Comparison of four chicken manure based bioponic solutions with conventional hydroponics in terms of lettuce quality and quantity produced, and the loss of nitrogen in bioponic systems

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Diplôme : Master en bioingénieur : sciences agronomiques, à finalité spécialisée
Année académique : 2021-2022
URI/URL : http://hdl.handle.net/2268.2/16300

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This work was carried out in “Centre de Recherche en Agriculture Urbaine” (C-RAU) in Gembloux Agro-Bio Tech (Gembloux, Belgium; University of Liège) and in the framework of the SWIM project, financed by the European Union.

Acknowledgements:

This Master’s thesis could not have been completed without the intervention of several people to whom I would like to send my most honest gratitude.

I would like to thank:

My supervisor, Professor Haissam Jijakli, for his assistance and many pieces of advice.

Iris Szekely for her assistance, her availability and her constant follow-up.

Professor Sébastien Massart, Professor Pierre Delaplace and Professor Gilles Colinet for accepting to take part in the jury of this thesis.

Jimmy Bin and Cyril Voss for their assistance in setting up the experiments.

Gembloux Agro-Bio Tech for giving me the opportunity to follow my major.

Centre de Recherche en Agriculture Urbaine (C-RAU) for allowing me to have access to the resources and material for the experimental phase.

My friends and family members for their moral and/or financial support for the entirety of my cursus.

RESUME

Suite à la diminution des financements alloués à la crise des réfugiés Sahraouis, le panier alimentaire moyen fut réduit en termes de quantité et de diversité, surtout en matière de fruits et légumes. Avec l'intention de promouvoir l'agriculture locale, des fientes de poules ont subi une digestion anaérobie, suivie d'une digestion aérobie pour créer quatre solutions bioponiques avec différentes concentrations initiales en minéraux, basées sur l'azote (N) (65mgN/L, 90mgN/L, 115mgN/L and 140mgN/L) pour la culture de laitues. Les efficiences de nitrification furent calculées, les causes de perte en azote furent discutées, plusieurs paramètres physiques furent suivis et la bioponie fut comparée à l'hydroponie conventionnelle.

La bioponie et l'hydroponie conventionnelle étaient égales en termes de quantité produite. Cependant, la bioponie a abouti à des laitues plus riches en calcium, magnésium, zinc, manganèse et sodium sur poids frais. Mais, celles produites en hydroponie étaient plus riches en azote.

De plus, il serait préférable de créer des solutions nutritives bioponiques avec des concentrations initiales en azote entre 65mgN/L et 90mgN/L, puis de fractionner les ajouts de la matière organique durant la culture. Effectivement, toutes les différentes concentrations ont abouti à des productivités similaires. Néanmoins, des concentrations initiales plus élevées ont conduit à des pertes de minéraux plus importantes et des efficiences de nitrification plus faibles.

Enfin, la perte d'azote est probablement liée à l'incorporation de cet élément dans la biomasse bactérienne, la sédimentation des résidus solides sur les systèmes NFT et les racines des plantes, et à la volatilisation de l'ammonium. Cette dernière pourrait être responsable de jusqu'à 30% des pertes en azote.

Mots clés: Bioponie; Hydroponie organique; Nitrification; Digestion aérobie; Digestion anaérobie; Fertilisant organique; Déjection de poules; Laitues; Pertes d'azote; Prélèvement de nutriments; Solution nutritive

ABSTRACT

Following the decrease in fundings for the Sahrawi refugees crisis, the average food basket has shrinked in terms of quantity and diversity, especially with fresh vegetables. With hopes of promoting local agriculture, chicken droppings underwent anaerobic digestion then aerobic digestion to create four bioponic solutions with different initial concentrations in minerals based on nitrogen (N) (65mgN/L, 90mgN/L, 115mgN/L and 140mgN/L) for lettuce culture. The nitrification efficiencies were calculated, the causes of the loss of nitrogen were discussed, several physical parameters were monitored and bioponics was compared to conventional hydroponics.

Bioponics and conventional hydroponics were equal in terms of the quantity produced. However the former led to lettuce richer in calcium, magnesium, zinc, manganese and sodium based on fresh weight. Conversely, those cultivated in hydroponics were richer in nitrogen.

In addition, it would be best to create bioponic nutrient solutions with initial nitrogen concentrations between 65mgN/L and 90mgN/L, then to add organic matter by fractions during culture. In fact, all the different concentrations led to similar productivity. However higher initial concentrations led to greater mineral losses, and lower nitrification efficiencies.

Moreover, it was speculated that most nitrogen losses were linked to the incorporation of this element into bacterial biomass, the sedimentation of solid residues on the NFT systems and plant roots, and ammonia volatilization. The latter could have been responsible for up to 30% of the losses.

Key words: Bioponics; Organic hydroponics; Nitrification; Aerobic digestion; Anaerobic digestion; Organic fertilisers; Chicken faeces; Lettuce; Nitrogen loss; Nutrient uptake; Nutrient solution

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List of abbreviations

AFAD: Association Femmes Action Développement

ANOVA: Analysis Of Variance

AOB: Ammonia Oxidising Bacteria

BOD5: Biochemical Oxygen Demand 5

COD : Chemical Oxygen Demand

CPAR: Centre Provincial de l'Agriculture et de Ruralité

C-RAU: Centre de Recherche en Agriculture Urbaine

DNA: Deoxyribonucleic Acid

DO : Dissolved Oxygen

DW: Dry Weight

EC: Electroconductivity

EU: European union

HPS: High Pressure Sodium

HSD: Honest Significant Difference

ISR: Induced Systemic Resistance

IUPPL: Integrated and Urban Plant Phytopathology Laboratory

NA: Not Available

NE: Nitrification Efficiency

NFT: Nutrient Film Technique

NOB: Nitrite Oxidising Bacteria

PVC: Polyvinyl Chloride

RNA: Ribonucleic Acid

TAN: Total Ammonia Nitrogen

TMN: Total Mineral Nitrogen

TSS: Total suspended solids

USD: United States Dollar

VFA: Volatile Fatty Acids

WFP: World Food Program

TABLE OF CONTENT

1. INTRODUCTION

1.1. CONTEXTUALISATION

This master thesis takes place in "Centre de Recherche en Agriculture Urbaine" (C-RAU) and is part of the SWIM project. The latter consists of a project financed by the European Union (EU) and titled "Innovative irrigation systems in Sahrawi refugee camps in Tindouf (South-west of Algeria)" (Figure 1.1).

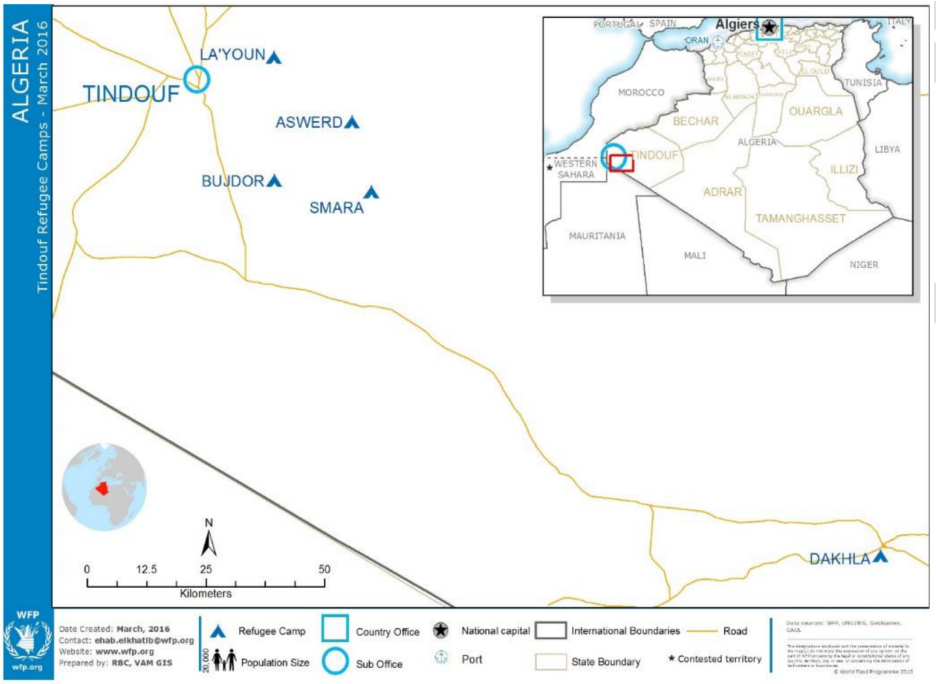

Figure 1.1: The locations of the Sahrawi refugee camps (Eyt, 2019)

With hopes of improving accessibility to fresh vegetables and reducing the Sahrawi's dependency for external aids, "Association Femme Action Developpement" (AFAD), Oxfam-Solidarité and the University of Liège, have collaborated to launch a project to implement alternatives to conventional agriculture in the Sahrawi refugee camps, including soilless culture techniques. It began in June 2019 and is projected to end in January 2023.

Figure 1.2: The Laayoun gardens (Eyt, 2019)

Conventional hydroponics could overcome some major challenges for agriculture found in the Sahara Desert. Namely, the harsh pedoclimatic conditions (Figure 1.2) and the poor water accessibility in the camps. However, access to inputs such as nutrient solution salts in this region is also a problem. Nonetheless, it is possible to create nutrient solutions by recycling organic matter such as animal faeces through bioponics. Therefore, the latter technology is being researched and developed simultaneously in Algeria and in C-RAU to be carried through in the Sahrawi refugee camps.

1.2. HYDROPONICS

Hydroponics is a soilless crop culture technique in which plants are irrigated using a nutrient solution containing all the essential elements for their healthy growth, while being rooted or not in a substrate, often inert (Resh, 2013). Several hydroponic systems exist and three of the most commonly used are the **Deepwater culture system** where the plants float on the nutrient solution in a raft, the **ebb and flow system** where nutrient solutions simulate tides and low tides and the **nutrient film technique** (NFT) system where roots are in contact with flowing water (Musa et al., 2019; Putra et al., 2018).

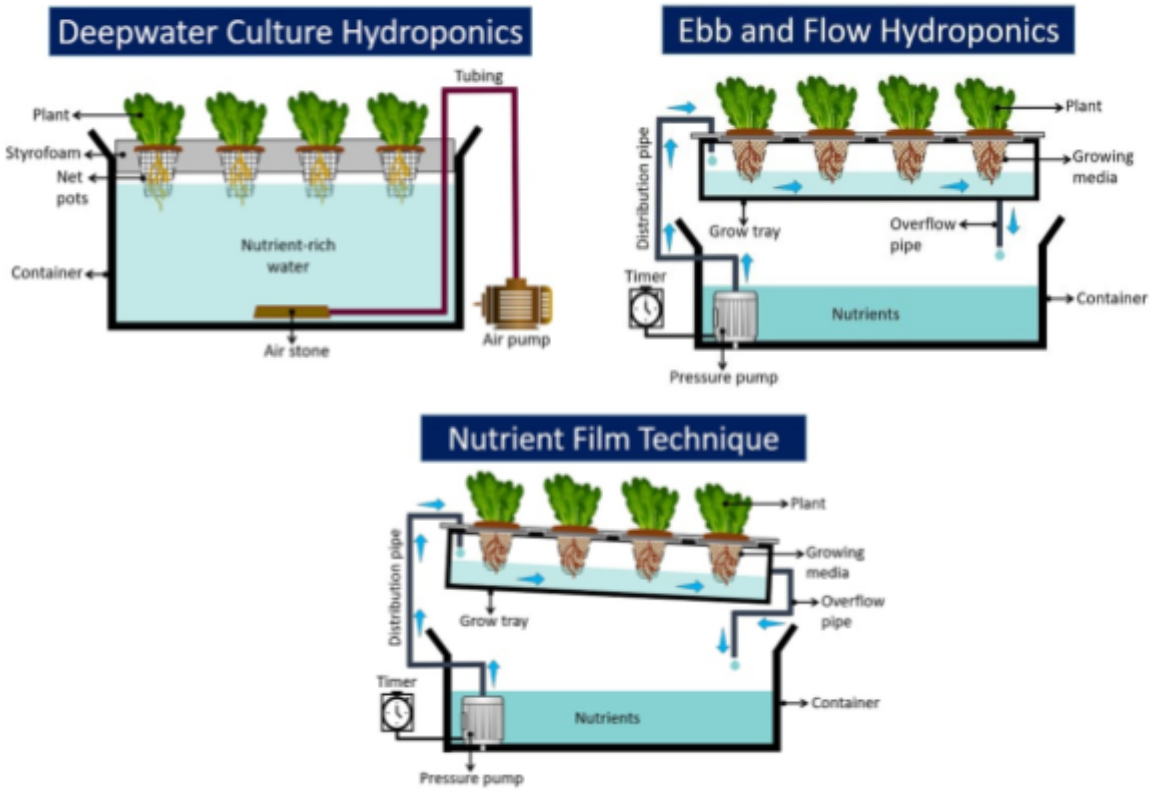

Figure 1.3: Illustrations of Deep water culture systems (top left), Ebb and Flow systems (top right) and Nutrient film technique (NFT) systems (bottom). Source: https://biologyreader.com/types-of-hydroponic-systems.html<

Hydroponics holds several advantages over conventional soil culture:

- First, hydroponics has the potential of increasing crop yields. Indeed, Majid et al. (2021) have shown that lettuce plants cultivated in hydroponic systems were 24-41% heavier than those grown in soil-based agriculture. According to Barbosa et al. (2015), lettuce yield per surface area could be 11 times greater in hydroponics than in soil-based agriculture.
- Secondly, this technique allows crop culture even in some of the least favourable areas, such as in deserts or highly polluted soils due the ability to control the environment (Pandey et al., 2009; Resh, 2013; Uchimura et al., 2014).
- Furthermore, hydroponics could save substantial amounts of water. In fact, hydroponics could reduce water usage by up to 95% in comparison with conventional agriculture (Pandey et al., 2009; Al-Karaki et al., 2011; Resh, 2013; Treftz et al., 2016).
- In addition, it is possible to reduce culture time. To illustrate, lettuce cultivated in hydroponics by Majid et al. (2021) required respectively 7 and 7.5 less days to

transplanting and to first harvest in comparison with soil-based agriculture (time required for first harvest was therefore reduced by 19%).

- Finally, weed nuisance is hardly a problem in hydroponics (Resh, 2013). .

However, hydroponics also has certain limitations such as the requirement for substantial amounts of inputs (synthetic salts, acids and bases) to create and maintain nutrient solutions. This renders the technology sensitive to the difficult access to inputs in the Sahrawi camps mentioned above.

In fact, fertiliser prices have almost quadrupled between the beginning of 2020 and march 2022 (Statista, 2022). This increases the difficulty of providing this raw material to the Sahrawi refugee camps. In addition, the manufacturing of conventional hydroponic fertilisers negatively impacts the environment as some elements are extracted from depletable mines such as phosphorus and potassium, and others, such as nitrogen, require burning natural gas (Yara, 2018).

Therefore, another soilless culture technique, bioponics, which shares similar advantages in regard to water usage as hydroponics, while also being less dependent on mineral inputs, must be taken into consideration.

1.3. BIOPONICS IN THEORY

1.3.1. In general

According to Fang et al. (2018), bioponics, also called organic hydroponics, stands for: "A contained and controlled growing system in which plants in growing media derive nutrients from plant-based, animal-based and mineral natural substances which are released by the biological activity of microorganisms".

In a nutshell, bioponics is therefore a soilless crop culture system where natural substances (such as animal wastes or plant residues) can be processed by microorganisms in order to create nutrient solutions. The latter's origin and composition are in fact the main differences separating conventional hydroponics and bioponics.

Bioponics offers an environmentally sustainable solution for the treatment of organic wastes. According to the World Food Program (WFP, 2020), around 1.3 billion tons of food (almost the equivalent of 1 trillion USD) are discarded annually and if assembled, would represent the third largest CO_2 emitter after the USA and China. Recycling food waste through the creation of nutrient solutions for crop culture could not only be beneficial for the environment, but also a step forward towards a circular economy (Stocknes et al., 2016). In addition, other organic wastes such as animal droppings, carcasses or even green wastes could be recycled for crop culture.

Bioponics holds several advantages over conventional hydroponics:

- To begin with, the ability to recycle organic wastes into nutrient solutions reduces the dependency of soilless agriculture for synthetic inputs. The latter are known to be harmful for the environment as they are either extracted from depletable mines, such as in the case of phosphorus (Reijnders, 2014) or produced in polluting factories (Mirlean et al., 2006).
- In addition to the environmental benefits, the ability to recycle organic wastes allows poor and remote populations to make use of soilless culture without worrying about acquiring substantial amounts of chemical fertilisers.
- Moreover, Bioponics can also provide biological benefits, namely the increase of the product's quality instead of simply improving the quantity produced, such as reduced moisture content while maintaining similar or higher yields and enhanced chlorophyll content (Ansari et al., 2015; Ragaveena et al., 2021). Some studies have demonstrated that bioponic systems lead to an enhanced root hair development and disease resistance (Shinohara et al., 2011; Fang et al., 2018; HSIEH et al., 2018). For example, bioponically cultivated lettuce has shown induced systemic resistance (ISR) against grey mould caused by Botrytis cinerea (Chinta et al., 2015). Plus, bioponic nutrient solutions could also contain biostimulants (Bergstrand et al., 2020).

It is important to note that even though bioponics outperform hydroponics in certain aspects, this technology comes with flaws and disadvantages:

- The first being the system's dependency for bacteria, especially nitrifying bacteria (Shinohara et al., 2011). Organic matter cannot be directly added to water to create nutrient solutions as with mineral salts in the case of conventional hydroponics. Organic matter, when added unprocessed in nutrient solutions is considered to be toxic to plants (Garland et al., 1993; Shinohara et al., 2011). This can probably be linked to the presence

of certain organic acids, some among which can be related to allelopathy (Rice, 2012). For example, Lee et al. (2006) have demonstrated that increasing the concentration of p-Hydroxybenzoic acid from 25µM to 50µM reduced lettuce fresh weights by 23%. In addition, nutrients in organic matter are generally found in large insoluble molecules (e.g. complex proteins in the case of nitrogen, DNA in the case of phosphorus, etc…) entailing their unavailability for plant roots (Weil et al., 2017). These molecules can be broken down by microorganisms and their enzymes to inorganic nutrients, which can be solubilised and become available to plants. This process is called **mineralization** (Weil et al., 2017). Microorganisms must be imported from external sources and cultivated in order to mineralise the organic matter as their presence in sufficient amounts cannot be taken for granted (Shinohara et al., 2011). They can be cultivated from different origins: soil, plant residues, animal faeces, composts etc… Nonetheless, the dependency on microorganisms calls for concessions to be made for certain physical parameters: pH, temperature and dissolved oxygen (DO). For instance, a specific method of creating bioponic nutrient solutions requires pH to be maintained between 7 and 8.5 (see section 1.3.2.2). However above neutral pH levels are considered to hinder plant growth in soilless systems as some elements become unavailable to plants as shown in figure 1.4. (Resh, 2013).

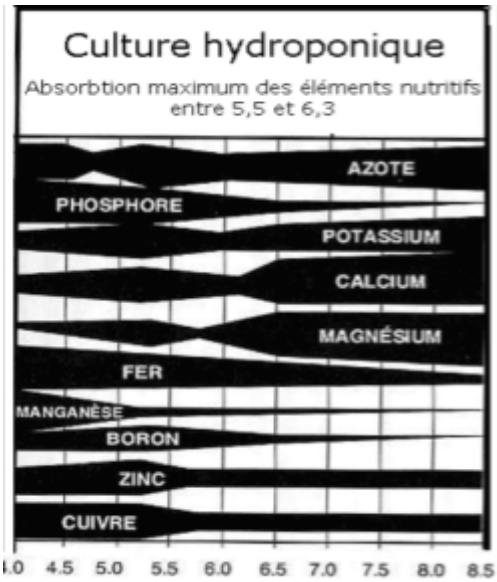

Figure 1.4: Nutrient availability according to pH in soilless culture systems (Jijakli, 2020)

- Moreover, mineral losses can be observed when creating bioponic solutions. For example, in the experiments conducted by Khronis et al. (2020), some of the bioponic solutions had lost more than half of their phosphorus concentration 3 days after the beginning of their creation. Other researchers have observed losses in nitrogen (Shinohara et al., 2011; Suhl et al., 2018; Delaide et al., 2019; Wongkiew et al., 2021). This part will be further developed in later sections.

Several methods exist for the creation of bioponic solutions. For instance, the organic matter can be digested aerobically, anaerobically, or both. Then, they can be of different origins. The main sources of organic matter used to create bioponic solutions are either animal or vegetal which have different biodegradabilities (Shinohara et al., 2011). Some of the raw materials used for organic fertilisers are listed in Table 1.1:

Organic Materials	N	P_2O_5	K_2O
Manures	- - - - - - - - - - - - - - - - - % dry weight basis - - - - - - - - - - - - - - - - -		
Beef	1.2	2.0	2.1
Dairy	2.1	3.2	3.0
Bat guano	6.0	5.0	3.0
Horse	2.1	3.2	2.0
Poultry	3.0	5.0	2.0
Sheep	1.6	1.2	2.0
Swine	2.5	2.1	1.0
Alfalfa hay	2.5	0.5	2.5
Blood meal	13.0	2.0	1.0
Bone meal, raw	3.0	22.0	0
Bone meal, steamed	1.0	15.0	0
Castor bean meal	5.5	2.0	1.0
Cotton seed meal	6.0	3.0	1.5
Fish meal	10.0	6.0	4.9
Kelp/seaweed	1.5	1.0	0
Peanut meal	7.0	1.5	1.2
Soybean meal	7.0	1.2	1.5
Tankage[2]	7.0	10.2	1.5

[1] *These are total concentrations and only slowly available over weeks, months, or years. Many materials will vary in composition due to moisture content and methods of handling.*
[2] *May contain high levels of chromium.*

Table 1.1: Approximate nutrient composition of various organic fertilizers (Rosen, 2005).

As we can see in Table 1.1, the composition of organic fertilisers is diverse whether they originate from manure or plant wastes. Focusing on animal wastes, their composition will mainly depend on the animal species and the farming system (Charoenpakdee, 2014; Rosen et al., 2005). Indeed, both factors impact the diet and different diets will lead to different manures.

1.3.2. Common methods to create bioponic solutions

In general, bioponic solutions are either created aerobically (Shinohara et al., 2011), or anaerobically (Delaide et al., 2019) or a combination of both. Whichever the case, the main purpose is to degrade organic matter into simple, soluble nutrients through mineralisation. Hansen et al. (1991) have concluded that aerobic and anaerobic breaking down of organic matter result in similar organic nutrient solutions. The difference lies therefore in the consumption of energy. Indeed, maintaining an aerobic environment in liquid solutions requires powerful air pumps (Delaide et al., 2019), which are not necessary in the case of nutrient mineralisation through anaerobic digestion. However, this is not entirely accurate: in contrast to aerobic digestion, anaerobically digested organic matter will mainly release nitrogen in the form of ammonium (NH_4^+) instead of nitrate ($NO3^-$) as will be seen in section 1.3.2.1.

1.3.2.1. Anaerobic digestion or fermentation

Anaerobic digestion is the breaking down of organic matter by microorganisms in anoxic conditions. This process involves 4 steps, at the end of which ammonium (NH_4^+), methane (CH_4), carbon dioxide (CO_2), hydrogen sulphide (H_2S) and water are produced. This technique is mainly used to produce renewable biofuel from low value feedstock all the while suppressing the odours and the pathogens of the digestate (Ronga et al., 2019). The digestate is the remaining material following anaerobic digestion and is the end product used for the creation of a bioponic solution.

At first, Macromolecules undergo **hydrolysis** and are broken down to monomers. Then, during the **acidogenesis** phase, these simple organic substances are fermented by bacteria, liberating volatile fatty acids (VFA), NH_4^+, CO_2, H_2S. The third step is **acetogenesis**, where acetic acid is produced from VFA and alcohols. Finally, the **methanogenesis** phase consists in the breakdown

of acetate molecules into methane and CO_2 (Delaide et al., 2019). During this last phase, hydrogenotrophic methanogenic bacteria (belonging to the Archaea domain) are able to reduce CO_2 to methane. All elements that have not been used to produce methane remain as "digestate" (Bernet, 2015; Orellana et al., 2019; Trably, 2002).

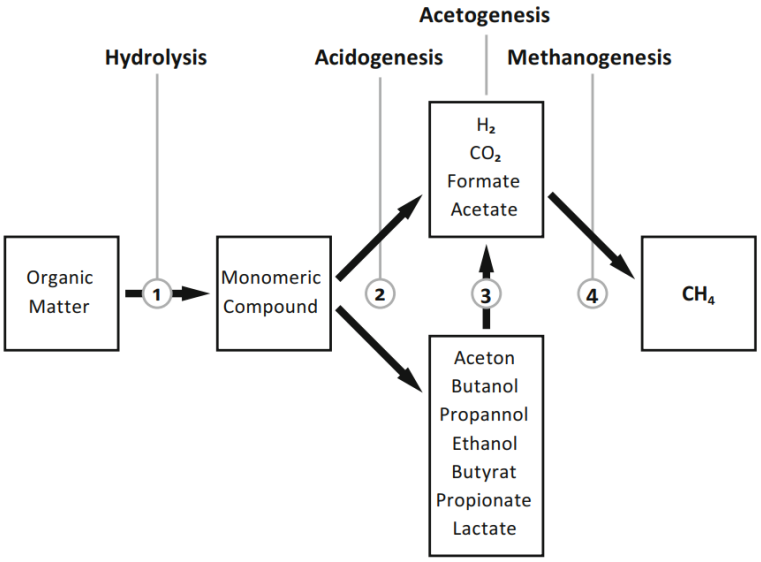

Figure 1.5: the process of anaerobic digestion (Delaide et al., 2019)

As we can see, nitrogen is liberated in the form of ammonium when organic matter is anaerobically digested. Even though plants are able to absorb NH_4^+, it is widely accepted that an exclusive NH_4^+ diet is toxic to most plants (Bergstrand et al., 2020; Shinohara et al., 2011). Therefore, at least part of the ammonium ions must be transformed into a more suitable source of nitrogen to plants. This is done through nitrification which oxidises NH_4^+ to nitrate (NO_3^-).

