to 1800 g (75% WHC) to account for increased plant biomass. Pots were randomly rotated at every irrigation to mitigate against environmental gradients in the glasshouse and arranged into rows of even spacing to minimise shading and competition effects.

Five days after transplanting, 10 plants were randomly selected for each treatment, and 100 mL of each treatment applied. Plants were harvested once statistically significant differences appeared in in-situ measurements between treatments consistently.

3.2.2 Environmental Conditions

Environmental conditions were measured using an automated Ektron-II C sensor unit (HortiMaX S.L., Netherlands) which gave hourly averages of air temperature, relative humidity, CO_2 and light. The probe was 2 m above the floor, located centrally between the two plant benches, which were 0.8 m high. Solar radiation (W m^{-2}) was estimated in terms of photosynthetically active radiation (PAR) by first converting into photons (μmol m^{-2} s^{-1}) using 1 W m^{-2} $\approx$ 4.57 μmol m^{-2} s^{-1} (Sager and McFarlane, 1997). Then, by working on the assumption that 49% of solar radiation is PAR (Szeicz, 1974), a conversion factor of 1 W m^{-2} $\approx$ 2.24 μmol m^{-2} s^{-1} was used .

The vapour pressure deficit (VPD) was calculated using

$$VPD = 610.78 \times e^{\frac{T}{T+237.3} \times 17.2694} \times \left(1 - \frac{RH}{100}\right),$$

where T is the air temperature (°C) and RH is the relative humidity (%).

3.2.3 Non-Destructive Measurements (In-situ)

Chlorophyll concentration

Chlorophyll concentration was measured using an MC-100 Chlorophyll Concentration Meter (Apogee Instruments, Logan, Utah, United States), which measures the ratio of transmittance of red (650 nm) and near infrared (900 nm) to infer chlorophyll concentration, with the former being heavily absorbed and the latter almost entirely transmitted by chlorophyll (Parry et al., 2014). This ratio can be used to infer chlorophyll concentration to a resolution of $\pm$ 10 μmol m^{-2} (Apogee Instruments Inc., 2022). Measurements were taken at least every 3-4 days on the terminal leaflet of the oldest true leaf. Since tomato plants reallocate nutrients, including N, from the oldest to youngest leaf (Pfenning et al., 2009), treatment differences would likely be seen first in the oldest leaves.

Stomatal conductance

Stomatal conductance (g_s) was measured using an AP4 Porometer (Delta-T, Cambridge, UK). This uses a small cup containing a humidity sensor, which is clamped to the leaf. Water vapour, emitted through the stomata of the leaf, causes a rise in humidity in the cup – the instrument measures the time taken for relative humidity to increase by 2.3%. To establish the optimum time for measurements and reveal any statistically significant differences between treatments, g_s was measured at two-hourly

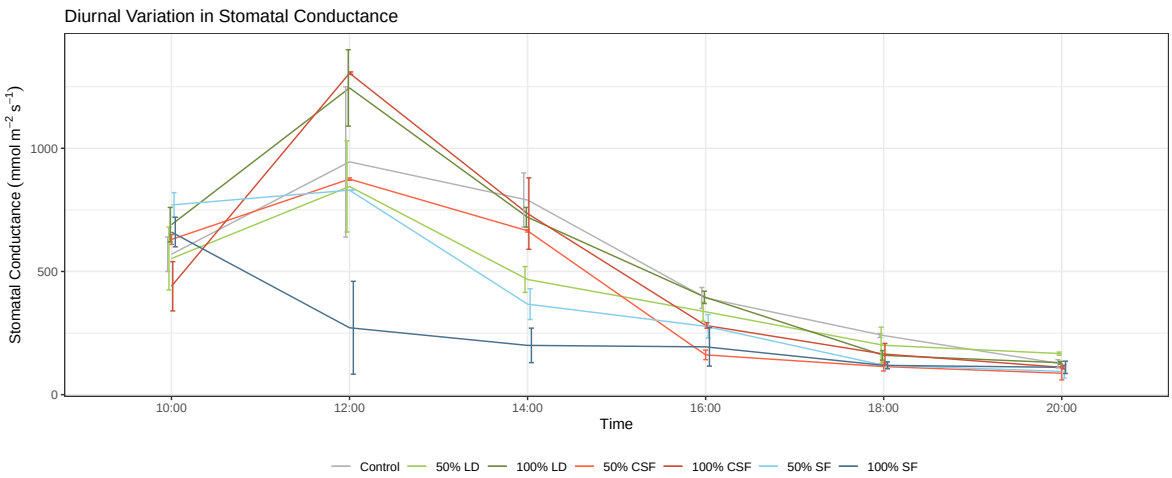

Figure 3.2: Diurnal variations in average stomatal conductance for tomato plants treated with a plant-based liquid digestate (LD), crop-specific fertiliser (CSF) and general synthetic fertiliser (SF) at half (50%) and full (100%) manufacturer's recommended concentration. n=4 for each treatment. Range bars denote standard error.

intervals for four replicates under representative conditions (Figure 3.2). Based on this, g_s was measured as close to midday as possible. To avoid shading effects, measurements were taken on the terminal leaflet of the youngest fully expanded true leaf, as in Barbieri et al. (2012).

Evapotranspiration

Plant pots were weighed individually before and after each irrigation event, according to a target weight outlined in Experimental Design (3.2.1). Water addition and losses were calculated based on weight, from which evapotranspiration was calculated.

3.2.4 Destructive Measurements (Ex-situ)

Plants were harvested once statistically significant differences in in-situ measurements were displayed consistently between treatments. Plants were first irrigated to ensure turgidity. Leaves were separated at the petiole insertion and passed through a LI-3100C Leaf Area meter (LI-COR Biosciences, Nebraska, USA) to determine total leaf area, as in dos Santos et al. (2016). Fresh weight of leaves and stem were weighed separately, before being transferred to paper bags. Samples were dried at 40 °C until a constant weight was reached and dry weight was recorded.

Fertiliser Nutrient Analysis

Water-extractable NH_4^+-N, NO_3^--N and PO_4^{2-}-P were determined using an AQ2 Discrete Analyzer (SEAL Analytical, Norderstedt, Germany) by an in-house laboratory technician, using methods based on EPA 350.1 v2, EPA 353.2 v2 and EPA 365.1 v2, respectively (USEPA, 1993). Samples were diluted to a ratio of 1:1000 with Milli-Q water, and an aliquot of 2 mL. After conversion, the nutrient concentrations were scaled to the dilutions recommended by the manufacturer to give nutrient concentration in mg L^{-1}.

3.2.5 Statistical Analysis and Hypothesis Testing

Statistical analysis was performed using R v4.2.1 on RStudio v2022.07.1.554. All the statistical tests used assume there is independence between observations within and between treatment groups, the data is normally distributed, and the within-group variance is the same across all treatment groups.

One-way analysis of Variance (ANOVA) and Tukey's Honest Significant Difference (HSD) test were used to identify whether there was a significant difference between groups. The statistically significant groups were identified on violin plots using compact letter displays; all groups with the same letter are not significantly different from each other at the 5% level.

For all fitted linear models, residuals were closely aligned with the diagonal line of their respective Q-Q plot and, when plotted as a histogram, resembled a bell-shaped curve. This indicated independent, normally distributed residuals and thus linear models were suitable for making coefficient estimates of the effect of different fertilisers and dose levels, as well as fertiliser-dose response. ANOVA, Tukey's HSD test, and linear modelling were performed using the `aov`, `TukeyHSD` and `lm` functions respectively.

Since the pattern of change over time for each individual plant was not of interest, longitudinal analysis was not formally tested and only considered using plots with error bars. Instead, measurements at specific time points were analysed separately, as the end point measurement was the focus of the study.

3.3 Results

In-situ

Chlorophyll concentration decreased over time in all treatments except the synthetic fertiliser at the manufacture's recommended concentration (100% SF; Figure 3.3a). By 16 days after transplanting, synthetic fertiliser at the manufacturer's recommended dose (100% SF) had a significantly higher chlorophyll concentration versus the control, but not other treatments (Figure 3.3). Linear modelling also found a significantly (p-value<0.05) higher chlorophyll concentration versus the control for liquid digestate at 16 days although this was not found in the pairwise comparison (Figure 3.3.By 30 days, synthetic fertiliser at both concentrations (50% and 100% SF) was significantly higher than the control and all other treatments (Figure 3.3.

Stomatal conductance (g_s) did not display consistent temporal trends across treatments (Figure 3.3b), and did not vary between groups (Figure 3.3e&f). Table 3.2 shows environmental conditions during porometry measurements.

Table 3.2: Minimum and maximum values of different environmental parameters during stomatal conductance measurements in Experiment 1.

Days after transplantation	**Vapour pressure deficit (VPD) Range (kPa) between 11:00 and 13:00**	**Solar Radiation (μmol m^{-2} s^{-1})**	**Photosynthetically Active Radiation (PAR) (μmol m^{-2} s^{-1})**
15	2.3-2.5	999 - 1370	490 - 671
25	1.8	987 - 993	484 - 486
30	2.1 - 2.2	1001 - 1018	491 - 499

Evapotranspiration increased over time for all treatments, with a plateau after 27 days (Figure 3.4). Cumulative evapotranspiration showed treatment differences – general synthetic fertiliser at full concentration (100% SF) was 27% greater than the control (p-value <0.001), and 31% greater than the liquid digestate treatment (p-value <0.001) - it was significantly higher than all treatments except its own half concentration (50% SF).

Ex-situ

Figure 3.5 shows that the general synthetic fertiliser at full concentration (100% SF) significantly (p-value <0.05) increased shoot fresh weight relative to all other treatments, and by nearly 34% relative to the control (p-value <0.001). Synthetic fertiliser at half concentration (50% SF) significantly (p-value <0.05) increased fresh weight versus both concentrations of liquid digestate (LD), while the crop-specific fertiliser at full

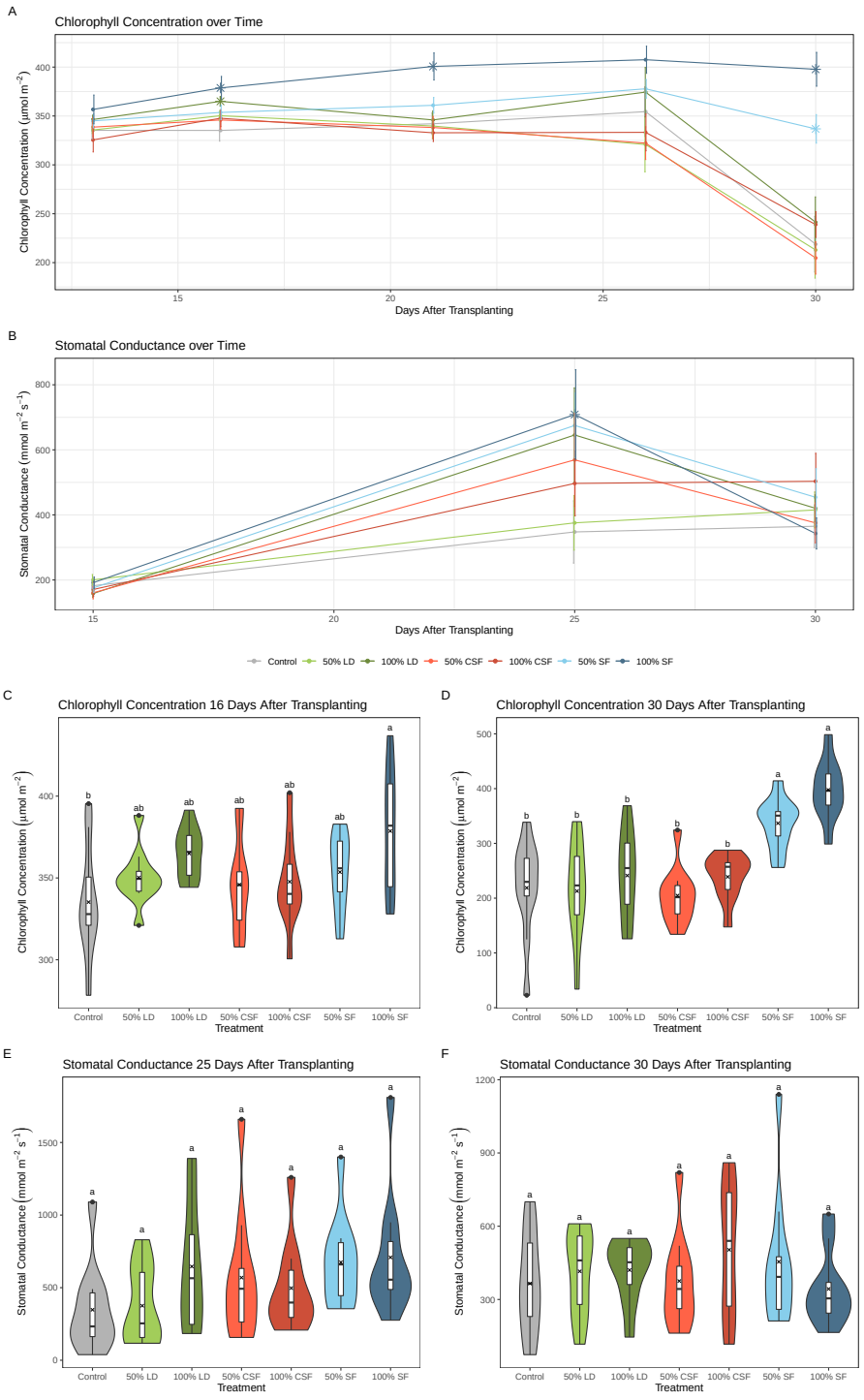

Figure 3.3: Plant physiology measurements for tomato plants treated with a plant-based liquid digestate (LD), crop-specific fertiliser (CSF) and general synthetic fertiliser (SF) at half (50%) and full (100%) manufacturer's recommended concentration. Statistical significance versus the control is denoted with * (A-B). Treatments that share a letter are not significantly different, and mean average is denoted with $\times$ (C-F). n=10 for each treatment.

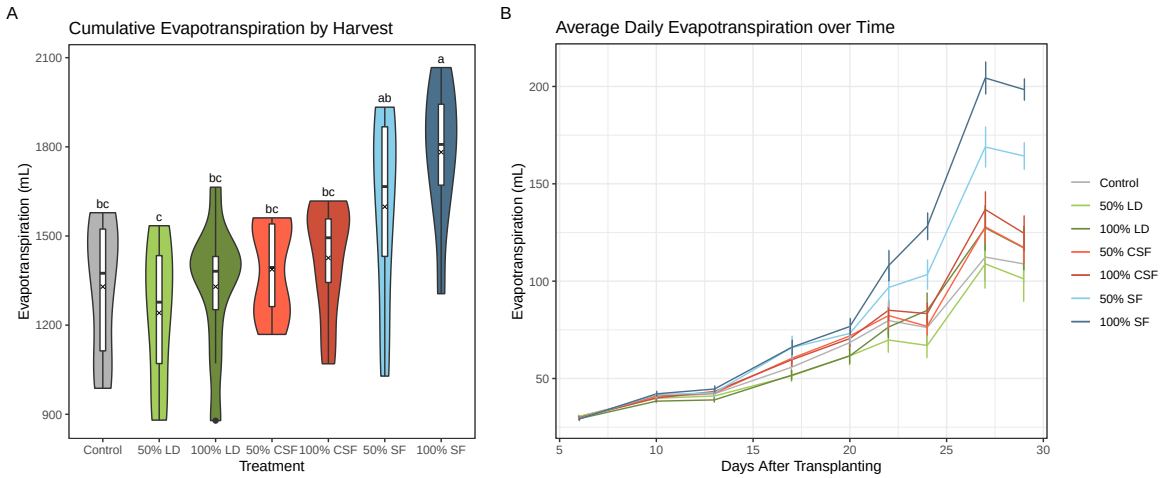

Figure 3.4: Cumulative (A) and average daily (B) evapotranspiration for tomato plants treated with a plant-based liquid digestate (LD), crop-specific fertiliser (CSF) and general synthetic fertiliser (SF) at half (50%) and full (100%) manufacturer's recommended concentration. Treatments that share a letter are not significantly different, and mean average is denoted with ×(A), while range bars display standard error (B). n=10 for each treatment.

concentration (CSF 100%) significantly (p-value <0.05) increased fresh weight relative to liquid digestate at half concentration (50% LD; Figure 3.5a). Shoot dry weight and leaf area showed similar trends, and liquid digestate did not significantly differ versus the control for either (Figure 3.5d). The crop specific fertiliser at full concentration (100% CSF) significantly increased leaf mass as a proportion of shoot fresh weight.

Dose Response

There was a significant dose response (p-value <0.001) across all fertiliser sources for fresh and dry weight, with the manufacturer's recommended concentration increasing by 31.4% and 12.9% compared to half the recommended, respectively. Leaf area was also increased significantly (p-value <0.001) by a higher concentration, but there was an interaction between fertiliser and dose: synthetic fertiliser (SF) and crop specific fertiliser (CSF) increased leaf area by 35.0% at the higher dose, while liquid digestate increased leaf area by 21.9% when concentration was doubled.

Nutrient Analysis

Table 3.4 shows the nutrient concentration for each treatment. From this, total application has been calculated. Leaf area increased significantly (p-value <0.05) with N and phosphate addition (Figure 3.5g&h).

