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Development of a Simple Bioponic Method Using Manure and Offering Comparable Lettuce Yield than Hydroponics

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Abstract: Using locally accessible organic materials as fertilizers in hydroponics can represent a sustainable alternative to the synthetic mineral fertilizers typically used. This study aimed at developing a simple bioponic method using chicken or goat manure on lettuce (*Lactuca sativa* var. Lucrecia) in the nutrient film technique, requiring few inputs. The first experiment compared nutrient solutions made from chicken or goat droppings and mineral fertilizers in terms of physico-chemical parameters, plant yields, and shoot mineral content. Organic solutions were produced in two main stages before being used on plants: (1) a simple manure maceration in water to produce stock solution, followed by (2) an aerobic digestion of the filtrated and diluted stock solution according to the total mineral nitrogen (TMN). The second experiment compared different concentrations of chicken manure stock solution (60, 80, 100, or 120 mg/L TMN) to a control mineral solution. In the first experiment, both organic treatments resulted in yields significantly lower than those of the control, probably due to nitrogen scarcity. In the second experiment, all organic treatments resulted in wet and dry shoot masses similar to those obtained with the inorganic control treatment. Produce quality was also improved, with lower shoot nitrate content. Important nitrogen losses occurred in the organic solutions during aerobic digestion, particularly in the goat treatment and in the highly concentrated treatments in stock solution during the 2nd experiment (~50–65% TMN losses). This was probably caused by the presence of residual organic matter, which resulted in excessive microbial development. It can be concluded that chicken and goat manure are suitable fertilizers for lettuce hydroponic production using this method; however, further research should be carried out to improve mineralization during digestion steps.

Keywords: bioponics; organic hydroponics; chicken manure; goat manure; biofertilizer; low-tech

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1. Introduction

Hydroponics is a soilless agriculture technique in which plants extract essential nutrients from a nutrient solution [1]. It allows to grow year-round crops in various contexts and regions [2–4], even those with limited access to land and/or facing extreme pedo-climatic conditions, e.g., degraded, polluted, or infertile soils, water shortages, strong seasonality with periods of severe droughts or floods [5,6]. Hydroponics could therefore provide an element of response to various challenges in agriculture, such as the reduction of arable land, the increase in land cost, or the increase in frequency and intensity of extreme weather events related to climate change [3,5–7]. However, nutrient solutions in hydroponics are typically made from synthetic mineral fertilizers. Their extraction and/or manufacture cause various environmental issues [8,9]. Furthermore, they are often not accessible in developing countries or remote areas due to their high cost and/or their absence on the local market [10,11].

The development of organic hydroponics, or "bioponics", using locally accessible organic materials as a source of fertilizer is therefore essential from a sustainability perspective. In addition to showing positive effects on plant disease mitigation and produce

quality, such as lower nitrate content in leafy vegetables, bioponics in closed-loop systems can be an interesting way of recycling nutrient-rich organic wastes, which are often sources of pollution if they accumulate in the environment [12–19]. Nutrients present in organic wastes are often locked up in large organic molecules. The materials must go through decomposition and mineralization processes supported by a multitude of microorganisms. The sources of organic materials used in bioponics are diverse, such as animal manure [14,20–24], plant residues [17], compost [13,25,26], agro-industrial or household waste [12,27–30] for instance. Compared to plant residues, manure has a relatively large proportion of nutrients already in mineral form, depending on the animal species, the diet, or the manure storage conditions [31–34]. Poultry droppings in particular usually contain high nitrogen (N) content, mostly in the form of urea, which mineralizes very quickly into ammonium (NH_4^+), thanks to the widely distributed urease enzyme in nature [31,33,34]. In bioponics, several authors have been able to use manure as a fertilizer source by making a so-called "tea" [14,20,22,35–37]. This technique consists of bathing manure or compost in a volume of water, aerated or not, for a few hours to a few days. However, mineralization in this technique remains limited, the process being of very short duration [30]. Negative effects on plants were shown in several studies, notably due to the presence of dissolved organic compounds that were not degraded during the process [38–41]. This can cause excessive microbial development in the rhizosphere, which asphyxiates the roots via oxygen consumption by the microbes [40,41]. Another method is to perform extensive anaerobic digestion (AD) of the organic residues. The resulting digestate is then used as a stock solution in the hydroponic systems [30]. Several studies obtained yields similar to a mineral solution on lettuce when the digestate was sufficiently diluted [15,42,43]. Conversely, solutions too concentrated in digestate were phytotoxic, notably due to the high concentration of NH_4^+ [42,44]. Indeed, mineral N in anaerobic digestates is mainly in the form of NH_4^+ rather than nitrate (NO_3^-). This phenomenon is due to the need for aerobic conditions for the microorganisms responsible for nitrification (oxidation of NH_4^+ into NO_2^- and then into NO_3^-) [45–47]. As opposed to N–NO_3^-, N–NH_4^+ can be deleterious to plant growth when present as the predominant mineral N source. This is associated with diverse biochemical processes, such as carbon scarcity caused by high root demand for carbon skeleton to detoxify excess NH_4^+, cation uptake inhibition, or excessive root acidification [48–50]. Hence, a maximum N–NH_4^+:N–NO_3^- ratio of ~25:75 can be recommended for most plants [51–54]. In this perspective, another type of method consists in carrying out an aerobic digestion of the organic materials or the digestates, notably to allow nitrification [12,30]. In Bergstrand et al. (2020)'s [16] and Pelayo Lind et al. (2021)'s [17] studies, digestates were nitrified in moving bed biofilm reactors internal and/or external to the hydroponic systems. The development of nitrifying bacteria and thus the nitrification rate can be enhanced by ensuring good oxygenation (at least 5 mg/L dissolved oxygen—DO), pH and temperature (T) ranges conducive to nitrification (pH ~ 7.5, T of 20–30 °C), a limitation in organic materials, and initial total ammonia nitrogen (TAN)–N in the form of ammonia (NH_3) and NH_4^+, which are in equilibrium as a function of pH–[47,55–58]. The addition of compost or active sludge from wastewater treatment plants also served as nitrifier inoculum in several bioponics studies [12,16,17,23].

Using organic fertilizers in hydroponics comes with several challenges: an imbalanced nutrient composition [16,21], the presence of potential compounds toxic to plants or humans, a more complex pH management [20], and having to deal with a living microbiota. The objective of this study was to develop a simple bioponic method requiring little input using manure from chickens or goats that could be implemented at a family scale in communities located in the Sahara Desert. The first experiment was conducted to assess the possibility of using both types of manure on lettuce and to compare the yields obtained with a mineral solution. Physico-chemical parameters were monitored during digestion processes. A second experiment was conducted to assess the best stock solution concentration to have for aerobic digestion and to better understand the biochemical mechanisms behind manure digestion. Manure from chickens and goats was chosen for the study as

both animal species significantly contribute to food security in smallholders and poor rural and/or urban communities, particularly in developing countries [59–61].

2. Materials and Methods

2.1. Experiment 1: Comparison of Nutrient Solutions Made from Chicken or Goat Manure and Mineral Fertilizers

2.1.1. Plant Material and Growing Conditions

The experiment was conducted in a greenhouse at the Integrated and Urban Plant Pathology Laboratory (IUPPL) of Gembloux Agro Bio-Tech, Gembloux, Belgium (50.56285, 4.69980), in the summer period of July–September 2021. The plants grew in natural light (daylight hour ~14.4 h), with an average daily temperature of 22.9 ± 6.1 °C and a relative humidity (HR) of 68.2 ± 15.7%. Seeds of Butterhead lettuce (*Lactuca sativa* var. Lucrecia rz, Rijk Zwaan) were sown in rockwool plugs of dimensions 36 mm × 36 mm × 40 mm (Grodan, Roermond, The Netherlands) soaked with tap water in the greenhouse. After 7 days of germination, seedlings with 2–3 true leaves were transferred with their rockwool plugs into 5-cm net pots, which were then inserted in the nutrient film technique (NFT) gullies in the greenhouse. Plants were harvested 42 days after transplanting.