In addition to nitrification, the anaerobic digestate often requires dilution before its use as it has an elevated Electroconductivity (EC), and high $NH4^+$ and organic matter concentrations. To illustrate, Pelayo Lind et al. (2021) had diluted their digestate 10 times and the product still hampered their crops. They had to further increase the dilution factor to obtain a solution with an EC of 2 mS/cm. For the sake of giving a reference, an EC above 2.5 mS/cm and 2.6 mS/cm can respectively hinder tomato yields (Sonneveld et al., 1988) and reduce lettuce growth (Andriolo et al., 2005). Focusing on $NH4^+$, the anaerobic digestate used by Botheju et al. (2010) had a concentration of $N\text{-}NH4^+$ of 1700 mg/L whereas 50mg/L $N\text{-}NH4^+$ was enough to reduce lettuce shoot fresh weight by 15% (Hoque et al., 2007).

Moreover, anaerobic digestate could contain inhibitory substances, such as organic acids which suppress root growth, and thus require additional treatment before it's use in crop culture, for instance aerobic digestion (Salminen et al., 2002). Salminen et al. (2001) have found that aerobic digestion of the digested material for a period of 6 hours had reduced acetic acids by 60% and other organic acids to less extents.

1.3.2.2. Aerobic digestion

1.3.2.2.1. In general

Aerobic digestion is the breakdown of organic matter in the presence of oxygen by aerobic microorganisms (Bernard et al., 2000; Demirbas et al., 2017). The latter consume organic matter for their survival and reproduction releasing CO_2 in the process (Demirbas et al., 2017). Aerobic digestion is influenced by temperature, where a higher temperature leads to quicker degradation (Bernard et al., 2000). However, Temperatures above 40℃ will completely inhibit nitrification (Matsch et al., 1977), which is an essential part of aerobic digestion, through which NH_4^+ is ultimately converted to NO_3^-. Therefore, when implemented in the frame of bioponics, aerobic digestion should be operated at temperatures between 20 and 40℃. The duration of aerobic digestion is variable in literature: Tikasz et al. (2019) aerated his solutions for 48 hours before filtering out the manure, while Shinohara et al. (2011) aerated their solutions for 48 days.

1.3.2.2.2. Nitrification

Nitrate is less toxic than its cation counterpart ($NH4^+$) (Li et al., 2013) and can even alleviate ammonia toxicity (Britto et al., 2002). Consequently, it is important to make sure that plants are acquiring at least around half of their nitrogen in the form of nitrate instead of ammonium (Weil et al., 2017). Thus, a nitrification process is important in bioponics.

Nitrification is the oxidation of ammonium to nitrite (nitritation) by ammonia oxidising bacteria (AOB), which is in turn converted to nitrate (nitratation) by nitrite oxidising bacteria (NOB) (Botheju et al., 2010). Nitrification can be represented by the following equations, the first two are accomplished by AOBs, and the third one by NOBs:

1) $NH_3 + O_2 + 2e^- \rightarrow NH_2OH + H_2O$
2) $NH_2OH + H_2O \rightarrow NO_2^- + 5H^+ + 4e^-$
3) $NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$

AOBs and NOBs are aerobic autotrophic organisms (Botheju et al., 2010). However, it is believed that some heterotrophic microorganisms are capable of nitrification under low oxygen conditions (Kampschreur et al., 2009; Niel et al., 1993).

Nitrification is a delicate process that takes place when certain physical parameters are found within specific ranges. The triad to remember are pH levels, temperature, and dissolved oxygen (DO). nitritation and nitratation occur at slightly different ranges of pH. However, if maintained between 7 and 8.5, the entire nitrification process can take place (Park et al., 2007). When it comes to temperature, AOBs and NOBs operate sufficiently at a range between 15 ℃ and 35 ℃ (Shammas, 1986). It is best if DO levels are maintained above 1 mg/L for nitrification to come about (Jianlong et al., 2004).

It is important to wait long enough for nitratation to complete because an accumulation of nitrite (NO_2^-) quickly renders the nutrient solutions toxic to plants (Phipps et al., 1970). Hoque et al. (2007) have found that even 5 mg/L of nitrite-nitrogen ($N\text{-}NO_2^-$) can reduce lettuce growth.

1.3.3. Nutrient mineralization

As mentioned previously, nutrients in organic matter are present in large insoluble molecules and are therefore inaccessible to plants. The main purpose of aerobic and anaerobic digestion is to breakdown and solubilize the organic molecules through nutrient mineralisation. This section will focus on the mineralization process of phosphorus, potassium and nitrogen.

1.3.3.1. Phosphorus mineralization

Organic phosphorus can be found mainly in three groups of compounds (Weil et al., 2017):

1) Inositol phosphates or phosphate mono-esters such as phytic acid, a compound used by plants for phosphorus (P) storage.
2) Phosphate di-esters which can be found in nucleic acids (DNA and RNA).
3) Phospholipids which constitute cell membranes.

Organic phosphorus can undergo hydrolysis with the help of phosphatases and phytases, both of which can be produced by microorganisms (Richardson et al., 2011). This releases phosphorus in the form of bioavailable soluble orthophosphate anions (HPO_4^{2-} and $H_2PO_4^-$) (Weil et al., 2017).

Phosphate ions are prone to immobilisation. Indeed, inorganic phosphorus compounds can readily react with calcium (Ca), iron (Fe), aluminium (Al) or manganese (Mn), creating substances with low solubility (Weil et al., 2017). The solubility of these compounds heavily relies on pH. Indeed, at alkaline pH, phosphates are fixated mostly as calcium phosphates. In contrast, at acidic pH, they are fixated by Fe, Al and Mn or their hydrous oxide forms (Figure 1.6) (Weil et al., 2017).

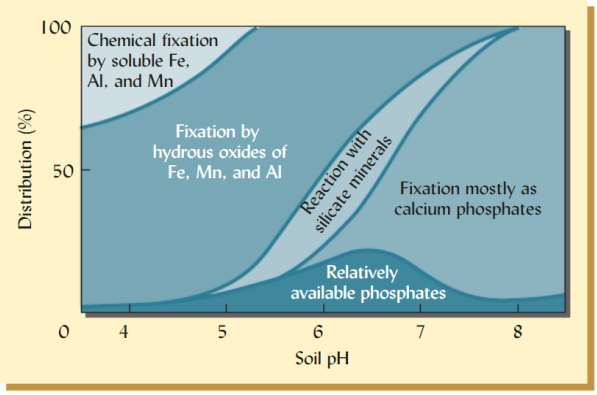

Figure 1.6: Phosphate inorganic fixation according to pH (Weil et al., 2017)

Microorganisms and certain plant species can increase the solubility of inorganic phosphate compounds by excreting phosphatases or by releasing organic acids (such as citric or malic acid) that either dissolve the calcium phosphates or chelate the metals, thus releasing the phosphates (Weil et al., 2017).

1.3.3.2. Potassium mineralization

Potassium is an element required in large quantities by plants (nearly as much as nitrogen) (Weil et al., 2017). However, potassium isn't incorporated into organic compounds and remains as an ion in living organisms (Resh, 2013). Therefore, no specific mineralization process is required for potassium release. Nonetheless, K can be trapped in exchangeable forms on colloïd surfaces in organic matter (Weil et al., 2017). Hence, it is expected that the mineralization of organic matter increases the amounts of potassium in a solution. It is not clear how microorganisms could affect the bioavailability of potassium. Still, uptake of K could be limited due to high concentrations in calcium and/or magnesium and can be estimated using Equation 1.1 (Weil et al., 2017).

$$\frac{[K^{+}]}{\sqrt{[Ca^{2+}]+[Mg^{2+}]}} \qquad \text{(Equation 1.1)}$$

1.3.3.3. Nitrogen mineralization

Focusing on nitrogen, complex nitrogen-containing molecules are broken down to amino compounds and amine groups. These undergo hydrolysis and nitrogen is released in the form of ammonium ions (NH_4^+) (Weil et al., 2017; Pelayo Lind et al., 2021). This process requires enzymes such as hydrolases and deaminases that are predominantly secreted by microorganisms but can also be produced by plant roots or some soil animals (Weil et al., 2017). Although nitrogen mineralisation can take place intracellularly, most often it occurs extracellularly (Weil et al., 2017). Ammonium can then be nitrified into nitrate (see section 1.3.2.2.2.).

Plants can absorb the inorganic nitrogen molecules present in a solution (NH_4^+,NO_2^- and NO_3^-). However, these molecules can be removed from a solution through the action of several processes, which will be discussed in the following section.

1.3.4. Nitrogen losses : a theoretical point of view

During the creation of bioponic solutions, researchers have found that nutrients, especially nitrogen, have been lost in the process (Shinohara et al., 2011; Suhl et al., 2018; Delaide et al., 2019; Wongkiew et al., 2021). For example, Salminen et al. (2001) have found that more than 50% of their solutions' nitrogen was lost after 7 days of aerobic digestion.

In a previous bioponic essay conducted in C-RAU from March 2021 to May 2021, total ammonia nitrogen (TAN) decreased by 35 to 60% with no increase in nitrite-nitrogen or nitrate-nitrogen (unpublished data). TAN is the sum of ammonium-nitrogen ($N\text{-}NH_4^+$) and ammonia-nitrogen ($N\text{-}NH_3$) and is used because during analyses, the pH of the sample is raised to high pH levels (around 11-12) where NH_4^+ is converted to NH_3.

In a later essay conducted from July 2021 to September 2021, around 32% of total mineral nitrogen (TMN) had disappeared after 11 days of aerobic digestion from a bioponic solution created using chicken droppings (unpublished data). These losses could have been due to one or more of the following phenomena : ammonia volatilisation where NH_3 is freed into the atmosphere, denitrification where nitrate and nitrite are reduced to gases which escape the

solution, microbial assimilation where nitrogen is trapped in microbial biomass and anammox where ammonium is oxidised into atmospheric nitrogen (N_2).

1.3.4.1. Ammonia volatilization

Ammonia volatilisation is the loss of nitrogen in the form of volatile ammonia (NH_3).

In general, ammonium (NH_4^+) and ammonia (NH_3) coexist in a solution. Their proportions are mainly influenced by the pH of the solution and follow the Henderson-Hasselbalch equation (Johansson et al., 1980). In fact, the higher the pH, the greater the proportion of NH_3 (Figure 1.7) with 9.2 being the pH level at which both proportions are equal (pka NH_4^+ is 9.2; Ninnemann et al., 1994). NH_3 in a solution is prone to volatilization leading to nitrogen losses.

Some wastewater treatment facilities eliminate nitrogen by raising the pH and temperature of the solution and ventilating it. This is in a nutshell the concept of ammonia stripping towers (figure 1.6; Zhu et al., 2017). The bubbles created by the aeration will transport ammonia out of the solution.

Ammonia volatilization is mainly influenced by solution pH (with an optimum of 12), temperature (with an optimum of 60 ℃), and air flow rate (with an optimum of 0.5 $m^3h^{-1}L^{-1}$). (Zhu et al., 2017).

In the context of bioponics, during aerobic digestion, aeration will eventually create bubbles which will increase the similarities between the bioponic systems and ammonia stripping towers as shown in Figure 1.8, possibly leading to nitrogen loss (Botheju et al., 2010; Raboni et al., 2013; Zhu et al., 2017).

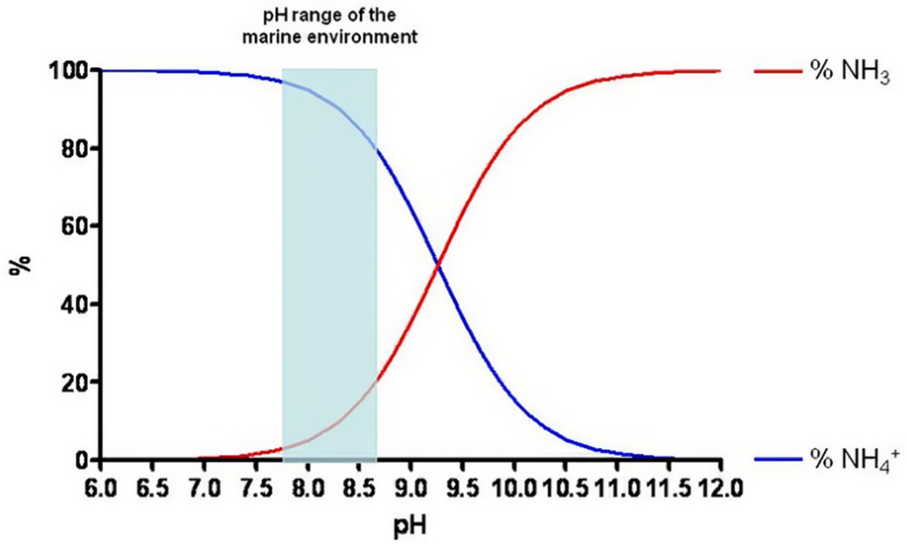

Figure 1.7: Evolution of ammonium and ammonia ratios with pH (source : https://www.shrimpoly.com/my/bacteria-in-the-freshwater-aquarium-2/)

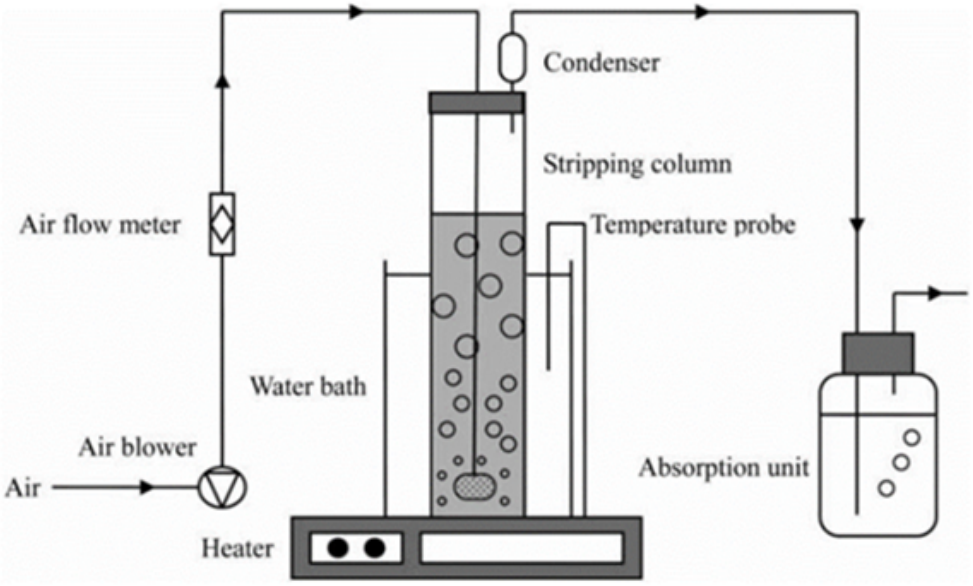

Figure 1.8: Ammonia stripping towers as illustrated by Zhu et al. (2017)

1.3.4.2. Denitrification

Denitrification is the reduction of nitrate sequentially to nitrite (NO_2^-), nitric oxide (NO), nitrous oxide (N_2O), and finally dinitrogen (N_2) (Knowles, 1982; Robertson et al., 1988). NO, N_2O and N_2 are gases that can escape the solution, leading to nitrogen loss. This process occurs predominantly in strictly anaerobic conditions. In fact, Oh et al. (1999) have demonstrated that a dissolved oxygen (DO) concentration of 0.09mg/L reduced the rate of denitrification by 35% in comparison with strictly anoxic conditions. However, some researchers have identified that some species (such as *Paracoccus denitrificans)* are capable of denitrification in aerobic conditions (Castignetti et al., 1984; Robertson et al., 1988, 1989, 1990, 1995). Aerobic denitrification allows these species to obtain electrons from nitrate instead of oxygen (Ji et al., 2015). The optimal range of DO for aerobic denitrification is between 3 and 5 mg/L (Ji et al, 2015). Therefore, maintaining DO above 5 mg/L could limit the effects of denitrification in general.

Besides oxygen, other parameters that control denitrification are pH and temperature. In fact, rates of denitrification are optimal when pH levels are 7-8 (Knowles, 1982) and temperature is around 25-30 ℃ (Saad et al., 1993). Overstepping both ranges one way or another markedly suppresses denitrification. Nonetheless, for some microorganisms the optimal pH for denitrification is 6 (Kiskira et al., 2017). Moreover, this phenomenon can still be observed at pH levels as low as 4 and as high as 11 or when temperature is as low as 5℃ (Knowles, 1982) or as high as 50℃ (Saad et al., 1993).

1.3.4.3. Microbial assimilation

Microbial activity and growth requires the production of protein. Nitrogen is an essential element when it comes to protein production. When nitrogen contained in the residues being

metabolised by microorganisms isn't sufficient, the latter will immobilise NH_4^+ and NO_3^- ions present in the solution and incorporate them into their biomass (Weil et al. 2017). Berntson et al. (2000) have shown that approximately 20% of nitrates added to hardwood forest soils were immobilised by microorganisms after 24h of the addition.

This same logic can be applied for other macronutrients such as phosphorus. Mclaughlin et al. (1988) have shown that 28% of the phosphorus added to soil in the form of plant residues was incorporated into microbial biomass after 7 days. Microorganisms compete with plants for phosphorus (Richardson et al., 2011) and probably other nutrients (Weil et al., 2017).

1.3.4.4. Anammox

Finally, anammox is a reaction where the oxidation of ammonium is coupled to the reduction of nitrate. The product is dinitrogen (Kimura et al., 2011). Usually, anammox is strictly anaerobic (Botheju et al., 2010). Studies have shown that a DO higher than 2.5 mg/L will entail a complete halt of this process (Kimura et al., 2011).

1.3.5. Plants, nutrition and bioponics

Different plants have been studied in bioponics. In fact, the same plants that seem to be adapted to aquaponics and hydroponics are also suitable for bioponics (lettuce, tomatoes, cucumbers…). Lettuce is particularly used in these types of agriculture due to the shortness of its growth cycle (Rakocy, 2012). For this reason, a recommended nutrient solution composition can be easily found in literature for lettuce culture. For example, the concentrations of different minerals recommended by Resh (2013) for hydroponic lettuce cultivation can be found in Table 1.3.

Resh (2013) also lists tissue nutrient level ranges for healthy lettuce plants, based on dry weights (Table 1.2).

Reference	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Fe (ppm)	Zn (ppm)	Mn (ppm)
Resh (2013)	3.0-6.0	0.8-1.3	5-10.8	1.1-2.1	0.3-0.9	130-600	60-120	20-150

Table 1.2: Ranges of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc and manganese concentrations found in typically healthy lettuce plants based on dry weights (Resh, 2013).

In hydroponics, reaching the desired concentration of a certain element can be done by simply mixing the right proportions of salts (Resh, 2013). However, in bioponics, it is at the moment difficult to reach a specific nutrient solution composition because an important amount of factors are involved (for example: the origin of the organic matter, the method of mineralisation and solubilisation, the microbial community etc…). Table 1.3 shows the mineral composition of some bioponic, aquaponic and hydroponic solutions used in literature for lettuce cultivation.

Reference	Nutrient origin	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)	Fe (ppm)	Zn (ppm)	Mn (ppm)
Mowa et al. (2018)	Goat manure	198	42.1	360	250	67	NA	NA	NA
Wongkiew et al. (2021)	Chicken manure	18.2	57.4	NA	NA	NA	NA	NA	NA
(Ezzedine et al., 2021)	Aquaponic sludge	121.7	58	185	124	17	1.2	0.83	0.02
(Ezzedine et al., 2021)	Inorganic	111	23	140	94	23	1.7	0.26	0.42
(Yang et al., 2020)	Fish excreta	164.2	27.1	114.1	20.4	2.4	NA	NA	NA
(Yang et al., 2020)	Inorganic	198.8	125.5	339	148.8	39	NA	NA	NA
Resh (2013)	Inorganic	190	50	210	200	40	5	0.1	0.5

Table 1.3: Comparison of different Bioponic, Aquaponic and Hydroponic nutrient solutions found in literature in terms of their concentration of nitrogen, phosphorus, potassium, calcium, magnesium, iron, zinc and manganese.

Even though the nutrient solution composition in bioponics diverges from the recommended conventional hydroponic solutions, researchers have obtained positive results. For example, Wongkiew et al. (2021) were able to effectively produce lettuce with no visible signs of deficiencies using chicken manure bioponic solutions even though their solution had less than 10% the recommended concentration in nitrogen. Elena et al. (2016) have found that the use of organic fertilisers in NFT systems entails a higher fresh weight in comparison with conventional hydroponics : an increase ranging from 6% and 36%, depending on the cultivar studied. Shinohara et al. (2011) have created a conventional hydroponic solution with a nutrient composition close to their bioponic nutrient solution in terms of nitrogen concentration and found that after 25 days of culture, their bioponic solution yielded lettuce heads 30% heavier.

1.4. Objectives of this work

The main goals of this work are to further understand the bioponic technique that was chosen and developed for the SWIM project and to optimise it for its use in the Sahrawi refugee camps.

Six specific objectives can be formulated, to:

1) Follow the evolution of plant macronutrients at each step of the essay and determine nutrient losses for the chosen bioponic technique.
2) Test several initial concentrations of nutrients in the nutrient solutions in order to determine and discuss the optimal one.
3) Evaluate the nitrification efficiency of each initial concentration of nutrients and compare them.
4) Evaluate and discuss the loss of nitrogen that was observed in the essays.
5) Monitor the physical parameters of the nutrient solutions as well as the biochemical and chemical demand in oxygen to deepen the understanding of the technique.
6) Compare the chosen bioponic technique with conventional hydroponics in terms of lettuce quality and quantity.

1.5. The bioponic essay of this study

In order to answer the general and specific objectives, lettuce needed to be cultivated using bioponic solutions originating from chicken droppings.

Goats and poultry can be found in the sahrawi refugee camps. However, previous essays conducted in C-RAU have shown that chicken droppings are a better source of organic matter for bioponics in comparison with goat faeces. Indeed, in an essay conducted from July 2021 to September 2021, average lettuce fresh weights for solutions created with chicken droppings were at least 25% higher than for those created with goat faeces (unpublished data). In addition, the creation of nutrient solutions from goat faeces was more time consuming (unpublished data). Hence, in the present work, the bioponic solutions were created using chicken droppings.

To understand the effect of the initial concentration of nutrients in the solution, it was determined that four different bioponic modalities (having different initial nutrient concentrations) would be studied and compared to conventional hydroponics.

1.6. The ammonia volatilization essay

The purpose of this small scale essay is to determine whether the conditions in which the bioponic nutrient solutions are can promote ammonia volatilization. As a reminder, this phenomenon is the loss of nitrogen in the form of volatile NH_3. This mechanism does not involve the presence of organic matter or living organisms. In fact, physical parameters on which the efficiency of ammonia stripping depends are pH, temperature, concentration of ammonium and air flow (Zhu et al., 2017).

2. Materials and methods

2.1. The Bioponic essay

2.1.1. The chosen method

The general method that was chosen and used in C-RAU to create bioponic solutions in the framework of the SWIM project consisted in a succession of an anaerobic digestion followed by an aerobic digestion.

First, the collected chicken droppings underwent an "**anaerobic**" digestion phase for nutrient mineralisation and solubilisation at lesser energy costs, where faeces were simply mixed with demineralised water and left at around 30℃. Though, it should be stressed that "anaerobic" in this essay has to be taken with precaution as the solutions were exposed to air and mechanically mixed when measurements or samples were taken. The product of this phase, after filtration, will be called a "stock solution".

The latter was diluted for the creation of the nutrient solutions for each modality. The coefficient of dilution was based on the desired concentration in total mineral nitrogen (TMN) because this same parameter was used in previous bioponic essays conducted in C-RAU. A total of four modalities were tested, each of which was repeated thrice.

Then, for all bioponic solutions, an "**aerobic**" digestion phase took place in order to promote nitrification. In this sense, the oxic conditions were created by circulating the nutrient solutions for 24 days in empty NFT gullies (i.e., in the absence of plants). In previous essays conducted in C-RAU, the circulation was enough to create the oxic conditions required for nitrification to occur (unpublished data). Therefore this same method was used in this study.

Microorganisms were imported by dipping **biomedia** (small circular plastic elements with increased surface area) for 9 days in a solution containing chicken faeces. This method was successful in previous internal essays, therefore it was reused for this study.

Samples of the bioponic solutions, as well as the hydroponic solutions were taken at key events and analysed by a third-party laboratory to determine their chemical composition and physical properties.

2.1.2. The installations

2.1.2.1. The greenhouse

The experiments were conducted in the "Sapristi" greenhouse of Gembloux Agro-Bio Tech (Gembloux, Belgium). Temperature and humidity are not controlled in this greenhouse. However, built in the latter are automatic trap windows which open whenever temperature exceeds 30 °C. The cardinal latitude and longitude are respectively 50.564380 and 4.698787. The main faceplate of the greenhouse is oriented towards the south-east.

The temperature and the relative humidity of the greenhouse were taken every 30 minutes using a data logger (MOINEAU Instruments, Chef-Boutonne, France) from the 17th of January to the 30th of March.

