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Abstract: Digestate was evaluated as an alternative and sustainable growing medium and nutrient solution in the hydroponic cultivation of baby leaf lettuce (Lactuca sativa L.). Nine hydroponic combinations of substrate and fertilization (agriperlite + standard solution, agriperlite + liquid digestate, solid digestate + standard solution, solid digestate + liquid digestate, soil + standard solution, peat moss + standard solution; peat moss + liquid digestate, pelleted digestate + standard solution and pelleted digestate + liquid digestate) were tested and compared for the cultivation of baby leaf lettuce, in three different experiments. During the crop cycles yield, as other agronomical and microbiological parameters were investigated. The combination of agriperlite + liquid digestate, solid digestate + standard solution and pelleted digestate + standard solution enhanced plant growth by affecting the root, the shoot, the total dry weight and SPAD parameters, in the all investigated experiments (+32%, +40%, +29%, +17% respectively). Based on the obtained results, digestate represents a sustainable and alternative growing media or nutrient solution for the cultivation of baby leaf lettuce cultivated in hydroponic system.

Ms. Ref. No.: HORTI21401

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Dear Editor,

here enclosed please find the revised version of the MS by Ronga et al., with track changes, on which all coauthors agree.

We enclose a separate letter with a precise rebuttal of all doubts and question of the reviewers reporting and tracking any change in the revised version of the manuscript.

We want to thank the Reviewers for their work, for appreciating the manuscript and for the suggestions they have given us to improve the work.

Hoping that the manuscript now meets the quality standards of the Journal, and that it can now answer all the concerns of the reviewers,

Kindest regards,

Sincerely,

Domenico Ronga

Reviewers' comments:

Reviewer #1:

Relevant comments:

1. The research topic of scientific work falls within the general scope of the journal.

2. The interpretations of the results are appropriate and justified by the data obtained. The bibliography used in the discussion supports the explanation of the results obtained. The conclusions that emanate from the work are solid.

3. Research contributes highlighted the possible use of digestates as growing media or nutrient solution to grow baby leaf lettuce with high yield and low microbiological contaminations. The digestate represents a sustainable and alternative growing media or nutrient solution for the cultivation of baby leaf lettuce cultivated in hydroponic system

The author must include the following considerations:

a. in the summary, only mention the factors that explains the treatments

A: Ok, thanks for the comment. According to Referee's suggestion only the factors that explains the treatments were mentioned in the summary

b. Line 64-65 and Line 67-68. Update information to the year 2016. This is the last update that appears in the FAOSTAT.

A: Correct, thanks for the observation. We modified the information. Lines 64-69.

c. Line 197. Surely the evaluation of the percentages of germination was made under some international regulations. As for example the ISTA. Cite source used as the basis for evaluations.

A: Thanks for the good observation. The reference was added. Line 216.

I want to congratulate the authors for the level of depth achieved in the work and the type of analysis performed on the results. The work shows statistical solidity, which support the results obtained.

I believe that by incorporating the suggestions made in the present evaluation, the work is suitable for publication.

Reviewer #2: This is an interesting paper dealing with the use of alternative Growing media and nutrient solution for cultivation of lettuce. The exposure of the authors is considerably clear. My view is that the manuscript could be improved significantly when authors explain last findings in research dealing with the use of organic materials as a substrate. They also are invited to update the references dealing with the use of liquid by-products when should be used as a nutrient solution. The conclusion section should be written down again.

Line 75: "agricultural residues" and "dedicated energy crops" concept: are they excluded each other?

A: Thanks for the observation. We modified the sentence. Lines 75-76.

Line 76. Biofuels is in plural, here? Are there other fuels rather than biogas?

A: Ok. Biogas is the only fuel. We modified the sentence. Line 78.

Line 83: WitH.

A: Sorry for the trivial error; we modified the word. Line 85.

Line 85. Please, revise "while, when"

A: Ok; we revised the sentence. Lines 86-87.

Line 97: "circular economy research" instead of "circular economy"?

A: Correct, thanks for the observation. We modified the sentence according to the Reviewer's suggestion. Line 98.

Line 98. "4" is subscript.

A: Sorry for the mistyped; we changed "4" as subscript. Line 99.

Line 107-108. These references are relatively recent…when this is a well-known concept. Can you include other references?

A: Ok; we included other references. Line 109.

Line 140: revise "regimen"

A: Sorry for the trivial error; we modified the word. Line 155.

Line 141: meaning of RCBD.

A: Thanks for the good observation. The meaning of RCBD was added. Line 156.

Line 144. "In the first": in another paragraph.

A: Correct, thanks for the observation. We added a new paragraph according to the Reviewer's suggestion. Line 161.

Line 148. Why not soil + LD in the first and second experiment sets?

A: Thanks for the good point. For this work we only used soil + standard solution (S + SS) as control in the first and second experiment sets. We added this information also in the manuscript. Line 164. However, your suggestion will be considered in future researches.

Line 157. What does mean 20%?

A: We apologise for the typo; we modified the sentence. Line 174.

Line 165. Explain the active compound of the commercial product that acidifies the medium.

A: Correct, thanks for the observation. We added the information. Lines 182-183.

Line 166. Explain "ca"

A: We apologise for the typo; we modified the sentence. Line 184.

Line 168 Explain briefly the system

A: The system was explained. Lines 186-188.

Line 170. Explain the system

A: The system was explained. Lines 189-191.

Line 233. Explain the main methods.

A: The reference was added. Line 253.

All the document. Please, search for all the document and review the use of "trait": what do you mean? Like "treatment"?

A: Correct, thanks for the observation. We chanced the word trait with parameter along the manuscript.

Line 291. Substitute dray by dry.

A: We apologise for the typo; we modified the word. Line 311.

Line 419. Again: the 4 of the ammonium formulae should be subscript.

A: Sorry again for the mistyped; we changed ”4” as subscript. Line 439.