Each system consisted of a grow bed made of one gully (GOPONIC, Agrilogic Systemes, Normandy, France) of 2.6 m length with 12 net pots and a plant spacing of 15.15 cm, as illustrated in Figure 1. Each grow bed was connected to a 25 L-capacity plastic bucket containing 22 L of nutrient solution. The latter had constant recirculation through the gully thanks to a 950 L/h submersible pump (SICCE, Pozzoleone, Italy).

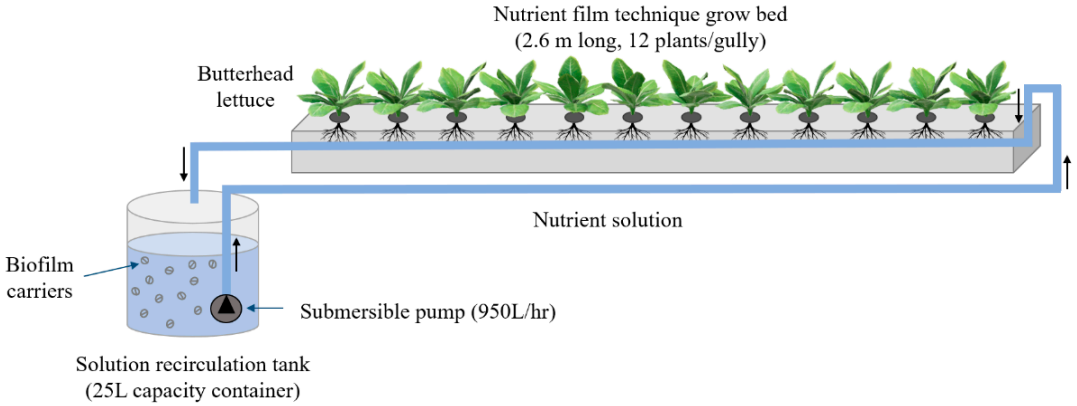

Figure 1. Schematic of the bioponic system used in the two experiments.

2.1.2. Production of Stock Solution from Chicken and Goat Manure

Chicken manure was collected at the farm "Volailles des serres de Sauvenières" (Gembloux, Belgium) on 10 June 2021, and had the following characteristics: dry weight (DW) 71.77%, carbon to nitrogen ratio (C/N) = 11, pH = 6.7, total organic matter = 87.51% DW, total N = 4.64% DW, ammonium nitrogen ($N–NH_4^+$) = 0.80% DW, P_2O_5 = 2.52% DW, K_2O = 2.64% DW, CaO = 2.44% DW, MgO = 1.01% DW, Fe = 424 mg/kg DW, Mn = 382 mg/kg DW, Cu = 86 mg/kg DW, Zn = 283 mg/kg DW. Goat manure was collected on 10 June 2021 in the farm "Chèvreries du moulin du Wez" (La Roche-en-Ardennes, Belgium) and had the following characteristics: DW 24.8%, C/N = 20.9, pH = 7.2, total organic matter = 87.67% DW, total N = 2.44% DW, $N–NH_4^+$ = 0.25% DW, P_2O_5 = 2.08% DW, K_2O = 1.69% DW, CaO = 2.80% DW, MgO = 1.06% DW, Fe = 829 mg/kg DW, Mn = 347 mg/kg DW, Cu = 55 mg/kg DW, Zn = 254 mg/kg DW. Both types of manure were then stored in closed plastic buckets at 4 °C before being used to create stock solutions on 30 June 2021.

For each type of manure, a 2.5% dry matter (DM) stock solution was prepared by simply letting the manure macerate in a large volume of water for 8 days. More specifically, 3.48 kg of fresh chicken manure were mixed with 96.52 L of demineralized water (de-water) in a 200-L bucket for the chicken manure-derived stock solution, and 15.12 kg of fresh goat

manure were mixed with 134.88 L of de-water for the goat manure-derived stock solution. A 200 W electric resistance (SuperFish, Aquadistri B.V., Klundert, The Netherlands) was immersed in each bucket and calibrated at 25 °C to promote microbial activity. Both solutions were manually mixed twice a day during the maceration process. The average temperature was 26.2 ± 2.4 °C in the chicken manure solution and 23.8 ± 1.2 °C in the goat manure solution.

After 8 days, the two stock solutions were filtered by sieving through a 250 µm mesh and analyzed for N, phosphorus (P), and potassium (K) content (Table 1). A part of the stock solutions was then diluted with de-water according to the total mineral N (TMN) solution content (Table 1) to obtain a total concentration of 60 mg TMN/L (comprising TAN, $N–NO_3^-$, and $N–NO_2^-$). In this way, chicken manure stock solution was diluted 6.93 times, while goat manure stock solution was diluted 2.1 times, the latter being less concentrated in TMN. The resulting diluted solutions were then introduced in the NFT systems for the aerobic digestion phase (corresponding to "day 1 of operation"). On their side, the remaining stock solutions were stored at 4 °C in closed plastic containers. The relatively low TMN concentration compared to the typical N concentration found in hydroponic solution (80–200 mg/L N) was chosen to limit TAN concentration, similar to what can be done in wastewater treatment plants to biologically remove N since free NH_3 can inhibit ammonia oxidizers and nitrifiers [55,62].

Table 1. Physicochemical properties in Experiment 1 of the chicken and goat manure solutions (i) before dilution in the NFT systems (stock solution), (ii) after dilution in the NFT systems (solution before aerobic digestion), and (iii) at the end of the "empty circulation" phase (solution after aerobic digestion), the day of transplant; and in the mineral control treatment, the day of transplant. pH; EC—electroconductivity (µS/cm); COD—chemical oxygen demand (mg/L); TSS—total suspended solid (mg/L); TAN—total ammonia nitrogen (mg/L); $N–NO_3^-$—nitrate–nitrogen (mg/L); $N–NO_2^-$—nitrite nitrogen (mg/L); TMN—total mineral nitrogen (summation of $N–NO_3^-$, $N–NO_2^-$, and TAN) (mg/L); $P\text{-}PO_4^{3-}$—phosphate–phosphorus (mg/L); K, potassium (mg/L).

Parameter	Chicken Manure-Based			Goat Manure-Based			Mineral Control Treatment
	Stock Solution	Solution before Aerobic Digestion	Solution after Aerobic Digestion	Stock Solution	Solution before Aerobic Digestion	Solution after Aerobic Digestion	
pH	5.6	6.2	7.3 ± 0.2	6.1	6.7	7.8 ± 0.2	6.3
EC	6170.0	937.3	613.7 ± 175.6	3010	1079.7	1019.3 ± 56.1	1301.0
COD	n.a. [1]	98.1	20.1 ± 8.9	n.a.	n.a.	32.6 ± 0.5	40.1
TSS	n.a.	590.3	277.4 ± 38.8	n.a.	2602.8	395.2 ± 56.3	69.0
TAN	415.0	63.2	0.8 ± 0.3	65.0	31.3	1.6 ± 0.2	9.3
$N–NO_3^-$	0.0	0.0	44.2 ± 16.5	61.0	26.5	25.3 ± 9.8	111.9
$N–NO_2^-$	0.0	0.0	0 ± 0.1	0.0	0.0	0.1 ± 0.1	0.0
TMN	415.0	63.2	45.1 ± 16.1	126.0	57.8	27.0 ± 9.9	121.2
$P\text{-}PO_4^{3-}$	170.0	24.5	18.9 ± 7.8	25.0	12.0	16.3 ± 3.8	44.0
K	550.0	79.4	75.8 ± 40.6	350.0	168.4	83.3 ± 5.8	186.7

Note: [1] n.a.—not available: the concentration exceeded the range of measurable values.