2.1.2.2. The bioponic systems

The system was created using three makeshift tables, each carrying five NFT PVC gutters (see Figure 2.1). The gutters were manufactured by GOPONIC® (Agrilogicsystemes, Normandy, France). The dimensions of the gutters are represented in Figure 2.1. They consist of three separable pieces. The upper part bears 12 circular holes, 4.5 cm in diameter, separated by 15.5 cm from one another. It has the purpose of carrying the plastic baskets in which lettuce is grown. The middle part is where the roots meet the nutrient film. The bottom part acts as a container that collects the nutrient solution. After a certain level, the excess proceeds to a 25L bucket through a hole connected to PVC pipes. At the elevated end of each NFT gutter lies a hose connected to an individual 16W Syncra silent pump (SICCE, Pozzoleone, Italy). Each pump was immersed in the 25L plastic bucket filled with a nutrient solution. The pumps were turned on around-the-clock for the entire duration of the experiment.

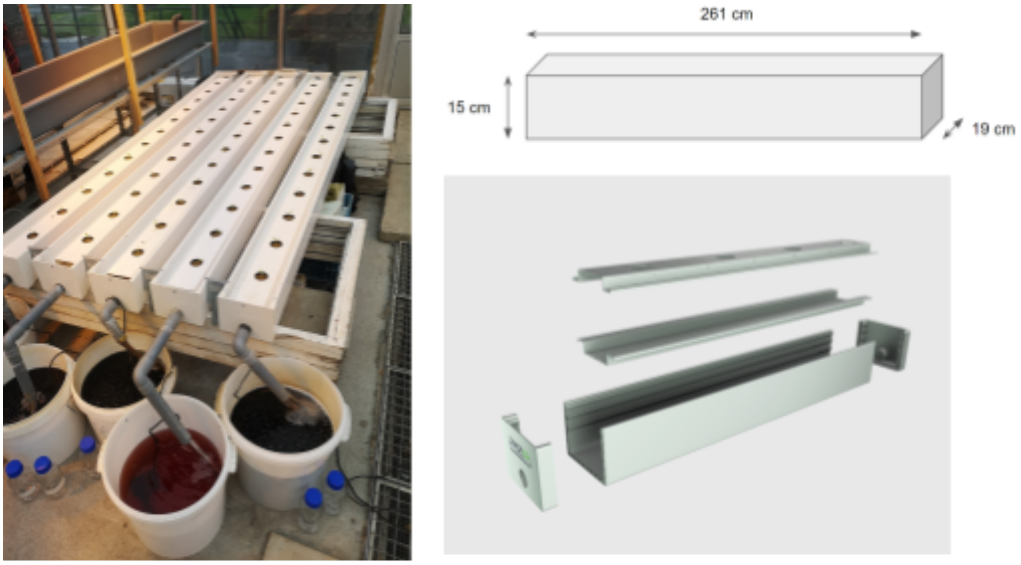

Figure 2.1: The bioponic systems (left side); The NFT gully composition (top right); The NFT gully dimensions (bottom right)

2.1.2.3. The lighting

Sunlight was the main source of light throughout the experiment. However, during cloudy or rainy days, IP 65 high pressure sodium (HPS) light bulbs were used (C LUCE SRL, Truccazzano, Italy). Given that HPS bulbs generate important amounts of heat, all artificial light sources were turned off during sunny days in order to avoid exceeding an air temperature of 26 °C, which is the upper limit of the optimal temperature range for lettuce growth (Ahmed et al., 2020). The HPS light bulbs were installed in four independently controllable rows of 6.

2.1.3. The chicken droppings

2.1.3.1. Collection

Chicken faeces were collected in an organic poultry farm named “Ferme du Rouchat” (Fernelmont, Belgium) on the 10th of January 2022.

2.1.3.2. Dry matter determination

The stock solutions were going to be built with a certain percentage of dry matter. Thus the dry matter percentage of the faeces needed to be determined. Therefore, fresh weight measurements were made using a “Mettler PM4600 DeltaRange” scale (N.V. Mettler-Toledo S.A., Zaventem, Belgium).

Then, dry weight (DW) measurements were made using the same scale after drying the faeces for 5 days at 105 ℃ in a Binder model 400 drying and heating chamber (BINDER GmbH, Tuttlingen, Germany.

2.1.3.3. Analysis by an external laboratory

To better understand the chemical composition of the faeces and for the purpose of comparing this essay with others, chicken droppings were sent to an external laboratory for the analysis of their chemical composition: “Centre provincial de l’agriculture et de ruralité” (CPAR; La Hulpe, Belgium).

2.1.4. Anaerobic digestion

2.1.4.1. Setup and monitoring

The anaerobic fermentation phase started the 18th of January and ended the 27th of the same month (It lasted 9 days). It took place in the bioponics room of the Integrated and Urban Plant Pathology Laboratory (IUPPL) in Gembloux Agro-Bio Tech (Gembloux, Belgium). In a 200L Plastic bucket, 9.696 kg of fresh chicken faeces (which represents 5.5 kg of dry matter) were mixed with 90.304L of demineralised water. Thus, the solution contained 5.5% of dry matter. Two 200W electric resistance heaters (SuperFish, Aquadistri B.V., Klundert, Netherlands) equipped with adjustable thermostats were set to 30℃ and immersed in the stock solution.

Figure 2.2: The anaerobic digestion solution

2.1.4.2. Filtration

Once this phase was completed, the stock solution was filtered using a 3-step sifting and filtering makeshift system (see Figure 2.3). The stock solution was first poured on a sieve, through which it dripped on an aquarium filter for coarse particles followed by another aquarium filter for fine particles. The filtered solution was then poured in a plastic canister and stored at 4℃.

Figure 2.3: The filtering system used after the anaerobic digestion phase

2.1.4.3. Spectrophotometer analysis

A Hanna HI83200 multiparameter spectrophotometer (HANNA instruments, Woonsocket, RI, USA) was used for nitrite, nitrate and TAN concentration measurements, using respectively HI 93707-01, HI 98728-01 and HI 93715-01 reagents. Measurements were made once a week on

the biomedia solutions and the nutrient solutions during the aerobic digestion phase, and once every other week on the nutrient solutions during the lettuce culture phase to monitor the presence of total mineral nitrogen (TMN). TMN is the sum of nitrate nitrogen, nitrite nitrogen and TAN expressed in concentrations (mg/L).

2.1.5. Aerobic digestion

As a reminder, the aerobic digestion phase was conducted to promote nitrification and to prolong nutrient mineralisation and solubilisation. It was performed by circulating the nutrient solutions in empty gullies. The circulation allowed the nutrient solutions to be mixed with air.

2.1.5.1. Stock solution dilution

The bioponic nutrient solutions were created on the 28th of January by diluting the anaerobically fermented stock solutions to reach four different concentrations in TMN, namely 65, 90, 115 and 140 mg/L. Higher TMN concentration can be generalised to higher nutrient concentration. The TMN concentration of the stock solution was 1954.5 mg/L. In detail, the concentrations of TAN, nitrate nitrogen and nitrite nitrogen were respectively 1854mg/L, 100mg/L and 0.5 mg/L. The desired volumes of nutrient solutions were 24L in each 25L bucket. The layout of the experiment is represented in Appendix 4.

Modality name	V_s	V_w
P65	0.800	23.20
P90	1.1	22.9
P115	1.41	22.59
P140	1.72	22.28

Table 2.1: The volumes of stock solution (V_s) and demineralized water (V_w) used to create each modality.

2.1.5.2. Biomedia

The biomedia (or biocarriers) used were PVC rings with an increased surface area that carry microorganisms which can then be transported in the solutions. On the 18th of January, they were immersed in three buckets filled with 15 L of a 0.5% dry matter solution. Each solution was created by mixing 132.2g of fresh chicken faeces with water.

Outlets extending from a 65 W AP100 Eco air pump (Aqua Forte, Fluidra Commercial S.A.U., Sabadell (Barcelona), Spain) were dipped in all three buckets to promote the growth of nitrifying bacteria by creating oxic conditions.

On days 3 and 7 of this phase, around 20 mL of H_2SO_4 10% were added to each of the three buckets as the pH was exceeding the upper extreme of the optimal range for nitrifying bacteria development (see section 1.3.2.2.2.).

This phase ended on the 27th of January: it lasted 9 days.

The biomedia were scooped out and weighed. Each bioponic nutrient solution received the same mass of biomedia while the latter were still moist.

2.1.5.3. Nitrogen concentration monitoring

Samples of the nutrient solutions were taken every Tuesday using 15 mL falcon tubes after mixing the solutions. All samples were then centrifuged using a Sigma 4-16KS refrigerated benchtop centrifuge (Sigma Laborzentrifugen GmbH, Osterode am Hartz, Germany) for 15 minutes at maximum velocity (13131 g). The aim of this step was to eliminate suspended solids from the samples. The centrifuged solutions were then extracted and analysed with the HANNA multiparameter spectrophotometer described above using the same reagents.

In addition, a TriOS optical sensor (TriOS Messund Datentechnik GmbH, Rastede, Germany) was used to measure the concentration of nitrate nitrogen ($N\text{-}NO_3^-$) of the nutrient solutions every Monday, Wednesday, and Friday.

2.1.5.4. Physical parameters monitoring

The pH, EC, Dissolved Oxygen (DO) and the temperature of the different solutions were measured using a Hach HQ40d portable multimeter (HACH Lange NV/SA, Nazareth, Belgium) with the corresponding probes. Measurements of the pH, EC and temperature were made every day except for the weekends. These same parameters were measured in addition to the DO every Monday, Wednesday, and Friday for the nutrient solutions.

In order to ensure the correct occurrence of nitrification and avoid an excessive pH (above 8.5), the latter was lowered using a 10% H_2SO_4 solution.

The TriOS optical sensor was used to measure the chemical oxygen demand (COD) and the total suspended solids (TSS) every Monday, Wednesday and Friday.

According to Boyles (1997), COD is "*a measure of the oxygen equivalent of the organic matter content of a sample that is susceptible to oxidation by a strong chemical oxidant.*"

2.1.5.5. Analysis by an external laboratory

At the first and last day of the aerobic digestion phase., samples of the bioponic solutions were taken and analysed by CPAR. At the end of the lettuce culture phase, samples of P90 and H solutions were taken and analysed by CPAR for the concentrations of all the macronutrients. Nitrogen concentrations were determined for the following forms: total nitrogen, ammonium, nitrate, and nitrite. The following physical parameters were also measured: pH, EC, Chemical Oxygen Demand (COD), Biochemical Oxygen Demand 5 (BOD5) and Total Suspended Solids (TSS).

BOD5 measures the amount of oxygen consumed by microorganisms in a sample during a five-day period (Boyles, 1997). This test can indicate the presence of microorganisms in a solution.

2.1.6. Lettuce culture

2.1.6.1. Germination

A total of 385 Rockwool cubes (3.5*3.5*4 cm) were sown with Butterhead lettuce seeds (*Lactuca sativa L., 1753 var. Lucrecia,* Rijk Zwaan) on the 8th of February. They were scattered across five 7X11 trays (see Figure 2.3). The latter were then placed in a greenhouse in Gembloux Agro-Bio Tech (Gembloux, Belgium) for thirteen days. The rockwool cubes were bottom watered using tap water.

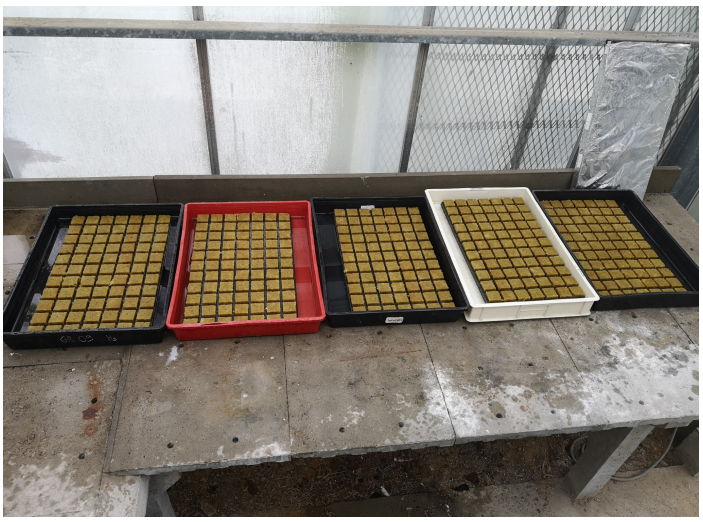

Figure 2.4: Rockwool cubes sown with Butterhead lettuce

2.1.6.2. Conventional hydroponic solutions

The conventional hydroponic nutrient solutions were created on the 21st of February by mixing equal amounts of hydro solution A and hydro solution B (HY-PRO, Friends B.V., Bladel, Netherlands) in demineralized water until an EC of 1300 mS/cm was reached. It is the reference EC used for lettuce culture in C-RAU.

Given the heat that the nutrient solutions were exposed to, a line representing initial solution heights was traced on each bucket to maintain the solutions' volumes when acquiring samples or when taking measurements.

2.1.6.3. Plantule introduction in the systems

On the 21st of February, the plantules that visually looked the most similar, along with their growing media (the rockwool cubes), were inserted in plastic baskets. The latter were conical with a large disc diameter, small disc diameter and height respectively of 4.5, 3.5 and 5 centimetres. Each basket was randomly placed in one of the holes of one of the NFT gutters.

2.1.6.4. Monitoring

Nitrogen concentration monitoring and physical parameter monitoring were performed in the same manner as during the aerobic digestion phase with one exception: nutrient solution samples were taken every other Tuesday instead of every Tuesday for analysis using the HANNA multiparameter spectrophotometer.

pH was lowered and maintained at 6 using a 10% H_2SO_4 solution, a 1M NaOH solution or both.

2.1.6.5. Deficiency management

Stock solution was added four times (on days 47, 49, 52 and 54) to the bioponic solutions in order to compensate for a deficiency in nitrogen observed at late stages. All bioponic solutions received 0.71L of stock solution, equally divided between the 4 days, which equates to 50 mg/L of nitrogen, mostly in the form of ammonium. In addition, 23.4 mg/L of phosphorus, 57 mg/L of

potassium, 19.22 mg/L of calcium and 11.26 mg/L of magnesium were added to the bioponic nutrient solutions as a consequence of the stock solution additions..

2.1.6.6. Rodent management

On the 10th of March, several lettuces were attacked by rodents (see Figure 2.4). Fatal rodent baits were scattered across the greenhouse as soon as the attacks were observed. One or two plants per gutter showcased rodent bites. These were marked so that they could be eliminated when harvested. Hence, the damaged lettuce plants were discarded.

Figure 2.5: Different individuals showing apparent damage

2.1.7. Lettuce analysis

2.1.7.1. Harvest and fresh weight measurement

As mentioned before, each gutter held 12 lettuces. The latter were attributed a number from 1 to 12 starting with the closest to the nutrient solution buckets (see Appendix 4). All lettuce were cut at the base of their stems and weighed immediately using a FKB 8K0.05 Kern scale (KERN & SOHN GmbH, Balingen-Frommern, Germany).

2.1.7.2. Analysis by an external laboratory

Lettuce in the second, fifth, eighth and tenth position were sent to an external laboratory (CPAR) to analyse the chemical composition of the leaves.

2.1.7.3. Dry weight measurement

The remaining lettuce was dried using a Memmert heating and drying oven (Memmert GmbH, Schwabach, Germany) for 7 days at 40℃. Dry weights were then measured using the Kern scale mentioned above.

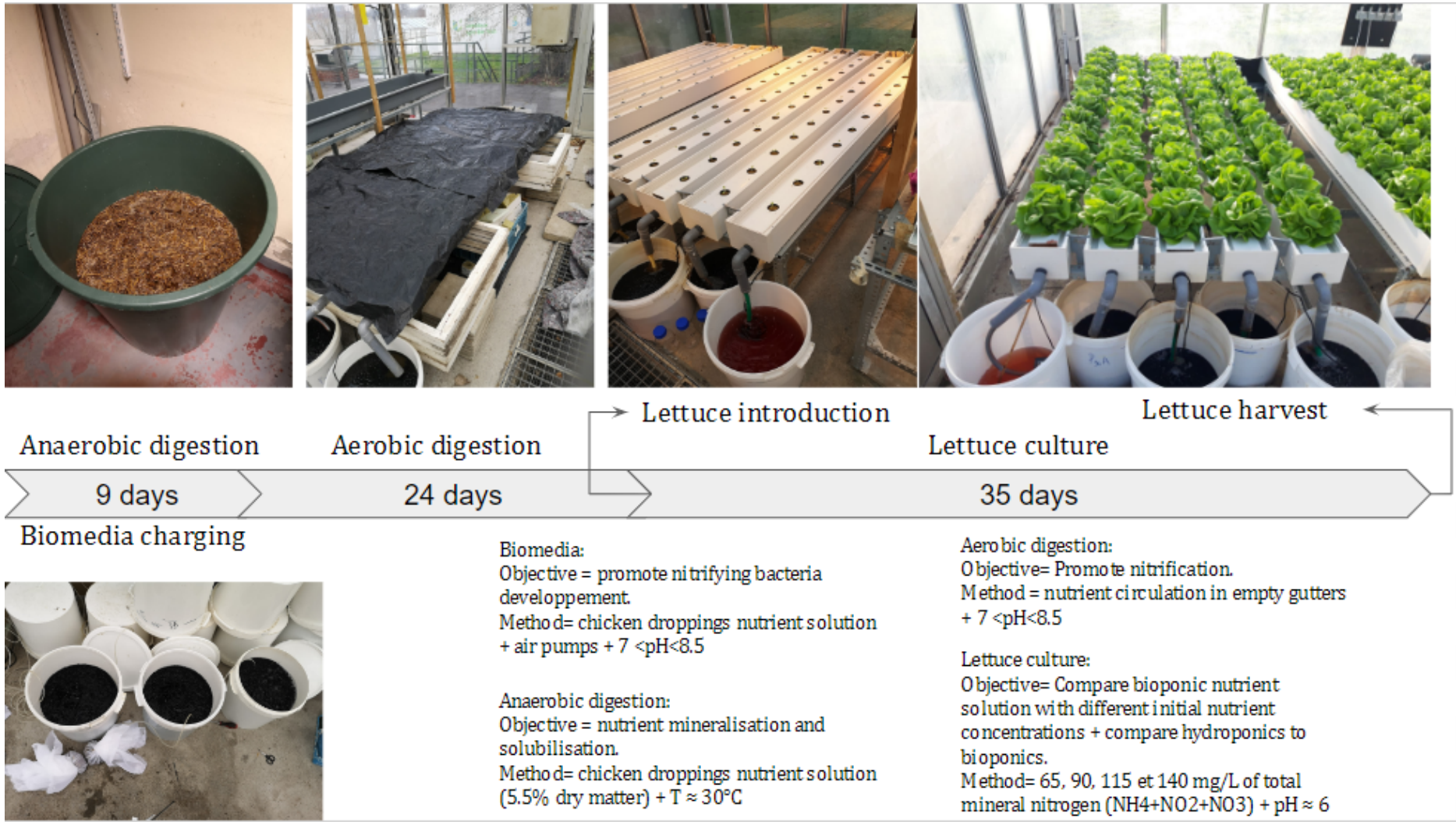

Figure 2.6: Timeline of the essay and the major phases

2.2. AMMONIA VOLATILISATION

2.2.1. Introduction

Part of the nitrogen lost during the creation of bioponic solutions could be due to the volatilisation of ammonia. Gas bubbles created in the solution because of the circulation can trap NH_3 molecules and carry them into the atmosphere. Given the pH levels reached during the aerobic digestion phase, presence of NH_3 is suspected.

Thus, having an idea of the impact of this phenomenon on nitrogen losses when creating bioponic solutions could help further understand the latter.

2.2.2. Setup

For this reason, solutions containing ammonia from a mineral source (NH_4OH) were created and maintained at pH levels representing both extremes of the range at which the bioponic solutions could find themselves during the aerobic digestion phase: 7 and 8.5.

In order to study the maximum amount of nitrogen that could be lost due to ammonia volatilisation in a situation similar to the bioponic essay, the concentrations of ammonium-nitrogen were set to the same levels as the P140 modalities (116 mg/L). This was done by mixing 3.1 g of a 28% NH_4OH solution with 3L of demineralised water in 5L buckets.

To avoid biological activities that could consume nitrogen, 30 mL of a 27.5% hydrogen peroxide (H_2O_2) solution was added to each bucket.

With the purpose of replicating the aeration mechanism in the bioponic essay, an air pump outlet was immersed in some buckets.

Given the heat that the nutrient solutions were exposed to, a line was traced on each bucket to maintain the solutions' volumes when acquiring samples or when taking measurements

Therefore, 4 modalities can be discerned for this essay:

- A7X: pH was set and maintained at 7 in the absence of air pumps
- A8.5X: pH was set and maintained at 8.5 In the absence of air pumps
- O7X: pH was set and maintained at 7 in the presence of an air pump
- O8.5X: pH was set at 8.5 in the presence of an air pump.

Where X represents the repetition of the modality (A, B or C).

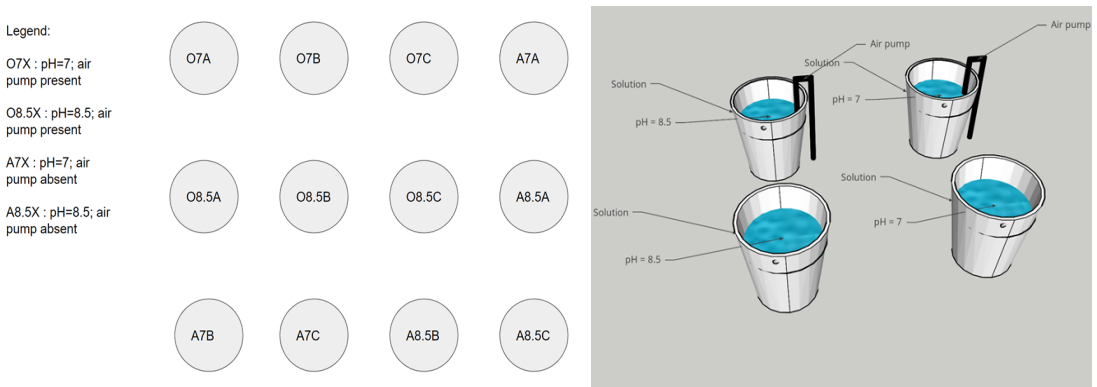

Figure 2.7: The four modalities illustrated on Sketchup (right side); The layout of the ammonia volatilization essay (left side).

2.2.3. Monitoring

pH measurements were made using the Hach multimeter every other day throughout the experiment except for the weekend. pH was adjusted using either a 10% hydrochloric acid (HCl) solution, a 1M sodium hydroxide (NaOH) solution or both. 30mL of a 27.5% H_2O_2 solution were added every other day to inhibit biological activity.

Samples of every solution were acquired on days 0, 3 and 7 to measure the concentrations of TAN. They were centrifuged using a Sigma 4-16KS refrigerated benchtop centrifuge for 15 minutes at maximum velocity (13131 g). Then, they were extracted and analysed in the Hanna multiparameter spectrophotometer. The latter was used for nitrite, nitrate and TAN concentration measurements, using the same reagents described above.

2.3. DATA ANALYSIS

2.3.1. Physical parameter and spectrophotometer analysis

The different physical parameters, as well as nitrite, nitrate and TAN concentrations were represented in charts built using RStudio.

2.3.2. Nitrification efficiency

In order to understand the effect of the initial solution concentration in nutrients on nitrification, the nitrification efficiency (NE) of each repetition was calculated using the following formula:

$NE = \frac{N-NO3}{N-NH4} \times 100$ (Equation 2.1)

Where:

- N-NO3 = the concentration in nitrate-nitrogen at the end of the aerobic digestion phase
- N-NH4 = the concentration in ammonium-nitrogen at the beginning of the aerobic digestion phase.

NE will identify the proportion of ammonia that has undergone and completed nitrification.

2.3.3. Nutrient losses

With the purpose of further understanding the aerobic digestion phase, nutrient losses were established as a percentage by comparing nutrient concentrations in the solutions on the last day of the aerobic digestion phase with those on the first day. Nutrient losses could cast light on certain mechanisms that are occurring during this phase. To illustrate, if nutrient concentration losses are disproportionate with nitrogen and phosphorus taking the lead, this could probably be the result of nutrient uptake by heterotrophic microorganisms which particularly require these two nutrients in large quantities (Todar, 2020).

2.3.4. Dry and fresh weight analysis

With the purpose of determining which initial concentration in nutrients would result in the best yields, a one-way analysis of variance (ANOVA) was conducted using the RStudio software (version 4.1.1) between the different modalities (mock included). Another one-way ANOVA was conducted between the repetitions of each modality in order to study the repeatability of the solutions. This took place once for dry weights and once for fresh weights. The ANOVA was either conducted using a linear model or a linear mixed-effects model. The former only considers the effect of the fixed variable (the bioponic solutions in this case) and was used when the homogeneity of the variances assumption was met among the repetitions or the modalities. However, whenever this was not the case, a linear mixed effects model was used to also take into consideration the random effects of the gutters and the blocs. This allows an ANOVA without worrying about the homogeneity of variances.

2.3.4.1. Checking the assumptions for the one-way ANOVA

The population should be normally distributed. This was tested thrice: once by observing the bell shape of a histogram built using the population, then by using the Shapiro-Wilk test (H0=ANOVA residues follow a normal population), and finally by observing a q-q plot of the residuals after the test.

The variances need to be equal. The homogeneity of the variance was verified using Bartlett's test (H0=The variances of the ANOVA residues are identical). This assumption is not needed in the case of a linear mixed-effects model.

The observations of each group need to be independent. The independence of the modalities and repetitions was inherent to the essay.