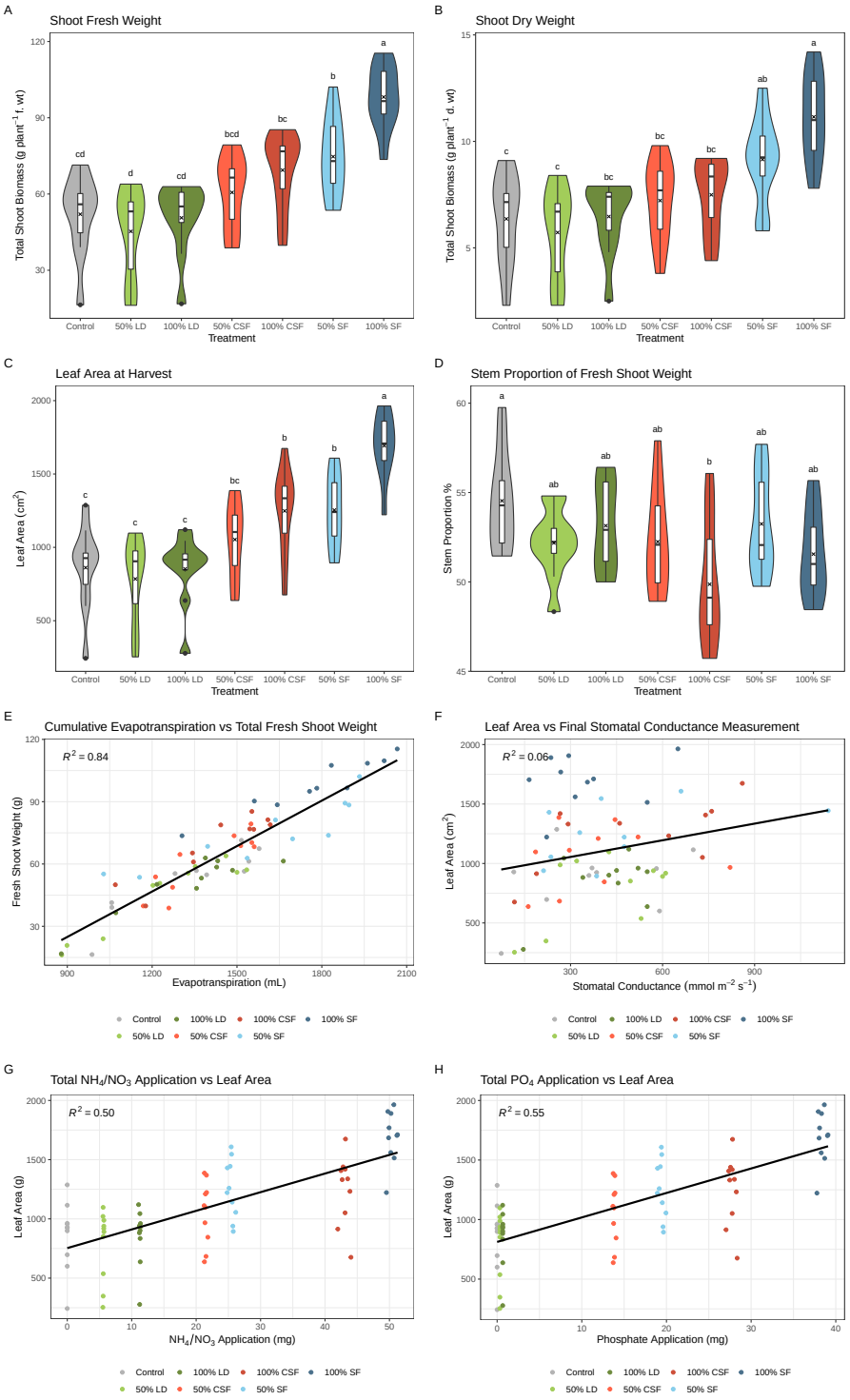

Figure 3.5: Biomass measurements for tomato plants treated with a plant-based liquid digestate (LD), crop-specific fertiliser (CSF) and general synthetic fertiliser (SF) at half (50%) and full (100%) manufacturer's recommended concentration. Treatments that share a letter are not significantly different, and mean average is denoted with ×(A-D). Linear regression models (E-F) show relationships between variables. n = 10 for all treatments.

Table 3.3: Proprietary nutrient analysis for the plant-based liquid digestate provided by 1Up Natural Ltd. Values are for the undiluted product, on a fresh weight basis.

Determinand on a Fresh Weight Basis	**Units**	**Results**
pH 1:6 [Fresh]		8.35
Oven Dry Solids	%	5.88
Total Kjeldahl N	% w/w	0.39
NH_4^+ - N	mg kg^{-1}	2113
NO_3^- - N	mg kg^{-1}	<10
Total Phosphorus (P)	mg kg^{-1}	535
Total Potassium (K)	mg kg^{-1}	6235
Total Magnesium (Mg)	mg kg^{-1}	88.7
Total Sulphur (S)	mg kg^{-1}	185
Total Copper (Cu)	mg kg^{-1}	1.08
Total Zinc (Zn)	mg kg^{-1}	4.06
Total Sodium (Na)	mg kg^{-1}	1350
Total Calcium (Ca)	mg kg^{-1}	836

Table 3.4: Nutrient concentrations, molar ratios and total application amounts for working solutions used in Experiment 1.

Treatment		**Concentration (mg L^{-1})**			**Per Application (mg)**			**Total Application (mg)**			**N:P Molar Ratio**
		NH_4	**NO_3^-**	**PO_4^{3-}**	**NH_4**	**NO_3^-**	**PO_4^{3-}**	**NH_4**	**NO_3^-**	**PO_4^{3-}**	
Liquid Digestate	Full	27.3	1.6	1.7	2.7	0.2	0.2	10.9	0.7	0.7	41.6:1
	Half	13.7	0.8	0.9	1.4	0.1	0.1	5.5	0.4	0.4	
Crop Specific Fertiliser	Full	52.4	57.7	70.9	5.2	5.8	7.1	21.0	23.1	28.4	3.77:1
	Half	26.2	28.9	35.5	2.6	2.9	3.6	10.5	11.6	14.2	
General Synthetic Fertiliser	Full	129.1	0.2	98.6	12.9	0.0	9.9	51.6	0.1	39.4	13.2:1
	Half	64.6	0.1	49.3	6.5	0.0	5.0	25.8	0.0	19.7	

3.4 Discussion

Based on consumer reviews, liquid digestate was anticipated to be both optimal at the manufacturer's recommended dose, and comparable with that of synthetic alternatives. However, growth of plants supplied with general synthetic fertiliser was greater than plants supplied with liquid digestate in almost every metric (Figures 3.3-3.5) when applied at the recommended concentration. Furthermore, liquid digestate did not significantly differ from the control. Full manufacturer's dose led to significantly higher plant growth than half dose.

3.4.1 Chlorophyll Concentration

The general decrease in chlorophyll concentration observed over time in Figure 3.3a is due to senescence, the coordinated dismantling of leaf structure to remobilise nutrients to other parts of the plant (Schippers et al., 2015). The variable rates of chlorophyll concentration across treatments likely indicates different degrees of nutrient stress. Nitrogen (N) constitutes a large portion of photosynthetic organelles and proteins; it forms an essential part of chlorophyll's chlorin magnesium ligand and RuBisCo, with the latter contributing 56% of the total soluble leaf protein in C_3 plants (Mae et al., 1983). Because of this high N concentration, chloroplasts are the first organelles to be degraded through senescence (Masclaux-Daubresse et al., 2010). As such, the lower N supply under the control and liquid digestate treatments (Table 3.4) may have contributed to some of the variation in rates of chlorophyll decrease and final concentrations observed in Figure 3.3a&d. Statistically significant differences were not found during the first set of chlorophyll concentration measurements (Figure 3.3c) as the substrate likely contained sufficient amounts of nutrients (including N) to support growth for all treatments. Eventually, a threshold may have been reached whereby plant N demand exceeded N supply. Differential amounts of N application across treatments (Table 3.4) may explain some of the divergence observed at harvest (Figure 3.3d), although nutrient analysis of plant tissue would be needed to confirm this.

Based on Table 3.4, PO_4^{3-} also varied considerably across treatments, and is also likely to explain some of the variation in chlorophyll concentration. Phosphate is a crucial component of organellar DNA and RNA and is heavily remobilised from leaf tissue (Himelblau and Amasino, 2001): its supply was over fifty times greater in the synthetic fertiliser compared to the liquid digestate at the manufacturer's recommended concentration (Table 3.4). In keeping with Leibig's Law of the Minimum, this may have had an influence on differences in chlorophyll concentration between treatments, if the substrate had become P-limited during the course of the experiment, particularly in combination with low soil NO_3^- (Frydenvang et al., 2015; Wongsnansilp et al., 2016). This is because phosphate is contained in adenosine triphosphate (ATP), which is the primary energy source (as well as ultimately product) of chlorophyll synthesis (Voon et al., 2021). Similarly to N, a nutrient analysis of P contained in plant tissues would be required to diagnose potential P deficiency.

Towards the end of the trial, some plants may have experienced transient water deficit stress in between irrigation events. The effect of water stress on chlorophyll concentration in tomato plants is disputed, with some studies (e.g. Zhou et al., 2017;

Zhou et al., 2020) finding elevated chlorophyll-*a* and -*b* concentrations in response to drought stress, while others observed an accumulation of reactive oxygen species leading to degradation of the chloroplasts(e.g. Yuan et al., 2016), although the former observed this change in the youngest fully expanded leaves, which was not measured in this experiment. While this was likely a uniform stress at the beginning of the experiment (i.e., before statistically significant differences in evapotranspiration), differing water requirements on account of diverging biomasses toward the end of the trial may have led to a greater degree of stress on larger plants between irrigation events.

3.4.2 Stomatal Conductance

Assuming a plant is not water-stressed, stomatal conductance (g_s) is linked to photosynthetic rate and, consequently, plants with a higher nutrient status generally display a higher g_s, and vice versa (Marques et al., 2022). Of the three days in which g_s was measured, there was only one instance of statistically significant difference from the control: the 100% SF. This may be due to greater nutrient delivery relative to other treatments, with Marques et al. (2022) observing stomatal closure in response to low P supply.

However, this statistical significance is not sustained on the other measurement days, and there is considerable variation within treatments (Figure 3.3e&f). This may suggest that other factors may also have affected g_s, including the aforementioned potential water stress. Under water stress, plants close their stomata to reduce transpiration and water loss, reducing conductance (Li et al., 2021). The values obtained on the first day of g_s measurements are consistent with the drought-stressed treatments of a study by (Li et al., 2021). Figure 3.5e demonstrates that larger plants had a larger evapotranspiration, either due to an elevated g_s or simply a larger leaf area - more likely the latter as there were no significant differences when total evapotranspiration was standardised for leaf area (data not shown). As such, the increased biomass production (and thus water demand) elicited through higher nutrient supply may have increased the degree of water stress, obfuscating the extent of different contributing factors to stomatal regulation.

Whilst water stress may explain some of the variations in g_s, there are several additional environmental factors which can cause plants to open or close their stomata. Transpiration is heavily affected by changes in the vapour-pressure deficit (VPD), with a higher deficit reducing g_s (Patanè, 2011). The optimum VPD for tomato plants depends on the growth stage: 1 kPa is recommended for the vegetative stage, increasing to 1.2-1.5

kPa for flowering; VPDs exceeding 2.2kPa can cause stress, by increasing evaporative gradient (Noh and Lee, 2022). In response to a mild step increase of VPD from 0.7 to 1.5 kPa over 20 minutes, McAdam et al. (2016) observed significant stomatal closure in tomato leaves. As Figure 6.1d shows, 40% of the study period was spent >1.5 kPa, and 8% was more than the stress threshold of 2.2 kPa, with only 25% within the optimum range of 1.0-1.5 kPa. Furthermore, VPD was variable during g_s measurements (Table 3.2): there were large fluctuations during the first and final set of measurements, but on the only set with significant treatment differences - 25 days after transplantation - VPD was low and stable. As a result, high VPDs and variations in VPD during measurements add an additional complexity to interpretation of g_s results.

Variations in sunlight also significantly affect g_s (Jolliet and Bailey, 1992). A step-by-step increase in light (0 to 60 to 130, 270, 510, 750 μmol $m^{-2}s^{-1}$) was observed to initiate an oscillation in g_s and introduce a time-lag lasting up to one hour by Kaiser and Kappen (2001). There were frequently fluctuation in glasshouse light intensity levels (Figure 6.1e&f) including, as Table 3.2 shows, on the first day of g_s measurements. While supplementary lighting was constant, differences in solar radiation likely impacted g_s. As a result of these external factors, confidence in any conclusions on fertiliser performance or plant health from g_s measurements are limited.

3.4.3 Evapotranspiration

As Figure 3.4 shows, daily water consumption increased for all treatments over time, before appearing to plateau in the final days prior to harvest. The daily plant water uptake displayed a similar trend and numerical value to a tomato-based study by Romero-Aranda et al. (2002), with a dip and plateau occurring after ∼40 days. As there were no significant differences between non-control treatments when cumulative ET was normalised against leaf area (data not shown), differences are likely explained due to higher leaf area, not transpiration rate. Therefore, the higher leaf area of plants grown under synthetic fertiliser at the recommended concentration (100% SF) likely explains differences in evapotranspiration at the plant level.

3.4.4 Biomass and Leaf Area

Differences in biomass, both fresh and dry, are likely due to differences in nutrient supply across treatments. This is particularly true for N application, which sustains biomass production, with Bénard et al. (2011) observing a halving in tomato plant biomass from a 10 day N starvation relative to ample N supply. Similarly, a decrease in biomass

was observed in response to P deficiency in tomato plants grown in a field study by Higo et al. (2020). Therefore, the higher rates N & P application under the synthetic fertiliser likely explain the significantly greater fresh shoot biomass of synthetic fertiliser at recommended concentration (100% SF) versus other treatments (Figure 3.5a). Furthermore, the higher nutrient application under the manufacturer's recommended concentration versus half dose explains the positive dose response observed in leaf area and biomass production. Leaf area is known to be particularly sensitive to P nutrition in vines, and Grant and Matthews (1996) observed a strong dose-response between the two variables: therefore the much higher, and more similar, absolute concentrations of PO_4^{3-} in the general synthetic and crop specific fertilisers versus liquid digestate may explain why there was a lower dose-response of leaf area in the latter (Figure 3.5h). Additionally, the significantly higher N:P ratio in liquid digestate relative to other treatments (Table 3.4) may have contributed to reduced leaf area and resulted in increased susceptibility to P limitation (Luo et al., 2016).

A larger total leaf area allows greater light interception, facilitating a higher gross photosynthesis; when tomatoes are grown to fruition, this can represent an improvement to fruit yield (Heuvelink et al., 2005). This improvement is particularly realised through maintenance of the older leaves, which are sometimes removed by growers to improve airflow to prevent disease (Heuvelink et al., 2005). Though leaves were not pruned in this experiment, the effect of differential senescence and abscission rates seen through chlorophyll concentration decline (Figure 3.3a) can be considered broadly equivalent. Thus, the significantly higher leaf area of plants supplied with synthetic fertiliser (50% SF & 100% SF) and the crop-specific fertiliser at recommended concentration (100% CSF) versus the control and liquid digestate (LD) likely facilitate greater gross primary production, forecasting a greater fruit yield. It is important to caveat this as photosynthesis rate can be inhibited by excessive vegetative growth leading to self-shading effects. However, based on the short duration of the trial, leaf overlapping was minimal, and leaf area is considered advantageous at this stage. Consequently, plants supplied with liquid digestate displayed inferior growth to synthetic fertilisers at the recommended dose.

While the ultimate objective of domestic tomato cultivation is generally fruit production - with vegetative growth often being viewed as wasted energy expenditure and subsequently pruned - the results suggest that it was insufficient supply, rather than excess, that generated the differences seen across fertilisers and concentrations. Consequently, general synthetic fertiliser at the recommended concentration (100% SF) offered comprehensively superior growth than other treatments, whereas plants sup-

plied with crop-specific fertiliser (CSF) were more mixed, and liquid digestate (LD) did not significantly differ from the control.

3.5 Conclusions

As a result of these data, the following conclusions are made:

- Liquid digestate does not offer comparable performance to a general synthetic fertiliser at the manufacturers' recommended concentration, and recommended dose must be increased.
- Liquid digestate did not significantly improve plant growth relative to the control at harvest.
- Plant growth was significantly enhanced at the manufacturers' recommended concentrations, and recommendations are likely not excessive, although a greater range of concentrations in different media would be required to assess this.

Based on the results of the experiment, Hypothesis I should be **rejected**, on account of the significantly greater biomass production and physiological activity of tomato plants grown using synthetic fertiliser versus liquid digestate. However, there did appear to be a significant dose response, and thus sub-hypothesis ii should be **accepted**.

Chapter 4

Comparing Commercial Liquid Digestate & Mineral Fertilisers at the Same Nitrogen Application Rate

4.1 Introduction

Nitrogen (N) supply was suspected to be one of the main reasons for significant differences between treatments in Chapter 3, with the application rate of liquid digestate likely too low to support optimal plant growth. Similar results have been reported in raised bed studies, with Liedl et al. (2006) observing a superior response in tomato plants treated with digested poultry liquid at double the recommended N. Furthermore, matching for nutrients, such as N, is necessary for a fair comparison between liquid digestate and synthetic alternatives. While matching for other nutrients (e.g. PO_4^+) may provide valuable information, previous tomato-based liquid digestate experiments have matched for N (none matched based on P to my knowledge), providing grounds for comparison (e.g. Liedl et al. 2004). Consequently, Chapter 3 will focus on comparing treatments matched for plant-available, mineral N (NH_4^+/NO_3^-).

While the high microbial load (particularly bacteria) contained in digestate has been promoted as beneficial to soil and plant health by digestate-fertiliser producers, literature presents a more mixed evaluation (e.g. Panuccio et al. 2021; Barduca et al. 2021). However, these experiments have typically considered microbial communities and plant growth in isolation (i.e. incubation mineralisation experiments) which, whilst

valuable from a mechanistic perspective, may not relate well to a more holistic approach. Positive effects on nutrient mineralisation following application have been reported by Panuccio et al. (2021), attributed to enhanced microbial activity. However, other studies have found opposite effects – such as net immobilisation and negative competitive effects (e.g. Reuland et al.2021*b*), or no effect at all (e.g. Barduca et al.2021).

Thus, there is a need to establish whether microbe communities in digestate significantly affect plant growth or physiological response. Through sterilisation (i.e. through autoclaving) of the liquid digestate fertiliser, the comparative effect on plant performance versus the original product can be demonstrated. In the instance of a significant difference between autoclaved and unautoclaved liquid digestate treatments, where nutritional composition remains the same, the effect of microbial communities contained within unautoclaved liquid digestate can be evaluated holistically. Conversely, the absence of a significant difference between forms may infer comparable performance; this could indicate that concentration, stabilisation or even dehydration of liquid digestate is possible without loss of performance as a fertiliser. The reduction in transport volumes and product stability issues could have benefits both environmentally and economically for producers.

As a result, the following hypotheses are presented:

(II) When matched for mineral nitrogen, liquid digestate offers comparable performance to synthetic fertiliser.

Sub-hypotheses

(i) Liquid digestate provides a predominantly nutritional benefit to plants, and the non-nutritional effects are not realised over single tomato cropping cycle.

4.2 Experimental Design and Methods

4.2.1 Experimental Design and Environmental Conditions

The experiment compared three fertiliser treatments matched for mineral nitrogen (assumed to be NH_4^+/NO_3^-) and a negative control of no fertiliser application, with ten replicates of each treatment. The fertilisers used were a liquid digestate (Will&Al's Natural Plant Food Company, Cumbria, United Kingdom) in its retailer-supplied and autoclaved forms, and a synthetic fertiliser (Levington® Miracle-Gro®, Cardiff, Wales).

The experiment was conducted under glasshouse conditions [22 °C/16 °C day/night, photoperiod of 14 hours at ∼400 μmol $m^{-2}s^{-1}$].

Table 4.1: Fertiliser concentrations of working solutions for each treatment in Experiment 2. Plants received four weekly applications of each treatment.

	Ammonium (NH_4^+-N) (mg L^{-1})	**Nitrate (NO_3^--N) (mg L^{-1})**	**Phosphate (PO_4^{3-}-P) (mg L^{-1})**
Liquid Digestate (LD)	121.9	7.3	7.7
Synthetic Fertiliser (SF)	129.1	0.2	98.6

Tomato seeds (*Solanum lycopersicum* cv. Ailsa Craig) were sown in cell seed trays in the same low-nutrient, commercially available John Innes Seed and Cutting Compost (Westland Horticulture, Tyrone, Northern Ireland) used in Chapter 3 on the 23rd December 2021. Trays were irrigated when the surface of the substrate began to dry. When seedlings had reached the second true leaf stage, the most representative specimens were transplanted on 19th January 2022 (27 days after sowing) into 2 l square black plastic pots (110 (L) x 110 (W) x 165 (H) mm) filled with a 1:1 by volume mixture of top-dressing sand (Boughton, Kettering, Northamptonshire, UK) and John Innes Seed and Cutting Compost. The mixture was sieved to <20 mm, and combined for 15 minutes in a cement mixer. An initial 200 mL tap water was given to each seedling to avoid transplant shock.