2.1.3. Aerobic Digestion within the Hydroponic Systems before Plant Cultivation—"Empty Circulation" Phase

The aerobic digestion process took place within the NFT systems before plant cultivation via an integrated moving bed biofilm reactor (MBBR). The latter consisted in 1.5 L of KNS biofilm carriers (surface area 836 m^2/m^3, Ø 12 mm, 7 mm height), placed in each nutrient solution tank (Figure 1). The biofilm carriers were disinfected with H_2O_2 beforehand. The development of nitrifiers thus happened naturally, not via prior artificial inoculation. The circulation of the nutrient solution between the tank and the gully was maintained constant until the end of the experiment, which allowed good oxygenation of the solutions. During this period, the concentrations of $N–NO_3^-$, $N–NO_2^-$ and TAN were measured regularly to monitor the nitrification process. Once $N–NO_2^-$ and TAN concentrations were below 5 mg/L in all bioponic systems, which corresponded to 27 days

of "empty circulation" (without plants), plant cultivation was initiated by transplanting the seedlings into all systems. During this "empty circulation" phase, pH was manually controlled three times a week around 7.5 via the addition of 10% H_2SO_4 or 10% NaOH to maintain a pH range conducive to nitrification. Both types of pH regulators were chosen so as not to interfere with the major essential nutrients that were being monitored in this study.

2.1.4. Treatments and Experimental Setup

The set-up of NFT systems with either chicken manure-based or goat manure-based nutrient solutions was compared in two different treatments (n = 3 repetitions per treatment). An additional treatment using commercial mineral fertilizer (HY-PRO, Friends B.V., Bladel, The Netherlands) was used as the reference treatment for plant cultivation (n = 3 repetitions). This mineral nutrient solution was created on the day the seedlings were transplanted into the NFT systems by adding equal amounts of mineral solution A (NPK 3-0-2, Ca 2%) and solution B (NPK 1-3-4, Mg 0.5%) in de-watering to reach an EC of 1300 µS/cm, i.e., the reference internal treatment for hydroponic lettuces. The physico-chemical characteristics of the three treatments (chicken, goat, and mineral solutions) at plant transplantation are given in Table 1. A total volume of 22 L in all nutrient solution tanks was maintained throughout the entire trial via the regular addition of de-water. During plant cultivation, pH was manually controlled thrice a week in all modalities between 5.5 and 6.5 via the addition of 10% H_2SO_4 or 10% NaOH to maintain an optimal pH range for mineral bioavailability [1]. During plant cultivation, chicken or goat manure stock solutions were added 4 times in 250-mL portions to the chicken treatment or in 875-mL portions to the goat treatment, respectively, adding a total of ~20 mg TMN/L per system (additions on days 41, 49, 53, and 56 of operation), whatever the manure. This also added 8 mg/L of P and 25 mg/L of K in the chicken treatment and 4 mg/L of P and 56 mg/L of K in the goat treatment.

2.1.5. Measurements

During the stock solution production phase, pH, EC, and temperature (T) were measured daily using a Hach HQ40d portable multimeter (HACH Lange NV/SA, Nazareth, Belgium). The TAN, N–NO_3^-, N–NO_2^-, P, and K concentrations of the sieved stock solutions were then measured using a Hanna HI83200 multiparameter spectrophotometer (HANNA Instruments, Woonsocket, RI, USA).

In the NFT systems, the pH, EC, and T of the nutrient solutions were measured using the same Hach portable multimeter. N–NO_3^-, chemical oxygen demand (COD), and total suspended solids (TSS) were measured with an optical sensor (TriOs Optical Sensor, TriOS Mess und Datentechnik GmbH, Rastede, Germany), while TAN and N–NO_2^- were measured with the spectrophotometer. In the greenhouse, ambient temperature and relative humidity were measured every 30 min using a data logger (MOINEAU Instruments, Chef-Boutonne, France).

Concerning the agronomic data, the mean fresh yield was determined for each treatment by weighing the shoot mass of each lettuce individually (n = 36 per treatment) directly after harvest. A composite sample of 3 shoot fresh lettuces per system (n = 3 per treatment) was then analyzed by an externally accredited laboratory [63], the "Centre provincial de l'agriculture et de la ruralité" (CPAR) (La Hulpe, Belgium), for shoot mineral content: NO_3^- on fresh weight (FW) via continuous flow method after cadmium reduction [64]; P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn on dry weight (DW) with nitric acid extraction of the ashes obtained by calcination at 450 °C and dosage by coupled plasma atomic emission spectrometry (ICP-AES) method [65]. The mean dry yield was determined for each treatment by weighing the shoot mass of the remaining lettuces (n = 27 per treatment) after drying at 40 °C for 7 days.

2.1.6. Statistics and Treatment of Data

Data of fresh weight and dry weight were analyzed on R Studio (v 4.2.1) with analysis of variance (ANOVA) on a linear mixed model using the lme4 package (fixed

factor = treatment; random factor = repetition). Differences between least squares means (LS-means) were determined with the Tukey multiple comparison test ($p < 0.05$) using the emmeans package. The analysis was verified via a histogram of residuals and a normal q-q plot for the normality of residuals assumption. Shoot mineral contents data were tested for differences using ANOVA followed by Tukey's multiple comparison test ($p < 0.05$).

2.2. Experiment 2: Comparison of Different Stock Solution Concentrations for Aerobic Digestion to a Mineral Control Treatment

2.2.1. Plant Material and Growing Conditions

The second experiment was conducted in the same IUPPL greenhouse as the first, in the period February–March 2022. The plants grew in natural light (daylight hour ~11 h), with an average daily temperature of 24.4 ± 5.4 °C and a relative humidity (HR) of 35.2 ± 7.4%. The same butterhead lettuce was used for this second experiment, as were the germination and transplantation methods. The plants were harvested 42 days after transplantation. The hydroponic systems were the same as for the first experiment (Figure 1).

2.2.2. Stock Solution Production from Chicken Manure

Chicken manure was collected at the farm "Ferme du Rouchat" (Fernelmont, Belgium) on 10 January 2022, and had the following characteristics: dry weight 51.7%, C/N = 8.8, pH = 7.8, total organic matter = 80.15% DW, total N = 5.29%, N–NH_4^+ = 0.78% DW, P_2O_5 = 3.76% DW, K_2O = 3.34% DW, CaO = 4.07% DW, MgO = 1.07% DW, Fe = 2474 mg/kg DW, Mn = 418 mg/kg DW, Cu = 96 mg/kg DW, Zn = 430 mg/kg DW. The manure was then stored in a closed bucket at 4 °C, before being used to create a stock solution on 18 January 2022. With a similar method as during the first experiment, a 5% DM solution was prepared by macerating 9.67 kg of fresh chicken manure into 90.33 L of de-watered for 9 days. The percentage of dry matter in this solution was doubled compared to the previous experiment with the aim of concentrating the resulting stock solution in nutrients. The average temperature was 23.4 ± 2.6 °C in the solution. The solution was then filtered with the 250 µm mesh and analyzed for TMN content (Table 2). A part of this stock solution was then diluted according to four different TMN concentrations: 60, 80, 100, and 120 mg/L, which corresponded to a dilution factor of 28.2, 21.1, 16.9, and 14.1, respectively. The resulting four diluted solutions were analyzed for their physico-chemical composition (Table 2) and introduced into the NFT systems for the aerobic digestion phase (corresponding to "day 1 of operation" in the NFT systems). On its side, the remaining stock solution was stored at 4 °C in a closed plastic container.

Table 2. Physicochemical properties in Experiment 2 of the chicken manure solution (i) before dilutions in the NFT systems (stock solution), (ii) after dilutions in the NFT systems (N60, N80, N100, and N120 solution before aerobic digestion), and (iii) at the end of the "empty circulation" phase (solution after aerobic digestion), the day of transplant, and in the mineral control treatment, the day of transplant. pH; EC—electroconductivity (µS/cm); BOD5—five-day biological oxygen demand (mg/L); COD—chemical oxygen demand (mg/L); TSS—total suspended solid (mg/L); DO—dissolved oxygen (mg/L); TAN—total ammonia nitrogen (mg/L); N–NO_3^-—nitrate–nitrogen (mg/L); N–NO_2^-—nitrite–nitrogen (mg/L); TMN—total mineral nitrogen (summation of N–NO_3^-, N–NO_2^-, and TAN) (mg/L); P—phosphorus (mg/L); K—potassium (mg/L); Ca—calcium (mg/L); Mg—magnesium (mg/L).