2.3.4.2. Tukey's HSD Post-Hoc test

The one-way ANOVA only provides information on whether there is a significant difference in the means or not. They do not indicate where this difference lies.

Therefore, Tukey's honest significant difference (HSD) Post-Hoc test was performed to compare the difference between the repetitions in the same modality. The groups provided in Tukey's HSD test were then transferred to a boxplot for a better visualisation.

2.3.5. Lettuce leaves chemical composition comparison

The data retrieved from CPAR concerning the chemical composition of the lettuce leaves were analysed using one-way ANOVAs for each element. The ANOVA assumptions were verified for all elements using a Shapiro-Wilk test and Bartlett's test.

A Tukey's HSD test was executed after each ANOVA and the groups were represented in a boxplot for each element.

2.3.6. Ammonia volatilisation data analysis

Data from the ammonia volatilisation essay was used to calculate nitrogen loss percentages which were analysed using a one-way ANOVA test to understand the effect of each modality on the volatilisation of ammonia. A two-way ANOVA could have been conducted; however, this would only be interesting to understand the impact of pH and the creation of oxic conditions on ammonia volatilization. This was not the objective of this essay. Indeed, this essay was meant to isolate the impact of ammonia volatilisation on the loss of nitrogen by replicating conditions similar to the bioponic solutions without organic matter or bacterial activity.

3. RESULTS

3.1. THE BIOPONIC ESSAY

3.1.1. The chicken droppings

3.1.1.1. Dry matter determination

The dry matter percentage of the chicken droppings used in this experiment was 56.72%.

3.1.1.2. Analysis by an external laboratory

The results given by CPAR can be found in Appendix 3. However, it is important to highlight that organic matter accounted for 80.15% of the dry matter. In addition, the carbon over nitrogen ratio (C/N) was 8.8. Table 3.1 shows the composition of the chicken droppings

Analysis	On fresh matter	On dry matter
Total ashes	10.26 %	19.85 %
Total organic matter	414 g/kg	80.15 %
Ammonia-nitrogen ($N\text{-}NH_4^+$)	4.01 g/kg	0.78 %
Total nitrogen (N)	27.37 g/kg	5.29 %
Phosphorus (P_2O_5)	19.41 g/kg	3.76 %
Potassium (K_2O)	17.26 g/kg	3.34 %
Calcium (CaO)	21.05 g/kg	4.07 %
Magnesium (MgO)	5.52 g/kg	1.07 %
Sodium (Na_2O)	4.49 g/kg	0.87 %
Iron (Fe)	1.279 g/kg	2.474 g/kg
Manganese (Mn)	0.216 g/kg	0.418 g/kg
Copper (Cu)	0.049 g/kg	0.096 g/kg
Zinc (Zn)	0.222 g/kg	0.430 g/kg
pH	7.8	NA*
C/N ratio	8.8	NA*

Table 3.1: The chemical composition of the chicken droppings used in this essay

** NA = Data is not available*

3.1.2. Anaerobic digestion

3.1.2.1. Physical parameters comparison

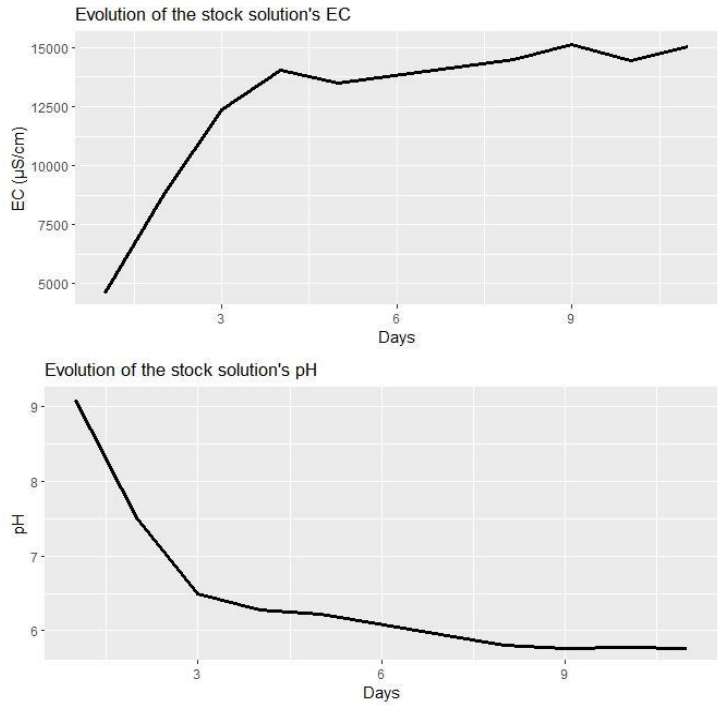

Figure 3.1: The evolution of the EC and pH of the stock solution during the anaerobic digestion phase

Electroconductivity (EC) and pH respectively underwent a sharp rise (from close to 5 mS/cm to 15 mS/cm) and decreased (from 9 to shy of 6) shortly after the beginning of the anaerobic digestion phase. This probably indicates the rapid occurrence of mineralisation due to acidogenesis as mentioned in section 1.3.2.1..

3.1.2.2. Spectrophotometer analysis

At the end of the anaerobic digestion phase and after filtration, the concentrations of nitrate-nitrogen, nitrite-nitrogen and TAN in the stock solution were respectively as follows: 100 mg/L ; 0 mg/L; 1854mg/L

As a result, the concentration of TMN in the stock solution was 1954mg/L.

3.1.3. Aerobic digestion and lettuce culture

3.1.3.1. Nitrite concentration measured by the spectrophotometer

In general, Figure 3.2 shows that P140 and P115 modalities experienced an increase in $N\text{-}NO_2^-$ up to one week later than P65 and P90 modalities. This means that ammonia oxidation took off in solutions with lower initial concentrations in nutrients before those with higher initial concentrations. $N\text{-}NO_2^-$ concentrations abruptly decreased after the introduction of the lettuce plantules (from up to ≈40 mg/L to less than ≈15mg/L). This is probably due to the artificial drop of pH to levels around 6, at which ammonia oxidation can be slowed down (Park et al., 2007). However, it is important to notice that for both P115B and P140C and PA40B, $N\text{-}NO_2^-$ was still at least twice as concentrated as in all other repetitions (respectively ≈14mg/L, ≈10mg/L and ≈5mg/L).

3.1.3.2. TAN concentration measured by the spectrophotometer

As can be seen in Figure 3.3, TAN levels decreased abruptly during the aerobic digestion phase until they reached levels close to null for all repetitions on day 18. Some TAN was introduced to the solutions when stock solution was added near the end of the experiment in order to avoid deficiencies.

On day 4, all solutions had lost between 36% (in the case of P65A) and 57.75% (in the case of P115B) of their TAN. However, nitrite spikes were not detected on the same day. This means that ammonium is following paths other than oxidation. Possible explanations for this decrease in TAN would be ammonia stripping and/or microbial assimilation.

3.1.3.3. Nitrate concentration measured by the TriOs probe

According to Figure 3.4, the first signs of NO_2^- oxidation appeared on day 17 of the aerobic digestion phase (February the 14th). As observed previously, the least initially concentrated solutions in nutrients were where nitrification took off the earliest. $N\text{-}NO_3^-$ concentration reached a plateau for most of the repetitions when lettuce plantules were introduced to the systems. This is probably linked to the artificial pH reduction to levels around 6 (Park et al., 2007). However, for repetitions P115B, P140B and P140C, concentrations in $N\text{-}NO_3^-$ still increased after the reduction of pH.

Conventional hydroponic solutions were twice to five times as concentrated in nitrate (≈118 mg/L) as any bioponic solution in this experiment.

The chart shows us that among bioponic solutions, both extremes belong to the P65 modality with P65A and P65B maintaining respectively the highest (≈46 mg/L) and lowest (≈23 mg/L) concentrations in $N\text{-}NO_3^-$ throughout the lettuce culture phase. This means that even with similar initial conditions, the composition of the nutrient solution was different. P90B shows concentrations in $N\text{-}NO_3^-$ lower (≈29 mg/L) than P90A and P90C (both ≈38 mg/L). Differences weren't as important for P115, P140 and H modalities. These results suggest that bioponic nutrient solutions with lower initial nutrient concentration are hardly predictable even if the initial conditions are known. This might be the result of the relyance on the action of microbial communities. It is possible that nitrifying bacteria were present in the microbial communities in increased proportions in some repetitions, leading to greater access to ammonium for nitrification. However, further research is needed to explain the reasons behind this

phenomenon happening with increased importance in solutions with lower initial concentrations.

The TriOs probe is susceptible to error in the presence of important amounts of solids. This could explain the sudden spike in $N\text{-}NO_3^-$ observed for P140B on day 49 of the essay (March the 18th), as this measurement took place after the addition of stock solution.

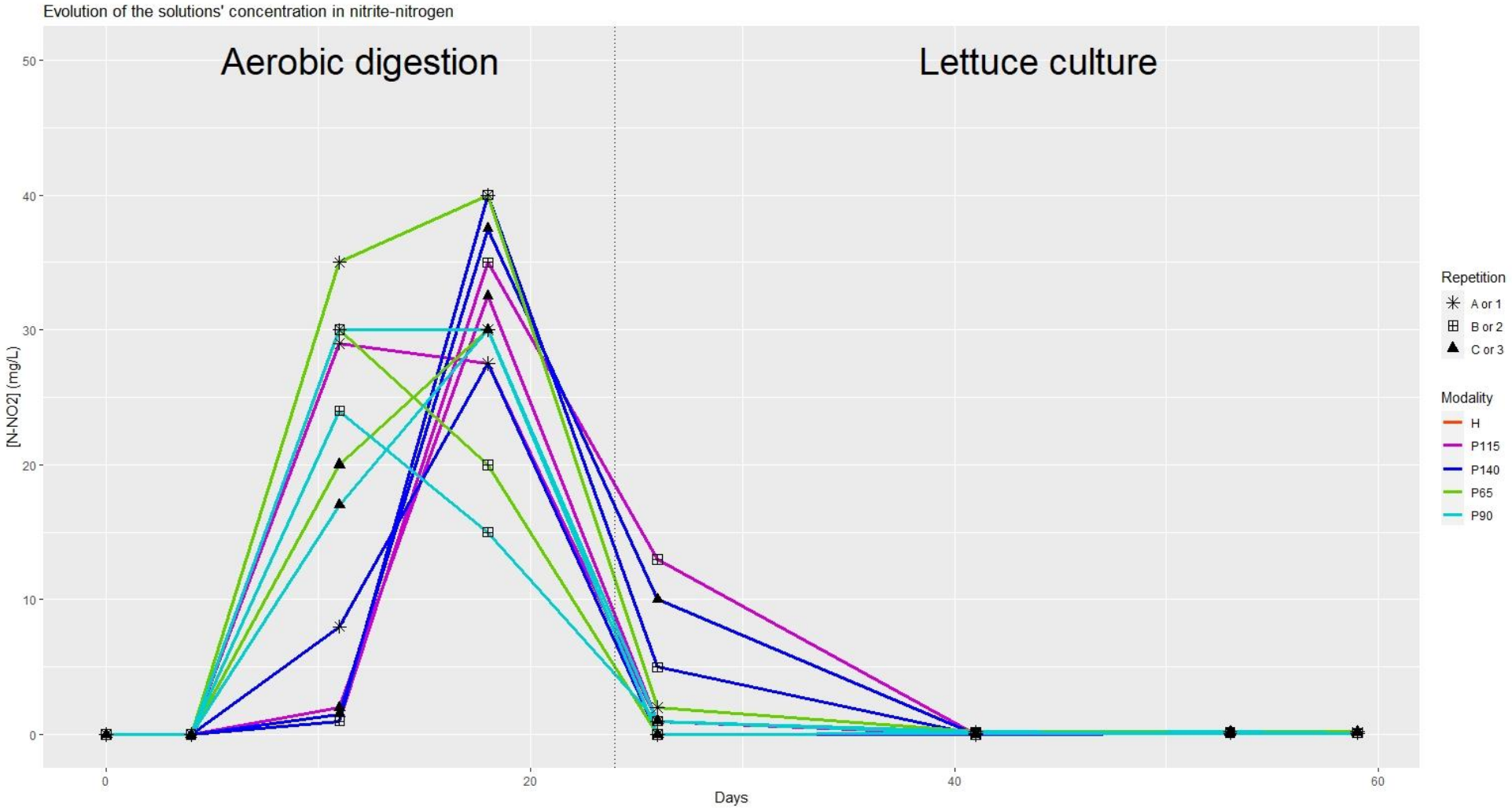

Figure 3.2: Evolution of the solutions' concentration in nitrite-nitrogen

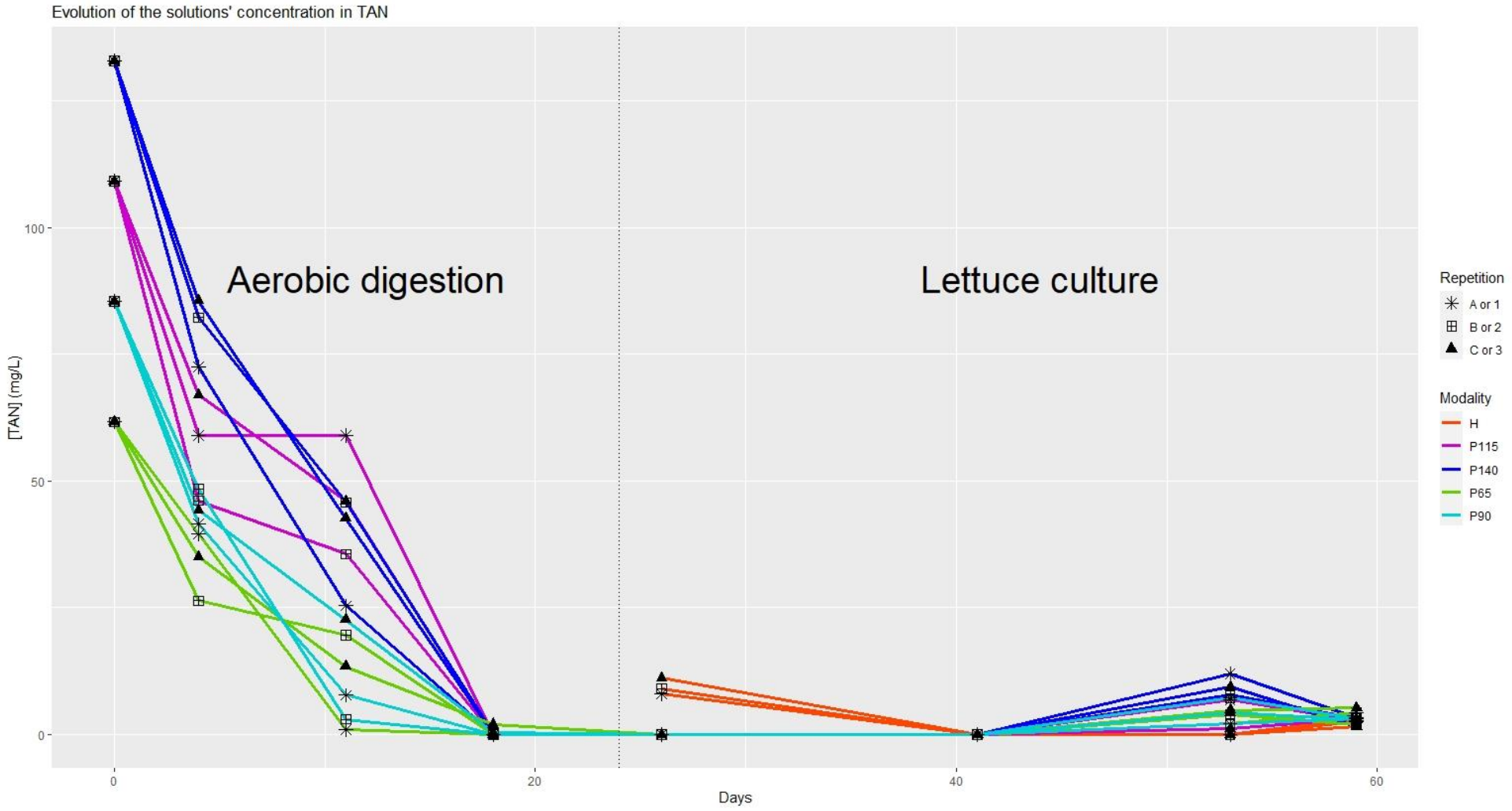

Figure 3.3: Evolution of the solutions' concentration of Total Ammonia Nitrogen (TAN)

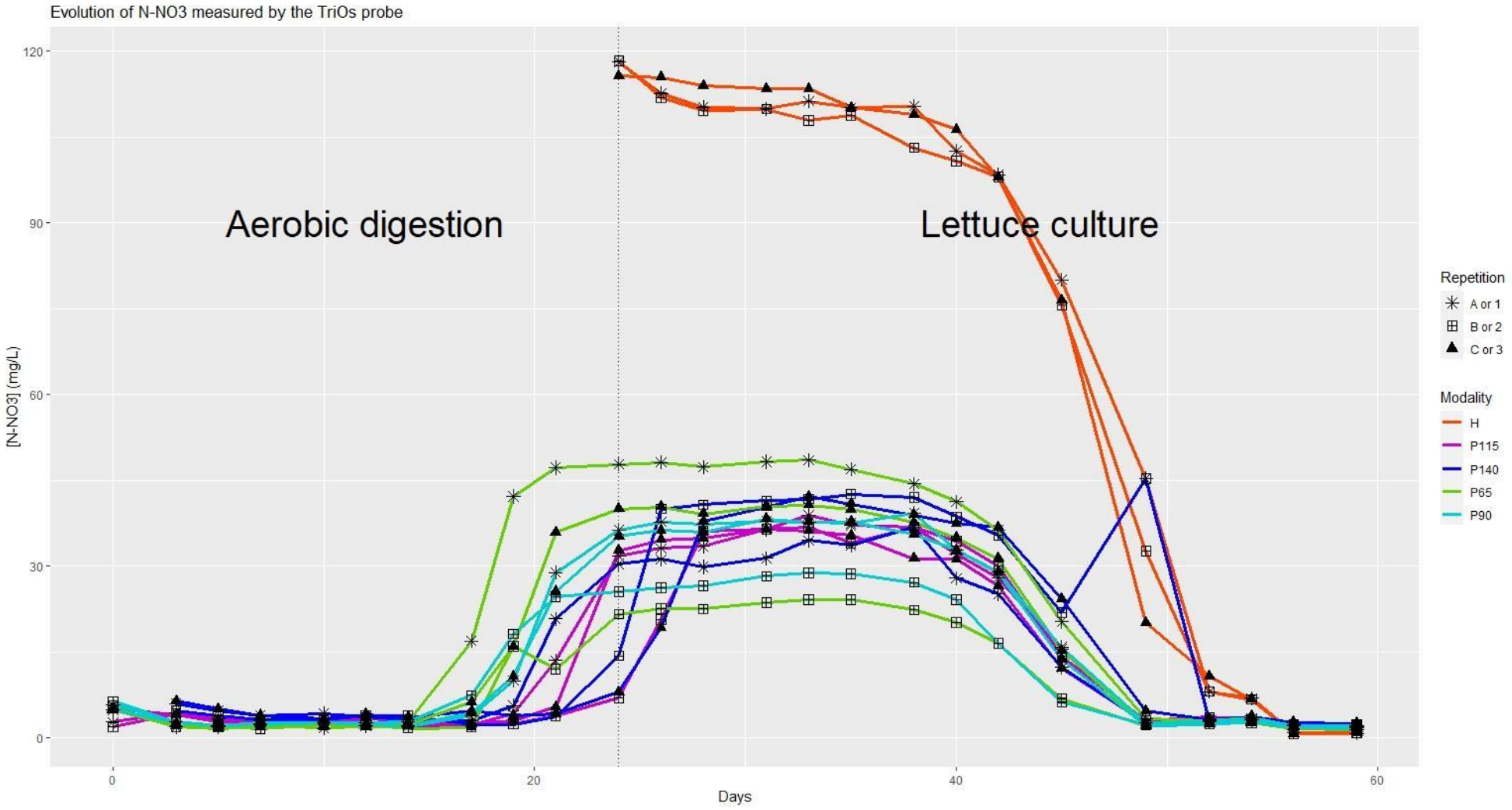

Figure 3.4: Evolution of the solution's concentration in nitrate-nitrogen

3.1.3.4. Solution pH

After observing Figure 3.5, in general, a pH spike of almost 3 units, followed by a steady increase to around 8.5 can be observed in the first couple of days of the aerobic digestion phase. On days ten to twelve of this same phase, a decrease in pH can be observed for the least initially concentrated modalities in nutrients (P65 and P90). It is important to state that the natural pH drop in P65B was less important than P65A and P65C and happened later. In contrast, except for P140A and P115A, no sudden decrease in pH was observed before the manual pH adjustment that happened on day 12 (February the 9th) in order to avoid harming the bacteria at such high pH levels (section 1.3.2.2.2.).

From day 2 to day 16 after the introduction of lettuce, pH levels seem to stabilise between 5.5 and 6.5, which is the optimal range for soilless crop culture (see figure 1.4).

Near the end of the lettuce culture phase, several spikes in pH can be observed which can be explained by the 4 additions of stock solutions to the bioponic solutions the 16th, 18th, 21st and 23rd of March (days 23, 25, 28 and 30 of lettuce culture).

3.1.3.5. Solution EC

For starters, it is important to understand that EC is difficult to interpret in bioponics as it can be altered by organic matter. Nonetheless, this section will explore the data retrieved and presented in figure 3.6 as tendencies can still be highlighted.

On day 17 of the aerobic digestion phase, the EC of P65, P90, P115 and P140 modalities had dropped on average by 33.1%, 44.4%, 44.4% and 48.3% , with initial values of ≈720µS/cm, ≈950µS/cm, ≈1200µS/cm, and ≈1430µS/cm respectively. Hence, there seems to be a positive correlation between the initial concentration in nutrients of a bioponic solution and the proportion of ions that are being lost during aerobic digestion. These ions could either be volatilized in the case of nitrogen, assimilated by microorganisms or even deposited on the NFT systems.