Line 480 Please, show data

A: The Table S2, containing the requested data, was added. Moreover, the sentence was rewritten. Line 500.

Line 498. Please, include new insights from the bibliography in which it is explained that ammonia, trough nitrification, can be transformated into nitrate to avoid these kind of problems (Waste Management 44, 72-81). In general, you should review other articles dealing with the use as a nutrient solution of liquid byproducts. Apart from the previous one: Agricultural Water Management 140, 87-95.

You also have to do more research on recent developments on alternative growing medium production through composting.

A: Thanks for the good suggestions. The reference (Waste Management 44, 72-81) was added. Lines 520-523. We also added other research that worked on the valorisation of the by-products to obtain innovative growing media and nutrient solutions. Lines 127-140.

Conclusions. I find that the conclusions are too general. Please, check the objectives and state appropriate and specific conclusions of your study.

A: The conclusions were rewritten according to the Reviewer's suggestions. Line 530-546.

Graphical Abstract

Highlights:

- Solid digestate might replace the common growing media in soilless cultivation
- Liquid digestate could replace fertilizer in soilless cropping systems
- Pelleted digestate is an interesting growing media for lettuce soilless cultivation
- The use of digestates are a sustainable approach for lettuce soilless cultivation

Effects of solid and liquid digestate for hydroponic baby leaf lettuce (*Lactuca sativa* L.) cultivation

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Keywords: digestate, lettuce, hydroponics, soilless, fertilizer, sustainability

Abstract

Digestate was evaluated as an alternative and sustainable growing medium and nutrient solution in the hydroponic cultivation of baby leaf lettuce (*Lactuca sativa* L.). Nine hydroponic combinations of substrate and fertilization (agriperlite + standard solution, agriperlite + liquid digestate, solid digestate + standard solution, solid digestate + liquid digestate, soil + standard solution, peat moss + standard solution; peat moss + liquid digestate, pelleted digestate + standard solution and pelleted digestate + liquid digestate) were tested and compared for the cultivation of baby leaf lettuce, in three different experiments. During the crop cycles yield, as other agronomical and microbiological ~~trait~~parameters were investigated. The combination of agriperlite + liquid digestate, solid digestate + standard solution and pelleted digestate + standard solution enhanced plant growth by affecting the root, the shoot, the total dry weight and SPAD parameters, in the all investigated experiments (+32%, +40%, +29%, +17% respectively). ~~Regarding the nitrate content and the aerobic mesophilic charge all the samples were below the threshold for the market (2500 mg kg^{-1} and 5.0E+05 CFU g^{-1} of fresh weight product, respectively).~~ Based on the obtained results, digestate represents a sustainable and alternative growing media or nutrient solution for the cultivation of baby leaf lettuce cultivated in hydroponic system.

Abbreviations

Abbreviations: AD – anaerobic digestion, AG – agriperlite, S – soil, SD – solid digestate, LD – liquid digestate, PD – pelleted digestate, PM – peat moss, VE – vermiculite, NS – nutrient solution, GM – growing media, SS – standard solution, MAC – mesophilic aerobic charge, MAC_{GMC} – mesophilic aerobic charge of growing media and nutrient solution combination, MAC_{L} – mesophilic aerobic charge of lettuce, SFC = spore forming charge, SFC_{GMC} – spore-forming charge of growing media and nutrient solution combination, CC_{L} – coliform charge of lettuce, HI – harvest index, RDW – root dry weight, SDW – shoot dry weight, TDW – total dry weight.

1. Introduction

The consumption of fresh-cut vegetables (including herbs) increased over the last 20 years in the European market, at the annual growth rate of about 4%; this is why food category is recognized to be as one of the most profitable in the fruit and vegetables segment. As the result of an upward trend observed during the last decade, lettuce (*Lactuca sativa* L.) and chicory (*Cichorium intybus* L.) are cultivated on a total area of ~1.2 M ha worldwide, with ~27~~5~~ M t of global production (FAOSTAT, 2016~~4~~). Italy ranks four~~six~~th in the world, with open-field lettuce and chicory covering a total area of 38~~15~~,542~~10~~ ha (31.7~~15.2~~% in the North, 10.0~~1.4~~ % in the Centre and 58.3~~73.4~~% in the South), and a total production of about 8~~3~~.1~~5~~ M t (AGRISTAT, 2016~~4~~a). Greenhouse production is also relevant, with a total area of 4,549~~264~~ ha (37.3~~23.2~~% in the North, 31.9~~5.0~~% in the Centre and 30.8~~41.8~~% in the South) (AGRISTAT, 2016~~4~~b). Alongside their wide market spread, leafy vegetables are considered the group of fresh foods with the highest concern for microbiological hazards. Among them, fresh-cut lettuce is frequently linked with food borne outbreaks (López-Gálvez et al., 2010); specifically, the bacterium *Escherichia coli* O157:H7 was found to be strongly associated with lettuce contamination (Franz et al., 2008). Therefore, fresh-cut vegetables might have a relatively short shelf-life which usually does not exceed 6–9 days.

Anaerobic digestion (AD) – or co-digestion – is a widely used process to treat various kinds of raw biomasses~~, ranging from organic wastes to agricultural residues and dedicated energy crops~~. The main goal of this technique is to efficiently convert a low-value feedstock into more bio-based products and renewable biofuel~~s~~, such as biogas. Besides that, reducing the dependence on fossil raw materials, AD has the advantage to limit odours and pathogens' charge of the remaining by-product, technically called digestate (Hijazi et al., 2016; Jolánkai et al., 2014; Nkoa, 2014; Uddin et al., 2016). As a consequence of the microbial activity that takes place during the AD, digestate enriches in nutrients already available in the feedstock – scientifically called ingestate – acquiring the following characteristics: low dissolved oxygen level, high levels of chemical and biological oxygen demand, rise in its content of suspended solids (Dosta et al., 2007). When compared with~~whit~~ solid digestate (SD), the liquid phase by-products (liquid digestate, LD) are characterized by lower levels of dry matter, total organic carbon (TOC), C/N ratio and viscosity; instead~~while~~, when compared with the ingestate, the liquid digestate ~~is compared with the ingestate, it~~ shows higher pH and ammonium percentages (Nkoa, 2014; Tambone et al., 2010).