Parameter	Stock Solution	N60		N80		N100		N120		Mineral Control Treatment
		Solution before Aerobic Digestion	Solution after Aerobic Digestion	Solution before Aerobic Digestion	Solution after Aerobic Digestion	Solution before Aerobic Digestion	Solution after Aerobic Digestion	Solution before Aerobic Digestion	Solution after Aerobic Digestion	
pH	5.8	6.3	6.0 ± 0.0	6.5	6.1 ± 0.0	6.1	6.1 ± 0.1	6.2	6.1 ± 0.0	6.2
EC	15,150.0	715.0	475.7 ± 48.6	953.3	528.0 ± 31.2	1190.0	671.7 ± 25.7	1424.3	755.0 ± 41.7	1302.3
BOD5	n.d. [1]	610.0	<2.0	773.0	<2.0	983.0	<2.0	1000.0	<2.0	5.0
COD	n.d.	1280.0	71.7 ± 10.6	1822.0	86.0 ± 6.2	2312.0	117.3 ± 23.5	2896.0	133.7 ± 21.6	48.1
TSS	n.d.	962.4	245.8 ± 19.7	1348.4	245.5 ± 11.6	1608.3	292.0 ± 18.9	1920.0	319.0 ± 51.1	178.9

Table 2. *Cont.*

Parameter	Stock Solution	N60		N80		N100		N120		Mineral Control Treatment
		Solution before Aerobic Digestion	Solution after Aerobic Digestion	Solution before Aerobic Digestion	Solution after Aerobic Digestion	Solution before Aerobic Digestion	Solution after Aerobic Digestion	Solution before Aerobic Digestion	Solution after Aerobic Digestion	
DO	n.d.	n.d.	8.0 ± 0.1	n.d.	8.0 ± 0.0	n.d.	7.7 ± 0.2	n.d.	7.7 ± 0.2	7.5
TAN	1590.0	53.1	0.1 ± 0	70.7	0.1 ± 0	92.6	0.1 ± 0	115.8	0.1 ± 0	9.9
$N–NO_3^-$	100.0	4.0	36.4 ± 13.5	5.0	32.3 ± 5.9	5.5	28.3 ± 9.2	7.0	25.1 ± 6.2	118.0
$N–NO_2^-$	0.0	0.0	0.0 ± 0	0.1	0.0 ± 0	0.1	8.6 ± 14.8	0.1	17.2 ± 16.1	0.0
TMN	1690.0	57.2	36.5 ± 13.5	75.7	32.4 ± 5.9	98.2	37.0 ± 6.1	122.9	42.4 ± 10.5	127.9
P	n.d.	27.2	20.3 ± 1.0	37.2	23.3 ± 3.4	43.4	31.1 ± 1.7	48.0	33.9 ± 1.3	46.6
K	n.d.	65.8	58.2 ± 1.2	86.3	71.1 ± 5.6	99.4	93.8 ± 3.0	132.5	111.4 ± 4.2	202.8
Ca	n.d.	22.8	38.1 ± 8.2	30.2	38.9 ± 1.3	33.7	44.7 ± 1.8	41.3	47.3 ± 3.5	86.1
Mg	n.d.	12.6	11.6 ± 0.3	17.1	13.9 ± 1.1	20.7	18.3 ± 0.9	25.6	21.0 ± 0.9	34.2

Note: [1] n.d.—not determined.

2.2.3. Aerobic Digestion Phase within the Hydroponic Systems before Plant Cultivation

Aerobic digestion was carried out in the same way as in the first experiment. During this "empty circulation" phase, pH was manually controlled around 7.5 via the addition of 2.5% H_2SO_4 or 10% NaOH. After 25 days of operation, plant cultivation was launched. Plant seedlings were thus transplanted into the systems, and the nutrient solutions were analyzed for their physico-chemical composition (Table 2).

2.2.4. Treatments and Experimental Setup

The four treatments consisting of NFT systems with the chicken manure-based solution concentrated at four different levels (N60, N80, N100, and N120 mg/L TMN) (n = 3 repetitions per treatment) were compared to a reference treatment (n = 3) made of the same commercial mineral fertilizer used in Experiment 1 for plant cultivation. This inorganic solution was created on the day the seedlings were transplanted into the NFT systems in the same manner as for Experiment 1. Table 2 shows the physicochemical composition of the nutrient solution for each treatment on the day of plant transplantation. The addition of de-watering in all nutrient solution tanks was made regularly to maintain the same total volume of nutrient solution (24 L). pH was manually controlled in all modalities between 5.5 and 6.5 via the addition of 2.5% H_2SO_4 or 10% NaOH. Towards the mid-end of plant cultivation, stock solution was added 4 times in 177-mL portions to each organic treatment, so as to add a total of 50 mg/L of TMN per system (additions on days 46, 50, 53, and 55 of operation). This also added 23 mg/L of P, 57 mg/L of K, 19 mg/L of Ca, and 11 mg/L of Mg to each treatment.

2.2.5. Measurements

As for the first experiment, pH, EC, and T were measured daily during the stock solution production phase using the same Hach portable multimeter. The TAN, $N–NO_3^-$, and $N–NO_2^-$ concentrations of the stock solution were then measured using the same Hanna spectrophotometer. The diluted solutions within the NFT systems on the 1st and on the last day of "empty circulation", i.e., before and after aerobic digestion, were then analyzed by the same accredited CPAR laboratory for the following parameters: five-day biological oxygen demand (BOD5) via seeding method using allylthiourea addition [66]; P via ammonium molybdate spectrometric method [67]; Ca, Mg, and K via inductively coupled plasma optical emission spectrometry [68]. BOD5 is a water quality parameter typically used in wastewater treatment plants that measures the oxygen consumed by aerobic microorganisms to break down organic compounds over 5 days of incubation at 20 °C per liter of water. This parameter thus reflects the concentration of biodegradable organic matter in water [69].

pH, EC, T, and dissolved oxygen (DO) of the nutrient solutions were measured three times a week using the same Hach portable multimeter. $N–NO_3^-$, COD, and TSS were measured on the same days with the Trios optical sensor. TAN and $N–NO_2^-$ were measured once a week with the Hanna spectrophotometer. During the trial, ambient temperature

and relative humidity were measured every 30 min using the same data logger as for experiment 1. Focusing on the agronomic data, the mean fresh and dry yield of each treatment was determined in the same manner as for Experiment 1 (n = 36 per treatment for fresh yield and n = 27 per treatment for dry yield). A composite sample of shoots of three fresh lettuces per system (n = 3 per treatment) was analyzed by the CPAR laboratory for shoot mineral content for the same minerals as in the 1st experiment, as well as for total N content, via combustion according to the Dumas principle [70].

2.2.6. Statistical Analyses and Treatment of Data

Data on fresh weight, dry weight, and shoot mineral content were analyzed in the same manner as for experiment 1.

3. Results and Discussion

3.1. Production of Stock Solution via Simple Manure Maceration

During the manure macerations of Experiment 1, the EC and the pH in both types of solutions followed the same evolution: the EC increased, while the pH was the opposite, as shown in Figure 2. This trend was the same for the chicken manure maceration in Experiment 2: EC and pH went from 4630 to 15,150 µS/cm and pH 9.10 to 5.77, respectively, in 9 days. This could be explained by the dissolution of the minerals present in the original manure as well as the mineralization of soluble organic compounds, which releases ions. The pH decrease reflects predominantly anaerobic digestion (AD). During the hydrolysis and acidogenesis stages of AD, complex organic molecules are reduced into soluble organic monomers (sugars, amino acids, and fatty acids), whose subsequent reduction into volatile fatty acids, CO_2, NH_4^+, and other minerals by fermentative acidogenic bacteria acidifies the medium [45,71,72].