Gravimetric water holding capacity (WHC) of the substrate was determined through the method outlined in Chapter 3.2.1. The threshold for irrigation was increased from 50% of WHC (in Chapter 3) to 70% of WHC (as per Panuccio et al.2021), to ensure adequate water supply, with irrigation every 1-3 days returning pots to 100% of WHC (saturation). Saucers were placed under pots to minimise nutrient leaching. Pots were covered with foam squares (110 (L) x 110 (W) x 12 (H) mm) to prevent evaporation from the soil surface, with a 40 mm diameter hole for the plant. Pots were randomly rotated daily to mitigate against environmental gradients in the glasshouse and arranged into rows of even spacing to minimise shading and competition effects.

Fertiliser treatments were prepared two days before application and stored in sealed Schott Duran bottles covered in foil in a cool dark cupboard: Liquid digestate (LD and ALD) and synthetic fertiliser were diluted to Table 4.1. The autoclaved liquid digestate was autoclaved at 121 °C at 30 kPa for 45 minutes and sealed immediately. On 6th February 2022 (45 days after sowing, 18 days after transplanting), 100 mL fertiliser treatments were applied. Plants were harvested once statistically significant differences consistently appeared between in-situ measurements, on 4th March 2022 (71 days after

sowing, 44 days after transplanting).

4.2.2 In-situ Measurements

Chlorophyll concentration and stomatal conductance (g_s) were calculated using the same methods outlined in Chapter 3 on the terminal leaflets of the oldest and youngest fully expanded true leaves, respectively. Due to the foam pot covering, water loss was calculated in the same way as Chapter 3, but assumed to result from transpiration only.

Non-destructive leaf area

Figure 4.1 shows the process for estimating leaf area in-situ. Using images taken on a mobile phone at a fixed height (60 cm) with a blue chroma key background of known dimensions (297 (L) x 210 (W) mm for first set of estimations, increased to (420 (L) x 297 (W) mm for second set) leaf area was estimated thrice during the experiment. These images were cropped to the background extent using Microsoft Lens (Microsoft Lens, Washington, USA) and processed for percentage of green cover using Canopeo (Patrignani and Ochsner, 2015). This percentage of green cover was then converted to cm^2 based on the known background size.

Figure 4.1: The process for leaf area estimation. First, images are cropped to the background extent (left), then green cover is analysed (right) using Canopeo (Patrignani and Ochsner, 2015).

Epinasty

Epinasty of the petioles was measured by quantifying the interior angle of the stem and abaxial surface of the petiole using a clear protractor on the second node above the cotyledonary node (Figure 4.2).

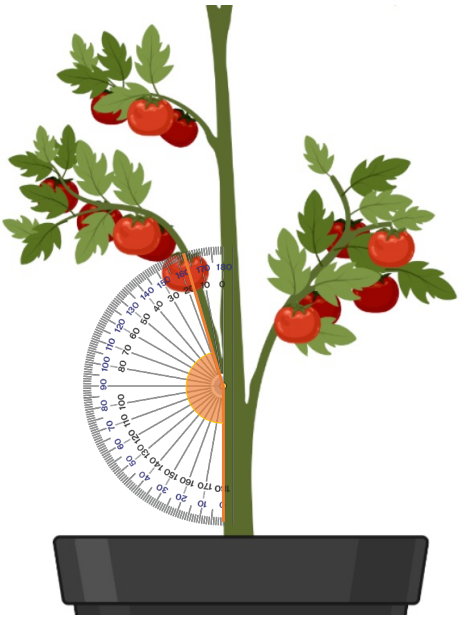

Figure 4.2: Schematic of epinasty measurement angle in this experiment.

4.2.3 Ex-situ

Water potential

To assess whether plants were water stressed, and if this was treatment specific, the approximate midday water potential was measured using a Scholander pressure bomb (Model 3000F01, SoilMoisture Equiment Corp., California, USA) as in Gavassi et al. (2020). Measurements were taken on the newest fully expanded leaf as close to midday as possible on 2nd March 2022.

Plant growth

Biomass and leaf area were determined using the methods outlined in Chapter 3. Harvest occurred on 4th March (44 days after transplanting). Plant tissues were dried at 70 °C for 48 hours until a stable weight was reached, and dry weight measured.

Root photography

During harvesting, mobile phone photographs were taken of specimens where the compost structure remained intact, from a distance of 30 cm, with a 30 cm ruler for scale. These images were then converted to black and white and contrast increased to the maximum to differentiate roots from potting media. Using the reference ruler, images were scaled for comparison.

4.3 Nutrient Analysis

4.3.1 Soil Nutrient Analysis

Soil nutrient analysis was carried out on air-dried soil sieved to <2 mm. For NH_4^+/NO_3^- analysis, a 5.0 g sample was combined in a 50 mL centrifuge tube with 25 mL of 2.0 M KCl and placed on an orbital shaker for 1 hour, as per McTaggart and Smith (1993). For PO_4^{3-} analysis, a 2.0 g sample was combined in a 50 mL centrifuge tube with 40 mL 0.5 M $NaHCO_3$ (pH 8.5) and placed on an orbital shaker for 30 minutes, as described by Olsen and USDA (1954). Both solutions were filtered through a Whatman No 42 filter and refrigerated until analysis. Analysis for NH_4^+/NO_3^- was conducted on a AA3 Autoanalyzer (SEAL Analytical, Germany, Method No G-102-93 Rev 2; Multitest MT7/MT8) using two colourimetric methods: ISO 11732:2005(en)) and ISO 13395:1996(en), respectively. Analysis for PO_4^{3-} was also conducted on a AA3 Autoanalyzer (Seal Analytical, Germany; Method No G-103-92 Rev1; Multitest MT7/MT8), conforming to ISO 6878:2004(en).

4.3.2 Plant Tissue Nutrient Analysis

For C/N analysis, dry samples of leaves and stem were ball-milled separately for 3 minutes until a fine powder. Samples of 10 $\pm$1.0 mg were weighed in rectangular tin boats, which were folded and placed on the carousel of a VarioCube (Elementar, Hanau, Germany). Samples were then combusted at 950 °C according to the Enhanced Dumas method ISO 19051:2015(en) and analysed for percentage content of N and C. Pairs of 3 $\pm$1.0 mg samples of acetanilide were used as standards for factorisation - as per ISO 19051:2015(en) - every 20 plant samples.

4.3.3 Plant Nitrogen Demand

Plant nutrient demand was estimated by using the relationship of leaf area and dry shoot biomass at harvest to infer dry shoot biomass when leaf area was measured. By combining these estimates with shoot N content, a total N demand was estimated.

4.3.4 Soil pH

Soil pH was determined on 10 g fresh weight substrate mixed with 25 mL milliQ water in 50 mL centrifuge tubes, placed on an orbital shaker for 30 minutes and measured

with a pH probe at the soil-water interface once sediment had settled, as per Allen (1989).

4.4 Statistical Analysis

Statistical analysis was performed as in Chapter 3.2.5, with ANOVA and Tukey's HSD test used to identify significant differences between treatments at the 5% level. As in Chapter 3.2.5, measurements at specific timepoints were analysed separately.

4.5 Results

4.5.1 Visual Observations

Initially, all plants developed similarly, and new growth was green and similarly sized. Visual differences appeared first in the control (C) with slight yellowing of the oldest leaves. By 1st March (41 days after transplanting) visual differences were apparent between the synthetic fertiliser (SF) and other treatments (Figure 4.3a). By harvest (44 days after transplanting) root density and length appeared to be greater both vertically and laterally for photographed samples of synthetic fertiliser versus other treatments, although it was not possible to determine this quantitively (Figure 4.3b).

Epinasty of the petioles was observed 33 days after transplanting (Figure 4.3c), but there were no significant differences between treatments (Figure 4.4d).

4.5.2 In-situ

Chlorophyll concentration decreased over time for all treatments (Figure 4.5a). Liquid digestate (LD), autoclaved liquid digestate (ALD) and synthetic fertiliser (SF) did not significantly differ from one another consistently until 32 days after transplanting, according to Tukey's HSD. From 35 days after transplanting, synthetic fertiliser had a significantly higher chlorophyll concentration than all other treatments, which did not significantly differ from one another By harvest, chlorophyll concentration was 1.3-fold higher than the control (p-value < 0.001), while neither liquid digestate nor autoclaved liquid digestate significantly differed from the control (Figure 4.5d).

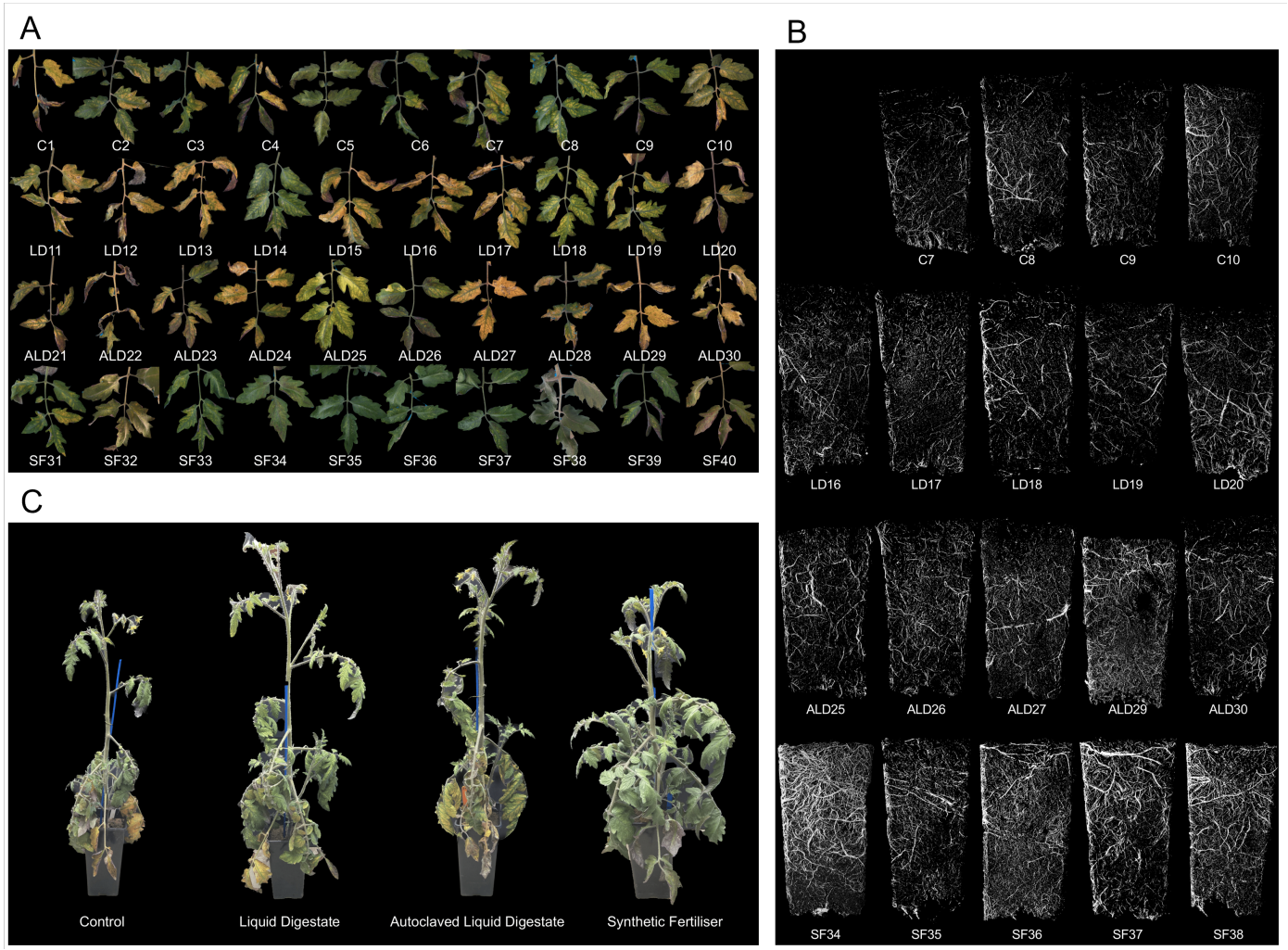

Figure 4.3: Visual comparison of (A) oldest true leaf grown under treatment by no additional fertiliser (control/C) liquid digestate (LD), an autoclaved liquid digestate from the same batch (ALD) or a synthetic fertiliser (SF), matched for plant available nitrogen application. (B) a side profile of roots of representative specimens and (C) side profile of shoots of a representative specimen.

Stomatal conductance (g_s) decreased over time for all treatments (Figure 4.5b). Neither liquid digestate nor autoclaved liquid digestate significantly increased g_s relative to the control. Synthetic fertiliser significantly (p-value < 0.001) increased g_s on the first day of measurements by 46% (Figure 4.5e) before converging with other treatments' values between 28 and 30 days after transplanting. On the last set of measurements (Figure 4.5f), only synthetic fertiliser had a significantly higher g_s than the control.

Table 4.2: Minimum and maximum values of different environmental parameters during stomatal conductance measurements in Experiment 2.

	19	**26**	**27**	**28**	**29**	**30**	**35**
Temperature (°C)	20.3 – 21.0	22.6 – 24.2	22.4 – 22.6	22.9 – 23.6	18.8 – 25.0	20.0 – 25.5	18.5 – 24.5
Humidity (%)	47.7 – 48.3	29.4 – 32.3	29.9 – 30.2	37.2 – 39.0	33.5 – 60.3	34.8 – 61.3	34.0 – 63.5
Vapour Pressure Deficit (VPD)	1.2 – 1.3	1.9 – 2.1	1.9	1.7 – 1.8	1.1 – 2.0	1.0 – 1.9	0.9 – 1.8
Solar Radiation (μmol m^{-2} s^{-1})	67 – 83	1443 – 1592	1517 – 1631	1380 – 1398	1510 – 1517	1381 – 1547	1428 – 1540
Photosynthetically active radiation (PAR) (μmol m^{-2} s^{-1})	33 – 41	707 – 780	744 – 800	676 – 685	740 – 743	677 – 758	700 – 755

Synthetic fertiliser displayed a significantly higher total transpiration than all other treatments, which did not differ from one another (Figure 4.4a). Average daily transpiration initially increased for all treatments until 27 days, after which synthetic fertiliser continued to increase while other treatments plateaued and eventually decreased (Figure 4.4b).

4.5.3 Ex-situ

Shoot fresh weight and dry weight were significantly higher when treated with synthetic fertiliser versus other treatments (Figure 4.6a&b), with an increase in fresh biomass of 46% relative to the control. Leaf area was also significantly higher (p-value <0.001) in plants treated with synthetic fertiliser, with neither liquid digestate nor autoclaved liquid digestate differing significantly from each other or the control (Figure 4.6c). Leaf area increased over time for all treatments, and leaf water potential was not significantly different across treatments.

4.5.4 Nutrient Analysis

Total shoot N was significantly higher under the synthetic fertiliser treatment than other treatments (which did not differ from one another) and was 85% higher than the control (p-value < 0.01) (Figure 4.6e). Shoot N partitioning was not significantly different between treatments, except synthetic fertiliser, which allocated 5.4% more of its N to its leaves than the control (p-value < 0.01), (Figure 4.6f).

Soil PO_4^{3-} was significantly higher under synthetic fertiliser relative to the control and liquid digestate, but not autoclaved liquid digestate (Figure 4.7). Similarly, soil NH_4^+ concentration was significantly greater in the control than other treatments (Figure 4.7a). Nitrate concentration was significantly higher (p-value < 0.01) in substrate treated with synthetic fertiliser relative to liquid digestate and autoclaved digestate, but not the control (Figure 4.7b).

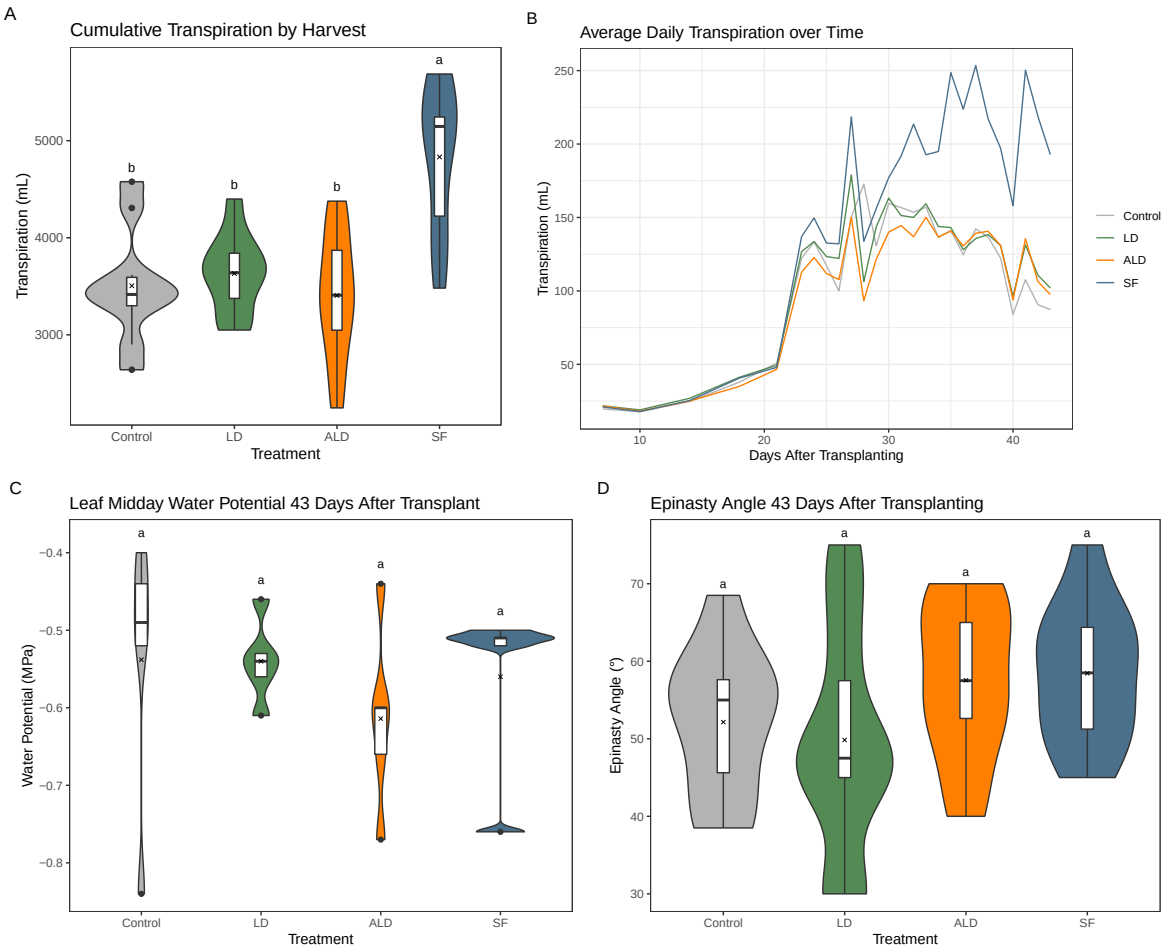

Figure 4.4: Cumulative transpiration (A), leaf water potential (C) and epinasty (D) measurements at harvest for tomato plants grown under treatment with liquid digestate (LD), an autoclaved form of the same liquid digestate (ALD), or synthetic fertiliser (SF), matched for nitrogen application, with a control (C) of no fertiliser application. Treatments that share a letter are not significantly different, and mean average is denoted with ×. A time series of average daily transpiration (B) for the same aforementioned treatments. n=10 for each treatment.