Previous studies have shown that digestate contains phythormones – above all, gibberellins, indole acetic acid, auxin-like and auxin-active molecules – dissolved in the organic matter, and other bioactive compounds that have the potential to promote plant growth, increasing the tolerance to biotic and abiotic stresses (Liu et al., 2009; Scaglia et al., 2017; Yu et al., 2010). Nevertheless, antithetical results about the phytotoxicity of digestate were reported in literature: several authors confirmed that digestate caused phytotoxic reactions (Abdullahi et al., 2008; Poggi-Varaldo et al., 1999; Salminen and Rintala, 2002). Other studies highlighted positive effects on germination and growth (Gell et al., 2011; Ronga et al., 2016; Sánchez et al., 2008;); in addition, a very recent report by Scaglia et al. (2017) suggested the use of digestate as an innovative bio-stimulant to increase the added value of the AD, positively reinforcing the circular economy research. The phytotoxicity of digestate after field application is related to the presence of NH_4^+-N ~~NH^{4+}-N~~ and organic acids;

however, no data about the duration of the phytotoxic effect in field conditions have been reported (Möller and Müller, 2012). The distribution of digestate in the soil might potentially spread both not pathogenic and pathogenic bacteria (i.e. *Salmonellae*, *Clostridia* and *Listeriae)* that may survive after the AD (Bonetta et al., 2011; Bonetta et al., 2014; Sidhu and Toze, 2009), causing soil and crop contamination (Bonetta et al., 2014). To overcome this inconvenient, Pulvirenti et al. (2015) demonstrated that the pelleting treatment of digestate can be a feasible solution for the elimination of any microbiological risk.

Modern protected horticulture recently shifted from soil-grown systems to soilless one (Martínez et al., 2013). Soilless systems might support efficient and intensive plant production (Barrett et al., 2016; Grafiadellis et al., 2000; Raviv and Lieth, 2008; van Os, 1999). Soilless growing media (GM) adopted in horticulture normally include both organic (e.g. peat moss) and inorganic (e.g. vermiculite, rockwool, perlite and/or sand) substrates.

Soilless media, fertilizer, irrigation, chemicals and greenhouse structure involve different level of fossil fuel inputs (Enoch, 1978; Stanhill, 1980). Moreover, the selection of substrate as growing medium is based on both agronomic performance and economic considerations (Barret et al., 2016). Peat moss (PM) is one of the most used organic component for the preparation of growing media, due to its agronomic, hydrological and physic-chemical characteristics (Herrera et al., 2008). However, peat moss is a non-renewable resource which is turning to be increasingly scarce and, when available, expensive; in fact, there is a lot of concern about the economic and environmental impacts related to the exploitation of peatland ecosystems, moreover resulting in fossil CO_2 mobilization (Schmilewski, 2008). In addition, peat-based substrates cause reduction of wetlands and loss of soil organic carbon (Carlile and Coules, 2013). Hence, the concern on the environmental impacts of some commonly used materials, such as peat-based growing media and chemical fertilizers, led researchers to identify and assess new environmental friendly products (Wallach, 2008). Another perspective to be considered is the request of sustainable products, by consumers

(Gül et al., 2007a; Gül et al., 2007b). So that, alternative substrates and nutrient sources for soilless horticulture need to be investigated in a perspective of circular economy and environment preservation (Herrera et al., 2008; Ronga et al., 2016). Interesting previous works assessed the valorisation of the by-products as innovative growing media and nutrient solutions. Gattullo et al. (2017) showed the suitability of a municipal solid waste compost (MSWC) and a sewage sludge compost (SSC) as components of growing media for the soilless cultivation of lettuce.

The use of composted agro-waste as growing media might be an efficient alternative to peat-based substrates for controlling diseases, also in soilless production (De Corato et al., 2016).

Regarding nutrient solutions by-product management from cheese industry can be a sustainable solution for the irrigation of horticultural crops, such as tomato (Prazeres et al., 2014).

Most crop nutrients might be derived from aquaculture (Tyson et al., 2011). Inf act, interesting studies showed the potential for crops to use the nutrient by-products of aquaculture as a nutrient solution (Adler et al., 1996, 2000; Lin et al., 2002).

As reported above, there are few published studies on the effects of the digestates on hydroponic production. Therefore, further studies on the use of the digestates on hydroponic production could be very useful to increase agricultural sustainability.

The aim of the present study was the evaluation of digestates as sustainable alternative growing media and nutrient solutions for baby leaf lettuce cultivation in hydroponic system. Accordingly, multiple experiments in controlled (growth chamber) conditions were set up, and the effects on yield together with other agronomic and microbiological ~~trait~~parameters were investigated to compare solid and liquid digestate with conventional growing media and nutrient solutions.

2. Material and methods

2.1. Plant material, experimental design and growing conditions

A baby leaf lettuce Batavia blonde type cultivar ‘Chiara’ (Isi Sementi S.p.A., Fidenza, Italy) was selected for cultivation. The genotype is a well-adapted fresh-cut lettuce characterized by a medium-short growing cycle (20-25 days), with tight erect blonde leaves, and high tolerance to tip burn. Lettuce 'Chiara' was sown into separated hydroponic discontinuous closed systems in three independent experiments (crop cycles) conducted in controlled conditions in a growth chamber of the University of Modena and Reggio Emilia (Reggio Emilia, Italy). Plants were grown under long-day conditions (15 h light, 9 h dark; light intensity 180 μmol m^{-2} s^{-1}). Relative humidity was maintained at 65%, while temperature regimen of the growth chamber varied in the different crop cycles. Each experiment was set up as a randomized complete block design (RCBD) factorial design with 16 replicates (corresponding to 16 pots with 20 lettuce seedlings each) per treatment. In total, five types of solid substrates (PM – peat moss, AG – agriperlite, S – clay-loam soil, SD – solid digestate, PD – pelleted digestate) were combined with two nutrient solutions (SS – standard solution, LD – liquid digestate).