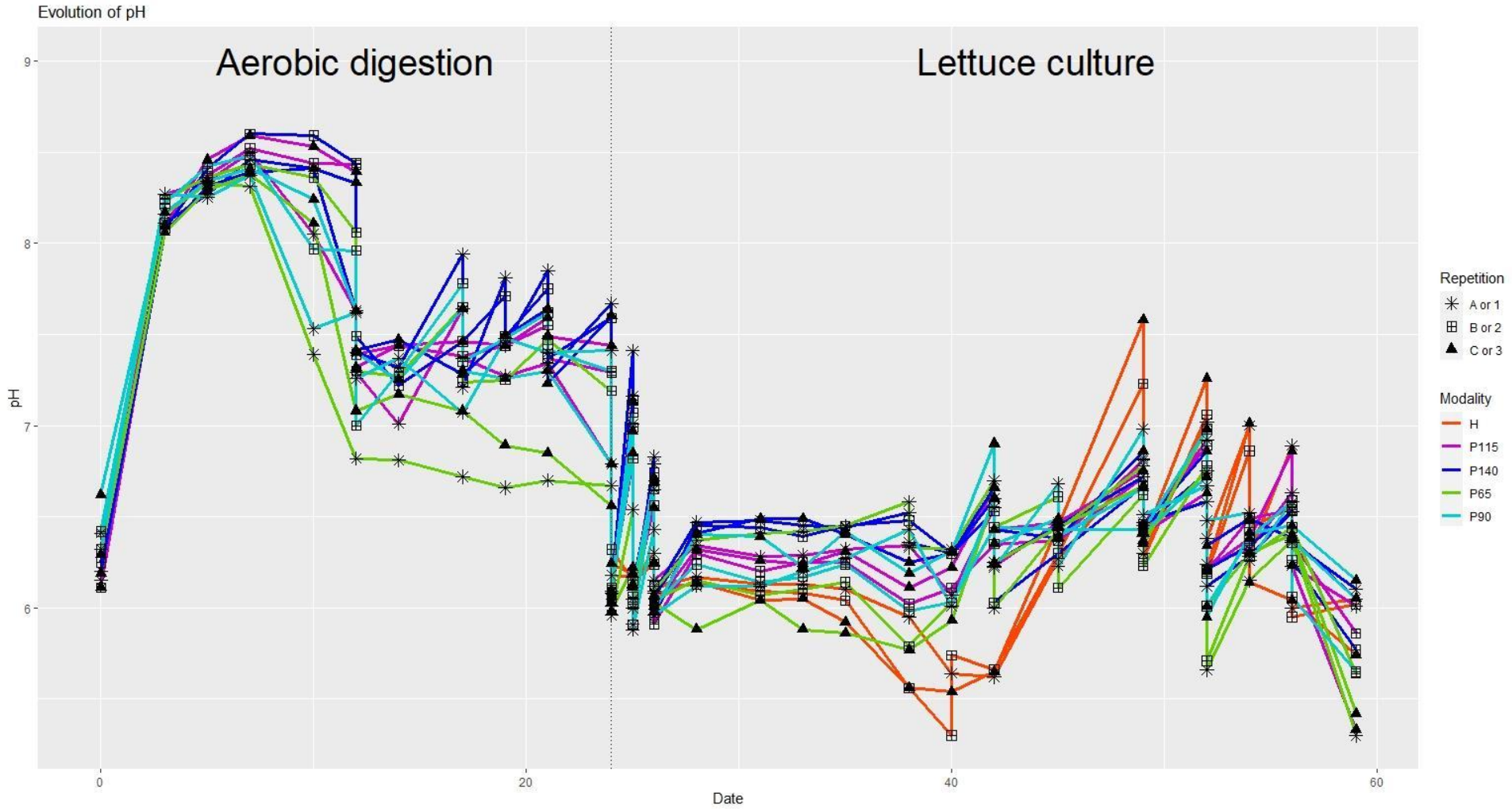

Figure 3.5: Evolution of pH in the nutrient solutions

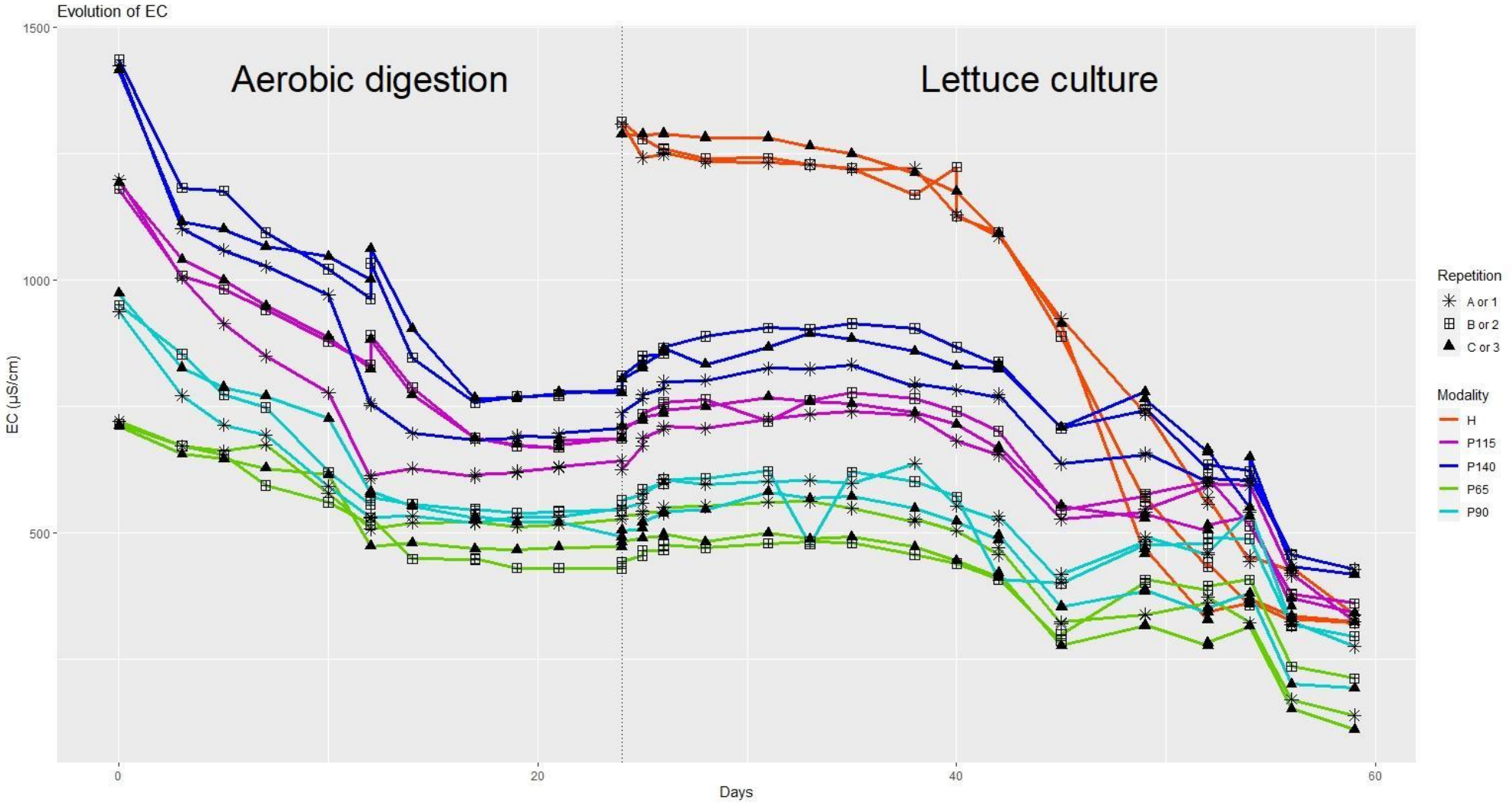

Figure 3.6: Evolution of EC in the nutrient solutions

3.1.3.6. Solution DO

On day 3 of the aerobic digestion phase, DO was close to zero for P140 (A,B and C) and below 2.5mg/L for P115 (A, B and C). This might have contributed to the belated nitrification (section 1.3.2.2.2.). This could indicate that even after three days, microbial and chemical activities were consuming a lot of oxygen. In fact, higher initial mineral concentrations led to increased concentrations in residual organic matter. This can promote the development of heterotrophic microbial communities which entails the consumption of oxygen and other minerals. It would be interesting to optimise the filtration mechanism in order to eliminate organic residues.

During the lettuce culture phase, DO concentration seems to be similar across all repetitions, remaining around 7.5 mg/L, until it plummeted to around 4 mg/L after the addition of stock solution. This possibly indicated a rise in biological and chemical activities.

For the majority of this essay, DO remained above the 2.5mg/L threshold required for the inhibition of anammox (Kimura et al., 2011).

3.1.3.7. Solution TSS

Figure 3.8 shows that by day 10, total suspended solids concentrations dropped for P65, P90, P115 and P140 by an average of 70.3%, 77.4%, 78.7% and 81.2% respectively. Two possible mechanisms could have contributed to this effect: mineralisation and the accumulation of residues in the bottom part of the NFT gutters and other system surfaces. However, the importance of each mechanism is unknown.

3.1.3.8. Solution COD

Chemical oxygen demand and TSS seem to be closely related when it comes to bioponic solutions, with an abrupt decrease in the beginning and a spike after the addition of stock solution (Figure 3.9). However, COD shows a clear difference between modalities where a higher initial concentration in nutrients leads to maintaining higher COD levels throughout the experiment. For recollection, COD reflects the organic matter of a solution (Boyles, 1997). Therefore, these results confirm the increased organic matter content of solutions with high initial mineral concentration. Hence, there is a need to optimise the filtration mechanism to avoid the development of heterotrophic microbial communities which consume oxygen and minerals.

In contrast, conventional hydroponic solutions show more important COD levels overall with the lowest concentrations in TSS and the highest concentrations in DO.

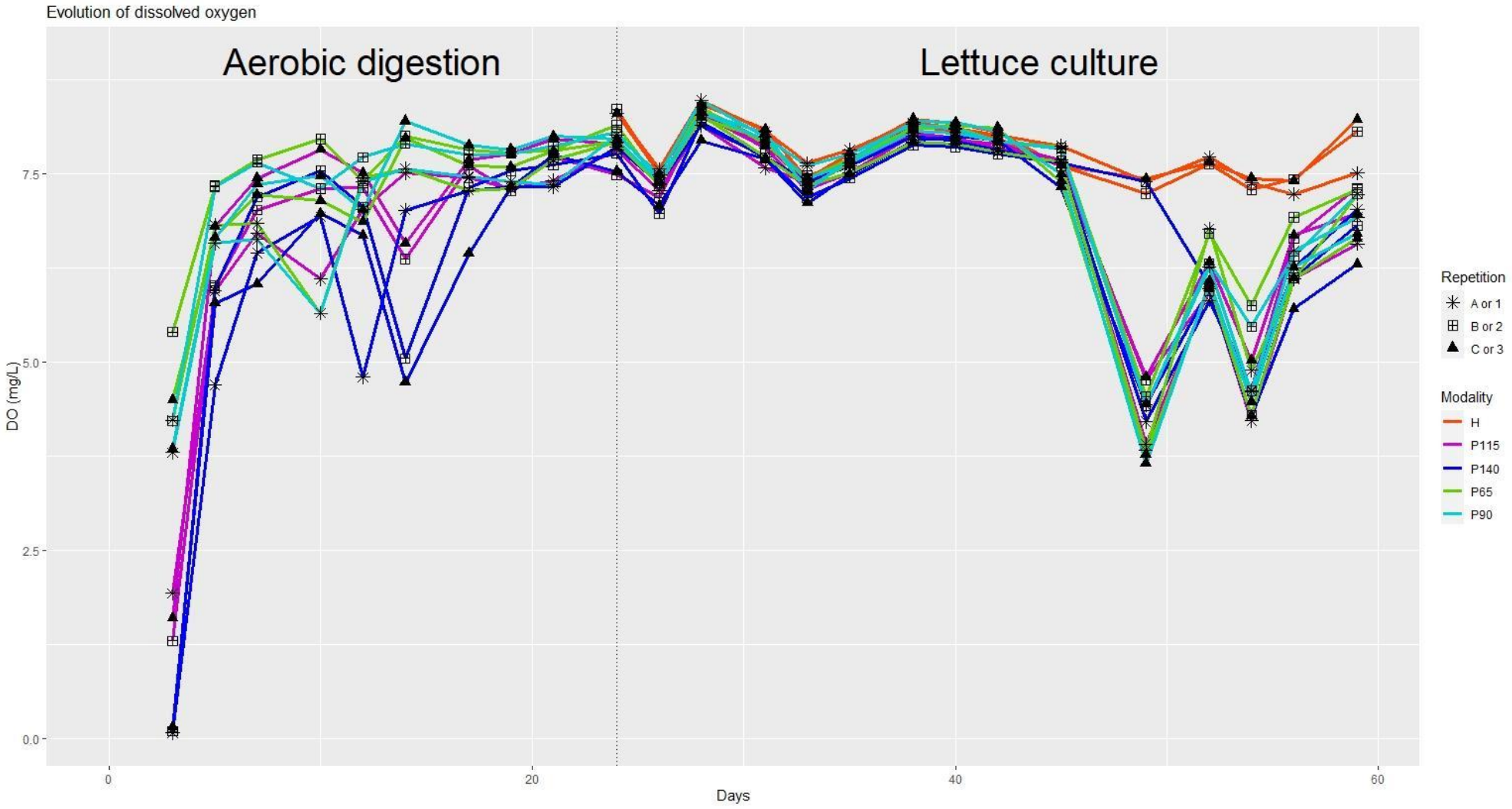

Figure 3.7: Evolution of DO in the nutrient solutions

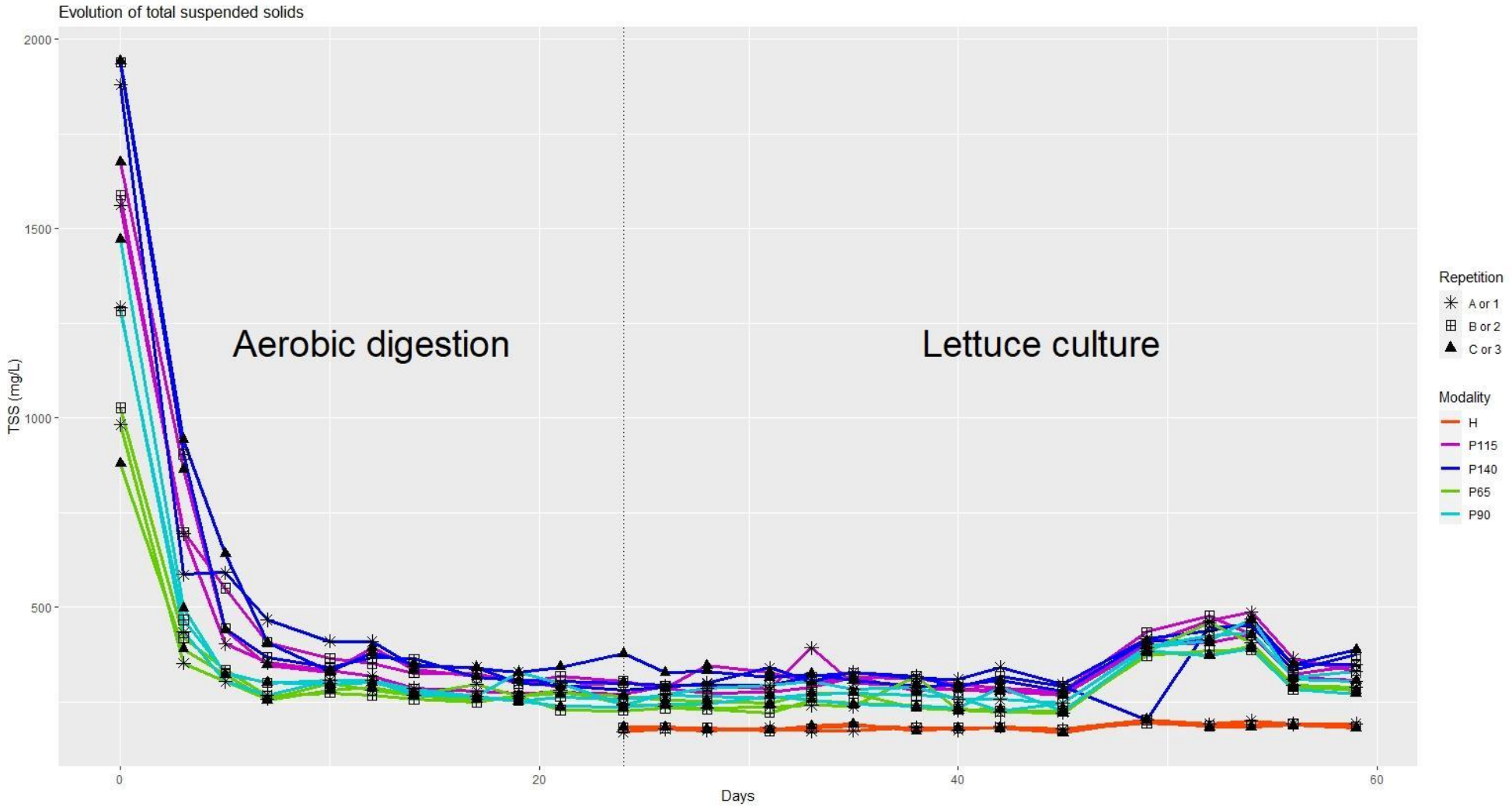

Figure 3.8: Evolution of TSS in the nutrient solutions

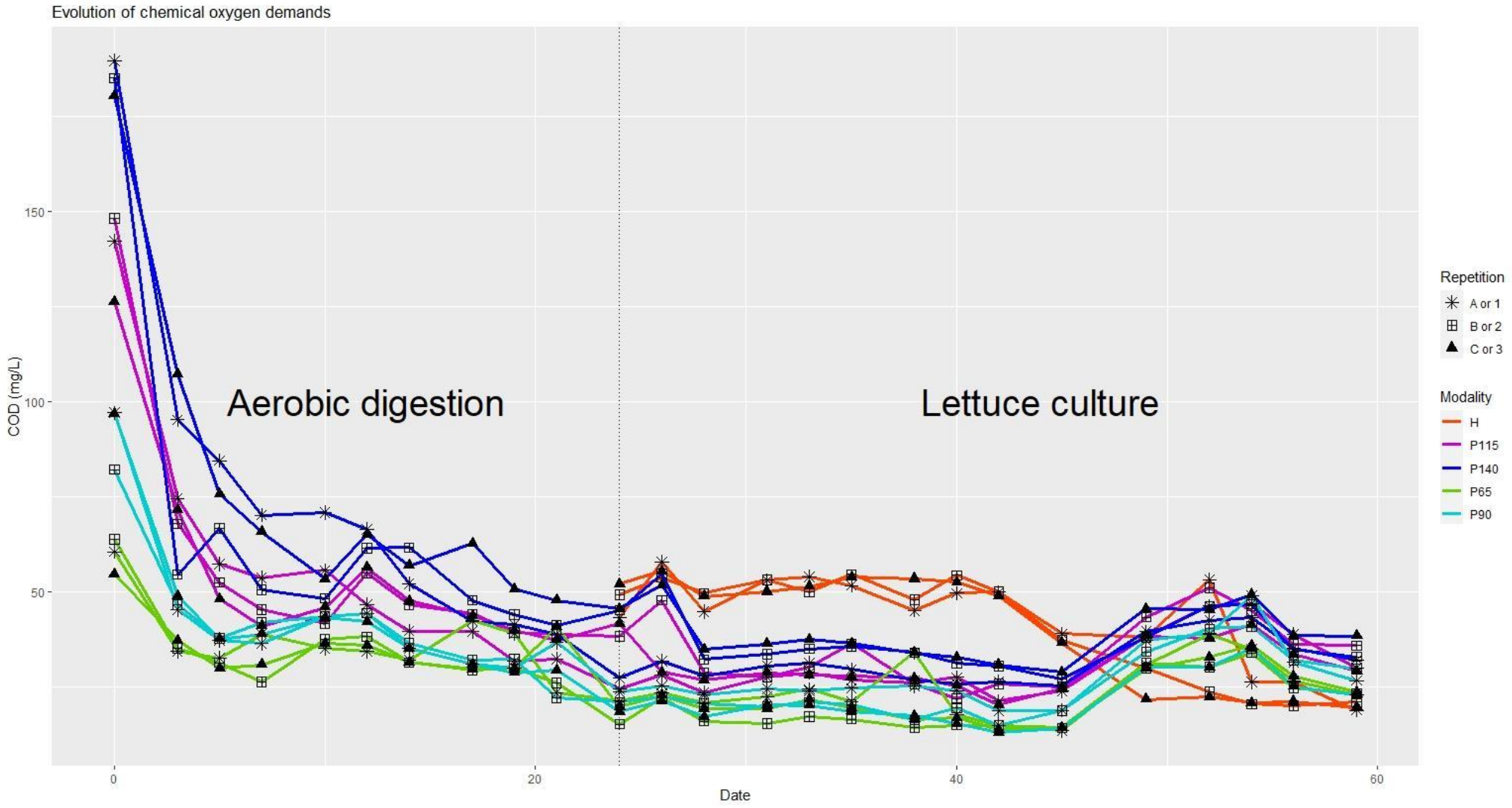

Figure 3.9: Evolution of COD in the nutrient solutions

3.1.3.9. Analysis by an external laboratory

Figures 3.10 and 3.12 are visual representations of the nutrient solution analyses that took place in CPAR using samples taken on day 1 of the aerobic digestion phase (January the 28th) and on day 1 of the lettuce culture phase (February the 22nd). Figure 3.11 shows a calculation of the nutrient losses between the first day of the aerobic digestion phase and the last day of it. Several observations can be highlighted:

For starters, all bioponic solutions have lost substantial amounts of total nitrogen during the aerobic digestion phase: respectively 43.8%, 63.6%, 69% and 72.6% for P65, P90, P115 and P140. The loss percentage seems to be correlated with the initial solution concentration in nutrients. P65A had only lost 24.1% of its total nitrogen.

In addition, all the TAN initially present in the solution were either nitrified or lost during the aerobic digestion phase as no TAN was still present at the end of the aerobic digestion phase, yet all repetitions except for P65A have lost more than 20% of their total mineral nitrogen. As a reminder, losses could have occurred either by ammonia volatilization, denitrification or through assimilation by microorganisms.

Then, all repetitions have suffered from a decrease in phosphorus, and potassium to a lesser extent. Indeed, phosphorus and potassium losses for P65, P90, P115 and P140 were respectively 25.5% and 11.5%, 37.3% and 17.7%, 28.3% and 5.6%, 29.3% and 15.9%. The pH levels of the solutions during sampling and analysis were well within the functional range for hydroponics (see Figure 3.12). Therefore, the contribution of phosphorus losses by crystallisation was likely small in this case. Hence, the losses could be the result of these elements' assimilation by bacteria, which disables their detection by a spectrophotometer.

In addition, BOD5, COD and TSS were significantly high at the beginning of the aerobic digestion phase but took a nosedive at the end of it . To illustrate, BOD5 at the end of the aerobic digestion phase, was less than 0.2% of this same parameter at the beginning of this phase (see Figure 3.12). This signals that microbial activity was high at the beginning but low at the end.

Table 3.2 compares the nutrient composition of the hydroponic solutions and the recommendations given by Resh (2013) for lettuce culture. It appears that our hydroponic solutions didn't meet the recommendations in nitrogen and calcium. In fact, our hydroponic solution contained 73.16%, 93.2%, 96.57%, 43.05% and 85.5% of the recommended nitrogen, phosphorus, potassium calcium and magnesium respectively. It is therefore important to keep in mind that our hydroponic nutrient solution was not optimal when analysing the productivity and quality of the lettuce heads.

	Nutrient origin	N (ppm)	P (ppm)	K (ppm)	Ca (ppm)	Mg (ppm)
Resh (2013)	Inorganic	190	50	210	200	40
H (1,2 and 3)	Inorganic	139.04	46.6	202.8	86.1	34.2

Table 3.2: Comparison the hydroponic solutions of this study (based on the results given by CPAR) with the recommendations of Resh (2013) in terms of their concentration of nitrogen, phosphorus, potassium, calcium and magnesium.

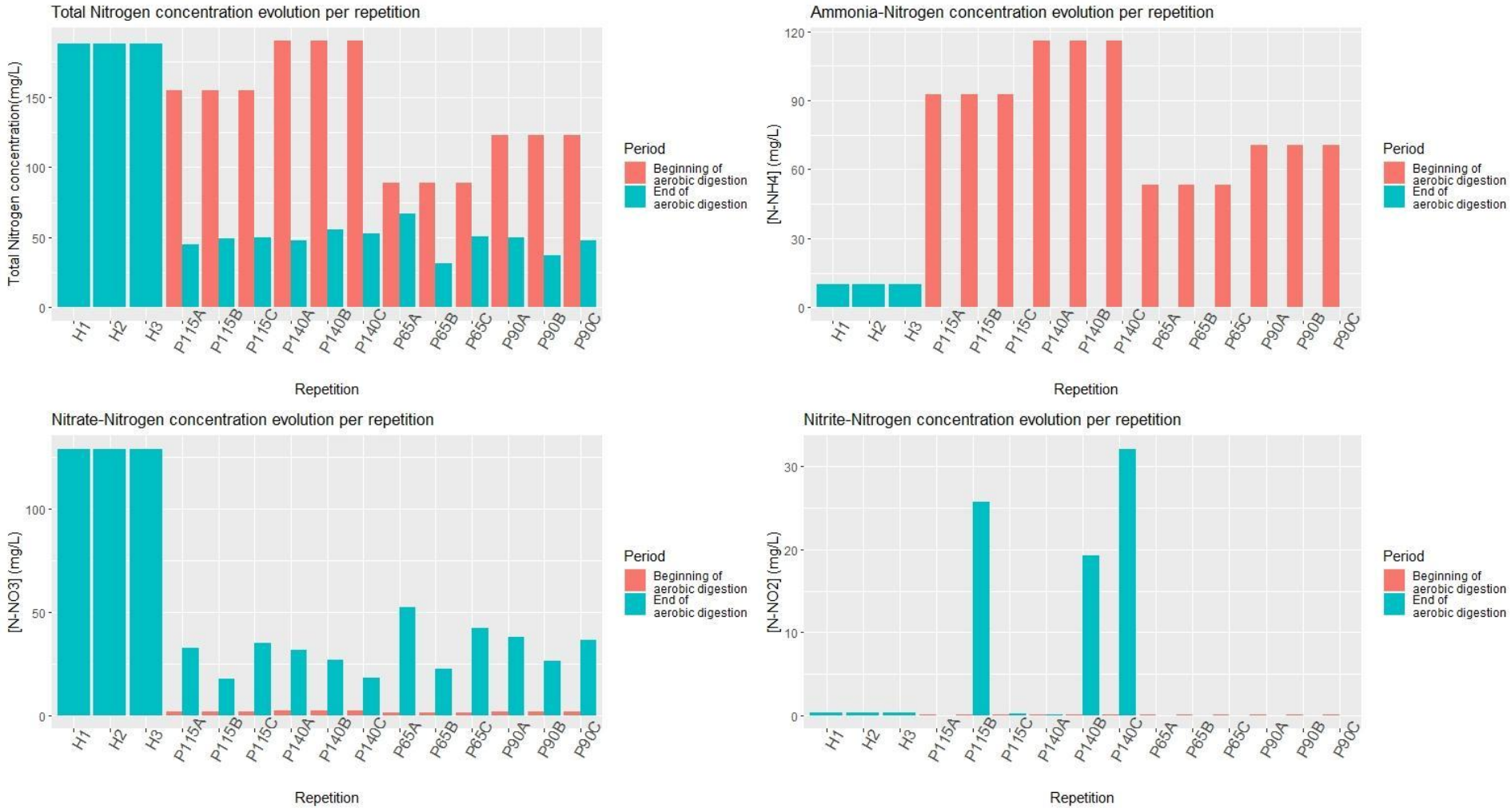

Figure 3.10: Comparison of the concentrations of total nitrogen, ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen between the beginning and the end of the aerobic digestion phase for each repetition.