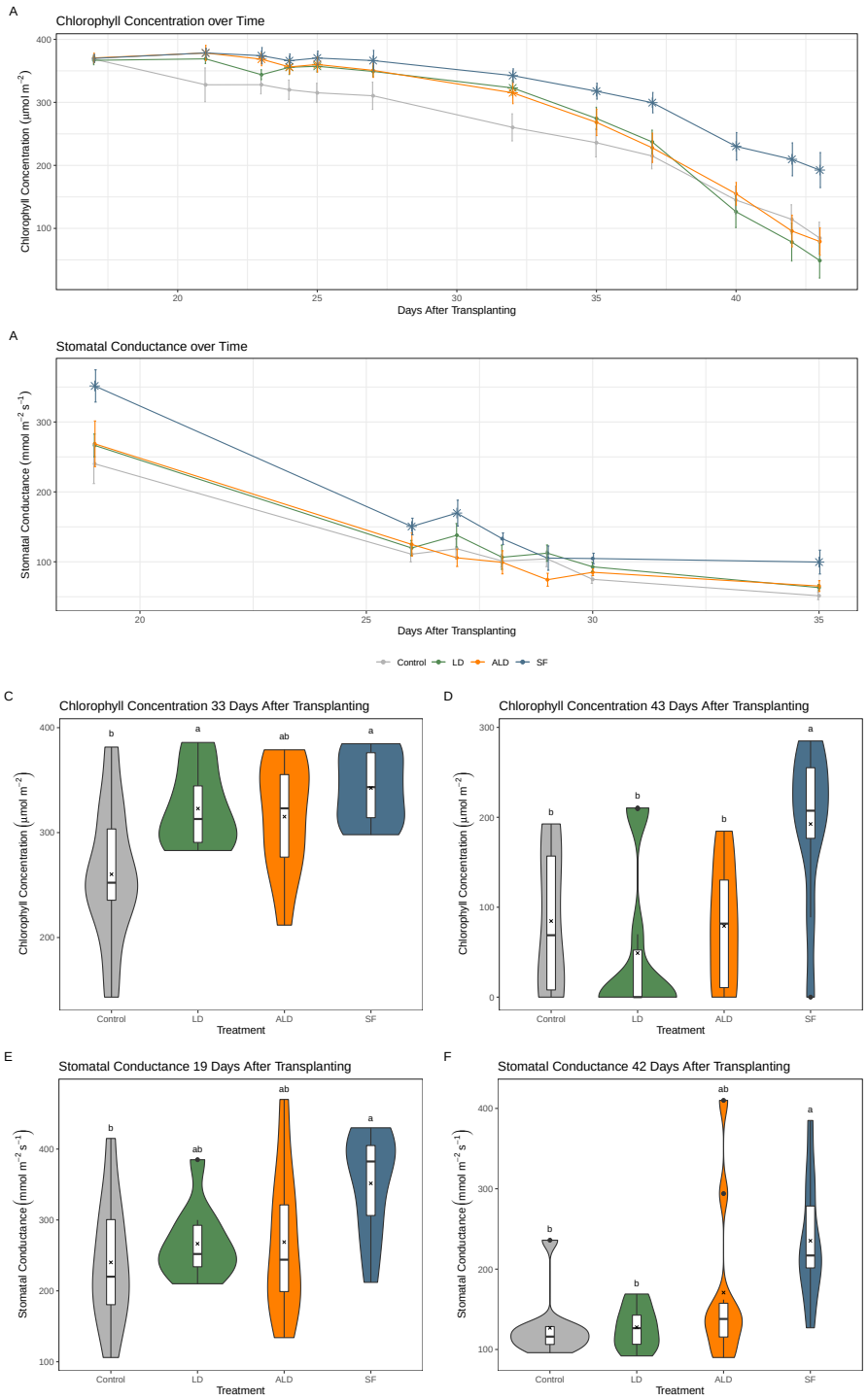

Figure 4.5: Plant physiology measurements of tomato plants grown under treatment with liquid digestate (LD), an autoclaved form of the same liquid digestate (ALD), or synthetic fertiliser (SF), matched for nitrogen application, with a control (C) of no fertiliser application. Statistical significance versus the control is denoted with * (A-B), with range bars displaying standard error. Treatments that share a letter are not significantly different, and mean average is denoted with × (C-F). n=10 for each treatment.

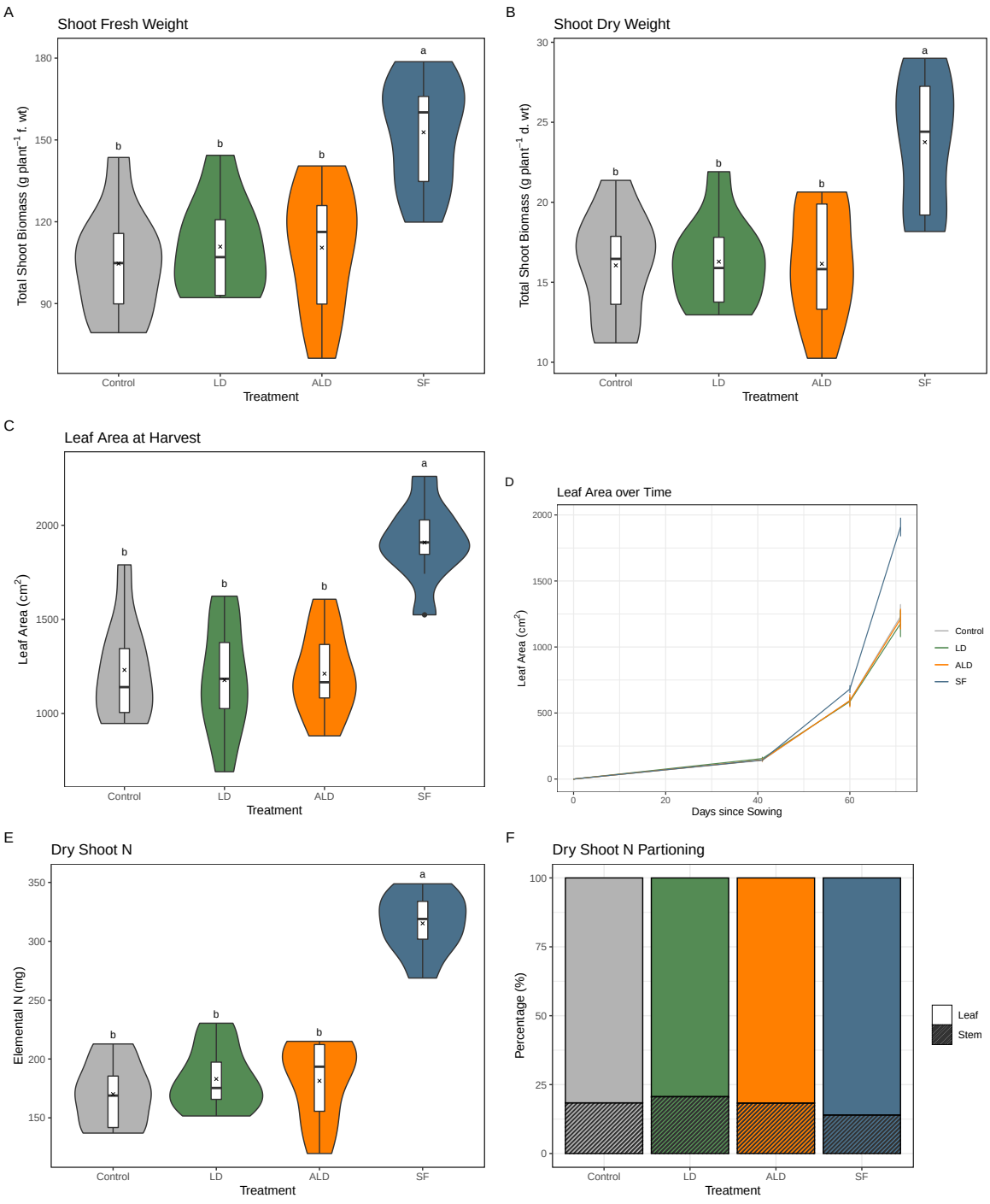

Figure 4.6: Biomass and plant nutrient analysis following harvest of tomato plants grown under treatment with liquid digestate (LD), an autoclaved form of the same liquid digestate (ALD), or synthetic fertiliser (SF), matched for nitrogen application, with a control (C) of no fertiliser application. Treatments that share a letter are not significantly different, and mean average is denoted with $\times$ (A-C and E). (D) A time series of inferred leaf area for the same treatments, with range bars displaying standard error. (F) Composite bar charts displaying the average partitioning of nitrogen to the stem and leaf. n=9 for each treatment.

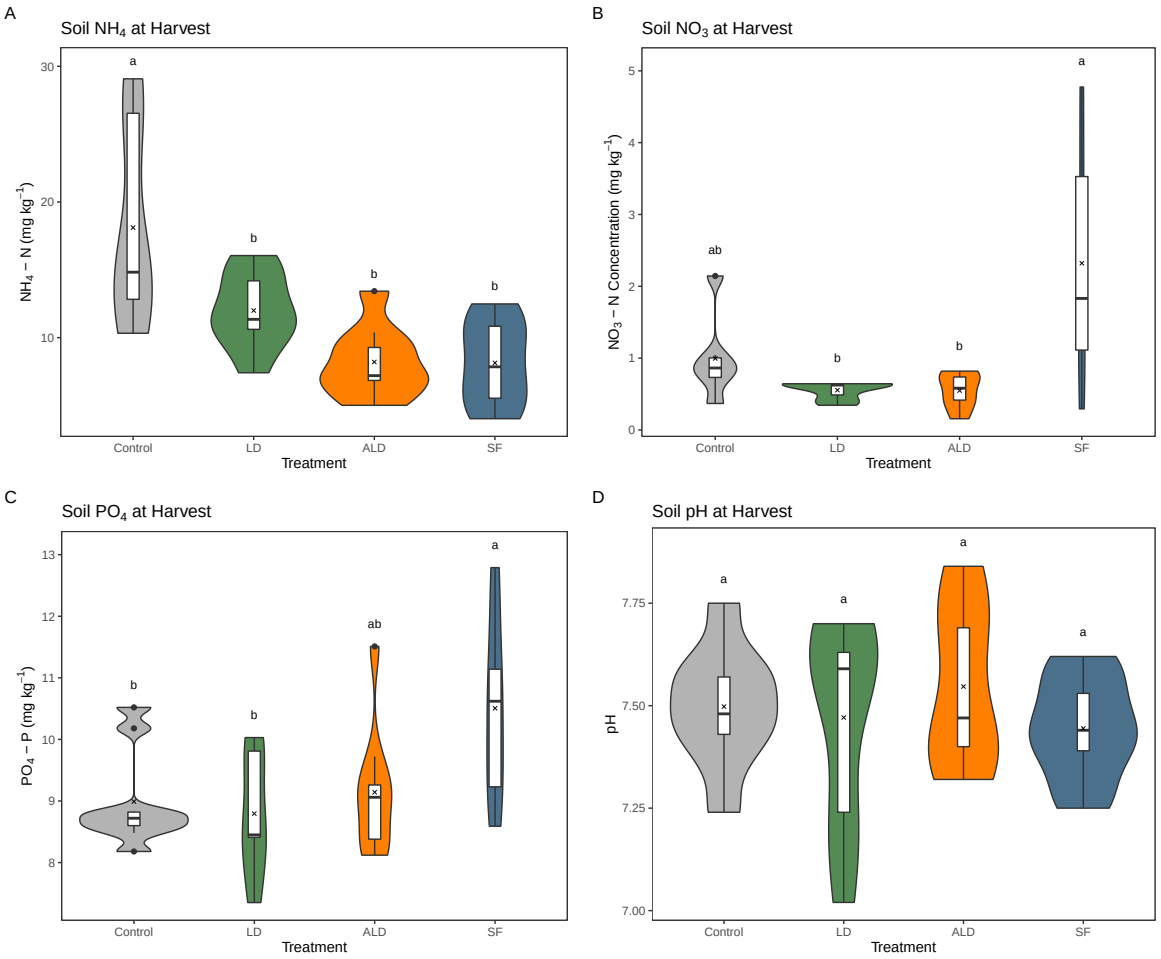

Figure 4.7: Soil nutrient analysis following harvest of tomato plants grown under treatment with liquid digestate (LD), an autoclaved form of the same liquid digestate (ALD), or synthetic fertiliser (SF), matched for nitrogen application, with a control (C) of no fertiliser application. Treatments that share a letter are not significantly different, and mean average is denoted with $\times$. n=9 for each treatment.

4.6 Discussion

Despite being matched for mineral N, synthetic fertiliser produced healthier and larger plants than liquid digestate which, in both autoclaved and unautoclaved forms, did not significantly differ from the control for any metric.

4.6.1 The impact of fertiliser source on plant physiology

Chlorophyll concentration displayed a similar temporal decrease to that observed in Chapter 3, as explained by N remobilisation to new growth (Masclaux-Daubresse et al., 2010). While liquid digestate (in both autoclaved and unautoclaved form) initially maintained comparable chlorophyll concentrations to synthetic fertiliser - likely on account of higher nutrient supply than in Chapter 3 - the significantly higher chlorophyll levels observed in synthetic fertiliser after 35 days (Figure 4.5c) were likely influenced by treatment differences in nitrogen use efficiency (NUE) (da Silva et al., 2014).

Although treatments were matched for mineral N, other nutrients, such as PO_4^{3-}, differed markedly (Table 4.1). The lower supply of PO_4^{3-} under liquid digestate possibly restricted N uptake, in keeping with Liebig's Law of the Minimum. Whilst PO_4^{3-} deficiency may have reduced N uptake, inadequate supply of P alone has not been found to reliably lower chlorophyll concentration or induce senescence (Khavari-Nejad et al., 2009), and so PO_4^{3-} deficiency is not likely to have directly caused the lower chlorophyll concentration (Crafts-Brandner, 1992). Deficiency in other nutrients, such as sulphur (S), can accelerate senescence - starting in the oldest leaves - even if N supply is ample (Eriksen et al., 2001; Eaton, 1951). Desulphurisation of biogas reactors using iron (Fe) triggers the formation of iron sulphide (FeS) precipitates; when combined with volatilisation of hydrogen sulphide (H_2S) during anaerobic digestion, this can produce low-S liquid digestate, even when derived from high-S feedstocks (Fontaine et al., 2020; Weimers et al., 2022). Based on Table 3.4, and the results of Weimers et al. (2022), it is possible that S deficiency occurred in liquid digestate treatments, although Weimers et al. (2022) studied *Brassica rapa* ssp. Chinesis, which is likely to have a higher S demand than the tomatoes grown in my experiment (Haneklaus et al., 1999).

Stomatal conductance (g_s) also followed a similar temporal decrease to plants in Chapter 3, albeit with lower absolute values (Figure 4.5b). Despite this, synthetic fertiliser significantly increased g_s which, like chlorophyll concentration, is likely explained by higher levels of other nutrients. While potassium (K) is a major osmoticum in guard cells, and can strongly affect g_s, it is ample in the digestate (Table 3.4) and thus does not explain differences between treatments (Mengel and Kirkby, 2001). Lower PO_4^{3-} levels

decrease root hydraulic conductivity, but as leaf water potential was similar despite differing P supply, it is assumed to be positively related to g_s (Turnbull et al., 2007; Simões et al., 2020; Farquhar and Sharkey, 1982; Radin, 1990). Phosphate is very low in the liquid fraction of digestate, particularly following addition of magnesium (Mg) and Fe salts for desulphurisation (Tuszynska et al., 2021; Weimers et al., 2022).

While water stress likely elicited stomatal closure in Chapter 3, leaf water potential values were within normal ranges in the current experiment, and thus the low g_s cannot be explained by water stress (Figure 4.4c). Vapour pressure-deficit (VPD) values were high and varied for several days (Table 4.2), which may explain some of these results. While foam pot lids were introduced to minimise evaporation and prevent water stress in between irrigation events, they may have also acted to reduce soil-atmosphere gas exchange. As a result, ethylene may have accumulated in the roots (Bradford and Dilley, 1978), potentially inducing stomatal closure in tomato (Jia et al., 2018). While ethylene concentration was not measured, epinasty (Figure 4.4d) – a symptom of ethylene overproduction – was comparable to the ethylene-stressed tomato plants treated with 1 mmol brassinolide in a study by Schlagnhaufer and Arteca (1985). However, epinasty was not treatment-specific (Figure 4.4d) and thus does not explain treatment differences.

Transpiration also followed a similar temporal trend to Chapter 3, with an initial increase of all treatments, followed by a drop at ∼27 days, whereby only synthetic fertiliser continued to increase daily transpiratory water losses (data not shown). Total transpiration also mirrored results found in Chapter 3. The higher transpiration rate under synthetic fertiliser reflects a higher growth rate and photosynthetic activity, again deriving from greater nutrient supply of nutrients other than N, on account of matching for N, such as enhanced hydraulic conductivity under a higher PO_4^{3-} regime (Mengel and Kirkby, 2001).

4.6.2 The impact of fertiliser source on plant growth

Both fresh and dry shoot biomass revealed similar trends to in-situ measurements, and the results in Chapter 3. Biomass accumulation is affected by PO_4^{3-} availability which is, in turn, controlled by pH (Higo et al., 2020). A N:P ratio of 14:1 was found to be optimal for tomato plants by Abduelghader et al. (2011): as Table 3.4 shows, the synthetic fertiliser is very close to this ratio, while the liquid digestate is overwhelmingly dominated by N. Furthermore, the neutral-alkaline soils and alkaline digestate (Table 3.3 & Figure 4.7) may have decreased the overall PO_4^{3-} availability, and thus the

absolute value of PO_4^{3-} addition in each fertiliser may be more important than the ratio. Leaf area is also significantly affected by PO_4^{3-} addition (Figure 4.6c), and a significantly higher area under synthetic fertiliser is likely in response to elevated supply of PO_4^{3-} versus digestate treatments, which facilitated greater water delivery to expanding leaves by enhancing root hydraulic conductance (Radin, 1990; Mengel and Kirkby, 2001). While not measured empirically, the root network of representative plants appeared to be larger under synthetic fertiliser than other treatments (Figure 4.3b), supporting this hypothesis. Sulphur deficiency, which was identified as a potential issue by Weimers et al. (2022), may have also contributed to reduced biomass production versus synthetic fertiliser, through reduction of S-based metabolites (Zhao et al., 1997).