In the first crop cycle the growing temperature was kept at 24 ± 2 °C and the following growing media and nutrient solution combinations were tested: agriperlite + standard solution (AG + SS); agriperlite + liquid digestate (AG + LD); solid digestate + standard solution (SD + SS); solid digestate + liquid digestate (SD + LD); soil + standard solution (S + SS), used as control. In the second crop cycle, the growing media and nutrient solution combinations were the same whereas the growing temperature was set at 27 ± 2 °C. In the third crop cycle the growing temperature was kept at 24 ± 2 °C and the following growing media and nutrient solution combinations were tested: peat moss + standard solution (PM + SS); peat moss + liquid digestate (PM + LD); pelleted digestate + standard solution (PD + SS); pelleted digestate + liquid digestate (PD + LD). All crop cycles had the same duration of 21 d.

2.2. Characteristics of the growing media and hydroponic cultivation

Solid substrates used as potting media in this study had the following technical characteristics: agriperlite (AG) Agrilit® 3, Perlite Italiana s.r.l. (Italy) – grain diameter 2–5.6 mm, pH 7.5, density 90 kg m^{-3} ± 20 %, and EC 0.1 dS·m^{-1}; soil (S) – clay-loam type, organic matter (1.5 %), total N (1.0

‰), pH 8.0 and EC 0.03 dS·m^{-1}. Solid digestate (SD) and pelleted digestate (PD) were obtained in an AD plant of the Reggio Emilia area (see below). Peat moss (PM) technic®, Free Peat B.V. (The Netherlands) – organic C (23 %), organic N (0.5 %), organic matter (46 %), pH 7.4, and EC 0.2 dS·m^{-1}. Finally, to facilitate lettuce germination, a Vermiculite (VE) Saint-Gobain PPC S.p.a. (grain diameter 0.5–4.0 mm, pH 8.0, density 105 kg ± 15 % g m^{-3}) layer was added to all substrates.

The standard nutrient solution (SS) was prepared dissolving ca. 30 g of Hydrofood KB, Scott® s.r.l., Treviso (Italy) – NPK(Mg) 17-16-11-(2) plus micronutrients fertilizer – in 20 L of distilled water with the addition of 20 mL of acidifying agent CIFOVIR 1 (N 5%, P_2O_5 17%, pH 2.5 and EC 2.5 dS m^{-1}), ©CIFO s.r.l., San Giorgio di Piano, (Italy). The liquid digestate (LD) solution was prepared by mixing ~~ca.~~ 1.25 L of LD with 18.75 L of distilled water and 20 mL of CIFOVIR 1.

A modified Wilma 16 pots hydroponic closed system Atami® B.V., Rosmalen (The Netherlands) was used; briefly the system consisted in a plastic tank (120 cm x 60 cm x 30 cm) containing the nutrient solution and the pump. The tank was completely covered with a plastic tray that supported the pots. The plastic tray has a hole suited to draining the nutrient solution. T~~t~~he dimensions of the pots were 100 mm and 70.7 mm (diameter and height, respectively). A pump Wave STREAM 700, IDROPONICA®, Player s.r.l., Roma (Italy) was used to ~~pump~~ facilitate the~~d~~ NS recirculation and irrigation.~~,~~ The ~~and~~pots~~ plants~~ were irrigated with the nutrient solution every 185 min using a low-flow dripper (2 L h^{-1}) for 5 minutes, apart S that was irrigated two times every day. The two NS were analysed on a daily basis with a portable multi-parameter instrument HI9813-6, Hanna Instruments® s.r.l., Padova (Italy) to evaluate temperature (°C), turbidity (ppm), EC and pH. Once a week, 20 l of each NS were replaced to maintain N content, EC and pH within appropriate ranges (260 – 290 mg L^{-1}, 2.0 – 2.5 and 6.0 – 6.6, respectively). Finally, one week before the harvest, each experiment was irrigated using only tap water.

2.3. Digestate production and properties

Digestate was produced in an AD plant owned by CAT–Cooperativa Agroenergetica Territoriale, Correggio, Reggio Emilia (Italy) as described by Pulvirenti et al. (2015). After solid/liquid separation of the fresh digestate, the chemical parameters of the liquid digestate were: TOC (3.74%), nitrogen (N 0.34%), potassium (K_2O 0.95%), EC 1.07 $dS \cdot m^{-1}$, and pH 8.03 (P_2O_5 was completely absent). Conversely, solid phase digestate showed: TOC (17.02%), nitrogen (N 0.74%), phosphorus (P_2O_5 0.60%), potassium (K_2O 0.76%), EC 0.23 $dS \cdot m^{-1}$, and pH 8.11. A small fraction of SD was also dried and pelleted accordingly to Pulvirenti et al. (2015), and PD contained: TOC (16.32%), nitrogen (N 0.93%), phosphorus (P_2O_5 1.94%), potassium (K_2O 1.94%), EC 4.17 $dS \cdot m^{-1}$, and pH 8.28.