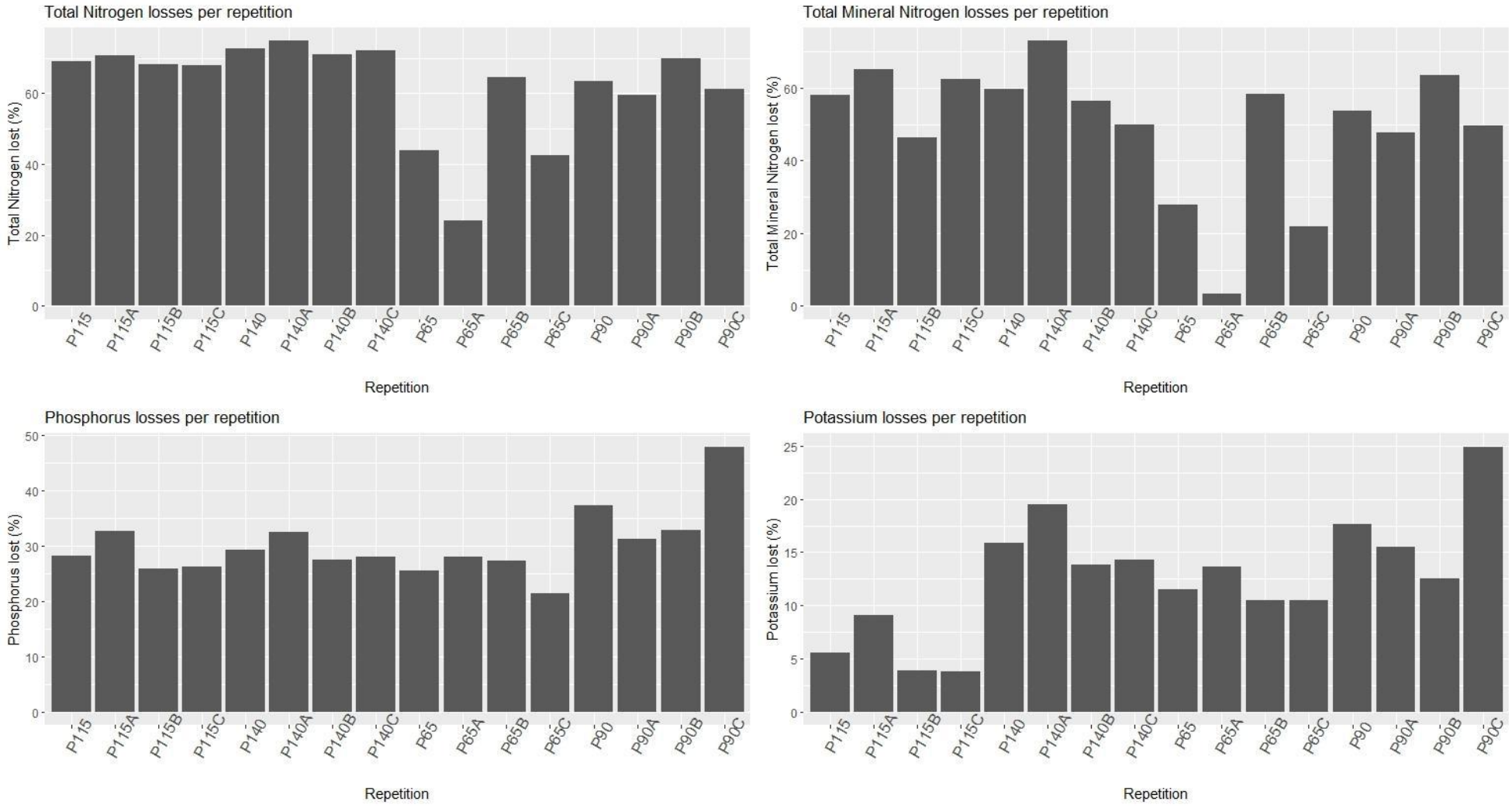

Figure 3.11: Loss percentage of total nitrogen, total mineral nitrogen, phosphorus and potassium per repetition and per modality during the aerobic digestion phase

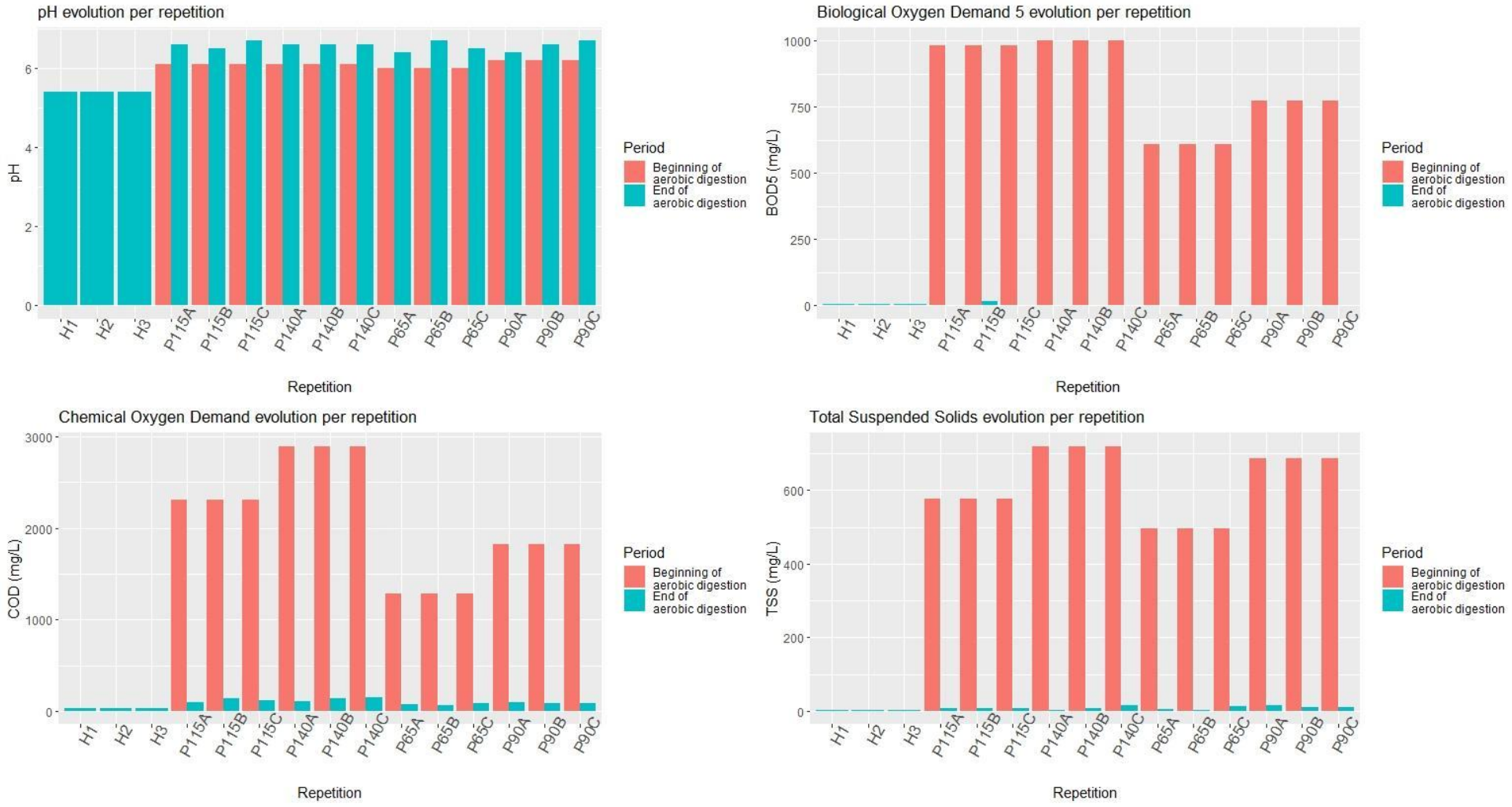

Figure 3.12: Comparison of pH, BOD5, COD and TSS between the beginning and the end of the aerobic digestion phase per repetition

3.1.3.10. Nitrification efficiency

As a reminder, nitrification efficiency in this study was calculated as a percentage of the TAN found in the solutions at the beginning of the aerobic digestion phase present as nitrate-nitrogen in the solutions at the end of the aerobic digestion phase (see Equation 2.1).

The nitrification efficiency was highest for the solutions with lower initial concentrations in nutrients: It was respectively 74.1%, 47.5%, 30.9% and 22.2% for P65, P90, P115 and P140. However, it is important to keep in mind that for P115B, P140B and P140C, the nitrification efficiency isn't representative of reality because at the time of the second sampling, substantial amounts of nitrite were still present in the solution (respectively more than 13 mg/L, 5 mg/L and 10 mg/L). The remaining nitrite was expected to undergo oxidation and increase nitrate concentrations. Moreover, P65A's nitrification efficiency was remarkably high (98.7%). This could be related to this repetition's total nitrogen loss being the lowest.

Although, it is important to recognise that the nitrification efficiency of all the solutions was probably slightly lower in reality than what is represented in Figure 3.13. In fact, during aerobic digestion, mineralisation was probably ongoing, releasing ammonium in the process. Therefore, the actual quantities of ammonium that were available for nitrification were probably higher than what was found in the solutions at the beginning of the aerobic digestion phase.

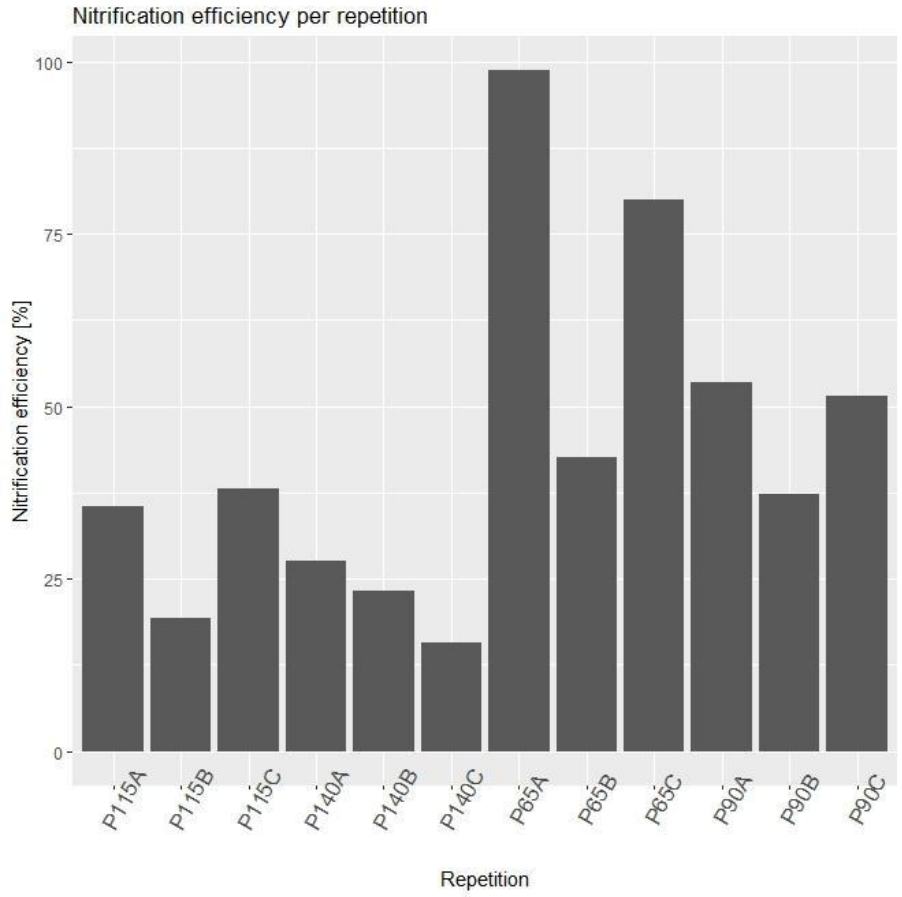

Figure 3.13: Nitrification efficiency per repetition

3.1.4. Lettuce analysis

3.1.4.1. Assumption verification

First, the independence assumption is implicit in this experiment as no interaction could occur between the repetitions.

Then, dry weight and fresh weight populations were both normally distributed. Both populations show regularly shaped bell curves (see appendix 5). Moreover, the shapiro-wilk tests for the fresh weight population and for the dry weight population had respectively p-values equal to 0.2485 and 0.06848, which in both cases is greater than 0.05. Residual q-q plots were also sketched and analysed and both seem to follow a normal distribution (see appendix 5).

When it comes to the homogeneity of the variances, on the one hand, Bartlett's test exhibited a p-value higher than 0.05 for fresh and dry weights grouped by repetitions (respectively 0.2867 and 0.05344). This means that the homogeneity of the variance between lettuces in the same gutter is verified in these cases and the analysis of variance can be executed between the repetitions using a linear model.

On the other hand when grouped by modality, Bartlett's test responded with p-values lower than 0.05 for both fresh weights and dry weights (0.02907 and 0.01586). This indicates that the variances between the repetitions are not equal in these cases. Therefore, a linear mixed-effects model had to be used in order to conduct an ANOVA on the different modalities. The populations seem to follow a normal distribution as can be seen in the residual q-q plots sketched in appendix 5.

3.1.4.2. Fresh and dry weight grouped by repetition

The means of lettuce fresh weights grouped by repetitions are highly significantly different as the p-value of the one-way ANOVA was equal to $2.43*10^{-8}$ which is substantially lower than 0.05.

Tukey's HSD test gave several groups which are represented in the boxplot below. In this test, repetitions sharing at least one letter are not significantly different from one another.

Except for P65, within the same modality, all repetitions share at least one letter according to Tukey's HSD test. This means that the repeatability of the experiment is solid. However, conclusions made about the P65 modality have to be taken with a grain of salt.

The same conclusion can arise by observing the Boxplot made using dry weights where the p-value of the one-way ANOVA was $1.348*10^{-5}$. The latter also indicates that the means of lettuce dry weights grouped by repetitions are significantly different.

3.1.4.3. Fresh and dry weight grouped by modality

Observing the results in the section above does not give a clear indication on whether the yields are different between the modalities. When grouped by modality, the p-values of the ANOVA test for fresh weights and dry weights were respectively 0.5485 and 0.7941. This indicates that there is no significant difference between the modalities (P65, P90, P115, P140 and H) neither in terms of fresh weights nor of dry weight

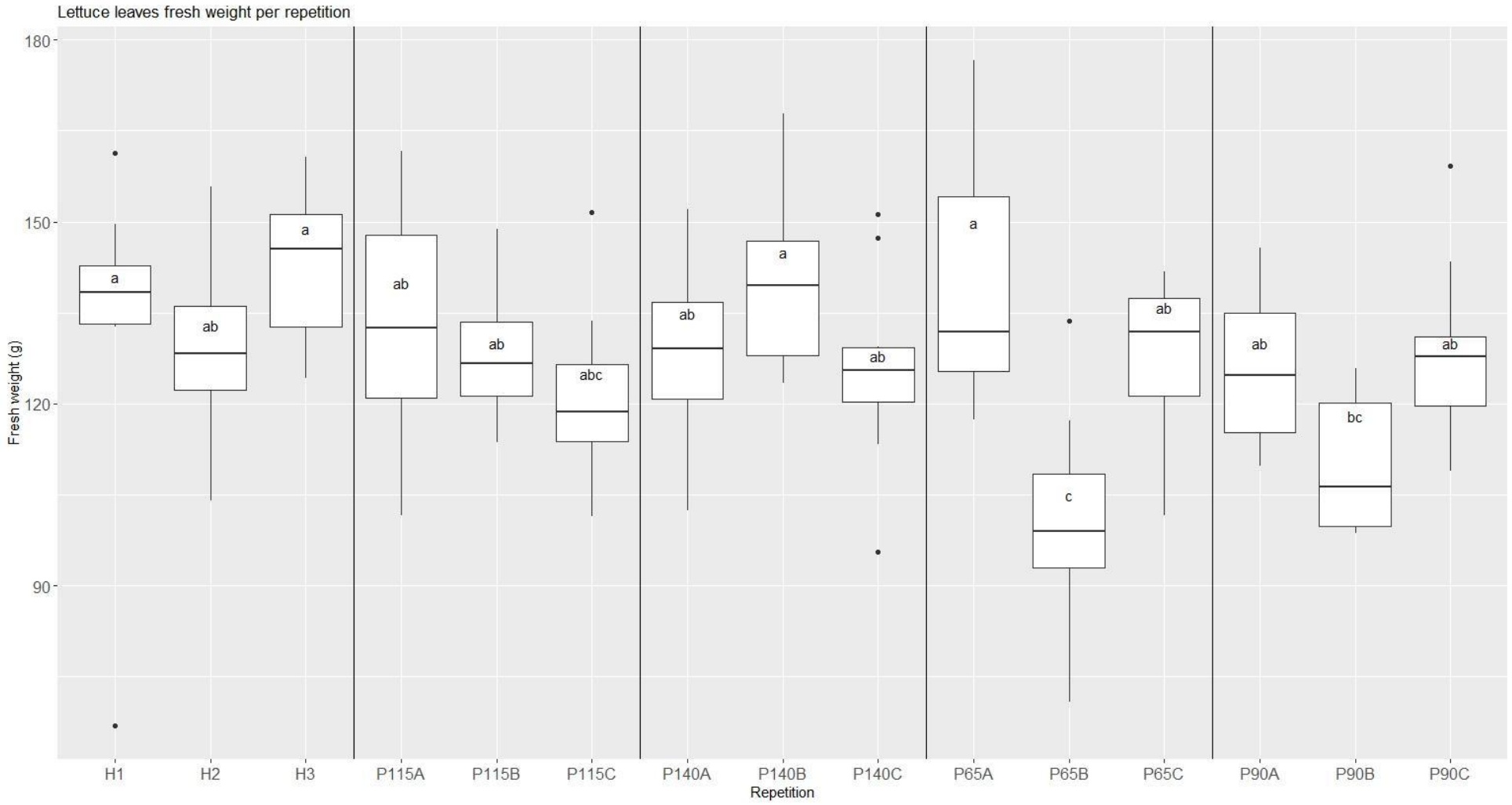

Figure 3.14: Boxplot of lettuce leaves fresh weight per repetition

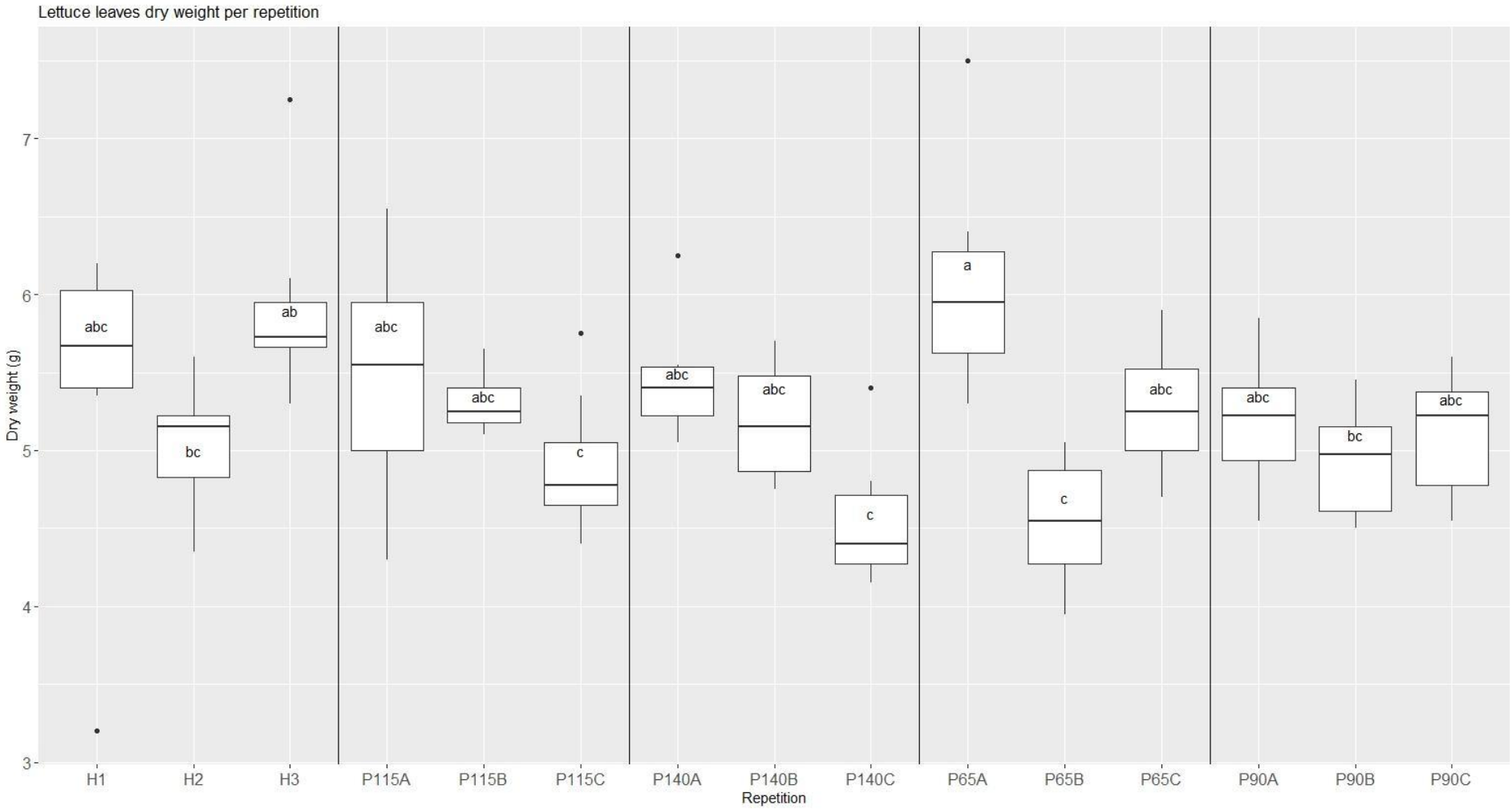

Figure 3.15: Boxplot of lettuce leaves dry weight per repetition

3.1.4.4. Lettuce chemical composition comparison

3.1.4.4.1. Within the essay

First, some of the tests for the ANOVA assumptions showed p-values higher than 0.05. However, given that the population sizes are similar (4 lettuce were sent per repetition), an ANOVA was still conducted in these cases as this test is fairly robust. However, conclusions must be taken with a grain of salt.

a) Total Nitrogen, Phosphorus, and Iron

For phosphorus, iron and total nitrogen, no significant difference was determined by the one-way ANOVA. Their respective ranges were 250-350 mg/kg, 2-6 mg/kg and 2-4%.

Nonetheless, it should be noted that conventional hydroponics appear to produce lettuce leaves with higher percentages of total nitrogen (an additional 0.5% to 1%) as shown in Figure 3.16.

b) Nitrate

Nitrate in lettuce cultivated with conventional hydroponics is around twice as present as in those bioponnically cultivated. Though, no significant difference in nitrate concentration was observed among bioponically cultivated lettuce, all being around 600 mg/kg.

All the lettuce cultivated had safe levels of nitrate for consumption by adults according to EU norms (Regulation (EU) No 1258/2011). To illustrate, the most restrictive threshold for fresh lettuce cultivated under shelter (excluding icebergs) is 4000 mg NO_3/kg. Figure 3.18 shows that all the lettuces harvested in this study respected the regulation (the maximum concentration of nitrate detected was just shy of 1600 mg/kg).

c) Potassium

Tukey's HSD test only shows a significant difference between P65 (ranging from 1600 and 2400 mg/kg) and H (ranging from 2750 and 3000 mg/kg) in leaf potassium concentration. However, by observing the boxplot, it is safe to state that the leaf potassium concentration is positively correlated with the initial solution concentration in nutrients.

d) Calcium, Magnesium, Sodium, Manganese and Zinc

It appears that lettuce cultivated bioponically is richer than conventional hydroponics in Ca (an increase of 28.9-111.5%), in Mg (an increase of 26.7-98.9%), in Na (an increase of 225.3-360%), in Mn (by factors ranging from 7.57-17.57) and in Zn (an extra of 142.4- 193.9%). P65 modality took the lead among bioponic solutions in the case of Calcium, Magnesium and Sodium with increases of at least 50.8%, 56.9% and 104.3% respectively.

3.1.4.4.2. With literature

When comparing the bioponic lettuce of this essay with the table given by Resh (2013) representing the nutrient levels that should be reached in healthy lettuce plants based on DW, it is clear that they are deficient in nitrogen (3%, 2.99%, 2.66% and 2.94% respectively for P65, P90, P115 and P140 versus the minimum of 3%), phosphorus (0.65%, 0.75%, 0.79% and 0.77% respectively for P65, P90, P115 and P140 versus the minimum of 0.8%) and iron (61.76ppm, 76.24ppm, 72.7ppm, 111.81ppm respectively for P65, P90, P115 and P140 versus the minimum of 130ppm). In addition, P65 and P90 are deficient in potassium (respectively 4.44% and 4.79% versus the minimum of 5%). However, zinc is in excess for all modalities (more than 181.96ppm versus the maximum of 120ppm). Manganese is only in excess for P115 and P140 (respectively 235.82ppm and 331.07ppm versus the maximum of 150ppm) . The hydroponic lettuce seems to be within the recommended ranges in all the minerals except for iron (97.7ppm) (see Table 3.3).