4.6.3 Treatment Impact on Soil and Plant Nutrient Status

Soil analysis displayed variable results. As liquid digestate is heavily NH_4^+-based (Table 3.4), volatilisation may have been an issue in both autoclaving of liquid digestate, and in air-drying of soil samples for nutrient analysis (due to training delays and equipment issues, it was not possible to assess N on fresh soil). However, the significantly higher NH_4^+ in the soil of the control (Figure 4.7a) versus the synthetic fertiliser may simply be a function of greater N uptake under synthetic fertiliser (Figure 4.6e). This does not explain, however, why digestate-treated soils contained significantly less NH_4^+ than the control (Figure 4.7a). Instead, it is possible that, for unautoclaved digestate, there was a period of N immobilisation into organic matter by soil microbes. Reuland et al. (2021*b*), who observed N immobilisation following digestate application, attributed this to high C/N ratios. Although C/N ratio was not measured for liquid digestate or synthetic fertiliser it is likely that, based on the organic origin of digestate and characterisation of liquid digestate by other studies, the former had a higher C/N ratio than synthetic fertiliser (Reuland et al., 2021*b*; Ye et al., 2018). As a result, the NH_4^+ in synthetic fertiliser was more readily assimilated by plants, despite matching for mineral N, due to the higher C/N ratio of liquid digestate. Finally, the reduced concentration of NH_4^+ contained in soils treated with autoclaved soils may be explained by N volatilisation, which will be discussed later in this section.

Analysis of soil nitrate (NO_3^-) supports the N immobilisation hypothesis: despite initially possessing a higher NO_3^- concentration than the synthetic fertiliser (Table 4.1), by harvest soils treated with synthetic fertiliser had a significantly higher NO_3^- concentration than liquid digestate (Figure 4.7b). The anoxic environments during liquid digestate storage favour denitrifying bacteria (Painter, 1970; Svehla et al., 2020);

when presented with an additional source of organic carbon (in this case the potting compost), this may lead to further denitrification of NO_3^- in the applied liquid digestate. However, the lack of statistical distinctness between liquid digestate treatments cannot be explained through this hypothesis, and thus further research would be required to characterise the presence and effect of denitrifying and nitrifying microbial communities in digestate.

The significantly higher PO_4^{3-} concentration in synthetic fertiliser soil relative to the control and unautoclaved liquid digestate treatment (Figure 4.7c) is due to a higher application rate (Table 4.1). While there were not significant differences between autoclaved liquid digestate and synthetic fertiliser, this appears to be due to the existence of an extreme value for autoclaved liquid digestate and does not fit the general distribution of other samples. This may be due to heterogeneity in nutrient concentration in the potting substrate or, perhaps more likely, a measurement error.

Plant N content was significantly higher in synthetic fertiliser applications (Figure 4.6e), likely because of greater N uptake through elevated supply of other nutrients – such as PO_4^{3-}, and, although not evaluated by this study, S (Weimers et al., 2022). Furthermore, N is likely more immediately available in the synthetic form, on account of a lower C/N ratio (Ye et al., 2018), preventing immobilisation in contrast to liquid digestate.

4.6.4 Effect of autoclaving on fertiliser efficacy

Autoclaving of digestate is practiced amongst some producers (but not the product in this study) of digestate-derived fertiliser, to reduce biogas production in containers during storage (W Tuer 2022, personal communication). While the effect of autoclaving digestates has not been studied, the autoclaving of feedstock to improve stability and hygienic quality is widely practiced in waste-based digestates, and has been found to reduce soluble N by up to 44% (Tampio et al., 2015). The effect of volatilisation was overlooked by my experiment, and thus it is likely that the autoclaved liquid digestate had a reduced concentration of N relative to other treatments. However, this provides an interesting comparison: unautoclaved liquid digestate did not perform significantly differently from the autoclaved form, despite likely having a higher N content. This may be explained by two mechanisms. Firstly, while N volatilisation reduces application amounts, autoclaving may improve NUE by reducing the N immobilisation observed following digestate application in other studies (e.g. Reuland et al.2022). Thus, despite the lower concentration of N in autoclaved liquid digestate, reduced immobilisation may

result in more immediate bioavailability, effectively ameliorating the effect of a reduced N supply. Additionally, and perhaps more importantly, autoclaving has little effect on phosphorus concentrations (Tampio et al., 2015). Therefore, if P supply was the limiting factor to growth, as is proposed in addition to N immobilisation, this would remain as important across digestate forms.

Nitrogen losses and unaltered P concentration following autoclaving will reduce the N:P ratio of digestate. Thus, this method could reduce the severity of two issues associated with liquid digestate application: excessive $NH_4{}^+$, as identified in Liedl et al. (2004); and insufficient P, as identified by Stoknes et al. (2016) by addressing the ratio between them. Further research on the impact of changing N:P ratios in digestate through autoclaving could yield valuable information.

4.7 Conclusions

As a result of these data, the following conclusions are made:

- Liquid digestate did not offer comparable performance to synthetic fertiliser, even when matched for mineral N.
  - This is likely due to differing concentrations of other essential nutrients, namely $PO_4{}^{3-}$.
- Despite likely reducing N concentration, autoclaving did not differ in performance to the unautoclaved, biologically-rich form of digestate.
  - This is likely due to microbial communities contained in digestate, which favour immobilisation of available N following application (e.g. Reuland et al.2022), or offer no impact (e.g. Barduca et al.).
- While this study did not directly measure microbial communities, there is no supporting evidence for enhanced plant growth as a result of the inherent microbial communities within liquid digestate.

As a result of these experimental data, there is strong evidence for the **rejection** of Hypothesis II. It is **not possible** to assess subhypothesis II(i), as liquid digestate did not significantly differ from the control plant for either form of digestate.

Chapter 5

Soilless Cultivation of Tomato Plants with Liquid Digestate as a Sole Nutrient Source

5.1 Background and Rationale

Following the results of Chapter 3 & 4, where liquid digestate failed to significantly differ from the control, there was a need to examine whether unamended digestate could provide a balanced nutrient source for the cultivation of tomato plants. When matched for N in Chapter 4, liquid digestate did not offer comparable performance to a synthetic fertiliser, even when autoclaved, suggesting that inferior plant growth was caused by deficiency in other essential nutrients. Deficiency in P and Mg have been reported in hydroponic studies cultivating tomatoes using liquid digestate while, conversely, NH_4^+ and Cl^- toxicity have also been encountered (Liedl et al., 2004, 2006; Stoknes et al., 2016). However, these studies have used manure and waste-based feedstock, and so may conceivably produce a product that is less suited to plants than an entirely plant-based digestate. Thus, an evaluation of whether less abundant nutrients can be supplied sufficiently before toxicity is reached in more abundant nutrients using liquid digestate as a sole nutrient source.

Soilless systems, or hydroponics, are of increasing interest to domestic horticulturalists, and represent a method of reducing water consumption, particularly in urban and water-stressed environments (Sheikh, 2006; Sankhalkar et al., 2019). They allow complete control over nutrient supply, and have been extensively used to grow tomatoes (Schwarz et al., 2014), including using liquid digestate (e.g. Liedl et al., 2004; Stoknes

et al., 2016), giving a potential for comparison. Therefore, I designed a soilless experiment suppling different concentrations of NH_4^+, to establish whether liquid digestate could be used as a sole nutrient source for tomatoes without alteration .

To evaluate this, the following hypothesis was devised:

(III) Liquid digestate derived from entirely plant-based feedstock is suitable as a sole nutrient source for tomatoes under protected horticulture.

5.2 Methods and Materials

5.2.1 Experimental Design

Tomato seeds (*Solanum lycopersicum*, cv. Ailsa Craig) were sown in cell seed trays in the same low-nutrient, commercially available John Innes Seed and Cutting Compost (Westland Horticulture, Tyrone, Northern Ireland) used in Chapter 3 and Chapter 4 on the 1st March 2022. Trays were irrigated when the surface of the substrate began to dry. Two-litre black square pots (110 (L) x 110 (W) x 165 (H) mm) were filled with top-dressing sand (Boughton, Kettering, Northamptonshire, UK), and the water holding capacity (WHC) calculated as in Chapter 3. Immediately prior to transplantation, each pot was supplied with two times the WHC in dilute fertiliser solutions at concentrations representing six nutrient regimes: 0, 1, 3, 6, 9 and 12 mmol NH_4^+. Each treatment had eight replicates. Seedlings were transplanted at the second leaf stage into 2 cm deep holes made using a dibber on 5th April 2022 (35 days after sowing). An additional amount of dilute fertiliser was applied slowly immediately after transplantation, equivalent to the water holding capacity (thus 3×WHC in total) and the electrical conductivity (EC) recorded using a W.E.T. Sensor (Delta-T, Cambridge, UK).

Daily fertigation treatments were applied, initially to 100% WHC, and then >100% WHC, to flush salts and prevent accumulation (once it was realised that a slight increase had occurred across treatments). Pots were randomly rotated daily to mitigate against environmental gradients in the glasshouse and arranged into rows of even spacing to minimise shading and competition effects. Diluted liquid digestate was stored in sealed opaque buckets at glasshouse temperature.

5.2.2 Non-destructive (in-situ) measurements

Chlorophyll concentration, evapotranspiration and leaf area estimation were calculated in-situ using the methods outlined in Chapters 3 and 4. Volumetric soil water content

(VSWC) and electrical conductivity (EC) were monitored using a W.E.T. Sensor (Delta-T, Cambridge, UK). Soil moisture did not differ between treatments, while electrical conductivity (6.3 in Appendix A) was statistically distinct between treatments (p-value <0.001). Both measures remained stable following fertigation for the duration of the trial.

5.2.3 Destructive (ex-situ) measurements

On 26th May 2022, the 5 replicates closest to the median chlorophyll concentration for each treatment were selected for harvesting. Leaf area and fresh shoot weight were measured as per previous chapters. A soil core (40 mm diameter, 80 mm height) was taken ∼20 mm from the plant stem for each replicate for analysis. Roots were then extracted carefully from the remaining substrate, rinsed initially with tap water followed by deionised water, before being blotted dry using blue roll. Plant samples were dried at 70 °C for 48 hours, until a consistent weight had been reached.

5.2.4 Nutrient analysis

Soil nutrient analysis was conducted for NH_4^+/NO_3^- on subsamples of the cores taken during harvest. Nutrient analysis was conducted as per Chapter 4.3.1, except for using fresh samples for ammonia analysis extracted within a day of harvest and frozen until analysis could be conducted.

Leaf, stem and root samples were measured for C/N separately using the method outlined in Chapter 4.3.1.

Fertiliser nutrient analysis was conducted for three different dilutions of the liquid digestate for validation data. Digestate was placed on an orbital shaker for 30 minutes and diluted with milliQ water to 25%, 50% and 100% (undiluted) strengths. Samples were then placed in a cool box and transferred via courier to NRM laboratories (Coopers Bridge, Bracknell, United Kingdom), where they were analysed according to standard operating procedures (SOP) JAS-510 / JAS-379, based on an aqua-regia digest and ICP-MS analysis, and conforming to BS EN ISO/IEC 17025:2017.

5.2.5 Statistical Analysis

Statistical analysis was performed as in Chapter 3 and 4. Ammonium (NH_4^+) concentration was treated as continuous data for linear modelling, while measurement

timepoints were considered independently from one another, as in previous chapters. Statistical significance was, as in previous chapters, calculated at the 5% level.

5.3 Results

5.3.1 Main Experiment

Visual observations

During the experiment, from around 35 days after transplanting, interveinal chlorosis was observed in the leaves of tomato plants at the higher concentrations of NH_4^+ (Figure 5.1). Growth of all plants were slower than in previous experiments.

Roots under 3 mmol NH_4^+ appeared to be minimally more developed than other concentrations, while there was a clear stunting of roots grown under 12 mmol NH_4^+.

In-situ

Chlorophyll concentration generally decreased over time, except for 9 mmol, which did not significantly differ between the first and last measurement. Figure 5.2b&c both increased initially with increasing concentration, before plateauing and then decreasing - giving a significant (p-value <0.001) negative quadratic linear model which peaked between 6 and 9 mmol NH_4^+. Chlorophyll concentration displayed a more pronounced curve at 25 days after transplanting (Figure 5.2b) than at harvest (Figure 5.2c).

Cumulative evapotranspiration displayed a weak negative correlation with concentration, however only 12 mmol L^{-1} NH_4^+ was significantly (p-value <0.05) lower than other treatments. Daily average evapotranspiration did not significantly vary across treatments, and appeared to remain largely consistent across time before increasing at $\sim$40 days.

Ex-situ

Fresh weight of the whole plant produced a negative quadratic linear model, and was highest at 6 mmol NH_4^+ (p-value <0.005). Dry weight displayed a similar curve and a significant difference between doses (p-value <0.05). Leaf area significantly (p-value $<$ 0.001) differed between treatments, with a negative quadratic curve best describing the relationship with NH_4^+ concentration, which peaked between 6 and 9 mmol L^{-1} NH_4^+.

Fresh and dry weight biomass allocations displayed a significant difference between no liquid digestate application (0 mmol NH_4^+) and all other treatments - which were

not significantly different from one another - with the former allocating 70% more of its dry weight toward the root versus the lowest root mass allocation at 3 mmol NH_4^+ (p-value <0.001).

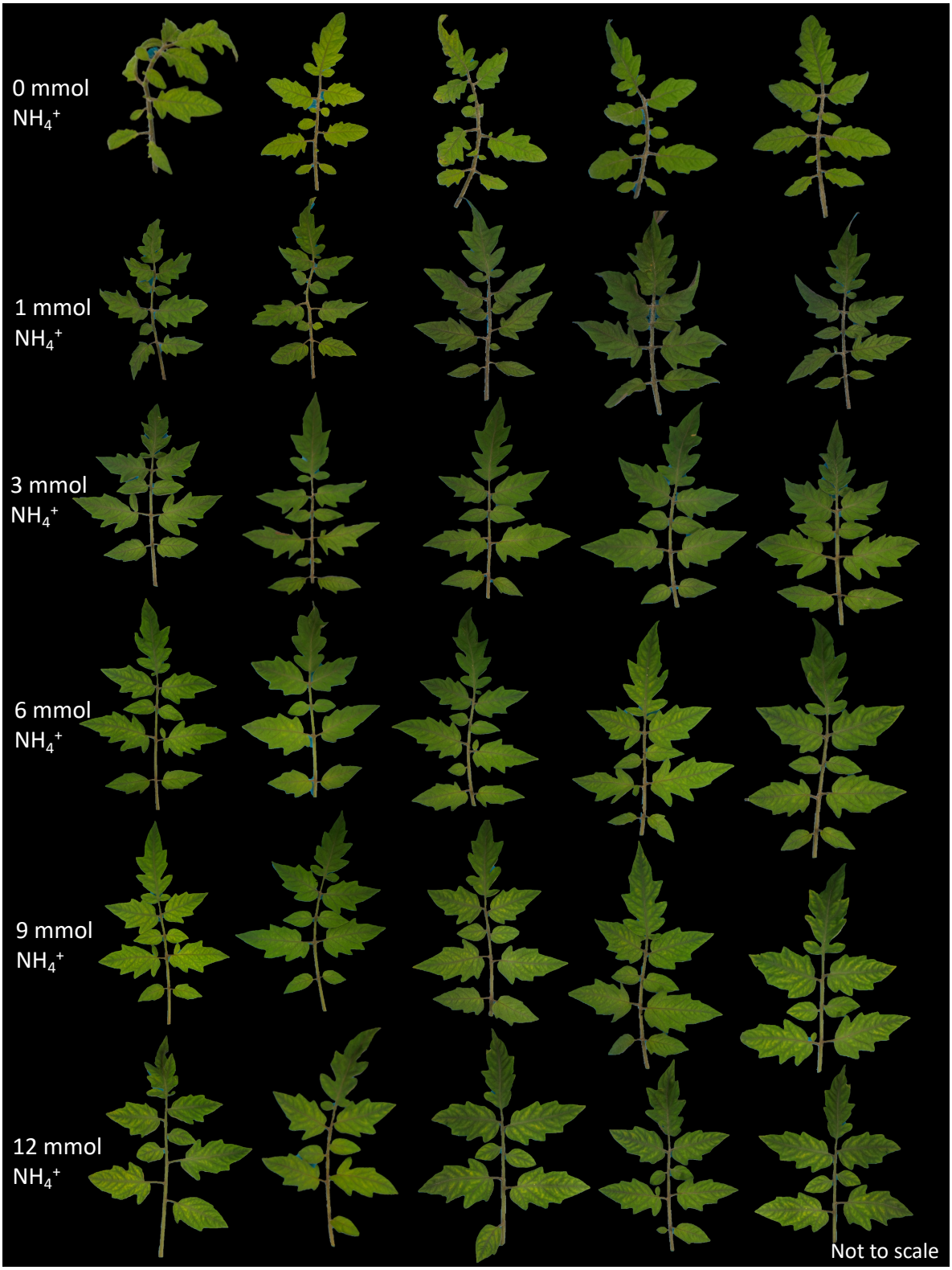

Figure 5.1: Oldest fully expanded leaf of tomato plants grown hydroponically using liquid digestate, under different concentrations of ammonium (NH_4^+).

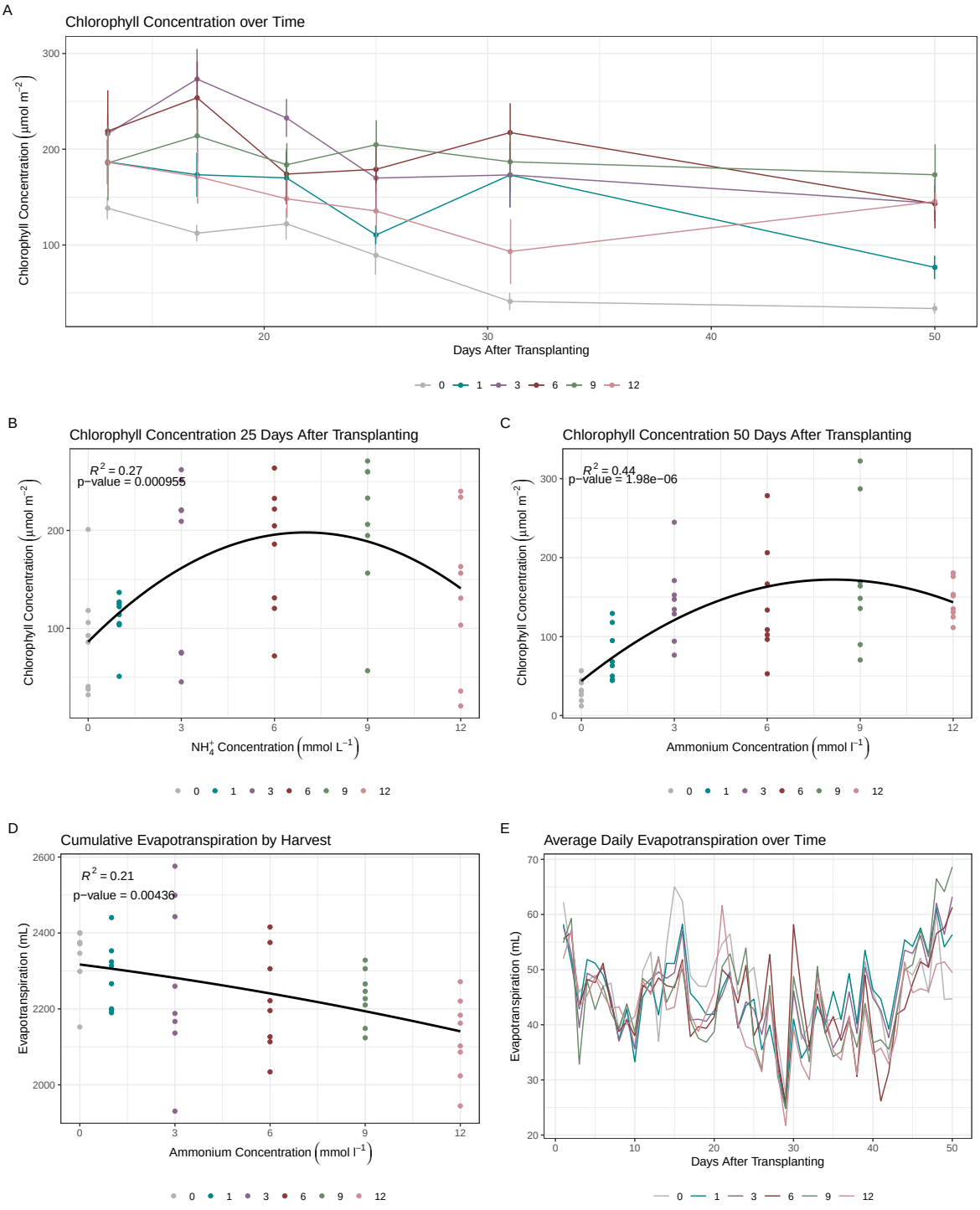

Figure 5.2: Plant physiology measurements of tomato plants grown under different concentrations of liquid digestate, in terms of ammonium (NH_4^+). (A) Range bars represent standard error, while statistical significance versus the control is denoted with *. (B-D) linear models of the same treatments, with R^2 and p-values for the line of best fit. n = 8 for each treatment.