2.4. Phytotoxicity test, microbiological analyses and agronomic ~~trait~~parameters

To evaluate the influence of the different growing media, of the nutrient solutions, and their combinations on lettuce's germination rate, a phytotoxicity test was performed following Zucconi et al. (1981). Briefly, 4 ml of each growing media water extract (50 g l^{-1}), of the nutrient solutions, and their combinations, plus a control treatment of only water were added to Petri dishes containing Whatman filter paper. Three replicates of 20 seeds were prepared, and the plates were incubated 36 h at 25 °C in a Binder ED53, Tuttlingen (Germany) heating chamber. The number of germinated seeds and the average length of roots were derived in order to calculate a Germination Index (GI%) according to the following formula (Tiquina and Tam, 1998):

$$GI\% = 100 \times (G_t / G_c) \times (R_t / R_c)$$

where,

G_t = number of germinated seeds of the treatment;

G_c = number of germinated seeds of the control;

R_t = average length (mm) of roots of the treatment;

R_c = average length (mm) of roots of the control.

Microbiological analyses were performed separately on the growing media, the nutrient solution, their combinations and baby leaf lettuce, as follows. Regarding the growing media (AG, SD, PM, PD, S), the NS (LD, SS) and their combinations, it was analysed the mesophilic aerobic charge (MAC) and the spore-forming charge (SFC) by mixing 10 g of each sample with 90 mL of peptone physiological solution in a sterile blender bag. The samples used for the determination of *Clostridium spp.* were thermally pre-treated (95 °C for 10 minutes) to activate the spores. Appropriate dilutions of the suspensions were plated onto Petri dishes and incubated at 30 °C for 24 h for MAC; in the case of SFC, the plates were incubated at 30 °C for 48 h in an anaerobic environment. The media used for the enumeration of MAC was Brain Heart Infusion Agar (BHIA, 70138, Sigma-Aldrich) and *Clostridium* reinforced agar (CM0149, Oxoid) for the spore-forming charge. Every sample was plated twice, and the test was repeated five times.

Bacterial charge was calculated after the incubation time according to the formula:

$\Sigma c / [(n1 + 0.1\ n2)\ d]$

Where:

Σc = sum of the number of total colonies;

n1 = number of plates used for the first dilution;

n2 = number of plates used for the second dilution;

d = dilution factor of n1.

As far as mesophilic aerobic charge (MAC_L), the *Coliform* charge (CC_L) was analysed as well in lettuce. The microbiological analyses of the baby leaf lettuce were performed as described: 25 g of samples were mixed with 225 ml of sterile peptone water using a stomacher bag. Appropriate dilutions of the solution formed were inoculated inside BHIA media, accordingly with the microbiological protocol: ISO 4833-1:2013 to determine the MAC_L. The same dilutions were also inoculated on Violet Red Bile Agar (VRBGA, CM0485, Oxoid), accordingly with the microbiological protocol: ISO 4831:2006, to determinate the Coliforms load (CL_L). Every dilution was plated twice; the test was repeated 5 times. Bacterial charge was calculated after the incubation time, as cited above.

At the end of each crop cycle the following agronomic ~~trait~~parameters were recorded. Before the harvest, plant height (H) was measured, and chlorophyll content was estimated by measuring three leaves by using SPAD-502, Minolta (Japan). A subsample of each treatment was used to detect leaf nitrate content (UNI EN 12014-2:1998) as suggested by Merusi et al. (2010). Shoot (SDW), root (RDW) and total dry weights (TDW) were measured after desiccation in stove at 65 °C. Harvest index (HI), fraction of biomass to root (FTR) and SDW/H ratio were calculated.

2.5. Statistical analysis

Factorial ANOVA was performed with GenStat 17.0th edition and factors' means were compared using Duncan's test at $P<0.05$ level. PCA models were used for biplots generation (Jackson, 1991; Wold et al., 1987), and since the considered variables had different scales, a pre-processing auto-scaling step was performed before calculating the PCA.

3. Results

Hydroponic cultivation might be one of the technical solutions to respond to the increasing demand of food, without the exploitation of new land, especially in the system of vertical (indoor) farms. However, alternative GM and NS are needed to improve the sustainability of traditional soilless cropping system that nowadays uses non-renewable substrate and nutrient solution.

Another important variable in the hydroponic cultivation is the temperature that plays a fundamental role on both the crop growth and the microorganism charge. Hence, in the present study the effects of different GM, NS and growth temperature were investigated in different experiments. In the first two experiments, the same variables were assessed, apart from temperature that was set at 24 ± 2 °C and 27 ± 2 °C, in the first and second crop cycles, respectively. Moreover, in the third experiment the effects of PM *vs* PD were tested using the same NS assessed in the first and second crop cycles.

3.1. Microbiological quality of GM and NS and their combinations

The microbiological analysis was performed on each GM and NS investigated (Table 1). The average values of MAC and SFC charges in term of CFU were 1.2E+06 g^{-1} and 1.6E+05 g^{-1}, respectively. Solid digestate highlighted the highest charge of MAC (8.3E+06 CFU g^{-1}) showing a content seven times higher than the general average, followed by PM (6.4E+05 CFU g^{-1}), while SS highlighted the total absence of MAC charge. Regarding another important microbiological parameter, such as the SFC charge, CFU values ranging from the total absence on one side, to 7.3E+05 CFU g^{-1} on the other; LD showed the highest value of SFC (7.3E+05 CFU g^{-1}) with a content four times higher than the general average, followed by SD (5.5E+05 CFU g^{-1}).

Medium and nutrient solutions[a]	**MAC (CFU g^{-1})**	**SFC (CFU g^{-1})**
SD	**8.3E+06 a**	5.5E+05 b
PM	6.4E+05 b	0 c
S	5.2E+05 c	1.3E+04 c
AG	5.2E+03 d	1.1E+03 c
LD	7.6E+03 d	**7.3E+05 a**
PD	1.5E+03 d	1.1E+03 c
SS	0 d	0 c
VE	1.1E+03 d	2.3E+02 c
Average	**1.2E+06**	**1.6E+05**

Table 1. Microbiological quality of the growing media and nutrient solutions. Means followed by the same letter do not significantly differ at $P<0.05$. AG = agriperlite; LD = liquid digestate; PD = pelletized digestate; PM = peat moss; S = soil; SD = solid digestate; SS = standard solution; VE = vermiculite; MAC = mesophilic aerobic charge; SFC = spore forming charge. See text for details.