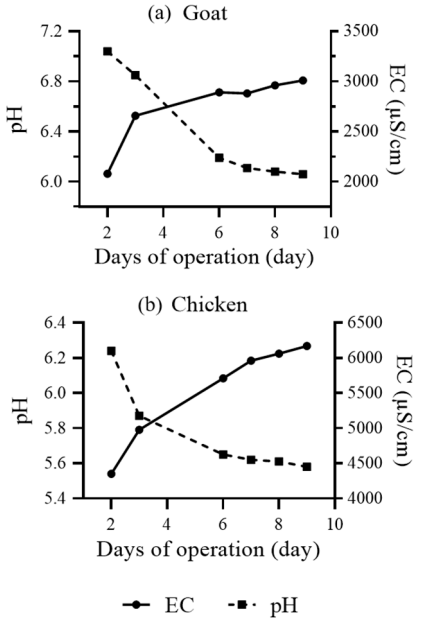

Figure 2. pH and electroconductivity (EC) dynamics during maceration of goat manure (**a**) and chicken manure (**b**).

In the 1st experiment, the chicken manure resulted in a macerate solution with an EC twice as large as that of the goat manure, a concentration in TAN and P-PO_4^{3-} ~ 6 times higher, and a concentration in K 1.5 times higher (Table 1). This could be explained by the naturally high nutrient content of the original chicken manure (total N 4.64% vs. 2.44%, P_2O_5 2.52% vs. 2.08%, and K_2O 2.64% vs. 1.69% DW), as well as its lower C/N ratio (11 vs. 20.9), which favors mineralization. Manure from forage-based diet animals tends to have lower soluble hydrolysable organic matter and mineral content than manure

from concentrate-based diet animals such as chicken [31,33,73]. In the specific case of P, orthophosphate ions are subject to immobilization processes, precipitation reactions, and fixation reactions, which are strongly influenced by pH. In alkaline conditions, dissolved P tends to react with Ca, forming insoluble calcium phosphate [45,47,74]. Studies have observed that P and Ca were released in an anaerobic reactor when pH was below 6 [75,76]. The initial manure pH (pH 7.2 in goat manure vs. 6.7 in chicken excreta) as well as the pH dynamics throughout the two macerations could thus partially explain the difference in P concentration (Figure 3) in the two types of macerate. Furthermore, goat manure macerate contained a higher amount of fine fibers and organic residues, which made the sieving process very long and laborious. This notably explains that only chicken droppings were used for the second experiment.

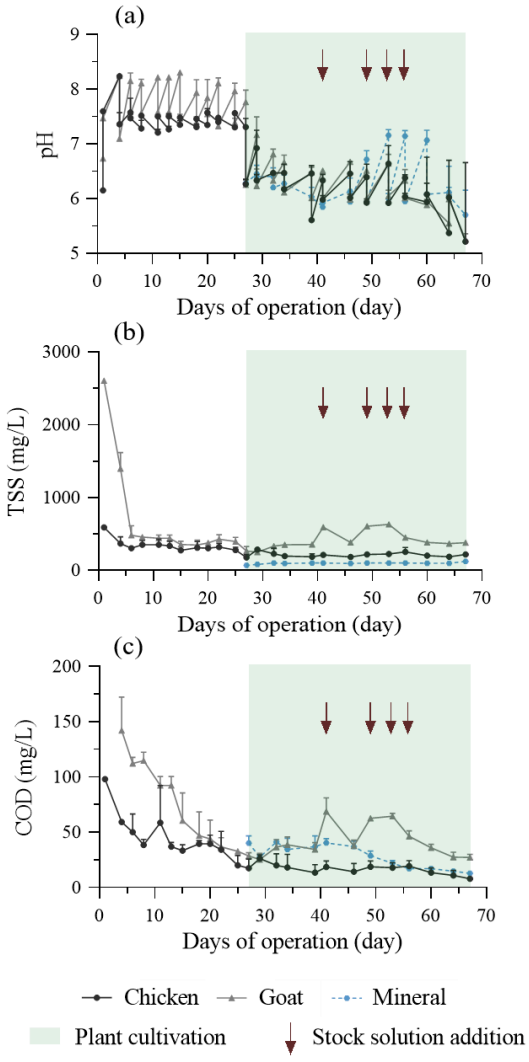

Figure 3. Comparison of chicken manure, goat manure, and mineral treatments in terms of pH (**a**), total suspended solids (TSS) (**b**), and chemical oxygen demand (COD) (**c**) during solution circulation in the NFT systems in Experiment 1. The error bar represents the standard deviation of each value (n = 3).

When comparing the two chicken manure macerations in the two experiments, maceration in experiment 2 resulted in EC and TMN concentrations ~2.5 and 4 times higher, respectively, than the ones observed during the first experiment. This could be explained by the solution dry matter concentration, which was doubled in the 2nd experiment, and by the original chicken manure, which had a higher nutrient content (P_2O_5 3.76% vs. 2.52%, K_2O 3.34% vs. 2.64%, Ca 4.07% vs. 2.44%, total N 5.29% vs. 4.64%) and a lower C/N ratio

(8.8 vs. 11). Relatively large disparities within the manure of the same animal species can exist due to differences in diet, state of the animal, or storage conditions [31,33,34].

3.2. Aerobic Digestion of the Organic Solutions with Respect to Physicochemical Parameters Dynamics

3.2.1. During the "Empty Circulation" Phase

At the introduction of organic solutions in the NFT systems in experiment 1, the goat and chicken modalities had similar levels of EC and pH (Table 1) while the TAN quantity was around 2 times higher in case of chicken manure (Figure 4). However, the levels of COD and TSS were much higher in the goat modality (COD > 450 vs. 98 mg/L, TSS 2602 vs. 590 mg/L), indicating a greater concentration of remaining non-mineralized organic compounds and suspended particles (Table 1). In Experiment 2, the more concentrated the treatment was in stock solution, the higher the levels of COD, TSS, and BOD5 (Table 2; Figure 5). This also shows that the stock solution had a relatively high concentration of remaining residues and non-mineralized organic materials despite filtration.

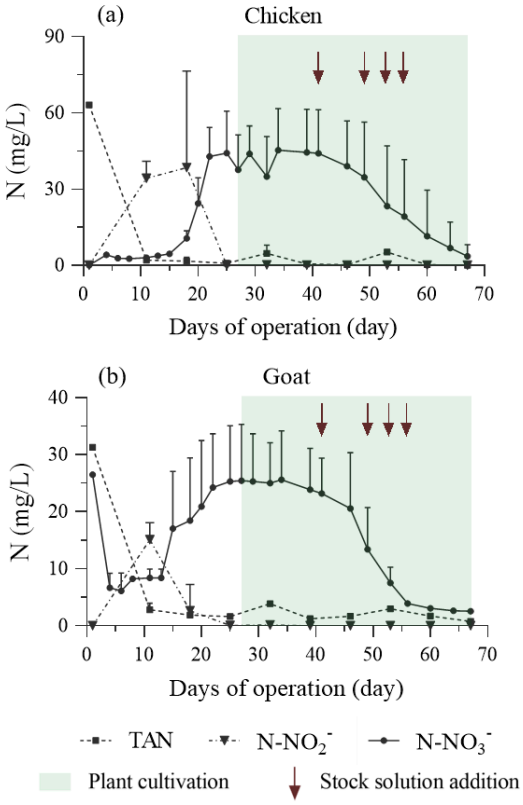

Figure 4. Total ammonia nitrogen (TAN), nitrite-nitrogen (N–NO_2^-) and nitrate-nitrogen (N–NO_3^-) concentration dynamics in chicken (**a**) and goat manure treatments (**b**) during solution circulation in the NFT systems in Experiment 1. The error bar represents the standard deviation of each concentration (n = 3).