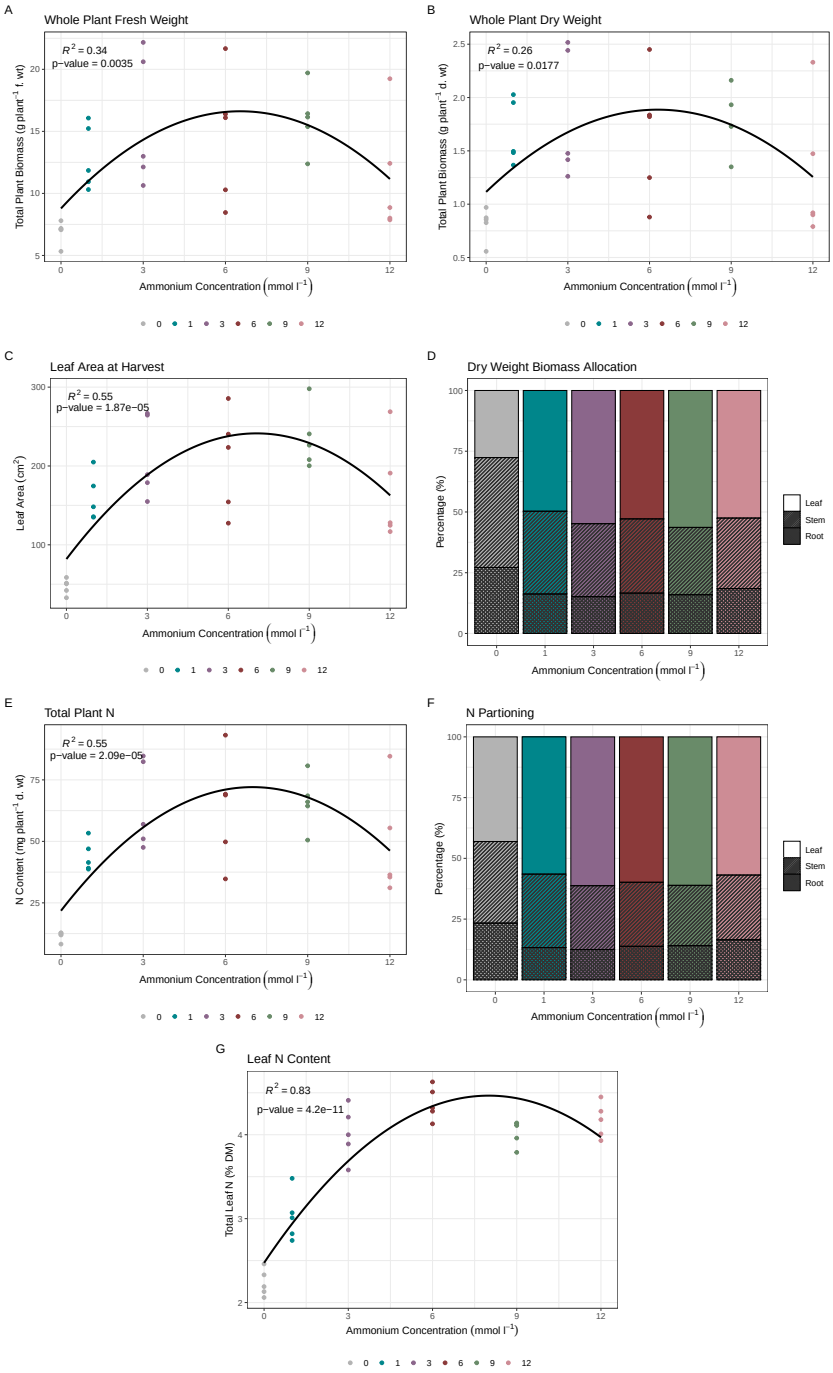

Figure 5.3: Biomass and nutrient measurements of tomato plants grown under different concentrations of liquid digestate, in terms of ammonium (NH_4^+). Linear models have been plotted where appropriate (A-C, E and G), with R^2 and p-values for the line of best fit. Composite bar graphs of dry weight (D) and nitrogen (F) partitioning to the leaf, stem and roots of the same treatments. n = 5 for each treatment.

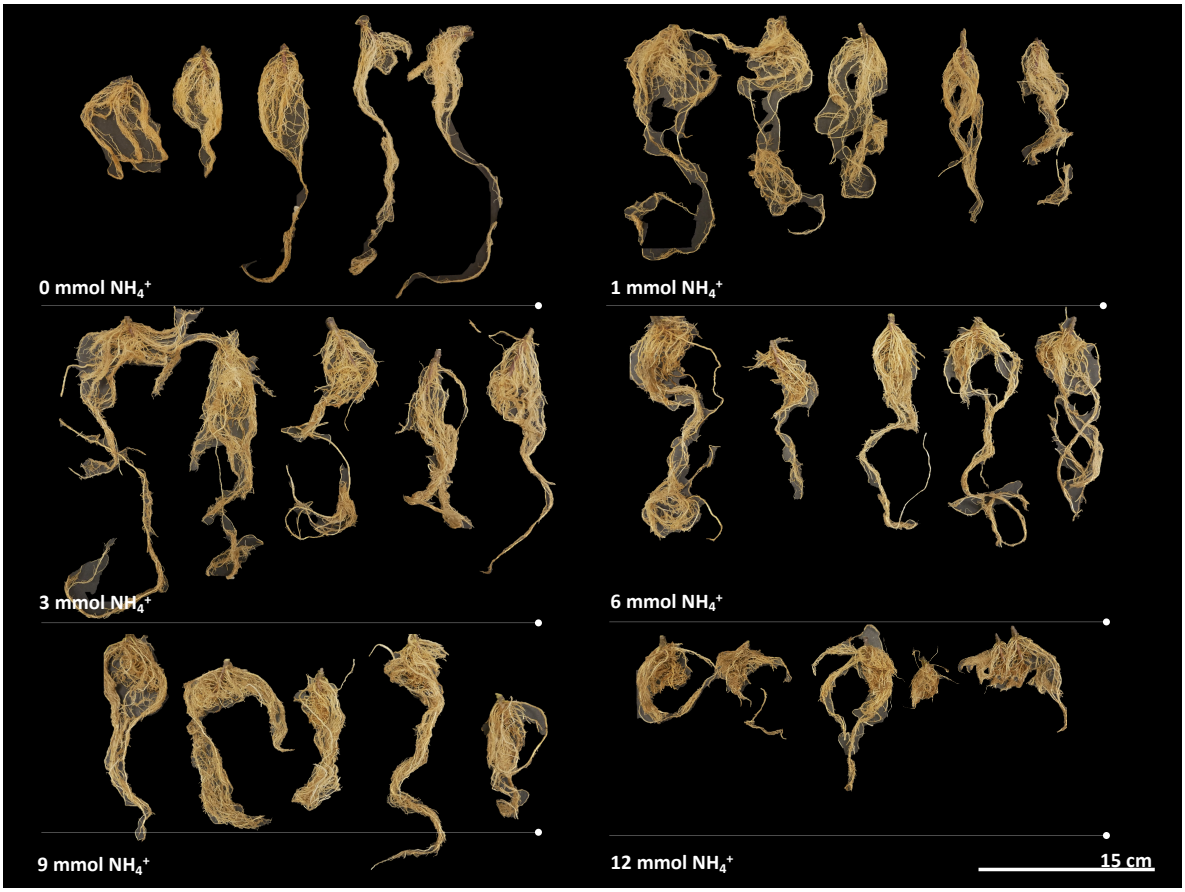

Figure 5.4: Roots of tomato plants treated with liquid digestate at different concentrations, presented in terms of ammonium (NH_4^+) concentration. n = 5 for each treatment.

Nutrient analysis

Total plant nitrogen content displayed a highly significant (p-value <0.001) difference between treatments, and a negative quadratic linear model best described relationship with NH_4^+, with a peak between 6 and 9 mmol NH_4^+. N allocation was significantly (p-value <0.001) higher toward the roots under 0 mmol NH_4^+ versus the lowest N allocation to roots in 3 mmol.

N content as a percentage increased in the leaves and roots with increasing concentrations of NH_4^+ (Figure 5.3g).

Table 5.1: Concentrations of various nutrients in working solutions of different concentrations of a plant-based liquid digestate.

NH_4^+ (mg L^{-1})	**1 mmol**	**3 mmol**	**6 mmol**	**9 mmol**	**12 mmol**
Total Phosphorus (P)	1.16	3.49	6.98	10.47	13.96
Total Potassium (K)	28.32	84.90	169.80	254.77	339.68
Total Copper (Cu)	0.01	0.02	0.04	0.06	0.08
Total Zinc (Zn)	0.03	0.08	0.15	0.23	0.31
Total Sulphur (S)	1.27	3.81	7.63	11.44	15.25
Total Calcium (Ca)	1.76	5.28	10.55	15.83	21.11
Total Iron (Fe)	0.30	0.91	1.81	2.72	3.62
Total Molybdenum (Mo)	<0.01	<0.01	0.01	0.01	0.02
Total Manganese (Mn)	0.03	0.10	0.21	0.31	0.42
Total Nickel (Ni)	<0.01	0.01	0.02	0.02	0.03
Total Selenium (Se)	<0.01	<0.01	<0.01	<0.01	<0.01
Total Cobalt (Co)	<0.01	0.00	0.01	0.01	0.02

5.4 Discussion

5.4.1 NH_4^+ Concentration Effect on Plant Physiology

As in previous chapters, chlorophyll concentration declined over time in the oldest leaves(Figure 5.2a), associated with remobilisation of N to new growth (Masclaux-Daubresse et al., 2010). The lower chlorophyll concentrations at higher NH_4^+ concentrations (Figure 5.2b&c) is likely explained by eventual NH_4^+ toxicity: Fernández-Crespo et al. (2015) used the same cultivar as my experiment (cv. Alisa Craig) and observed an elevated chlorophyll concentration under 'mild' NH_4^+ toxicity (5 mmol NH_4^+) versus the control, but chlorosis beyond 8 mmol NH_4^+. This can be attributed to oxidative stress eventually overwhelming the plant's ability to assimilate additional NH_4^+ (Fernández-Crespo et al., 2015). Figure 5.2b&c concurs with these findings, and demonstrates that, under extreme NH_4^+, plants succumb to toxicity, resulting in lower chlorophyll concentrations. Visual observations of interveinal chlorosis at $\geq$9 mmol NH_4^+ appear to confirm this (Figure 5.1). Chronic exposure to NH_4^+, even at lower concentrations, is enough to induce accelerated senescence due to accumulation of reactive oxygen species (ROS) (Pappi et al., 2021). Thus, under different degrees of NH_4^+ stress chlorophyll response differs, and so a holistic approach is required to understanding response.

Furthermore, the threshold of NH_4^+ toxicity is affected by substrate pH: a more acidic pH was found to induce toxicity at lower NH_4^+ concentrations than nearer neutrality by Feng and Barker (1992). The alkalinity of liquid digestate (Table 3.4) may have buffered some of the effects of this toxicity under moderate conditions, and explain why chlorophyll concentration was highest between 6 and 9 mmol NH_4^+, slightly higher than the 5 mmol observed by Fernández-Crespo et al. (2015), who adjusted nutrient solutions to pH 6.0. This is supported by Stoknes et al. (2016), who found that digeponic systems generally operated best at higher pHs, which they attributed to enhanced nitrification. However, this study used already nitrified (both microbially, mechanically and supplementary) liquid digestate reducing the problem of NH_4^+ toxicity. Additionally, while Fernández-Crespo et al. (2015) and I used the same tomato cultivar, different varieties of tomato can have very different NH_4^+ tolerances: Barreto et al. (2016) observed declining chlorophyll concentration beyond 1 mmol NH_4^+ in cv. 'Money Maker' tomatoes, whilst Horchani et al. (2010) observed elevated chlorophyll concentration (cv. Rio Grande) at 10 mmol NH_4^+, the highest concentration they studied; despite both hydroponic experiments being matched at pH 5.8.

Cumulative evapotranspiration (Figure 5.2d) indicates a response to NH_4^+ toxicity;

at the highest dose (12 mmol L^{-1} NH_4^+), the significantly lower evapotranspiration - likely a combination of reduced leaf area and/or stomatal closure - limits further N uptake in the shoot (Torralbo et al., 2019). Ammonium (NH_4^+) toxicity has also been shown to limit water uptake by disrupting root membranes and preventing uptake of calcium (Ca) (Pill and Lambeth, 1977). Daily evapotranspiration (Figure 5.2e), and its delayed increase over time, may represent a response to nutrient imbalance: an increasing concentration of NH_4^+ likely depressed water uptake even under mild stress, and the considerably lower evapotranspiration relative to other chapters (Chapter 4, Section 4.5.2) may be explained by deficiency in other nutrients (such as P) from growing in an inert medium (Mengel and Kirkby, 2001).

5.4.2 NH_4^+ Concentration Effect on Plant Growth and Nutrient Status

Leaf area and biomass (fresh and dry) revealed similar trends to in-situ measurements, with a peak between 6 and 9 mmol NH_4^+ (Figure 5.3a-c). As established in previous chapters, both deficiency and excess N – particularly in the form of NH_4^+ – reduce biomass production (Roosta et al., 2009). Liquid digestate appears to provide excessive NH_4^+ supply for hydroponic tomato cultivation and other species (e.g. *Physalis peruviana*), stunting growth (Stoknes et al., 2016). While at lower NH_4^+ concentrations there appears to be a near-linear positive relationship between concentration and biomass - consistent with studies on cucumbers (Roosta et al., 2009) - there is a threshold, beyond which plant growth is suppressed (Lugert et al., 2001; Britto and Kronzucker, 2002). Siddiqi et al. (2002) found a 67% reduction in fresh biomass production at 11 mmol NH_4^+ versus a control of 11 mmol NO_3^-. This is likely caused by competitive inhibition of K^+, Ca^{2+} and Mg^{2+} uptake through high levels of NH_4^+, and reduced to photosynthetic rates (Siddiqi et al., 2002; Torralbo et al., 2019). However, the exact point of extreme NH_4^+ toxicity is difficult to isolate and is dependent on a number of genetic and environmental factors – particularly light, which causes a great oxidative stress (Magalhaes and Wilcox, 1984).

The significantly higher percentage of biomass (Figure 5.3d) allocated to the roots in the control treatment than the NH_4^+ treatments was likely a response to severe nutrient deficiency (Hermans et al., 2006). Conversely, NH_4^+ toxicity in the treatments may have caused plants to halt root production, to limit NH_4^+ uptake (Vega-Mas et al., 2017). Although not quantified, Figure 5.4 shows that root length differed across treatments - and was particularly stunted under the highest concentration (12 mmol

NH_4^+). However, the root:shoot ratios observed in my experiment under high NH_4^+ concentrations (Figure 5.3d) are still similar to the control treatments of Ghanem et al. (2011), indicating that root biomass allocation was within normal values. Vega-Mas et al. (2017) observed maintenance of root biomass - even at high NH_4^+ concentrations in Agora Hybrid F1 - owing to greater root plasticity, indicating that partitioning response differs across varieties.

Despite a longer experiment duration than previous chapters, biomass at all concentrations was significantly lower (Figure 3.5 & 4.6). Although 6-9 mmol produced the greatest biomass and leaf area, this does not represent an "optimum" concentration so much as it represents the limit of NH_4^+ tolerance under sole treatment with the liquid fraction of digestate. Under lower concentrations of NH_4^+, where toxicity was less of an issue, biomass production was likely limited versus Chapter 4 due to deficiency in other nutrients – such as P. Stoknes et al. (2016) observed enhanced yield of tomatoes grown commercial compost versus inert media, and attributed this to increased PO_4^{3-} availability in the compost. Thus, even though plants in Stoknes et al. (2016) were suppled with a higher EC (2.3 mS cm^{-1}) of nitrified liquid digestate, derived from food waste, than the highest concentration in my experiment ($\sim$1.7 mS cm^{-1}), PO_4^{3-} deficiency may still have occurred without additional sources.

The low P content of liquid digestate is due to precipitation with Fe, which is added to biogas reactors for desulphurisation (Weimers et al., 2022), and causes much of the P contained within whole digestate to be within the solid fraction (Tambone et al., 2017). Furthermore, the limited supply of P in the liquid fraction is restricted in bioavailability due to digestate's alkaline pH, which favours precipitation as complex Ca-P compounds - in particular hydroxyapatite (HAP, $Ca_5(PO_4)_2OH$) (Güngör et al., 2007). Liquid digestate consists of two major forms of phosphate: HAp and struvite ($MgNH_4PO_4 \cdot 6H_2O$; Güngör et al., 2007). While both forms are largely insoluble, the latter is nearly 8 times as plant-available as the former (Achat et al., 2014). The ratio of HAp to struvite is a largely a function of Ca:Mg and Ca:P, with a value exceeding 2:1 favouring HAp formation (Toor et al., 2005; Liu and Wang, 2019). As Table 3.4 shows, while the Ca:P (1.62) ratio of the liquid digestate I studied does not meet this threshold, Ca:Mg (9.96) is comprehensively exceeded, favouring precipitation as plant-unavailable HAp. While necessary to avoid salt accumulation, daily flushing of pots with working solutions is likely to have prevented P in unavailable forms (e.g. HAp or organic P) from becoming bioavailable, either by phosphate solubilising microbes (PSM) or organic acid exudation by plant roots. It is possible that, over longer treatment interludes, P availability may have been improved.

A combination of low P availability and high NH_4^+ explains the low overall biomass observed at all concentration levels, highlighting a nutrient imbalance when liquid digestate is used as a sole nutrient source (Stoknes et al., 2016; Ronga et al., 2019). While tissue P content was not measured, total plant N was correlated with physiological and growth measurements (Figure 5.3e). While larger plants assimilated more N, initial increases in relative leaf N with increasing NH_4^+ concentration (Figure 5.3g) likely represent the additional NH_4^+ assimilated in leaves. Consequently, the eventual plateau is likely in response to release of NH_4^+ through deamination when toxicity levels were reached (Magalhaes and Wilcox, 1984).