Nevertheless, the combination of GM and NS plays an important role on the contamination of cultivated crop. Hence, the mesophilic aerobic and spore forming charges due to the combinations of GM and NS are reported in Table 2. In the first and second crop cycle, the same combinations were used and SD + LD recorded the highest charges of both MAC_{GMC} and SFC_{GMC} (3.6E+06 CFU g^{-1} and 1.3E+05 CFU g^{-1}, respectively) sowing ca. a double charges compared to the general average, respectively; while the combination of AG + SS showed the lowest ones (7.4E+05 CFU g^{-1} of MAC_{GMC} and 3.0E+03 CFU g^{-1} of SFC_{GMC}) (Table 2A). As far as the different combinations investigated in the third crop cycle (Table 2B), PD + LD displayed the highest charge of the two microbiological parameters analysed (1.4E+08 CFU g^{-1} of MAC_{GMC} and 9.1E+04 CFU g^{-1} of SFC_{GMC}, ca. two and three times higher compared to the average charges, respectively), followed by PD + SS (8.7E+07 CFU g^{-1} of MAC_{GMC} and 2.7E+02 CFU g^{-1} of SFC_{GMC}).

Hydroponic system[a]	**MAC_{GMC} (CFU g^{-1})**	**SFC_{GMC} (CFU g^{-1})**
A. 1st and 2nd crop cycles		
AG + SS	7.4E+05 c	3.0E+03 c
AG + LD	1.3E+06 b	2.5E+03 c
SD + SS	8.6+05 c	1.5E+03 c
SD + LD	**3.6E+06 a**	**1.3E+05 a**
S + SS	4.0E+05 c	8.0E+04 b
Average	**1.4E+06**	**4.3E+04**
B. 3rd crop cycle		
PM + SS	4.5E+05 c	7.7E+02 b
PM + LD	3.9E+05 c	8.0E+02 b
PD + SS	8.7E+07 b	2.7E+02 b
PD + LD	**1.4E+08 a**	**9.1E+04 a**
Average	**5.7E+07**	**2.3E+04**

Table 2. Microbiological quality of the growing media and nutrient solution combinations. Means followed by the same letter do not significantly differ at $P<0.05$. AG = agriperlite; LD = liquid digestate; S = soil; SD = solid digestate; SS = standard solution; PM = peat moss; PD = pelletized digestate; MAC_{GMC} = mesophilic aerobic charge of the growing media and nutrient solution combinations; SFC_{GMC} = spore forming charge of the growing media and nutrient solution combinations. See text for details.

3.2. Baby leaf lettuce - agronomical and microbiological results

Innovative GM or NS obtained by the valorisation of by-products should be evaluated before being used for crops cultivation due to their possible phytotoxic or bio-stimulation effects, caused by their chemical and microbiological content. The results reported in Table S1 on germination assay, demonstrated that all GM, NS and their combinations showed values higher than 50% which might be considered the phytotoxicity threshold reported by Zucconi et al. (1981). In particular, the combinations of water both using LD and SD proved the highest value of germination index (105% and 101%, respectively), while the lowest one was reported using PM (65%).

Growing media and NS performances were assessed on baby leaf lettuce in term of agronomical and microbiological parameters, in different experiments (three) and hydroponic systems (nine). In Table 3 are reported the production, nutrition status and microbiological ~~trait~~parameters recorded at the harvest time, regarding the first crop cycle using temperature at 24 ± 2 °C. Interesting statistical differences were observed for all the ~~trait~~parameters apart from shoot dry weight-height ratio and SPAD index. Focusing the attention on the most important ~~trait~~parameter such as shoot dry weight, the hydroponic systems SD + SS (0.85 g plot^{-1}) and AG + LD (0.82 g plot^{-1}) displayed the highest values (+42% and +37% compared to the general average, respectively). Moreover, the hydroponic system AG + LD recorded also the highest value of root (1.30 g plot^{-1}) and total dr~~a~~y weight (2.12 g plot^{-1}) (+86% and +56% compared to the general average, respectively). Finally, the hydroponic system AG + LD showed a drastic reduction of MAC_L (-76% respect to the average value of all others) and total absence of CC_L; similar microbiological results were recorded by the hydroponic system AG + SS, used as control in the present study. The hydroponic systems SD + SS and AG + SS showed the highest value of leaves height (+25 cm and +22 cm compared to the general average, respectively), S + SS one (used as another control), recorded the highest harvest index (+25% respect to the general average).

Hydroponic system[a]	**H (cm)**	**SDW (g pot^{-1})**	**RDW (g pot^{-1})**	**TDW (g pot^{-1})**	**HI**	**SDW/H (g cm^{-1})**	**SPAD**	**MAC_L (UFC g^{-1})**	**CC_L (UFC g^{-1})**
AG + SS	**9.00 a**	0.64 ab	0.87 ab	1.52 ab	0.43 bc	0.07 n.s.	11.33 n.s.	**0 c**	**0 d**
AG + LD	6.67 b	**0.82 a**	**1.30 a**	**2.12 a**	0.39 c	0.13 n.s.	13.80 n.s.	**9.65E+01 c**	**0 d**
SD + SS	**9.17 a**	**0.85 a**	0.83 ab	1.68 ab	0.53 ab	0.10 n.s.	13.03 n.s.	3.00E+02 b	8.50E+02 c

SD + LD	7.07 b	0.45 b c	0.37 bc	0.82 bc	0.55 ab	0.06 n.s.	13.93 n.s.	1.60E+03 a	6.10E+03 a
S + SS	4.83 c	0.22 c	0.12 c	0.34 c	**0.64 a**	0.04 n.s.	12.17 n.s.	5.00E+01 c	3.10E+03 b
Average	**7.35**	**0.60**	**0.70**	**1.36**	**0.51**	**0.08**	**12.85**	**4.09E+02**	**2.01E+03**

Table 3. Production, nutrition status and microbial charge of baby lettuce grown on different substrates and nutrient solutions in the first crop cycle (24 ± 2 °C). Means followed by the same letter do not significantly differ at P<0.05; n.s. = not significantly different; AG = agriperlite; SS = standard solution; LD = liquid digestate; SD = solid digestate; S = soil; MAC_L = mesophilic aerobic charge of lettuce; CC_L = Coliform charge of lettuce; H = plant height; SDW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; HI = harvest index; SPAD. See text for details.