Focusing on the pH, all organic solutions showed a rapid and strong pH increase in the first hours and days of circulation, going from pH 6–7 to pH exceeding 8 in less than 4 days. In the case of the goat modality in experiment 1, the pH remained close to 8–8.5 for almost all empty circulations, i.e., 27 days, despite the pH control thrice a week at 7.5. The chicken modality, on the other hand, saw its pH decrease naturally after 6–8 days of operation, and it continued to decrease until the end of the empty circulation (Figure 3). This was also the case for the least concentrated chicken modality in experiment 2 (Figure 5). Conversely, the most concentrated modalities (N100 and N120) had an overall higher pH (pH 7.5–8), despite pH control at ~7–7.5.

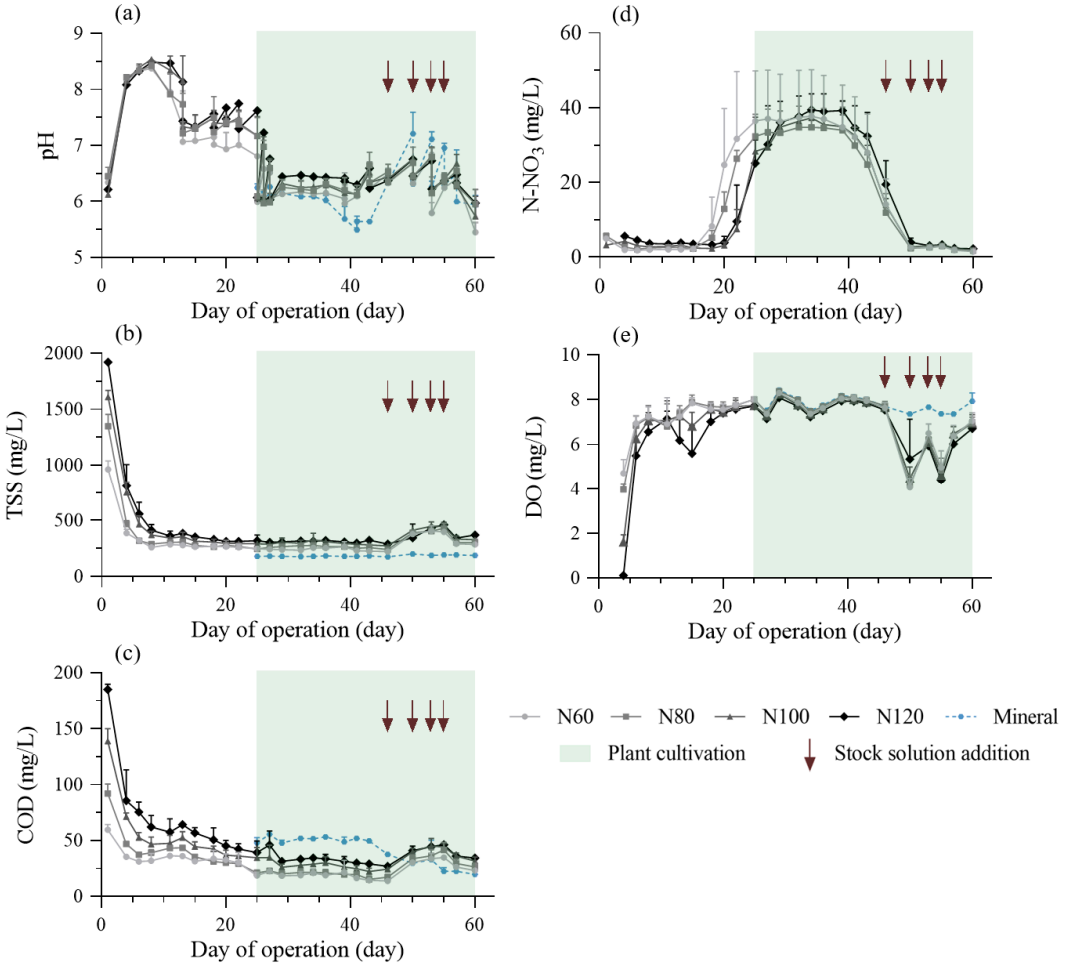

Figure 5. Comparison of chicken manure, goat manure, and mineral treatments in terms of pH (**a**), total suspended solids (TSS) (**b**), chemical oxygen demand (COD) (**c**), nitrate-nitrogen ($N–NO_3^-$) (**d**), and dissolved oxygen (DO) (**e**) during solution circulation in the NFT systems in Experiment 2. The error bar represents the standard deviation of each value (n = 3).

The initial increase in pH observed at the beginning of aeration could be due to a variety of biochemical processes: ammonification (mineralization of organic N into NH_4^+), which consumes H+; the removal of fatty acids via mineralization, and/or the removal of CO_2 resulting from the transformation of carbonate ions (CO_3^{2-}) and protons H+ into CO_2 and H_2O [45,77]. In aerobic conditions, the rate of mineralization and ammonification is considerably faster in comparison to that in anaerobic conditions. Organic matter decomposition in these conditions is carried out by a multitude of heterotrophic microorganisms. In oxygen-deprived conditions, a more restricted and less efficient microbiota is involved [58,77]. The high pH, along with the strong COD, TSS, and BOD5 reductions observed during oxygenation, probably reflect intense microbial activity that took place in the systems thanks to aeration, caused by the presence of remaining organic matter. DO values measured in experiment 2 reinforce this hypothesis, since the more the modality was concentrated in stock solution and therefore the more it contained remaining organic matter, the lower the DO was at the beginning of aeration (Figure 5). It reflects important oxygen consumption by intense microbial development [40,41]. In modalities N120 and N100, DO even reached a concentration lower than 1.5 mg/L, whereas it never went below 4 mg/L for modalities N60 and N80.

On the other side, the subsequent pH decreases could be due to nitrification, as H+ is released during the process. Heterotrophic aerobic respiration might also have contributed to it, as aerobic oxidation of organic matter results in the release of dissolved CO_2 in water,

which forms carbonic acid and lowers the pH [71]. The evolutions of N–NO_2^-, TAN, and N–NO_3^- in experiment 1 (Figure 4), and the evolution of N–NO_3^- in experiment 2 (Figure 5), confirm that nitrification took place in all organic solutions, thanks to the natural development of nitrifiers. Peaks of N–NO_2^- were measured 11 to 15 days after the start of empty circulation, while N–NO_3^- concentration plateaus were observed after approximately ~25 days in both experiments. In experiment 2, evolutions of N–NO_3^- in the four organic treatments suggest nitrification started earlier in the least concentrated modalities (N60, N80) (Figure 5). This could be explained by natural variations between the organic modalities caused by the non-artificial development of nitrifiers, but it could also be explained by the higher amount of residual organic matter in the more concentrated treatments. The intense heterotrophic microbial development that occurs in this case can hinder nitrifiers development as lower DO concentrations are available [55,56,58]. Being very sensitive to environmental conditions, it is well known that autotrophic nitrifying bacteria are often outcompeted by heterotrophic microorganisms [56].

Although nitrification occurred, the overall TMN concentration decreased during this aerobic digestion, where the most losses were detected in the goat modality (−53 ± 17% TMN) in the first experiment in comparison to the chicken modality (−29 ± 26% TMN). In experiment 2, the more concentrated the treatment was in stock solution, the greater the losses in TMN, resulting in similar TMN and N–NO_3^- at transplantation day, whatever the treatment (Table 2). Other mineral losses occurred, i.e., reductions of 6–53% K in both experiments and of 6–26% Mg in Experiment 2. This is also visible by the overall decrease in EC during this period in all types of solutions (Tables 1 and 2). Those mineral losses could be explained by the excessive microbial development that occurred during aeration, caused by remaining organic matter. In this case, the minerals released during the intense mineralization are quickly consumed and assimilated by the heterotrophs, as are the minerals already present in solution [40,41]. This can explain why the modalities with the highest concentration of organic matter had the highest mineral losses. Furthermore, mineral losses were probably reinforced by the formation of thick biofilms on the different surfaces of the systems and on organic biomass agglomerates [78]. TSS went from 2602 to 480 mg/L in less than 6 days in the goat modality and from 1950–1650 mg/L to 450 mg/L in less than 8 days in the N120 and N100 modalities of Experiment 2, suggesting an accumulation of solid organic residues within the systems. This was confirmed at the end of the experiments, with aggregates of organic residues located in the bottom of the NFT gutters. Thick biofilms can represent nutrient sinks by entrapping, precipitating, or absorbing minerals and/or by creating anaerobic zones, which can lead to denitrification and hinder nitrification [27,40,41,79]. Furthermore, pH values reaching ~8.5 were observed in all treatments in the first days of circulation (Figures 3 and 5). At this pH level, up to 10% of TAN in solution can be present in the form of free NH_3, thus exposing the solution to potential N mineral loss via NH_3 volatilization (estimation at ~20 °C) [80].