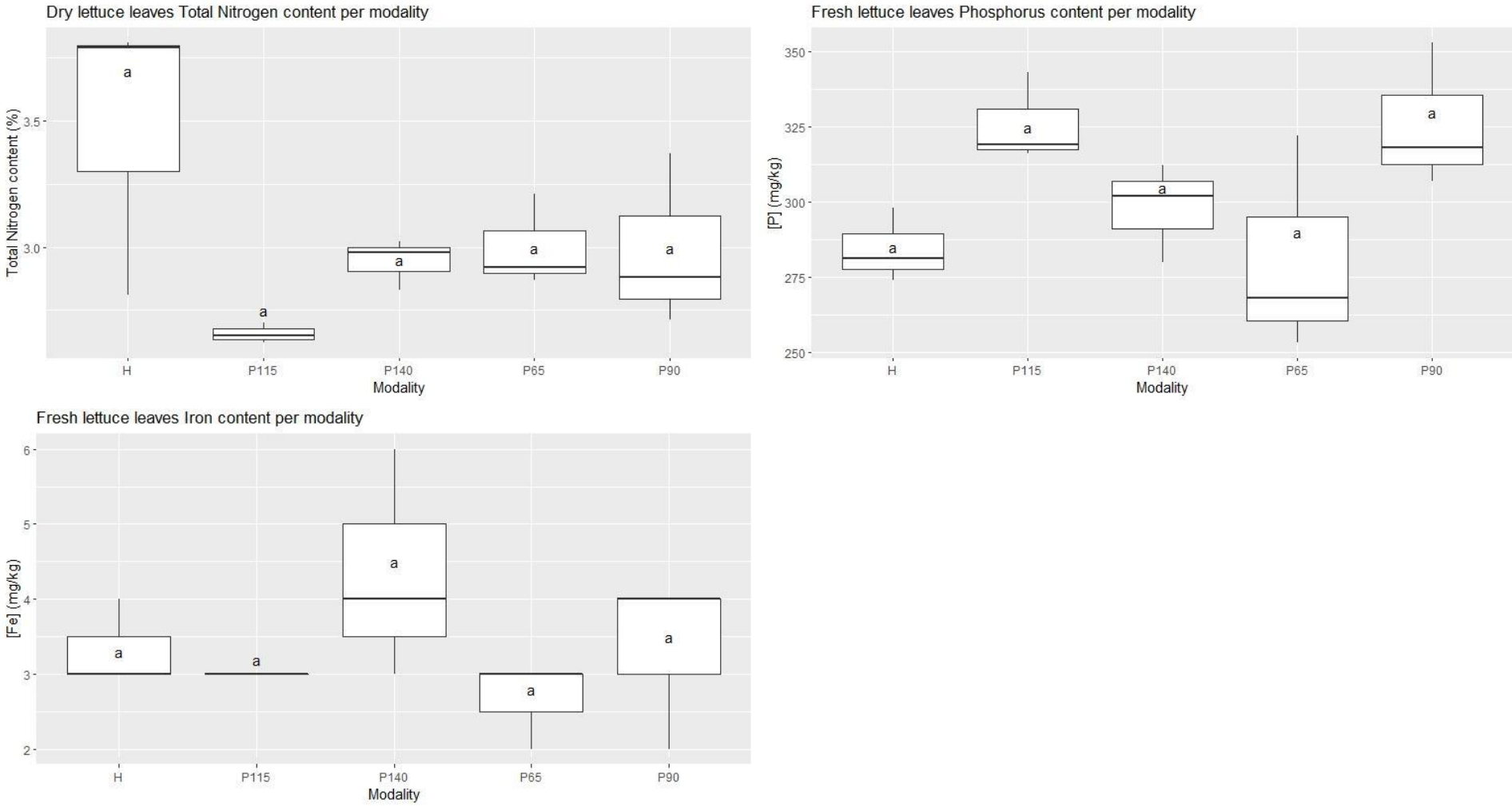

Figure 3.16: Boxplots of dry lettuce leaves total nitrogen content, fresh lettuce leaves phosphorus content and fresh lettuce leaves iron content per modality.

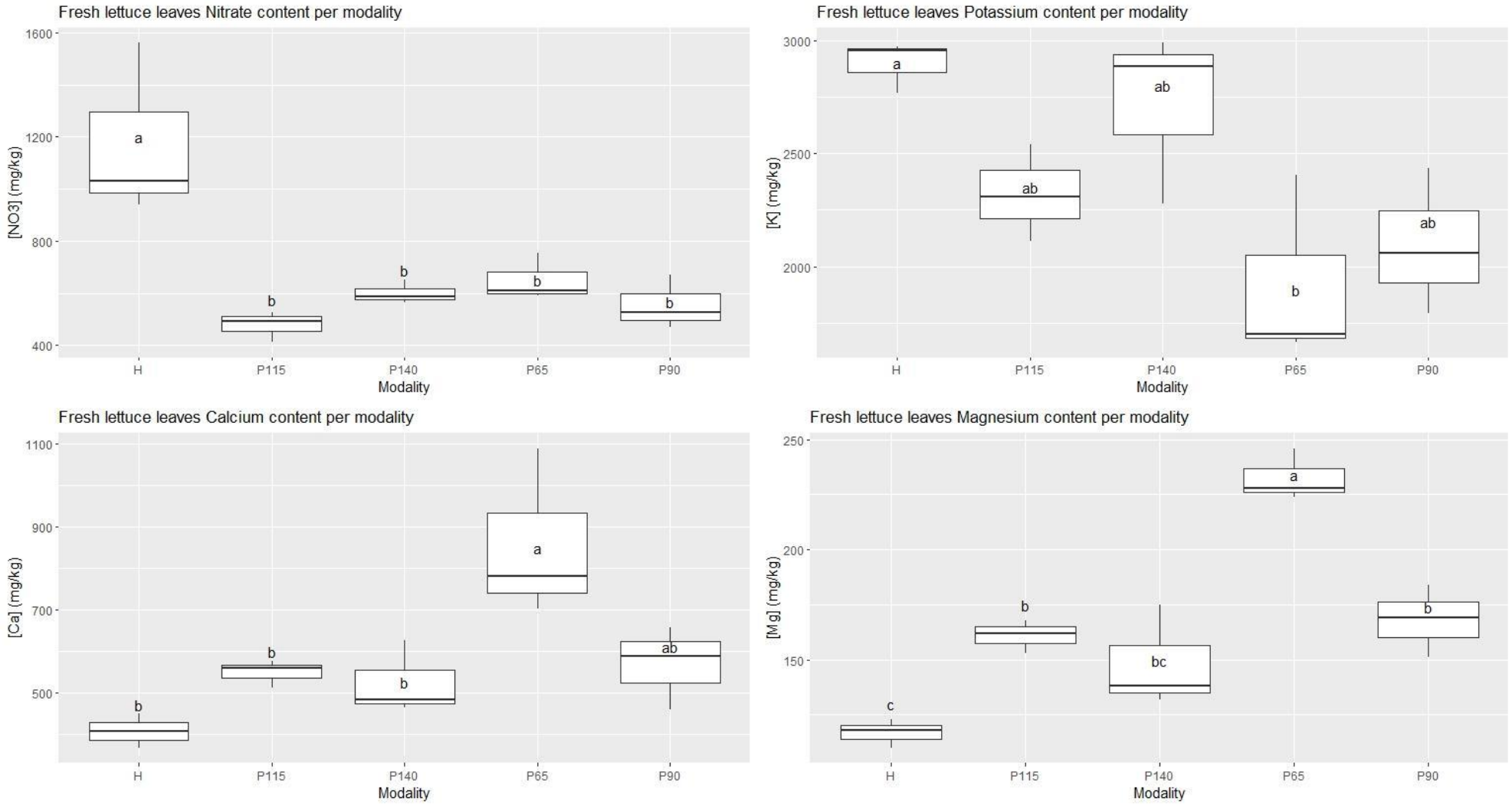

Figure 3.17:Boxplots of fresh lettuce leaves nitrate, potassium, calcium and magnesium content per modality.

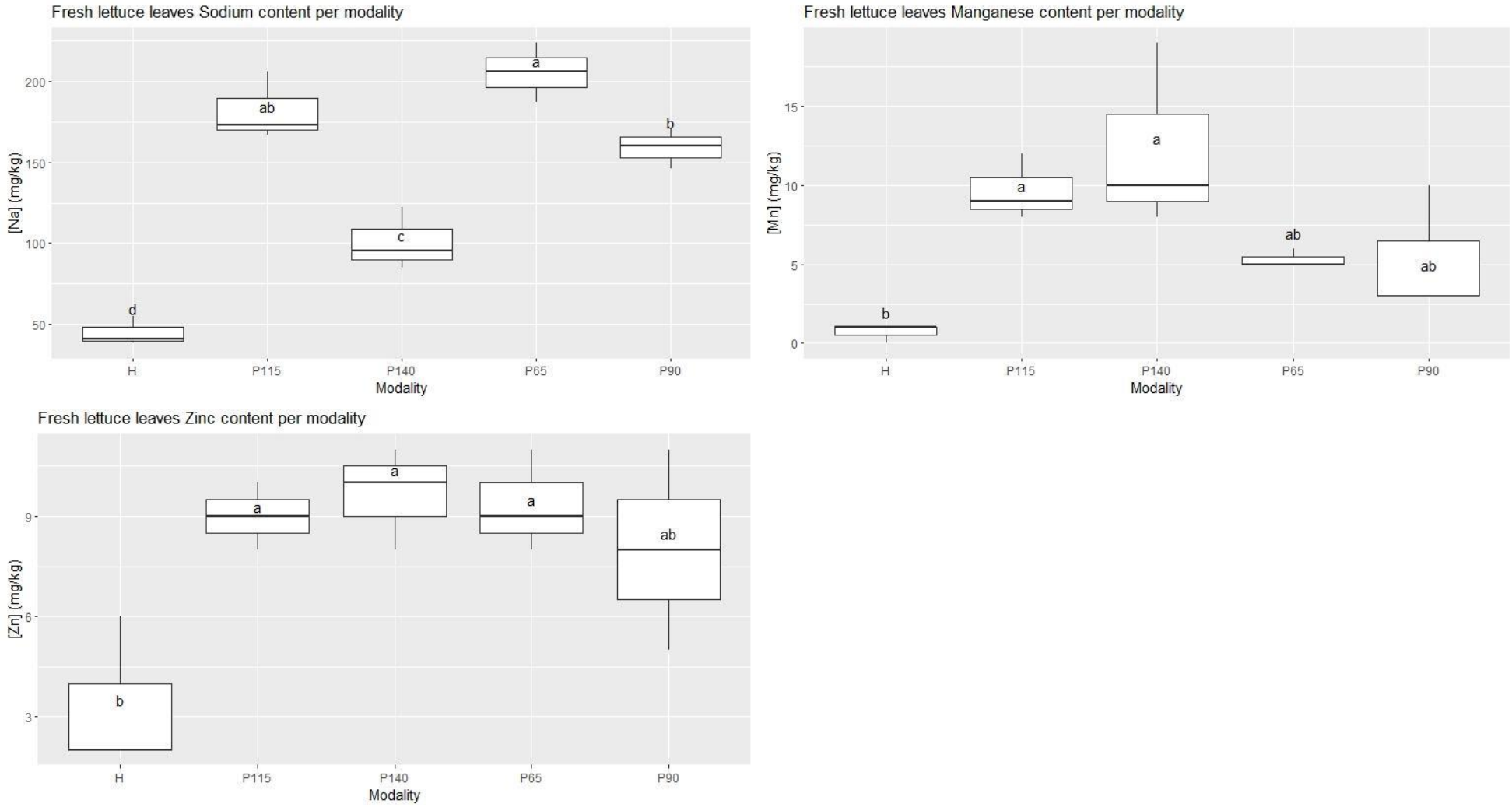

Figure 3.18: Boxplots of fresh lettuce leaves sodium, magnesium and zinc content per modality

Element	R*	YKA**	YKH***	P65	P90	P115	P140	H
N (g/kg)	30-60	42.7	45.3	30	29.9	26.6	29.4	34.7
P (g/kg)	8-13	21.2	26.9	6.5	7.5	7.9	7.7	8.4
K (g/kg)	50-108	66.7	69.3	44.4	47.9	56.1	71	85.5
Ca (g/kg)	11-21	3.5	3.4	19.7	12.9	13.3	13.7	12
Mg (g/kg)	3-9	2	2.6	5.4	3.8	3.9	3.9	3.4
Fe (ppm)	130-600	NA	NA	61.76	76.24	72.7	111.81	97.7
Zn (ppm)	60-120	NA	NA	215.76	181.96	218.73	252.64	100.63
Mn (ppm)	20-150	NA	NA	122.97	119.53	235.82	331.07	28.6

Table 3.3: Comparison of the chemical composition of lettuce leaves of this study with Resh (2013) and Yang et al. (2020)
**R= Resh (2013)*
***YKA = Yang et al. (2020) aquaponic solution*
****YKH = Yang et al. (2020) hydroponic solution*
NA= Not Available

3.1.4.5. Solution residual nutrients

Figure 3.19 shows the percentage of each measured macronutrient (except for sulphur) that remained in the P90 and H solutions after harvesting the lettuce. Almost no nitrogen or potassium could be found. However, more than 30% of phosphorus, calcium and magnesium were still present, probably as a result of the addition of stock solutions near the end of the experiment to compensate for nitrogen deficiencies.

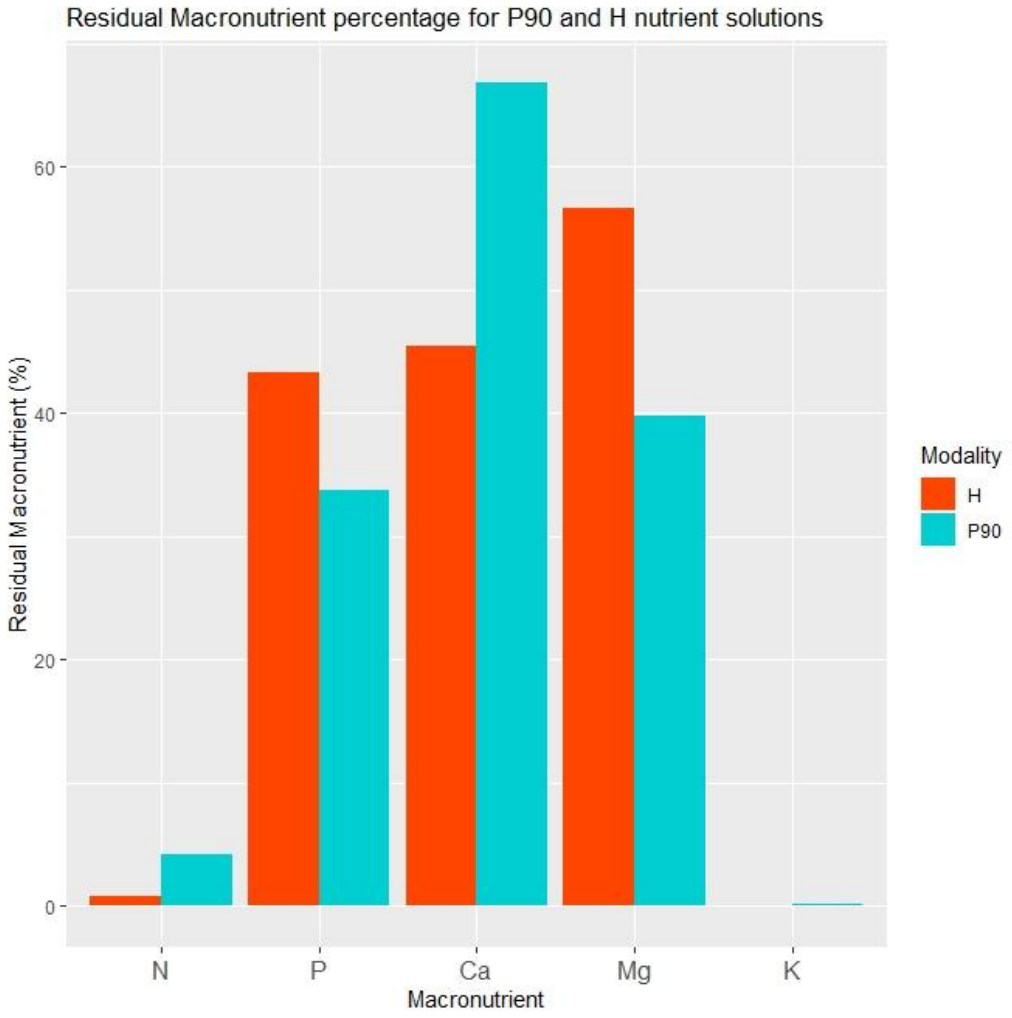

Figure 3.19: Percentage of macronutrients residing in the nutrient solution at the end of the essay

3.1.4.6. Root visual aspects

Root development was more important in bioponics than hydroponics. This is congruent with the studies that testified better root development in bioponics (Shinohara et al., 2011; Fang et al., 2018; HSIEH et al., 2018).

Lettuce roots were holding considerable amounts of residues. This might have contributed to the loss of nitrogen and other minerals.

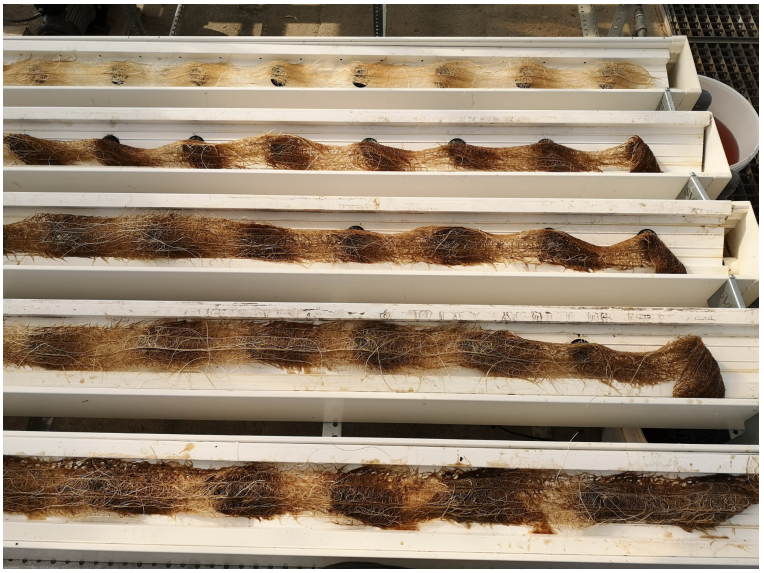

Figure 3.20: Root of H2, P65B, P115B, P90B and P140B in a top to bottom order.

3.1.4.7. Residues in the gullies

When cleaning the gullies after harvest, We have discovered that substantial amounts of solids were stagnating as can be seen in Figure 3.23. The stagnating solids could have contained part of the nutrients that were initially present and could have provided support for microorganisms to consume the nutrients. The residues could have also promoted the proliferation of biofilms and denitrification (Strayer et al., 1997)

Figure 3.21: Solid residues deposited on the P65C gully.

3.2. AMMONIA VOLATILISATION ESSAY

As a reminder, this essay was conducted in order to understand the effects of ammonia volatilisation on the loss of nitrogen in conditions similar to the bioponic methods implemented in the framework of the SWIM project.

This test shows that in a timelapse of seven days, up to 25-30 % of the present ammonia could be volatilized in conditions similar to the bioponic essay above.

Despite the ANOVA test showing no significant difference between the modalities, it appears that oxygenated modalities lost 5 to 15% more nitrogen.

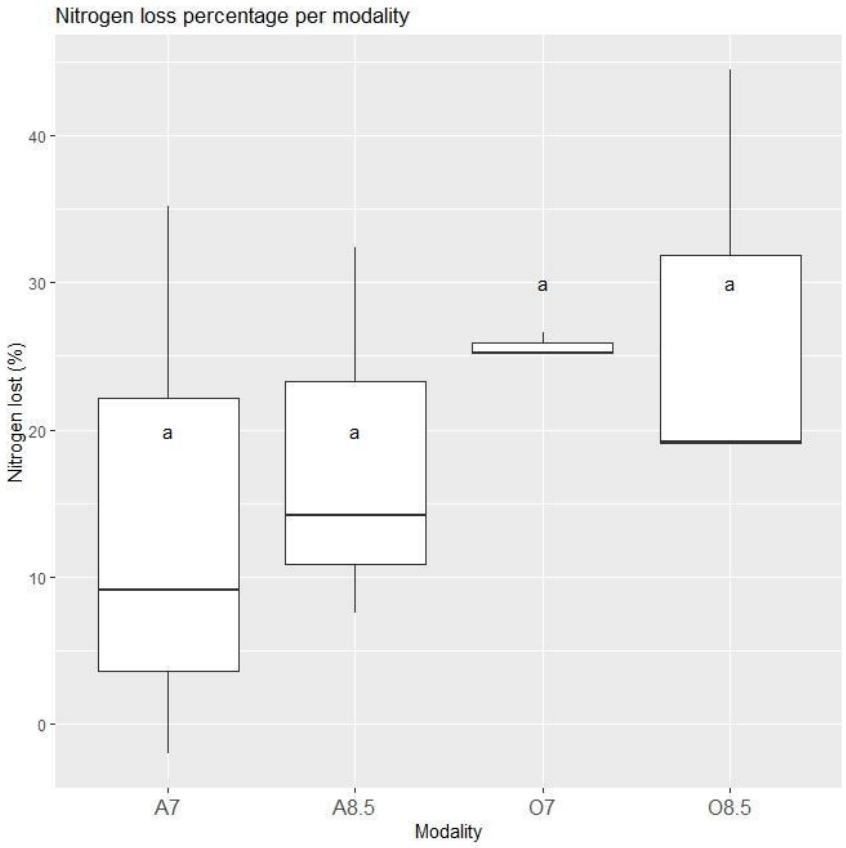

Figure 3.22: Nitrogen loss percentage during the ammonia volatilization essay per modality.

4. Discussion

4.1. Bioponics compared to hydroponics

4.1.1. In terms of productivity

This essay showed close productivities between bioponic and conventional hydroponic solutions. In fact, the productivity of bioponics in terms of fresh weight was between 89 and 97% of that of hydroponics. According to the linear mixed effect model, no significant difference could be found among the different modalities or between bioponics and hydroponics

The important productivity in this essay would have unlikely been reached without the addition of stock solution near the end of the crop culture. Therefore, it might be interesting to add a certain volume of stock solution by default near the end of each crop culture using this bioponic technique. According to the results of Shinohara et al. (2011), the stock solution is unlikely to show phytotoxic effects at this stage. This could be because the microbial communities (including nitrifying bacteria) are well established to degrade organic matter and nitrify ammonium.

4.1.2. In terms of nutritional value

Lettuce cultivated in conventional hydroponics is around twice as rich in nitrate. Elena et al. (2016) have observed similar results, and even lower nitrate concentrations than in the present study.

However, the opposite is true for calcium, magnesium, sodium, manganese and zinc which are present respectively one and a half, twice, thrice, ten times and almost four times as much in bioponically cultivated lettuce than in hydroponically cultivated ones. These results correspond with Fang et al. (2018) where bioponically cultivated lettuce was richer in magnesium, sodium and calcium than hydroponically cultivated ones.

All in all, even though bioponics outperformed our hydroponic solutions in terms of lettuce mineral concentrations, it is hard to generalise this statement because the mineral concentrations of the hydroponic solution used in this study are lower than those recommended in literature (see Table 3.2). Further research is required where optimal hydroponic solutions need to be compared with bioponics.

4.1.3. In terms of economy

To begin with, Bioponics consumed fewer industrial inputs than conventional hydroponics in total. By definition, the creation of a nutrient solution in conventional hydroponics requires chemical fertilisers such as salts. These can be expensive and/or inaccessible in certain regions. In comparison, bioponic nutrient solutions were created using collected animal faeces. In addition, throughout the experiment, bioponic solutions consumed less acids and bases than conventional hydroponics solutions (results not shown).

Then, the quantity produced was similar with conventional hydroponics and bioponics. Thus, the increased lettuce nutritious quality obtained in bioponics can be appreciated using labels or other certifications, leading to higher prices, hence greater turnovers per gully. Nonetheless, further research using optimal hydroponic solutions is necessary.

On the other side, bioponic nutrient solutions required a total of 35 extra days for their creation using the method chosen in this essay in comparison with conventional hydroponics. Those days required substantial amounts of labour for monitoring purposes. The impressive results that were obtained were partly due to the monitoring as it allowed avoiding nitrogen deficiencies in bioponic solutions (as a reminder, stock solution was added four times to all bioponic solutions).

In addition, extra space and equipment were necessary such as air pumps, filters, and resistance heaters. Moreover, filtering the stock solution after the anaerobic digestion phase is labour intensive if done manually. Most of the tasks could be automated, however this would require substantial investments. Therefore, for regions where labour cost is high, the cost of production of bioponic solutions as described in this study might be considerably higher than the cost of production of conventional hydroponic solutions.

Nonetheless, in regions such as the Sahrawi refugee camps, where opportunities of employment are low (leading to low labour costs) and accessibility to industrial inputs is expensive and limited, the creation of bioponic solutions should not be an economical obstacle.

Finally, the aerobic digestion phase lasted 24 days, during which around 2-3 litres of water were added to each bucket every day. Therefore, it is important to consider the increased water requirements in comparison with conventional hydroponics.

4.1.4. In general

On the whole, the use of bioponics as described in this study is justified in regions similar to the Sahrawi refugee camps where labour cost is low and chemical inputs are expensive or hardly accessible. In addition, the increased nutritional quality of the bioponically cultivated lettuce is valuable in such regions where a balanced diet is sought for.

However, at an industrial scale and in regions such as Belgium, crop culture with bioponics might not be the optimal choice in the present as it can increase the initial investments in equipment and/or labour expenses. Nonetheless, if industrial fertilisers' costs keep climbing as they have been for the past years, and/or if phosphate and potassium mines grow close to depletion, a further optimised version of this bioponic method (better filtering mechanisms, reduced nutrient losses…) could quickly become more advantageous than conventional hydroponics. In such a case, the improved plant quality could be appreciated.

4.2. THE FATE OF NITROGEN

4.2.1. Nitrification

Nitrification took place in all bioponic solutions. However, the more a solution was initially concentrated in nutrients, the longer it took for ammonia oxidation to be observed and the lower the nitrification efficiency. This might be due to several reasons.

First, an important solution initial concentration in nutrients came with important amounts of organic matter. This organic matter required and consumed considerable amounts of oxygen as was shown by the BOD5, COD and DO levels. The latter shows us that less oxygen was available for nitrifying bacteria on the first several days in solutions with high initial concentrations (P140 and P115) than in those with lower initial concentrations (P65 and P90) in nutrients. Hence, the starting point of nitrification might have been set back for this reason.

Moreover, the increased BOD5 levels in highly concentrated solutions in nutrients (≈1000mg/L for P140 versus ≈625mg/L for P65) indicated higher bacterial activity. Bacteria, as any living organism, require nitrogen for their growth and reproduction. Hence it is possible that greater proportions of nitrogen were incorporated into bacterial biomass in such solutions in comparison with more diluted ones. This statement can be further sustained by the behaviour of other nutrients in the solutions. Among the macronutrients studied in this work, heterotrophic bacteria require nitrogen, phosphorus, and potassium respectively the most (Todar, 2020). These three elements took the lead in this same order when it comes to nutrient loss during the aerobic digestion phase (see Figure 3.11). This is an indication that nutrients are being incorporated into bacterial biomass, whether it be nitrifying bacteria or other heterotrophic or autotrophic bacteria.