Accumulation of NH_4^+ in the roots (Figure 5.3f) displayed a similar increase with shoot NH_4^+ concentration; at moderate concentrations this likely reflects N assimilation, whilst the plateau at higher concentrations represents the roots' insufficient ability to assimilate further NH_4^+ (Cruz et al., 2006; Vega-Mas et al., 2015).

5.5 Conclusions

- Unamended plant-based liquid digestate is not suitable for use in hydroponic systems, due to high NH_4^+, which causes toxicity symptoms and prevents application in suitable quantities for other nutrients (e.g. P and Mg).
- Liquid digestate must be nitrified, and likely amended, before it is a suitable nutrient source for tomatoes.

As a result of these experimental data, there is strong evidence to **reject** Hypothesis III, and thus liquid digestate is unsuitable as a sole nutrient source for tomato plants.

Chapter 6

General Discussion

This project sought to understand and characterise the suitability of the liquid fraction of a plant-based digestate for application within domestic horticulture. As such, experiments were designed to mimic realistic domestic practices whilst yielding scientific insights within a short growing period. Successive trials progressively tested the nutritional benefits of the digestate by initially using an advertised lower-nutrient substrate in Chapter 3, which was further depleted by blending with turf dressing sand in Chapter 4, and then switching to liquid digestate as a sole source of nutrient addition in Chapter 5. By comparing growth of plants receiving liquid digestate versus synthetic alternatives, the research explored the role that liquid digestate could play in meeting the emerging market of domestic horticulture, while also examining its potential relevance to commercial growers.

As a result of these trials, I found that:

- Liquid digestate did not offer comparable performance to synthetic fertiliser at the manufacturer's recommended concentration.
- Liquid digestate did not offer comparable performance to synthetic fertiliser when matched for nitrogen concentration.
- Liquid digestate is not suitable as a sole nutrient source.

The following discussion presents these findings in a wider context, and outline further research that would be required to improve performance.

6.1 Liquid Digestate as a Fertiliser in typical horticultural systems

Based on the findings of the first two experiments, liquid digestate does not represent a suitable fertiliser for complete nutrient supply of plants without amendment. This is largely on account of low PO_4^{3-} levels, which likely explained some of the stunted growth seen in Figure 4.6 relative to the much higher P-containing synthetic fertiliser. While this has been observed in previous studies (e.g. Stoknes et al., 2016), these studies almost exclusively used animal-derived products, and so it is perhaps more intuitive that these were unsuitable for plant growth than the plant-based liquid digestate I studied, on account of original feedstock nutrient composition. However, the experimental results obtained in prior chapters demonstrate that, while a more homogenous product can be obtained through controlling feedstocks (based on standard deviation as a proportion of the mean in Table 2.1 & 6.1), the nutrient ratios of liquid (high NH_4^+ and K, low P) and presumably solid fractions (low NH_4^+ and K, high P) remain similarly unsuitable for tomato cultivation, when used independently.

Furthermore, as Chapter 3 showed, current application concentrations recommended by the manufacturer are far too low, and should be increased. Other studies applying liquid digestate reported similar results, with enhanced fresh weight yield in tomatoes at double the recommended concentration (Liedl et al., 2006). However, due to the imbalance of nutrients in liquid digestate for tomato (excess NH_4^+, insufficient P and Mg), combined with the nutrient heterogeneity of commercial potting composts, it is difficult to give an optimum concentration for application. However, as Chapter 5 shows, recommendations should be based on NH_4^+ concentration, as this limits growth when applied in excess (Stoknes et al., 2016). While moderate applications of liquid digestate likely limit plant-available P, this may not be a problem in the domestic horticulture market, where risk-averse suppliers ensure that growing media have ample inherent nutrition, including P. In fact, excess P was found in urban garden soils as a result of composting (Small et al., 2019)). Consequently, the P limitation I likely observed (Figure 4.6) by using a lower-nutrient media is not typically encountered. Beyond home horticulture, the P content of liquid digestate may actually be advantageous in areas that: (a) already have a high P content or (b) are vulnerable to P, as it prevents overapplication and facilitates better fertiliser management.

Solid digestate typically has a high PO_4^{3-} level (Reuland et al., 2021*b*), due to precipitation through Fe-sowing in the reactor, which could help to elevate P levels when applying liquid digestate. These reserves remained high in raised beds, even a year after

Table 6.1: Average nutrient concentration for the plant-based liquid digestate from all nutrient analyses received and conducted in this study. Asterisks (*) denote parameters where n <3.

Determinand on a fresh weight basis	**Units**	**Average ±SD (where possible)**
pH 1:6 [Fresh]		8.35 *
Oven Dry Solids	%	4.77 ±0.70
Total Kjeldahl Nitrogen	% w/w	0.44 ±0.10
Ammonium Nitrogen (NH_4^+-N)	mg kg^{-1} FW	3073.00 ±551.55
Nitrate Nitrogen (NO_3-N)	mg kg^{-1}	211.00*
Phosphate (PO_4-P)	mg kg^{-1}	221.00*
Total Phosphorus (P)	mg kg^{-1}	406.40 ±119.24
Total Potassium (K)	mg kg^{-1}	6662.67 ±619.85
Total Magnesium (Mg)	mg kg^{-1}	83.90 ±5.19
Total Sulphur (S)	mg kg^{-1}	309.05 ±88.94
Total Copper (Cu)	mg kg^{-1}	1.66 ±0.39
Total Zinc (Zn)	mg kg^{-1}	6.16 ±1.57
Total Sodium (Na)	mg kg^{-1}	1350.00*
Total Calcium (Ca)	mg kg^{-1}	546.00 ±193.62
Total Iron (Fe)	mg kg^{-1}	78.43 ±3.30
Total Molybendum	mg kg^{-1}	0.32 ±0.00
Total Manganese	mg kg^{-1}	8.74 ±0.21
Total Nickel	mg kg^{-1}	0.69 ±0.01*
Total Selenium	mg kg^{-1}	0.08 ±0.00
Total Cobalt	mg kg^{-1}	0.41 ±0.41

switching nutrient source to liquid digestate and growing tomato, potato and broccoli digestate (Liedl et al., 2006). This may also represent an additional caveat to the results presented in this thesis: over longer study periods, the P contained in non-bioavailable forms - such as HAp and inorganic P - may be made available through biological or chemical weathering, and thus may positively support plant growth in a way which was not attained in the short cropping cycles of my experiment. In light of this, and in recognition of the different release rates of nutrients in different digestate fractions, it may be both commercially and nutritionally sensible to recommend co-applying the liquid and solid fraction of digestate. The solid-liquid nature of digestate may also prove advantageous, as different fractions could be applied under different conditions.

The solid fraction, as it is less vulnerable to leaching (on account of temporary immobilisation due to high C content; Egene et al.2021) may be applied earlier in the year, and the liquid used to supplement growth during the drier seasons. Economically, this could represent a year-round source of income to biogas operators; environmentally this could reduce leaching events. Although society generally assumes that a higher microbial content of organic fertilisers will inherently benefit plant growth, often microbial communities contained in digestate favour immobilisation of available nitrogen following application (e.g. Reuland et al.2022), or have no impact (e.g. Barduca et al.2021). Comparing liquid digestate in unautoclaved and autoclaved forms indicated no beneficial effects of microbial communities in digestate, even with a potentially higher N supply (Figure 4.6). While additional studies would be required to characterise these communities and their longer-term impacts, and more accurately match for N, this suggests that liquid digestate could be concentrated without loss of performance. This would have several commercial benefits: reduced transport costs, increased product stability and potency (a common customer issue on Amazon was application amount and frequency). These economic benefits likely translate into environmental savings too, in terms of transport-related emissions; and improved water recovery, enhancing circularity. Industry-led attempts at concentrating digestate are already starting to be applied: HRS Heat Exchangers report an 80% reduction in water volume and an improved odour (through conversion of NH_4^+ into ammonium sulphate) through using surplus heat from CHP/biogas boilers to superheat digestate in a vacuum (HRS Heat Exchangers, 2022).

6.2 Liquid Digestate in Hydroponic Systems

Unamended liquid digestate does not appear to be suitable for hydroponic culture, as nutrient imbalance caused NH_4^+ toxicity (Chapter 5). Other studies have reported similar effects using digestate-hydroponic set-ups (sometimes referred to as "digeponic") for tomato cultivation (Liedl et al., 2004; Stoknes et al., 2016). Furthermore, the high concentration of NH_4^+ in digestate prohibits increasing application concentration to level that would supply less plentiful nutrients (especially P) at sufficient amounts. Thus, nutrient toxicity and deficiency occur together, although different elements are involved. While some cultivars of tomato (e.g. cv. 'Rio Grande'), are more tolerant of NH_4^+ than others (e.g. cv. 'Money Maker') (Horchani et al., 2010; Barreto et al., 2016) - it is highly unlikely that any varieties of tomato would be sufficiently tolerant to allow the necessary quantities of other nutrients to be provided using the liquid digestate.

A potential solution to this issue would be the nitrification of liquid digestate, through aeration or use of a biofilm (Weimers et al., 2022). This would increase NO_3^- concentration relative to NH_4^+, providing a more favourable N source to plants, and allowing a higher concentration of liquid digestate to be applied before NH_4^+ toxicity is encountered. This approach was suitable to grow lettuce and parsley (Stoknes et al., 2016). However, even following nitrification liquid digestate may not be a balanced fertiliser: Weimers et al. (2022) found deficiency in P, S and boron (B) when growing pak choi (*Brassica rapa,* ssp. Chinensis) hydroponically using a biofilm-nitrified liquid digestate derived from household and animal waste.

Consequently, it may still be necessary to supplement nitrified liquid digestate with additional nutrients to ameliorate deficiency in tomato plants. Liedl et al. (2004) reported comparable results of a digestate poultry liquid to a commercial hydroponic solution when supplementing with magnesium sulphate ($MgSO_4$) to raise magnesium (Mg) concentration to ∼94 mg L^{-1} and tricalcium phosphate ($Ca(NO_3)_2$) to raise NO_3^- to ∼272 mg L^{-1}. Experiments on other crops have also been successful: Weimers et al. (2022) were able to increase yields of pak choi (*Brassica rapa, ssp. chinensis*) relative to a standard mineral fertiliser following matching for P, S, Mg, manganese (Mn), molybdenum (Mo), and B by addition of phosphoric acid ($H3PO_4$), magnesium sulphate ($MgSO_4$), manganese sulphate ($MgSO_4$), sodium molybdate (Na_2MoO_4) and boric acid (H_3BO_3), respectively.

In an exploratory trial using three specimens from Chapter 5, nutrient source was switched at each NH_4^+ concentration to either (a) liquid digestate amended with 200 mg L^{-1} potassium phosphate monobasic (KH_2PO_4) or (b) synthetic fertiliser with the (c) original unamended liquid digestate treatment for comparison. While insufficient sample size prevented any statistical analysis, plants appeared to respond to this supplementation (Appendix C -6.4), and thus amending the liquid digestate used in this study merits further attention.

Finally, the alkaline pH of liquid digestate may be problematic in hydroponic setups, as the optimum growth response of tomato is between pH 5.5-6.5 (Short et al., 1998). While studies typically adjust pH to within this range, the impact of pH on yield in digeponic systems is mixed: Stoknes et al. (2016) observed higher yields of parsley at a lower pH (pH 8.1 reduced to 6.5), but a reverse trend for lettuce. Optimal nitrification under alkaline conditions to some extent compensates for above-optimal pH for plant growth (Stoknes et al., 2016). In cucumbers, Tyson et al. (2008) recommends maintaining pH between 7.5-8.0 to maximise total yield, despite a depression of early yield. Whilst studying the effects of altering digestate pH may yield interesting results, it may

not be practical for rudimentary or domestic digeponic systems, as aerating digeponic systems naturally reduces dissolved CO_2 and increases pH (Stoknes et al., 2018), meaning that near-continuous adjustment would be required. However, supplementation to address nutrient deficiency may unintentionally lower pH: Liedl et al. (2004) increased P supply through addition of phosphoric acid (H_3PO_4), increasing total P supply to between 233.2 and 308 mg L^{-1}, and reducing pH to 5.5-6.8. Therefore, with alteration, liquid digestate may form the basis for a viable nutrient solution in hydroponic systems.

6.3 The Future of Digestate-Based Fertilisers and Further Research

The widespread adoption of digestate-based fertilisers will be determined by availability and efficacy, as well as consumer attitudes towards it. Thus, the nutritional, socioeconomic and environmental credentials must be examined more thoroughly. While anaerobic digestate is broadly assumed to be a renewable source of energy and fertiliser, this is a function of circularity. For instance, the system ceases to be renewable if additional fertiliser is required to produce enough feedstock for the process, or there are substantial losses to the atmosphere. More academic work is required in understanding the role in which anaerobic digestion can play in meeting future energy and fertiliser demand, as well as refining the process to ensure optimum circularity. Emerging methods of digestate concentration represent interesting new avenues for utilising surplus heat from the system, to reduce overall water use, and increase the nutrient concentration of digestate. Odour reduction and conversion of NH_4^+ into ammonium sulphate ($(NH_4)_2SO_4$) may also make the product more saleable (HRS Heat Exchangers, 2022). However, to my knowledge, this has yet to be comprehensively evaluated, and thus a study aimed at making liquid digestate more visually and aromatically appealing may yield commercial benefits.

During efforts to improve performance, consideration must also be given to effects on sustainability. While supplementing with mineral nutrients may enhance crop growth, there exists a threshold whereby liquid digestate is no longer more "environmentally-friendly" than synthetic alternatives. A method of overcoming this, as Liedl et al. (2004) proposed, may be to incorporate the struvite contained within the solid fraction of digestate into the liquid fraction (e.g. through acid-dissolution (Ariyanto et al., 2012)), minimising the amount of additional inputs required to make liquid digestate successful as a fertiliser. This could additionally work for the organic P contained in digestate: Xu

et al. (2023) observed disruption to organic P flocculates following combination with ascorbic acid in sludge, increasing availability. However, this approach must again be validated by further study.

While liquid digestate occupies a far greater volume than the solid fraction, making it more difficult to store on-site, the solid fraction may be more economically valuable in the home horticulture market, particularly as a result of the proposed ban of horticultural peat in the UK by 2024 (Department for Environment, 2022). However, a holistic approach may prove mutually advantageous: advertising the liquid fraction in conjunction with compost made from the solid fraction may make it easier to recommend crop-specific optimum application rates of liquid digestate, and the deficient nutrients in one are likely to be ameliorated by the other (and vice versa). Similarly to the liquid fraction, it is likely that solid digestate must be refined in order to be optimal for plant growth: either through composting or, as Stoknes et al. (2016) advocate, vermicomposting. Following aeration and mixing of the digestate with sawdust to reduce compaction, Krishnasamy et al. (2014) was able to achieve significant N mineralisation as NO_3^- to produce nutrient-rich vermicompost.

Significant barriers (or conversely facilitators) to widespread adoption of digestate-based fertilisers in the domestic horticulture market include, but are not limited to: regulation, energy prices (and analogously fertiliser prices), subsidy provision, transportation and storability, efficacy, and marketing. These are dynamic factors, and thus continual monitoring and high adaptability is necessary for long-term success. Digestate, in both fractions, is an interesting alternative to synthetic fertiliser, and merits further study.

6.4 General Conclusions and Recommendations

By conducting empirical experiments and reviewing existing literature, the following recommendations are made:

- Current dose recommendations for commercially-available liquid digestate are too low for soil-based systems, and should be increased in order to perform better versus synthetic alternatives.
- Unamended liquid digestate is not a complete nutrient solution and should instead be considered a N-K fertiliser.
- The high NH_4^+ concentration in liquid digestate restrains plant growth if applied

at high concentrations, and the proportion of NO_3^- must be increased before it is suitable for use in hydroponics.

- If unamended liquid digestate is to be used in either soilless or soil-based systems, it should be applied in conjunction with a high P source, such as the solid fraction of digestate and/or an immediately bioavailable P source.
- Liquid digestate could be improved as a fertiliser through supplementation with additional nutrients, or nitrification through aeration or treatment with a biofilm.
- Liquid digestate can likely be concentrated without losing performance, although further study is required to confirm this.

Appendix

A. Environmental Conditions

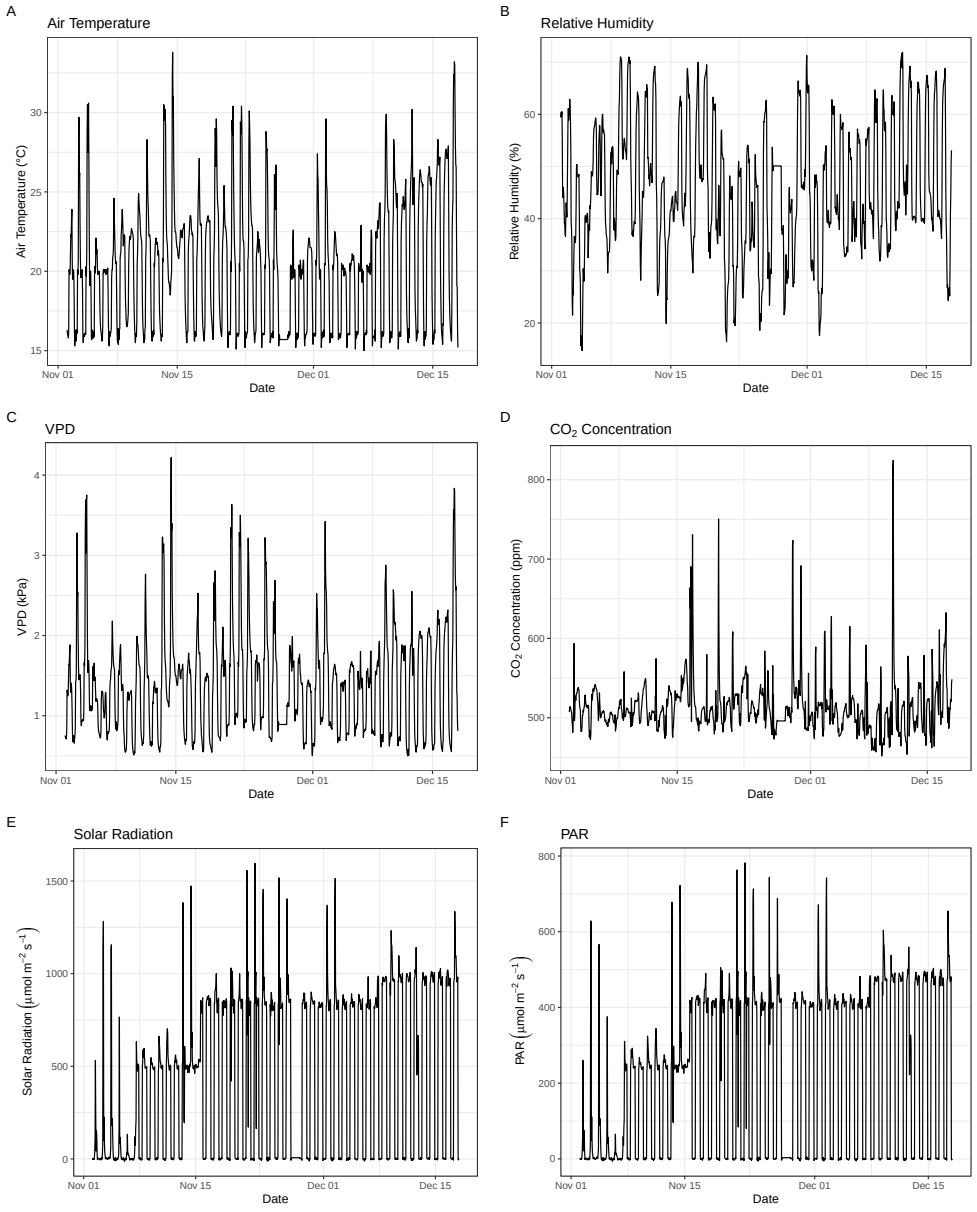

Figure 6.1: Environmental conditions for the duration of Experiment 1 in Chapter 3. (A) Air temperature (°C), (B) relative humidity (%), (C) vapour pressure deficit (VPD), (D) CO_2 concentration, (E) Solar radiation (μmol $m^{-2}s^{-1}$) and (F) photosynthetically active radiation (PAR) (μmol $m^{-2}s^{-1}$)