Overall, despite mineral losses, the effects of this aerobic digestion step before plant cultivation are rather positive. It drastically reduced the high TAN concentrations obtained during maceration, which could have been toxic to plants, while increasing N–NO_3^- concentrations, the preferential N form for plants. It also improved the solution stability by greatly reducing the residual organic matter that had not been mineralized during the previous maceration step, i.e., reducing at least 70% of COD, 50–86% of TSS, and almost 100% of BOD5 (Tables 1 and 2). Remaining organic matter in the nutrient solution during plant cultivation would have caused excessive microbial development, which could have negatively affected the plants via root asphyxiation due to DO consumption. The aesthetic quality of the nutrient solutions was also improved, as it resulted in odorless and relatively transparent solutions.

3.2.2. During Plant Cultivation

During plant cultivation, the temperature within the solutions was 21.4 ± 1.2 °C in the first experiment and 25.0 ± 5.7 °C in the second experiment. At seedling transplantation

in experiment 1, in terms of nutrients, the concentrations of TMN, P, and K were more than 50% lower than those of the inorganic control treatment (Table 1). The goat modality in particular had only about 22% of the TMN concentration of the inorganic treatment. In experiment 2, this percentage was ~25–33% for the four organic treatments. Focusing on the other nutrients (P, K, Ca, and Mg), the concentrations varied from ~30 to 70% of the control inorganic treatment. The more concentrated the treatment in stock solution, the higher the concentration of P, K, Ca, and Mg, while this was not the case for TMN, with similar concentrations between the treatments (Table 2). Although inferior to the inorganic reference, treatments N100 and N120 had P and K concentrations within normal ranges typically found in hydroponics, i.e., P 15–50 mg/L and K 100–200 mg/L [1,81]. The supplementation of nutrients via stock solution additions in organic treatments towards the second half of cultivation was therefore necessary, although it did not compensate for the differences with the controls, except for P and K in experiment 2.

COD, TSS, and BOD5 concentrations in organic treatments were similar to those of the inorganic controls at plant introduction, highlighting the importance of the pre-aerobic digestion step (Table 2). Higher concentrations were measured during stock solution additions; however, they remained well below those observed during the first days of aeration (Figures 4 and 5). This highlights the importance of making these additions in small quantities and distributing them over time to avoid sudden excessive microbial development during plant cultivation.

The pH varied between 5.3 and 6.8 in both experiments, which is close to the general pH range recommended for hydroponic cultivation, i.e., 5.8–7 [1,81]. Towards the mid-end of cultivation, pH tended to drop in all organic-based solutions, while it was the opposite for inorganic treatments. This could be explained by the absorption of different forms of N by plants during their growth peak. In the organic treatments, the stock solution additions provided N to plants mainly in the form of N–NH_4^+, while it was mainly in the form of N–NO_3^- in the inorganic treatment. Absorption of N–NH_4^+ lowers the medium pH via excretion of H+ by the roots, whereas it is the opposite for N–NO_3^- absorption, with excretion of OH- and carbonate ions [1,81,82].

3.3. Plant Growth and Shoot Mineral Content

In the first week of cultivation after transplantation, all organic treatments showed slower growth than the control treatments (visual observation). This trend was also observed in Pelayo Lind et al.'s (2020) study [17] on lettuce cultivation using nitrified biogas digestate. This could be explained by the need for the seedlings to acclimatize in the bioponic environment, going from a tap water medium to a nutrient-concentrated solution that also contains organic compounds. In response to this observation, the cultivation period was extended from the usual 35 days to 42 days for both experiments.

In experiment 1, yields of the chicken and goat modalities were significantly lower than the control treatment, representing 66.7% and 50.7%, respectively, of the yield of the reference treatment in terms of fresh weight and 73.7% and 65.1% of the control yield in terms of dry weight (Table 3). In experiment 2, there was no significant difference between the organic treatments and the mineral control for both fresh and dry yield. Bioponics, therefore, performed as well as conventional hydroponics in this experiment. The presence of bioactive substances (e.g., phytohormones, humic acids, vitamins, and nucleic acids) and plant growth-promoting microorganisms (e.g., K and P solubilizing bacteria) from the original organic matter could explain the good yields obtained, as observed in several bioponics and aquaponics studies [12,13,23,27,83–87].

Within experiment 2, all four organic treatments performed similarly. This is probably due to the mineral losses that occurred during the empty circulation phase, notably in N, which were particularly high in the most concentrated modalities (N120 and N100). In comparison to experiment 1, more nutrients were provided during the plant growth peak in experiment 2 via stock solution additions (e.g., 20 mg TMN/L in

experiment 1 vs. 50 mg TMN/L in experiment 2). This could explain the yield differences between the two experiments compared to their respective inorganic control treatments.

Table 3. Mean shoot mass (fresh and dry) of the lettuce in the different treatments of experiments 1 and 2.

Experiment	Treatment	Fresh Weight (g/Lettuce)	Dry Weight (g/Lettuce)
Experiment 1	Mineral	137.3 ± 25.0 a	5.4 ± 1 a
	Chicken	91.6 ± 27.5 b	4.0 ± 0.8 b
	Goat	69.6 ± 23.2 b	3.5 ± 0.7 b
Experiment 2	Mineral	135.5 ± 18.0 a	5.5 ± 0.8 a
	N60	123.2 ± 23.7 a	5.3 ± 0.8 a
	N80	121.4 ± 14.8 a	5.1 ± 0.4 a
	N100	127.1 ± 15.3 a	5.2 ± 0.6 a
	N120	131.3 ± 15.3 a	4.6 ± 0.4 a

Notes: Values reported as mean ± standard deviation (n = 36 for fresh weight and n = 27 for dry weight). Values within columns and experiments that do not share a letter are significantly different ($p < 0.05$).

Regarding shoot nutrient content, all treatments had values within normal ranges for healthy plants reported by other work [1,81,88,89], except for N in treatments N80, N100, and N120 of experiment 2 and Fe in the goat modality (Table 4), with slightly lower levels. In experiment 2, however, all lettuces appeared healthy. For experiment 1, the N content had not been determined.

Table 4. Nutrient concentration in the lettuce shoot of the different treatments in experiments 1 and 2. P, N, Ca, Mg, and K are expressed in g/kg of dry weight; NO_3^- is expressed in g/kg of fresh weight; and Fe, Mn, Cu, and Zn are expressed in mg/kg of dry weight.