Bacteria will compete for the available nutrients and oxygen. This also includes nitrogen.

At first, heterotrophic bacteria would have been consuming most of the dissolved oxygen in the solutions, leaving very few for nitrifying bacteria (less than the recommended 1mg/L in the case of P140; Jianlong et al., 2004) which were therefore unable to oxidise the ammonium. The latter is the favourite form of nitrogen for most heterotrophic bacteria and can promote their fast growth (Wang et al., 2016). This led to substantial amounts of ammonium being incorporated into heterotrophic bacteria biomass. In fact, by comparing the evolution of TAN and that of nitrite-nitrogen, it is clear that for P115 and P140 repetitions, TAN levels were decreased considerably during the first few days (≈47.4% and ≈40% respectively) while nitrite levels were barely increasing signalling an absence of ammonia oxidation.

However, at some point, the nutrient solutions might have run out of carbon to fuel heterotrophic bacteria activities. This allowed nitrifying bacteria to consume the available oxygen and start the nitrification process rapidly as nitrifying bacteria were cultivated before the aerobic digestion phase and imported into the nutrient solution using biomedias at the beginning of it. Nonetheless, considerable proportions of ammonium would have already been incorporated into bacterial biomass. This might have ultimately led to lower nitrification efficiencies for solutions with higher initial concentrations in nutrients.

In the meantime, solutions with lower initial concentrations in nutrients did not experience low DO at the initial stages of the aerobic digestion phase. Therefore, nitrification can quickly take place in this case and greater proportions of ammonium could undergo this process.

Particular attention must be drawn to the low nitrification efficiency of P65B (42.57% versus 98.7% for P65A and 79.87% for P65C). In fact, by observing the evolution of nitrite-nitrogen, despite a solid start, ammonia oxidation started dropping earlier than the other repetitions of

the same modality. In addition, this repetition of P65 has lost the most important proportion of total nitrogen during the aerobic digestion phase (64.56% versus 24.15% for P65A and 42.66% for P65C). By observing the evolution of TAN, it seems that ammonia concentration was already significantly lower in P65B than the other two repetitions of this modality after the first weekend of the aerobic digestion phase (24.7 mg/L versus 39.5 mg/L for P65A and 35.1 mg/L for P65C). This indicates that nitrogen loss was important in this repetition for reasons which will be explored in the following section.

4.2.2. Loss

In order to understand the nitrogen loss observed in this study and in the previous essays that took place in Gembloux, losses should be separated between mineral nitrogen loss and total nitrogen loss.

First, the previous essays almost exclusively used the spectrophotometer to measure the concentrations in nitrogen. However, as mentioned earlier, substantial amounts of nitrogen might have been incorporated into bacterial biomass during the aerobic digestion phase. This nitrogen was hence undetectable by the spectrophotometer and was translated as a loss in nitrogen. However, this doesn't explain the loss in total nitrogen.

At the end of the essay, substantial amounts of residues were deposited on the gullies as explained in section (3.1.4.7). It is believed that these residues contained nitrogen and therefore contributed to the loss of total nitrogen observed in the solutions. A possible solution is to increase the flow rate in the NFT systems. Pelayo Lind et al. (2020) have increased the flow rate to 3.5 L min^{-1} in order to avoid the deposit of solids in the gullies.

In addition, part of the available nitrate might have been undergoing denitrification in the solutions even in oxic conditions (Robertson et al., 1988, 1989, 1990, 1995). However, the impact of denitrification in the present bioponic solutions is unlikely to be important. Denitrification was probably suppressed as the DO concentration in this study was around 7 mg/L, well above the threshold of inhibition for aerobic denitrification (3-5 mg/L; Ji et al, 2015) and for anaerobic denitrification (5.6; Oh et al., 1999). However Denitrification cannot be excluded entirely as the residues on the gullies could have promoted the production of biofilms and denitrification (Strayer et al., 1997).

Finally, part of the initially present ammonia might have been volatilized. The ammonia volatilisation essay showed that in bioponic systems similar to the one used in this study, ammonia can be volatilized within short periods of time. Nonetheless, the 25-30% losses shown in the ammonia volatilization essay have to be taken with precaution. Indeed, contrary to the ammonia volatilization essay, the ammonium in the bioponic solutions were following other processes at the same time, such as incorporation into bacterial biomass and nitrification. This must have reduced the amount of ammonia available for volatilization.

4.3. Optimal initial bioponic solution concentration in nutrients for lettuce culture

4.3.1. In terms of productivity

No significant difference was observed in terms of fresh weights or dry weights among the different bioponic modalities. In addition, all lettuce were visually similar and offered no significant difference.

Therefore, when considering exclusively productivity, all bioponic solutions were similar.

4.3.2. In terms of nutritional value

When taking a look at the chemical composition of lettuce, the P65 and P90 Modalities were either on par or bested the other two bioponic solutions except for arguably Manganese, Iron and Potassium. This means that lettuce cultivated using bioponic solutions with lower initial concentrations in nutrients are either as rich or richer in minerals, thus more nutritious.

Therefore, from a purely nutritious standpoint, nutrient solutions with initial concentrations in mineral nitrogen lower than 115 mg/L should be favoured.

4.3.3. In terms of economy

Creating solutions with higher concentrations in nutrients requires increased quantities of organic matter and labour to collect the latter as well as to filter the stock solutions. Though higher concentrations seem to be leading to slight (although not significant) enhancements in productivity but important decreases in nutritious quality. In addition, greater proportions of nutrients are being lost in bioponic solutions with higher initial concentrations in nutrients.

Moreover, the duration of the aerobic digestion phase was extended due to the delayed nitrification observed in the more concentrated modalities as the concentrations in nitrite were still too high for some of these modalities. Hence, increasing the initial concentration in nutrients of bioponic solutions also increases the period of production, thus the cost of it.

Therefore, it is not economically justified to increase the concentration of nutrients in the bioponic solutions for lettuce culture.

As an example, the volume of stock solution that was used to create a single P140 repetition in this work could have been used for the creation of two P65 repetitions with slightly shorter production periods.

4.3.4. In general

All in all, increasing the initial concentrations of nutrients in the bioponic solutions leads to similar productivity, lesser nutritional values and greater nutrient losses and costs of production. Therefore, it is best to aim for initial concentrations of nitrogen in bioponic solutions lower than 115mg/L, otherwise, it would only lead to a waste of time, money and nutrients. However, the amount of nutrients in such bioponic solutions was not sufficient and organic matter had to be added during lettuce culture. The added organic matter didn't appear to be phytotoxic. The same observation was made by Shinohara et al. (2011). However, further research is required to optimise the quantities that should be added, as well as the time lapse between the additions.

4.4. PERSPECTIVES

4.4.1. Chicken droppings quality

According to the speculations of this study, an important presence of carbon in the bioponic nutrient solutions might lead to an increased activity of heterotrophic bacteria which ultimately leads to lower nitrification efficiency and greater nutrient losses. Therefore, the faeces that should be used in this particular method of bioponics should almost be pure and free of straw or other plant residues. For example, in the case of chicken droppings, it would be better to source them from an industrial poultry rather than an organic poultry.

4.4.2. Lettuce consumption

Lettuce produced in our bioponic systems were deficient in nitrogen, phosphate, iron and sometimes potassium, and excessive in zinc and manganese (see section 3.1.4.4). In addition, it is unclear whether they contain pathogens or not as their roots are in contact with animal excreta. Therefore, further research is required to make sure that it is safe to consume plants grown in similar bioponic methods.

4.4.3. Further research

First, up to this point, research has been done based on the culture of lettuce. However, it could be interesting to compare the bioponic method chosen for the SWIM project with hydroponics for the culture of other vegetables such as legumes, tomatoes, or other leaf vegetables.

Then, the steps taken in this study to create bioponic nutrient solutions are functional. However, during the aerobic digestion phase, the initial concentration of the nutrient solutions should be set between 65mgN/L and 90mgN/L. Then during the lettuce culture phase, organic matter should be added to the solutions. It would therefore be interesting to optimise the additions of organic matter in terms of quantities and in terms of time lapse between the additions.

5. CONCLUSION

Promoting local agriculture in the Sahrawi refugee camps is crucial for improving their diet. However, the pedoclimatic conditions impose significant challenges for conventional agriculture. Therefore, by implementing soilless culture, these can be overcome. Nonetheless, conventional hydroponics heavily depends on inputs to generate nutrient solutions, which are hardly accessible and expensive in this area of the world. Thus, bioponics is an interesting alternative where the nutrient solutions can be created using organic wastes such as chicken droppings. In the framework of the SWIM project, a specific bioponic method was chosen and developed and several essays were conducted for its optimization. Every trial led to substantial losses in nitrogen. The aim of this study was to further understand the chosen bioponic technique and to continue its optimization process.

In order to achieve this goal, the effect of increasing the initial concentration of nutrients in the bioponic solutions was tested, the nitrification efficiencies for each concentration were calculated, the causes of the loss of nitrogen were discussed, several physical parameters in addition to DO, COD and BOD5 were monitored and the bioponics technique was compared to conventional hydroponics.

This study allowed the production of similar quantities of lettuce with bioponics and conventional hydroponics. In terms of quality, it has shown that bioponics outperforms conventional hydroponics. However further research is required using optimal conventional hydroponic nutrient solutions.

In addition, this work showed that increasing the initial concentration of nutrients in a bioponic solution does not translate into better performance. In fact, higher initial concentrations led to similar productivity, but also came with greater mineral losses, and lower nitrification efficiencies.

Moreover, this study partly elucidated the causes of nitrogen losses. Indeed, the maximum losses related to ammonia volatilization were estimated and it was speculated that most nitrogen losses were linked to the incorporation of this element into bacterial biomass and the sedimentation of solid residues on the gullies and plant roots.

To conclude, for the Sahrawi refugee camps, the creation of bioponic nutrient solutions can be conducted using the same methods of anaerobic and aerobic digestions used in this essay. The initial concentrations of the bioponic solutions in the aerobic digestion phase should be set between 65mgN/L and 90mgN/L. During the lettuce culture phase, organic matter should be added. The conditions of the additions require further research. Moreover, studies on crops other than lettuce, as well as on the potential presence of pathogens should be conducted beforehand.

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7. APPENDICES

APPENDIX 1: SOIL ANALYSIS OF THE DAJLA GARDEN, THE EXPERIMENTAL PARCEL, THE LAAYOUNE GARDEN AND THE SMARA GARDEN IN A CLOCKWISE ORDER STARTING FROM THE TOP LEFT.

- ***Nature de l'échantillon :*** *Sol N°01.*
- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 122/2021.*

Déterminations	Résultats	Référence et méthode d'analyse
Préparation du sol selon :	/	NF ISO 11464
pH	*07,88*	NF ISO 10390
Teneur en matière organique	*0,98%*	Calcul
Azote totale	*0.001%*	NF ISO 11261
Carbone Organique	*0,56%*	NF ISO 14235
C/N	*560*	Calcul
Phosphore	*03,21%*	NF X31-108
Potassium	*0,19 meq/100g*	NF X31-108
Calcium	*08,11 meq/100g*	NF X31-108
Magnesium	*03,11meq/100g*	NF X31-108
C.E.C	*02,31 meq/100grs*	NF X31-130

- ***Nature de l'échantillon :*** *Sol N°02.*
- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 123/2021.*

Déterminations	Résultats	Référence et méthode d'analyse
Préparation du sol selon :	/	NF ISO 11464
pH	*07,98*	NF ISO 10390
Teneur en matière organique	*0,97%*	Calcul
Azote totale	*0.001%*	NF ISO 11261
Carbone	*0,56%*	NF ISO 14235
C/N	*560*	Calcul
Phosphore	*02,98%*	NF X31-108
Potassium	*0,17 meq/100g*	NF X31-108
Calcium	*08,02 meq/100g*	NF X31-108
Magnesium	*03,11meq/100g*	NF X31-108
C.E.C	*02,52 meq/100grs*	NF X31-130

- ***Nature de l'échantillon :*** *Sol N°03.*
- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 124/2021.*

Déterminations	Résultats	Référence et méthode d'analyse
Préparation du sol selon :	/	NF ISO 11464
pH	*07,78*	NF ISO 10390
Teneur en matière organique	*0,54%*	Calcul
Azote totale	*00%*	NF ISO 11261
Carbone	*0,31%*	NF ISO 14235
C/N	*00*	Calcul
Phosphore	*01,12%*	NF X31-108
Potassium	*03,21 meq/100g*	NF X31-108
Calcium	*37,36 meq/100g*	NF X31-108
Magnesium	*31,65meq/100g*	NF X31-108
C.E.C	*02,03 meq/100grs*	NF X31-130

- ***Nature de l'échantillon :*** *Sol N°04.*
- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 125/2021.*

Déterminations	Résultats	Référence et méthode d'analyse
Préparation du sol selon :	/	NF ISO 11464
pH	*07,77*	NF ISO 10390
Teneur en matière organique	*0,45%*	Calcul
Azote totale	*00%*	NF ISO 11261
Carbone	*0,26%*	NF ISO 14235
C/N	*00*	Calcul
Phosphore	*01,11%*	NF X31-108
Potassium	*01,58 meq/100g*	NF X31-108
Calcium	*19,87 meq/100g*	NF X31-108
Magnesium	*16,58meq/100g*	NF X31-108
C.E.C	*02,11 meq/100grs*	NF X31-130

APPENDIX 2: WATER ANALYSIS OF THE DIFFERENT WELLS IN THE SAHRAWI REFUGEE CAMPS IN TINDOUF.

Jardín regional de Dajla

- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 118/2021.*

Paramètre recherché	Unité	Résultat
pH	/	7,65
Carbonate	mg/L en $CaCO_3$	00
Bicarbonate	mg/L en $CaCO_3$	300
Electroconductivité	ms/cm	04,30
Ammonium	mg/L	0,033
Dioxyde d'azote	mg/L	0,11
Nitrate	mg/L	0,023
Phosphore	%	0,234
Salinité	mg/L	02,2
Potassium	mg/L	0,12
Sodium	mg/L	16,24
Magnésium	mg/L	24,02
Calcium	mg/L	23,56

Jardín regional de Smara

- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 120/2021.*

Paramètre recherché	Unité	Résultat
pH	/	7,89
Carbonate	mg/L en $CaCO_3$	00
Bicarbonate	mg/L en $CaCO_3$	210
Electroconductivité	ms/cm	07,72
Ammonium	mg/L	0,175
Dioxyde d'azote	mg/L	0,13
Nitrate	mg/L	0,125
Phosphore	%	0,521
Salinité	mg/L	04,30
Potassium	mg/L	0,56
Sodium	mg/L	16,82
Magnésium	mg/L	37,01
Calcium	mg/L	24,35

Jardín regional de Laayoune

- ***Prélevé le*** *: 12 Février 2021.*
- ***N° D'inscription*** *: 121/2021.*

Paramètre recherché	Unité	Résultat
pH	/	7,78
Carbonate	mg/L en $CaCO_3$	00
Bicarbonate	mg/L en $CaCO_3$	510
Electroconductivité	ms/cm	03,20
Ammonium	mg/L	0,084
Dioxyde d'azote	mg/L	0,06
Nitrate	mg/L	0,072
Phosphore	%	0,324
Salinité	mg/L	01,7
Potassium	mg/L	0,32
Sodium	mg/L	08,21
Magnésium	mg/L	11,54
Calcium	mg/L	13,11

APPENDIX 3: THE CHEMICAL COMPOSITION OF THE CHICKEN DROPPINGS.

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BULLETIN D'ANALYSE D'AMENDEMENT ORGANIQUE. (BA N° AO22/0003)

Date d'échantillonnage: **17/01/2022** Références de l'échantillon: **P1** N° d'analyse: **AO22/0003**
Date de réception: **17/01/2022** Catégorie: **Fientes**
Date d'envoi: **4/02/2022** Echantillonneur: **Le demandeur**
Dates d'analyses: **Du 17/01/2022 à 4/02/2022** Etat de l'échantillon à la réception: **Bon**

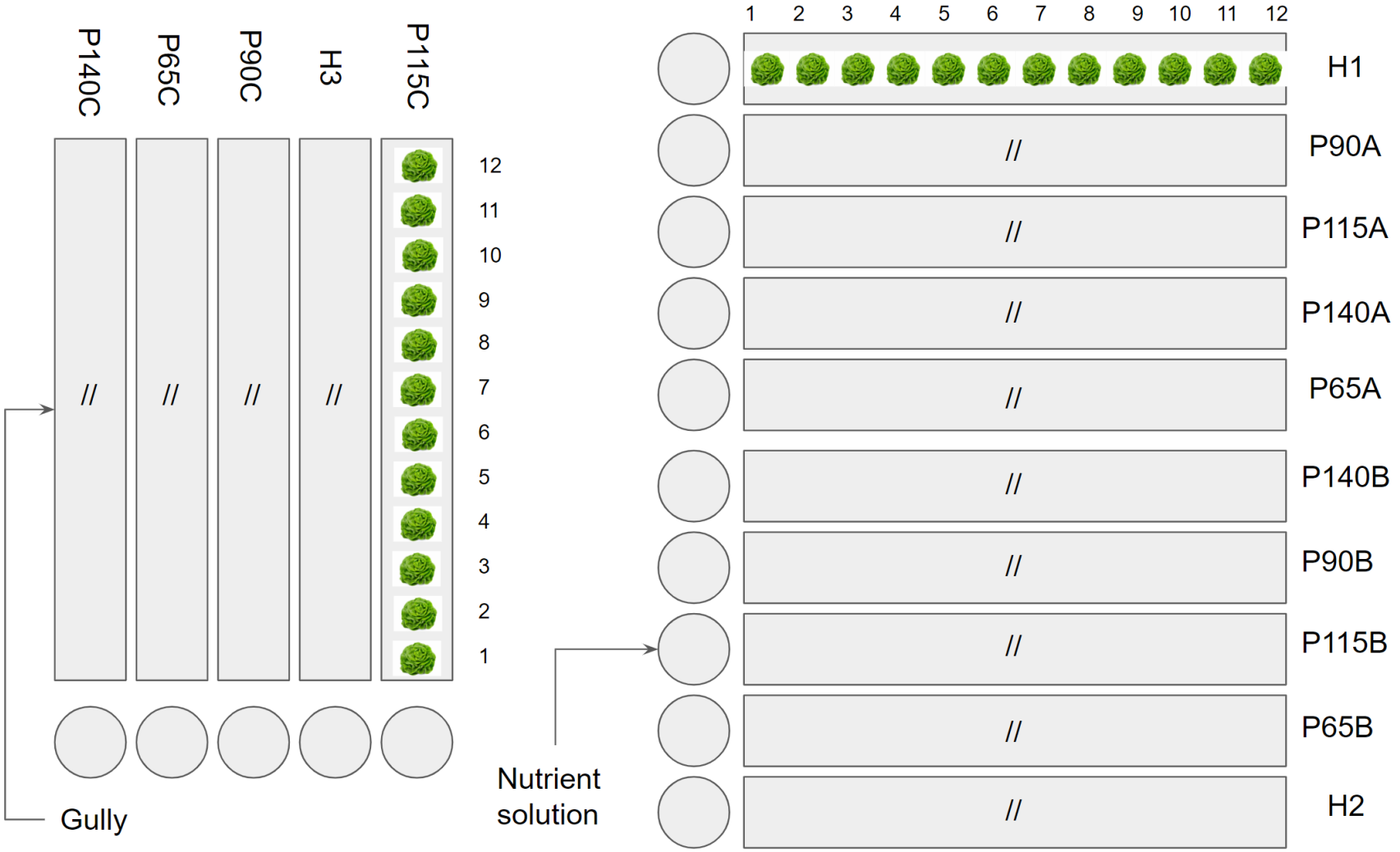
Les données présentées en gris dans ce bulletin ont été communiquées par le client.

Déterminations		sur matière fraîche		sur matière sèche		Statistiques ** (Moyennes)	
Matière sèche		51,7	%			55,49	%
Cendres totales		10,26	%	19,85	%	25,27	%
Cendres insolubles		1,25	%	2,42	%	3,28	%
Chlorure		0,36	%	0,69	%	0,47	%
Matière organique totale		414	kg/T	80,15	%	74,73	%
Azote ammoniacal	N-NH4+	4,01	kg/T	0,78	%	0,57	%
Azote total	N	**27,37**	kg/T	**5,29**	%	**4,71**	%
Phosphore	**P2O5**	**19,41**	kg/T	**3,76**	%	**3,36**	%
Potassium	**K2O**	**17,26**	kg/T	**3,34**	%	**3,72**	%
Calcium	CaO	21,05	kg/T	4,07	%	6,98	%
Magnésium	MgO	5,52	kg/T	1,07	%	1,53	%
Sodium	Na2O	4,49	kg/T	0,87	%	0,46	%
Fer	Fe	1.279	mg/kg	2.474	mg/kg	1444,34	mg/kg
Manganèse	Mn	216	mg/kg	418	mg/kg	466,46	mg/kg
Cuivre	Cu	49	mg/kg	96	mg/kg	93,89	mg/kg
Zinc	Zn	222	mg/kg	430	mg/kg	411,78	mg/kg
pH		7,8				7,7	
Rapport C/N *		**8,8**				9,6	

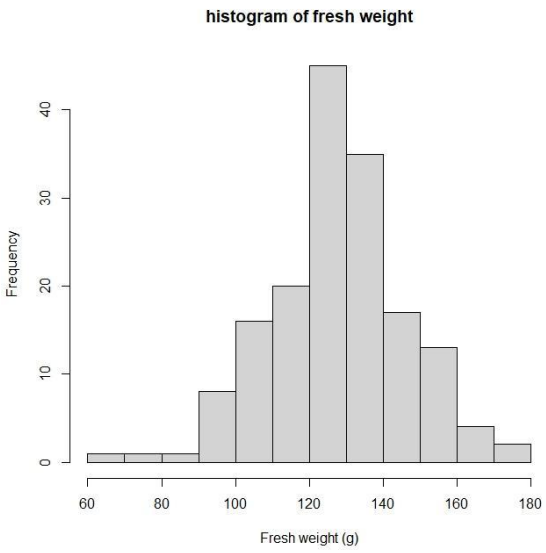
* Rapport C/N: Indice dont la valeur rend compte de la qualité de la matière organique
Rapport C/N <15:Matière à faible rapport C/N, produit peu d'humus mais décomposition et libération rapide d'azote

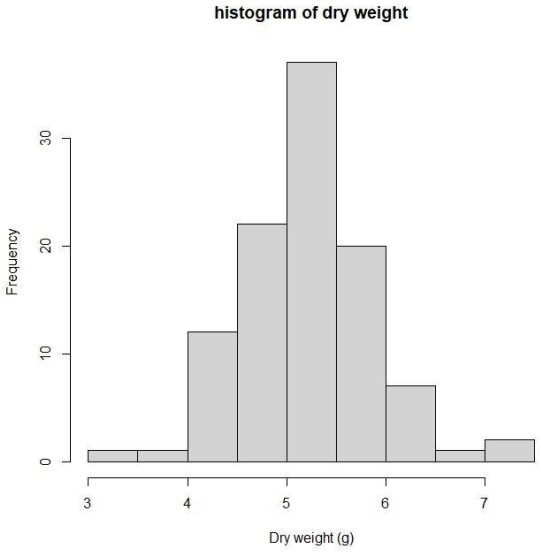
** Statistiques internes au laboratoire, valeurs moyennes exprimées sur la matière sèche.

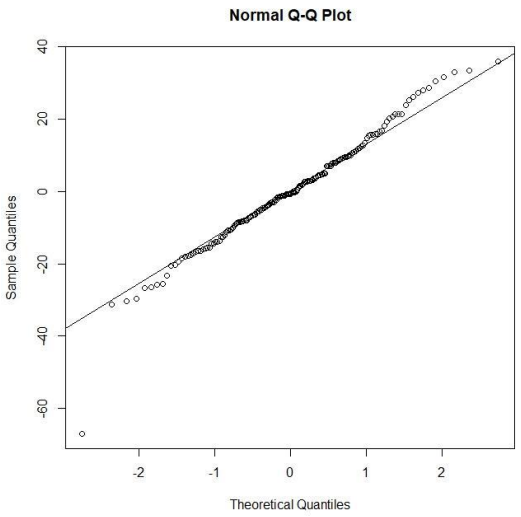
Appendix 4 : The layout of the bioponic essay.

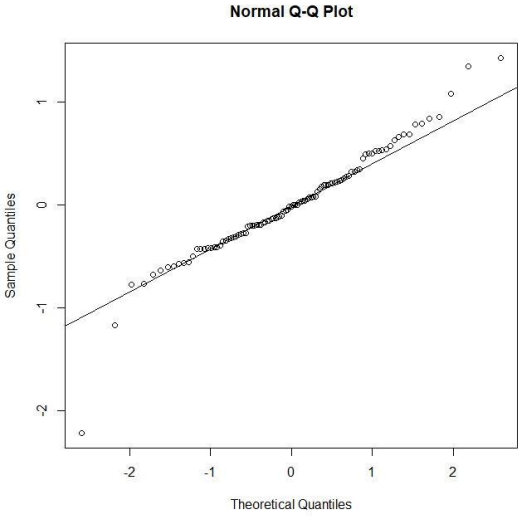


APPENDIX 5 : HISTOGRAMS AND Q-Q PLOTS FOR THE ANOVA ASSUMPTIONS.









APPENDIX 6 : HISTOGRAM OF DRY WEIGHT PER MODALITY.