In the second crop cycle an increase of three degrees Celsius during the baby leaf lettuce cultivation was investigated. In Table 4, are reported the production, nutrient status and microbiological charges of baby leaf lettuce grown at 27 ± 2 °C. Also, in the second crop cycle, interesting statistical differences were observed for all the ~~trait~~parameters recorded at the harvest time, apart from shoot dry weight-height ratio and HI. The hydroponic systems SD + SS and AG + LD recorded the highest shoot dry weight (0.74 g plot^{-1} and 0.72 g plot^{-1}, respectively) performing as well as in the first experiment. However, some interesting differences were highlighted. In the second crop cycle, the hydroponic system SD + SS showed the highest values of both root dry weight (0.34 g plant^{-1}) and total dry weight (1.08 g plant^{-1}) (+48% and +35% compared to the general average, respectively) and total absence of microbiological charge both for MAC_L and CC_L, and similar low microbiological charges were showed by AG + SS. Finally, the hydroponic system SD + SS recorded also the highest value of leaves height (+20% respect the general average), and the higher values of SPAD index (+12% compared to the general average), and similar indices of SPAD were recorded by AG + SS and AG + LD.

Hydroponic system[a]	**H (cm)**	**SDW (g pot^{-1})**	**RDW (g pot^{-1})**	**TDW (g pot^{-1})**	**HI**	**SDW/H (g cm^{-1})**	**SPAD**	**MAC_L (UFC g^{-1})**	**CC_L (UFC g^{-1})**

AG + SS	9.67 b	0.54 ab	0.13 b	0.67 bc	0.82 n.s.	0.06 n.s.	**12.90 a**	0 c	1.10E+01 c
AG + LD	9.67 b	**0.72 a**	**0.31 a**	1.03 ab	0.71 n.s.	0.07 n.s.	**13.77 a**	3.50E+02 b	2.60E+01 b
SD + SS	**11.17 a**	**0.74 a**	**0.34 a**	**1.08 a**	0.69 n.s.	0.07 n.s.	**13.20 a**	**0 c**	**0 d**
SD + LD	10.00 b	0.53 ab	0.27 ab	0.80 abc	0.68 n.s.	0.05 n.s.	9.17 b	4.95E+03 a	4.00E+03 a
S + SS	5.83 c	0.31 b	0.13 b	0.43 c	0.66 n.s.	0.05 n.s.	9.83 b	4.00E+02 b	1.00E+01 cd
Average	**9.27**	**0.57**	**0.23**	**0.80**	**0.71**	**0.06**	**11.77**	**1.14E+03**	**8.09E+02**

Table 4. Production, nutrition status and microbial charge of baby lettuce grown on different substrates in the second crop cycle (27 ± 2 °C). Means followed by same letter do not significantly differ at $P<0.05$; n.s. = not significantly different; AG = agriperlite; SS = standard solution; LD = liquid digestate; SD = solid digestate; S = soil; MAC_L = mesophilic aerobic charge of lettuce; CC_L = Coliform charge of lettuce; H = plant height; SDW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; HI = harvest index; SPAD. See text for details.

The results of the third crop cycle, regarding the assessment of PM *vs* PD as growing media and liquid digestate and standard solution as nutrient solution, are reported in Table 5. The hydroponic system PD + SS, displayed the highest values of shoot, root and total dry weights, shoot dry weight-height ratio and SPAD index (+23, +53, +29, +88, 21% compared to the general average, respectively), and similar value of SPAD index was recorded by PM + SS (+9%). Moreover, PD + SS showed the lowest value of leaves height (-26% respect the general average) and the lower harvest index (-5% compared to the general average) and similar value of HI was reported by PD + LD (-6%).

Hydroponic system[a]	**H (cm)**	**SDW ($g\ pot^{-1}$)**	**RDW ($g\ pot^{-1}$)**	**TDW ($g\ pot^{-1}$)**	**HI**	**SDW/H ($g\ cm^{-1}$)**	**SPAD**	**MAC_L (UFC g^{-1})**	**CC_L (UFC g^{-1})**
PM + SS	9.50 a	0.66 bc	0.10 b	0.76 c	0.87 a	0.07 c	15.95 a	7.00E+02 n.s.	1.20E+02 n.s.
PM + LD	8.66 b	0.54 c	0.08 b	0.62 c	0.86 a	0.06 c	11.43 b	6.00E+02 n.s.	5.00E+01 n.s.
PD + SS	5.60 d	**0.85 a**	**0.26 a**	**1.11 a**	0.77 b	**0.15 a**	17.63 a	3.00E+02 n.s.	3.50E+01 n.s.

PD + LD	6.33 c	0.72 ab	0.22 a	0.94 b	0.76 b	0.11 b	13.33 b	8.00E+02 n.s.	1.00E+01 n.s.
Average	**7.52**	**0.69**	**0.17**	**0.86**	**0.81**	**0.08**	**14.59**	**6.00E+02**	**5.38E+01**

Table 5. Production, nutrition status and microbial charge of baby lettuce grown on different substrates in the third crop cycle (24 ± 2 °C). Means followed by same letter do not significantly differ at $P<0.05$; n.s. = not significantly different; PM = peat moss; SS = standard solution; LD = liquid digestate; PD = pelletized digestate; MAC_L = mesophilic aerobic charge of lettuce; CC_L = Coliform charge of lettuce; H = plant height; SDW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; HI = harvest index; SPAD. See text for details.