Nutrient	Experiment 1			Experiment 2					Normal Ranges in Healthy Plants [1]
	Mineral	Chicken	Goat	Mineral	N60	N80	N100	N120	
P	7.4 ± 0.4 a	6.4 ± 0.5 a	7.1 ± 0.8 a	8.4 ± 0.8 a	6.5 ± 0.9 b	7.4 ± 0.2 ab	7.8 ± 0.5 ab	7.7 ± 0.5 ab	3.5–13.0
N	n.d. [2]	n.d.	n.d.	34.7 ± 5.7 a	30.0 ± 1.9 a	29.9 ± 3.4 a	26.6 ± 0.4 a	29.4 ± 1.0 a	30.0–60.0
Ca	12.9 ± 0.5 a	20.6 ± 7.7 a	13.0 ± 1.3 a	11.9 ± 1.0 b	19.6 ± 3.8 a	12.9 ± 1.8 ab	13.2 ± 1.3 ab	13.7 ± 4.0 ab	6.0–21.0
Mg	4.4 ± 0.4 a	4.7 ± 0.7 a	3.1 ± 0.3 b	3.4 ± 0.8 b	5.4 ± 0.2 a	3.8 ± 0.2 b	3.9 ± 0.6 b	3.9 ± 1.1 b	2.5–9.0
K	65.2 ± 5.2 a	37.8 ± 6.0 b	55.7 ± 7.1 a	84.2 ± 6.8 a	44.3 ± 9.2 c	47.7 ± 5.6 bc	55.8 ± 3.1 bc	70.7 ±16.1 ab	29.0–108.0
NO_3^-	1.1 ± 0.2 a	0.8 ± 0.3 ab	0.5 ± 0.1 b	1.2 ± 0.4 a	0.7 ± 0.1 b	0.6 ± 0.1 b	0.5 ± 0.1 b	0.6 ± 0.0 b	n.a. [3]
Fe	144 ± 11 a	126 ± 11 ab	92 ± 26 b	96 ± 16 a	64 ± 12 a	73 ± 16 a	74 ± 7 a	114 ± 35 a	100–600
Mn	27 ± 6 b	135 ± 28 b	418 ± 114 a	19 ± 4 b	122 ± 4 ab	121 ± 88 ab	236 ± 60 ab	324 ± 195 a	20–500
Cu	3 ± 1 b	6 ± 0 a	5 ± 1 a	2 ± 0 c	8 ± 1 b	10 ± 1 ab	11 ± 1 ab	12 ± 2 a	5–17
Zn	40 ± 11 b	152 ± 27 a	102 ± 19 a	98 ± 82 a	209 ± 35 a	182 ± 59 a	219 ± 38 a	254 ± 66 a	25–300

Notes: Values reported as mean ± standard deviation (n = 3 for both experiments). Values within lines and experiments that do not share a letter are significantly different ($p < 0.05$). [1] Resh [1], Hartz et al. (2007) [81], Jones [74], Kabata et al. (2007) [82]; [2] n.d.—not determined; [3] n.a.—not available.

In experiment 1, the two organic modalities generally had similar or significantly higher shoot nutrient content than the inorganic control ones, except for Mg and Fe in the goat modality and K in the poultry modality. Similar observations were made in experiment 2 (Table 4). This could be explained by imbalances in the nutrient solutions but also potentially by the varying $N–NH_4^+$:$N–NO_3^-$ ratio of the solutions. Organic treatments that decrease pH can increase P and micronutrient solubility and consequently favor their bioavailability [83]. Higher P, Cu, Mn, and Zn shoot content in lettuces was also observed in Pelayo Lind et al.'s (2020) bioponic study [17]. The authors used anaerobic digestate rich in $N–NH_4^+$ as a stock solution during plant cultivation. More generally, the N forms can influence other macronutrient uptake via ion antagonism as well as plant metabolism and nitrate accumulation [90]. Focusing on NO_3^- content, all organic treatments had significantly lower values than their respective control treatments. This was also observed in various bioponic studies with leafy vegetable cultivation when using organic based nutrient solutions [12,14,15,42,84]. This is a quality sign, as NO_3^- accumulation in leafy vegetables can pose a potential threat to human health in cases of excessive ingestion, such

as higher risks of cancer and methemoglobinemia [91]. That being said, all treatments, including inorganic, were well below the upper value set by UE of 3 g/kg FW of NO_3^- (CE No 1258/2011).

4. General Discussion and Perspectives

The experiments enabled the development of a rather simple bioponics technique, with the production of an organic nutrient solution from chicken or goat manure. Yields similar to those obtained using synthetic mineral fertilizers were achieved in the second experiment with a chicken manure-based solution. Lettuces derived from organic fertilizers also had lower shoot nitrate content, which is a quality sign.

The technique developed in the present study can be divided into three key stages: (1) maceration of manure in a volume of water; (2) aerobic digestion of the resulting macerated solutions; and (3) hydroponic crop cultivation, as illustrated in Figure 6.

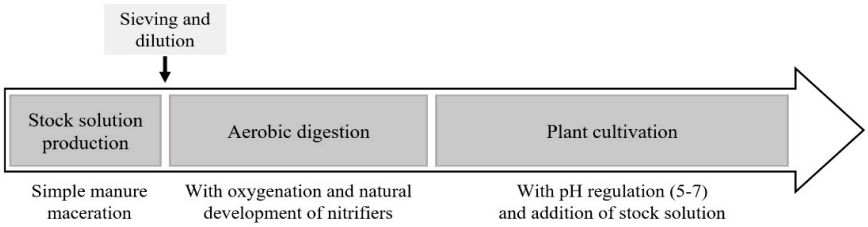

Figure 6. Schematic of the bioponic method developed in the present study.

The first step resulted in the dissolution of the minerals already present in the droppings and a more or less advanced mineralization of the organic matter without any external inputs. The macerate was then filtrated, diluted, and oxygenated for 3–4 weeks to carry out aerobic digestion of the solution and allow nitrification. This step reduced the solution $N–NH_4^+$:$N–NO_3^-$ ratio and the residual organic matter concentration, which could have negatively impacted plant growth. However, it also resulted in a relatively important nitrogen loss. Once nitrification was established, the resulting oxygenated solution was used on plants.

In the context of these trials, the pre-aerobic digestion step was carried out directly in the hydroponic systems via the presence of biofilm carriers in the nutrient solution tank. However, this step could also be carried out outside the system in an external aerobic reactor, so as not to occupy the hydroponic system and thus allow continuous plant production [30]. In the present study, the aerobic digestion phase also proved to be variable in terms of mineralization and nitrification: solutions loaded with organic residues, i.e., the goat manure modality or the highly concentrated chicken manure modality in stock solution, faced significant mineral losses, particularly in N, probably due to excessive microbial development. Variability in mineralization was already observed in other studies [30]. The latter can notably lead to mineral assimilation by microorganisms and anaerobic zones, which favor denitrification and inhibit nitrification [40,41,79]. Filtration after the maceration step is therefore essential, and it is more advantageous to add the stock solution gradually in small quantities, both during the pre-aerobic digestion phase and during plant cultivation, rather than starting with a high stock solution concentration, as shown in experiment 2. The use of low C/N ratio manure, such as chicken droppings rather than goat droppings, also allows greater mineralization of the organic matter, limiting nutrients assimilation by microorganisms. More generally, chicken manure was richer in nutrients, contained fewer fibers and residues, which are difficult to filtrate, and was therefore more interesting to use as fertilizer in bioponics than goat feces. The mineralization that took place during the simple maceration step could also be intensified with the integration of an actual anaerobic bioreactor, which can be achieved in a low-tech manner. It would also have the advantage of producing methane biogas, providing an additional resource to the operator. The management of pH can differ and be more complex than in conventional hydroponics [20,92]. In our systems, pH was manually controlled via strong acids or base

additions. Further research should be undertaken to assess the possibility of completely replacing these synthetic inputs with other regulatory mechanisms, by adjusting the supply of stock solutions, for instance. During pre-aerobic digestion, the addition of small amounts of stock solution could counter the pH drop created by nitrification, as achieved in other bioponic studies using anaerobic digestate [16,17]. During plant cultivation, the addition of nitrified solution would increase the pH via $N–NO_3^-$ root uptake, while the reverse would be the case for the addition of stock solution via $N–NH_4^+$ root uptake. These mechanisms would further minimize the use of external inputs in this bioponic method, relying solely on locally accessible organic materials. Furthermore, further studies should be made on the microbiological and nutritional quality of the lettuces produced with the present technique. As well, other plant species should be tested.

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