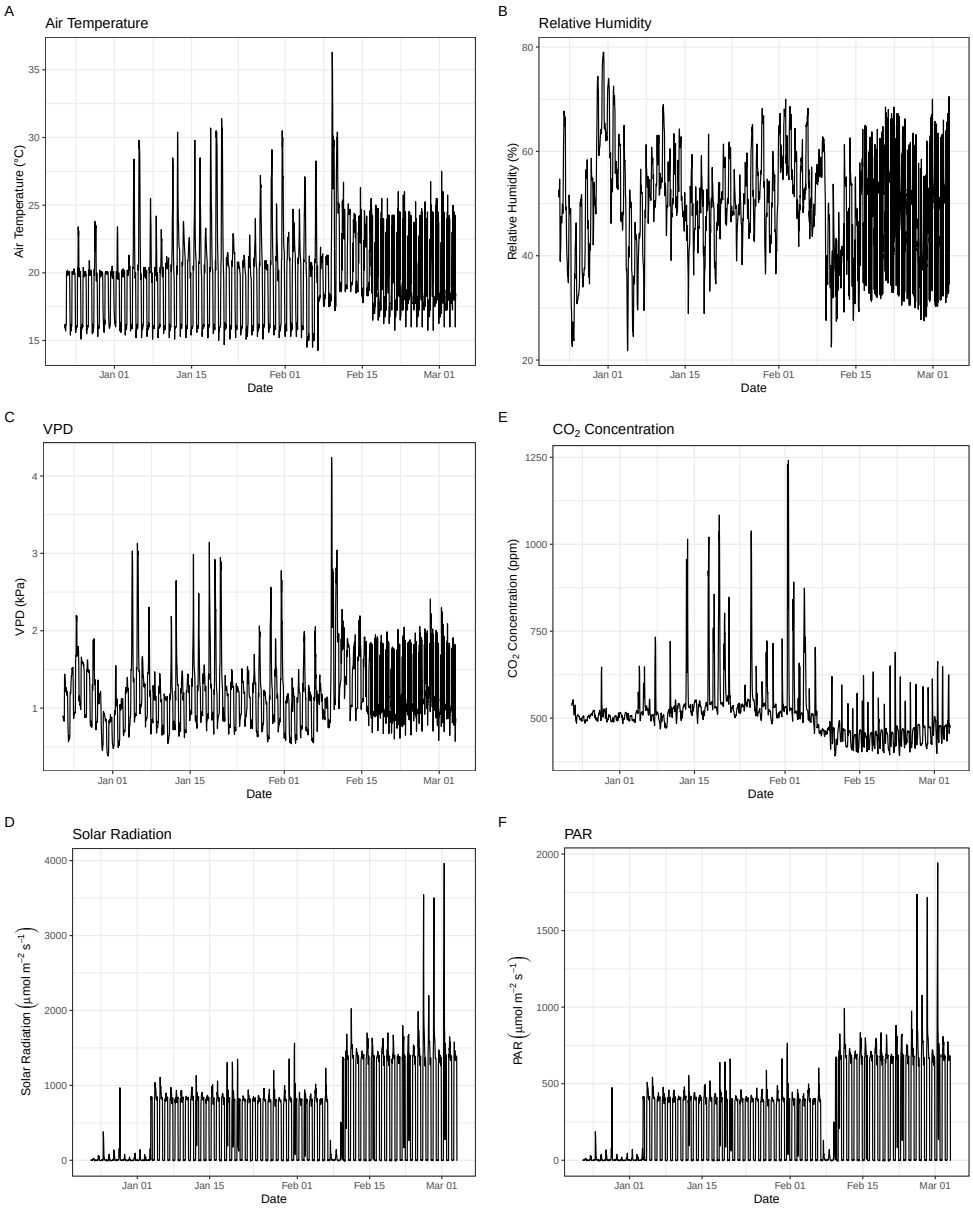

Figure 6.2: Environmental conditions for the duration of Experiment 2 in Chapter 4. (A) Air temperature (°C), (B) relative humidity (%), (C) vapour pressure deficit (VPD), (D) CO_2 concentration, (E) Solar radiation (μmol $m^{-2}s^{-1}$) and (F) photosynthetically active radiation (PAR) (μmol $m^{-2}s^{-1}$)

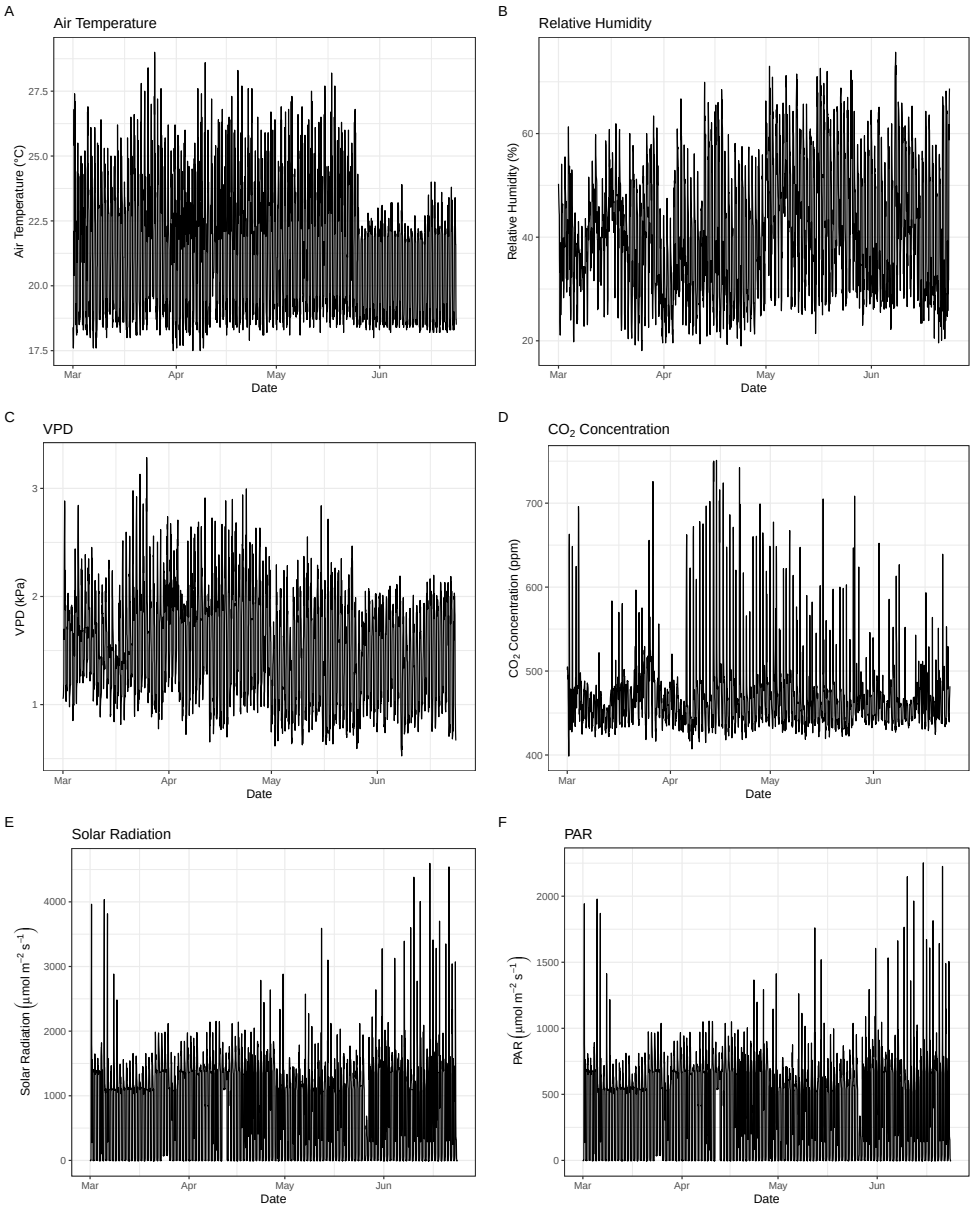

Figure 6.3: Environmental conditions for the duration of Experiment 3 in Chapter 5. (A) Air temperature (°C), (B) relative humidity (%), (C) vapour pressure deficit (VPD), (D) CO_2 concentration, (E) Solar radiation (μmol $m^{-2}s^{-1}$) and (F) photosynthetically active radiation (PAR) (μmol $m^{-2}s^{-1}$)

B. Nutrient Analysis

Table 6.2: Nutrient solutions of De Kreij et al. (1997) and average liquid digestate composition of Table 2.1, normalised for $NH_4{}^+$.

Nutrient/element	**Starter solution**		**Root environment (range)**		**Refilling solution**		**Average Liquid Digestate from Energy Crops and/or Manure**	
	Recommended	Normalised $NH_4{}^+$	Recommended	Normalised $NH_4{}^+$	Recommended	Normalised $NH_4{}^+$	Recommended	Normalised $NH_4{}^+$
NO_3	23	230	30.5	101.7	10.75	10.75		
$NH_4{}^+$	0.1	1	0.3	1	1	1	51.5	1
K	8	80	10.6	35.3	6.5	6.5	80.7	1.6
Ca	10	100	13.25	44.2	2.75	2.75	44.4	0.86
Mg	4.5	45	4.5	15	1	1	9.6	0.19
SO_4	6.8	68	6.75	22.5	1.5	1.5		
P	1	10	1	3.3	1.25	1.25	80.7	1.6
HCO_3	< 1		0.5	1.7				
Na	< 12		6.1	20.2	0	0		
Cl	< 15		7.6	25.2	0	0		
Fe	2.5×10^{-2}	2.5×10^{-1}	3.5×10^{-2}	1.0×10^{-1}	1.1×10^{-2}	1.1×10^{-2}	3.19	6.2×10^{-2}
Mn	5.0×10^{-3}	5.0×10^{-2}	3.0×10^{-4}	1.0×10^{-3}	1.0×10^{-3}	1.0×10^{-3}	2.6×10^{-1}	5.1×10^{-3}
Zn	7.0×10^{-3}	7.0×10^{-2}	1.1×10^{-2}	3.5×10^{-2}	6.5×10^{-3}	6.5×10^{-3}	4.0×10^{-1}	7.7×10^{-3}
B	5.0×10^{-2}	5.0×10^{-1}	1.3×10^{-2}	4.4×10^{-2}	2.8×10^{-3}	2.8×10^{-3}		
Cu	7.5×10^{-4}	7.5×10^{-3}	4.5×10^{-3}	1.5×10^{-2}	1.0×10^{-3}	1.0×10^{-3}		
Mo	5.0×10^{-4}	5.0×10^{-3}	6.8×10^{-3}	2.3×10^{-2}	1.5×10^{-3}	1.5×10^{-3}		

C. Exploratory Supplementation Trial

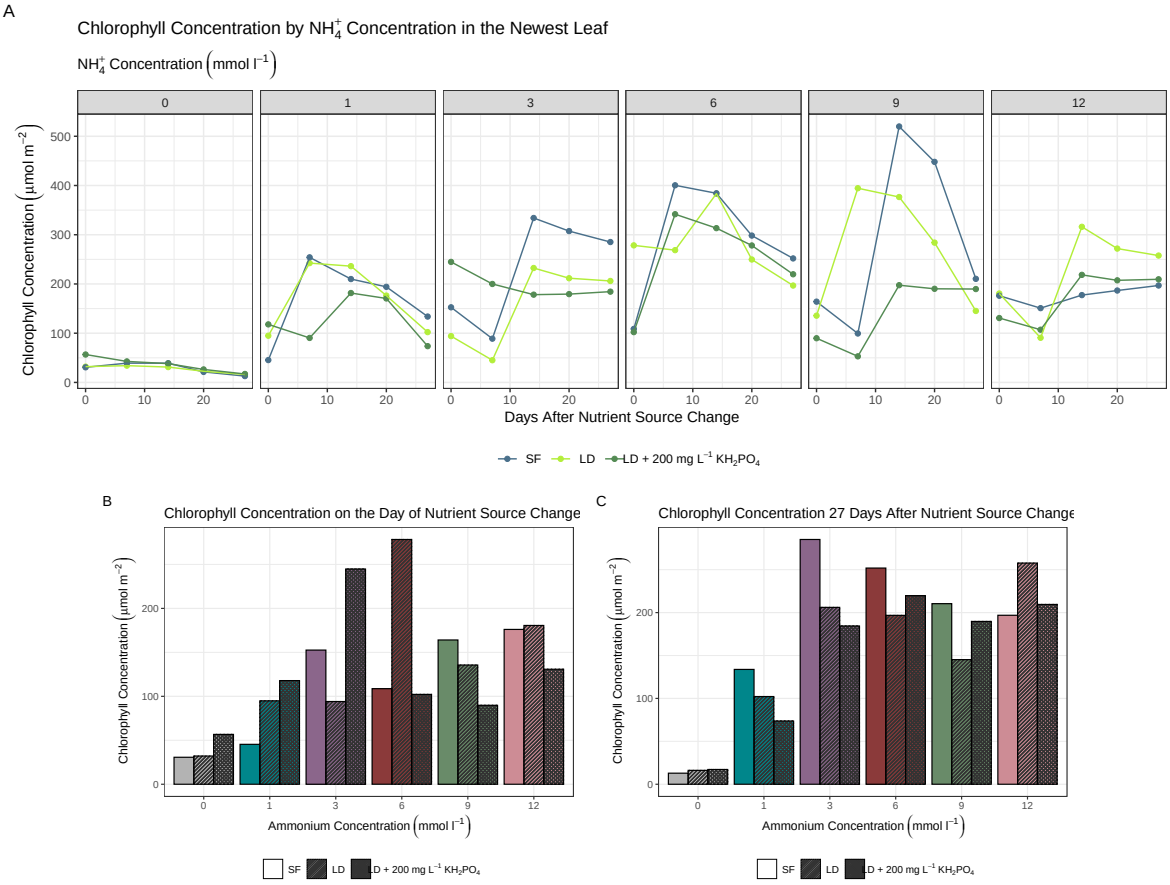

Figure 6.4: Physiology measurements under different mmol L^{-1} NH_4^+ concentrations of synthetic fertiliser (SF), liquid digestate (LD) and liquid digestate amended with 200 mg L^{-1} KH_2PO_4 (LD + 200 mg L^{-1} KH_2PO_4).

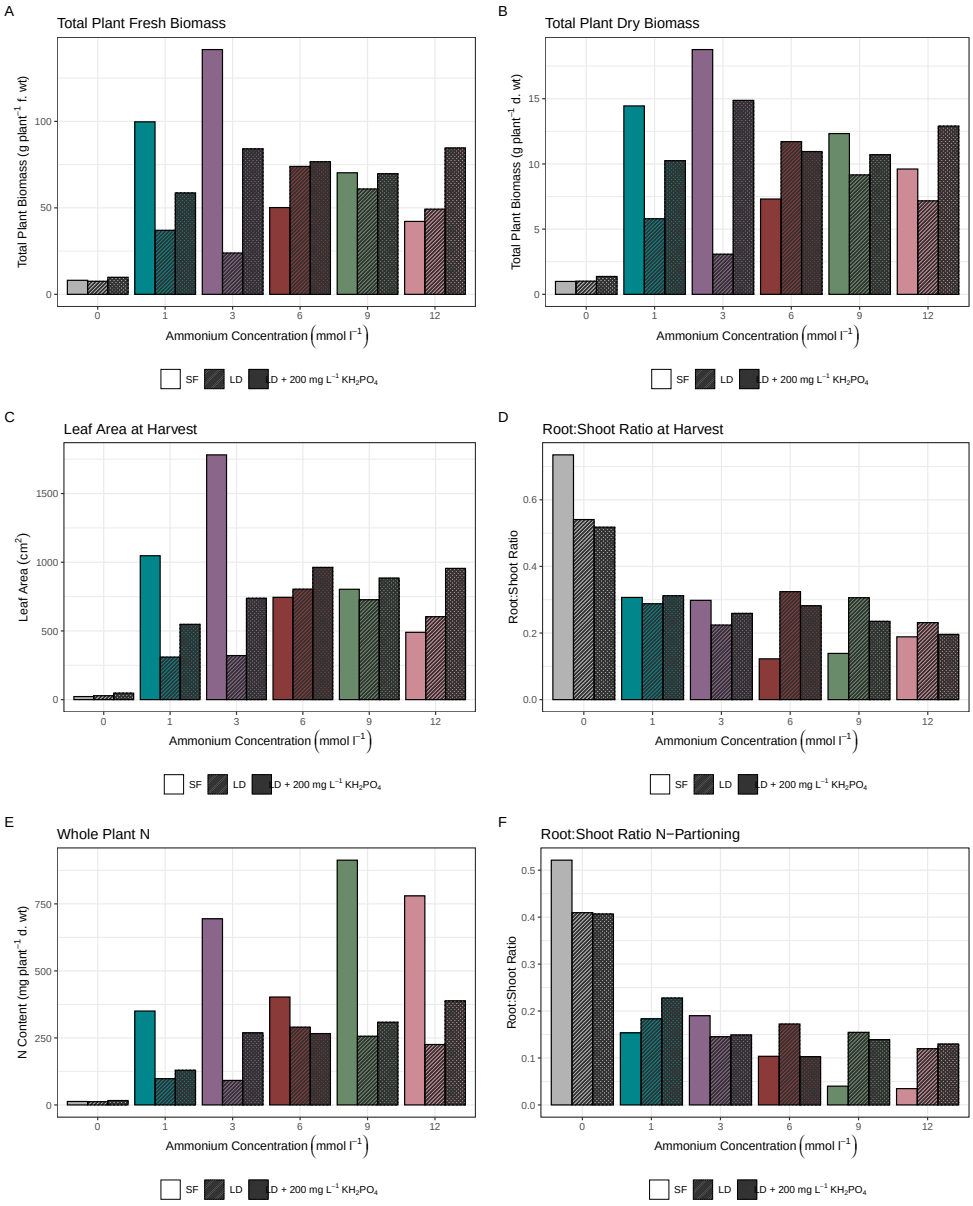

Figure 6.5: Plant growth and nutrient status under different mmol L^{-1} NH_4^+ concentrations of synthetic fertiliser (SF), liquid digestate (LD) and liquid digestate amended with 200 mg L^{-1} KH_2PO_4 (LD + 200 mg L^{-1} KH_2PO_4).

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