3.4 Relationships between recorded parameters and hydroponic systems

The correlations between data of the hydroponic systems variables measured on baby leaf lettuce were studied by PCA analysis. Figures 1, 2 and 3 report ordination biplots of the PCA output modelling for the three crop cycles investigated in the present work. For the first crop cycle, PC1 accounted for 59.09% of the variance, and PC2 accounted for 23.41%, and their sum explained 82.50% of total variance (Figure 1). The hydroponic systems $T2_1$ (AG + LD) and $T3_1$ (SD + SS) were positively associated with the descriptive ~~trait~~parameters regarding biomass such as shoot, root and total dry weight, leaves height, biomass fraction to root and shoot dry weight-height ratio and negatively associated with baby leaf lettuce nitrate content and microbiological parameters. While, the hydroponic system $T4_1$ (SD + LD) was closely associated with microbiological parameters such as CC_L, MAC_{GMC}, MAC_L, SFC_{GMC} and SPAD index. Finally, hydroponic system $T5_1$ (S + SS) was associated to HI.

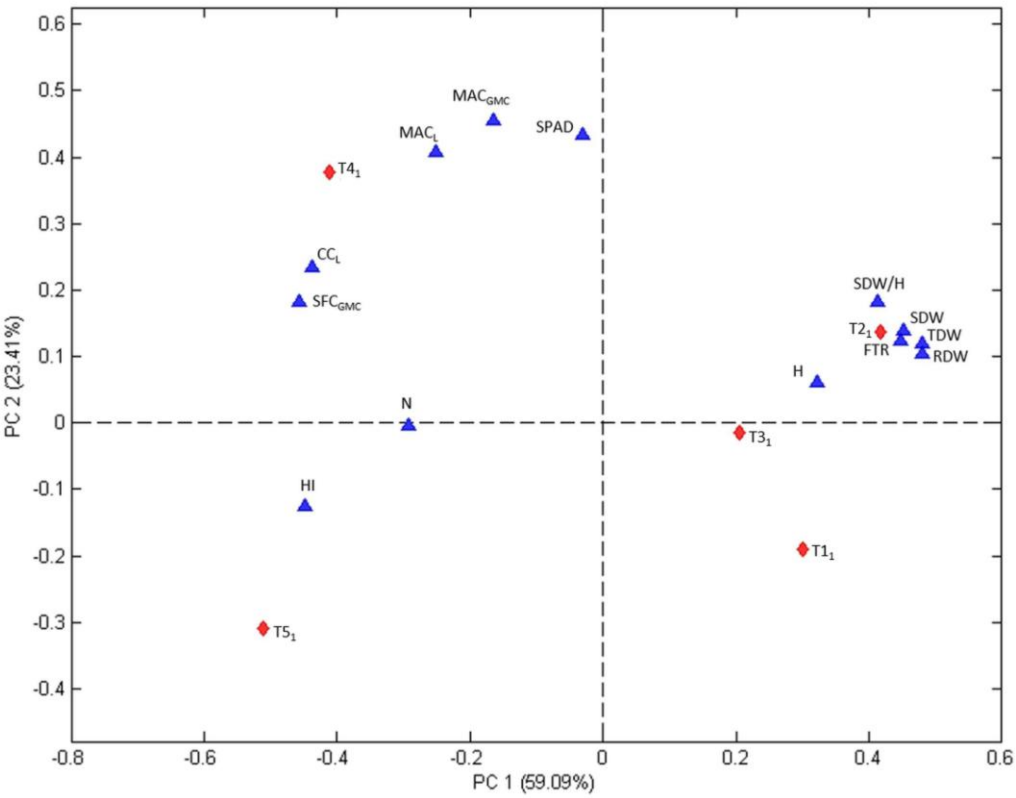

Figure 1. Ordination biplot of principal component analysis of the first crop cycle (24 ± 2 °C). Labels in the graph represent the investigated parameters: CC_L = clostridium charge of baby leaf lettuce; SDW= shoot dry weight; RDW= root dry weight; TDW = total dry weight; H= plant height; HI = harvest index; SDW/H = shoot dry weight-height ratio; FTR = biomass fraction of dry weight to root; MAC_{GMC} = mesophilic aerobic charge of the growing media combinations; MA_{CL} = mesophilic aerobic charge of baby leaf lettuce; SFC_{GMC} = spore-forming charge of the growing media combinations; SPAD; N = baby leaf lettuce nitrate content; T1-T5 = the different hydroponic systems: T1 = AG + SS; T2 = AG + LD; T3 = SD + SS; T4 = SD + LD; T5 = S + SS. AG = agriperlite; LD = liquid digestate; S = soil; SD = solid digestate; SS = standard solution. Number 1 following the hydroponic systems investigated indicate the corresponding crop cycle (the first one).

Regarding the second crop cycle investigated, PC1 accounted for 47.54% of the variance, and PC2 accounted for 37.00%, and their sum explained 84.54% of total variance (Figure 2). The hydroponic systems $T2_2$ (AG + LD) and $T3_2$ (SD + SS) were closely associated with the descriptive ~~trait~~parameters regarding biomass (shoot, root and total dry weight, leaves height, shoot dry weight-height ratio and SPAD index); on the contrary, it was negatively correlated with baby leaf lettuce nitrate content and microbiological parameters, as well as showed in the PCA profile of the first experiment, except for SPAD index and biomass fraction to root. Also, the hydroponic system $T4_2$

(SD +LD) confirmed its association with microbiological parameters such as CC_L, MAC_{GMC}, MA_{CL} and SFC_{GMC} as highlighted in the PCA result of the first experiment. Finally, hydroponic system $T1_2$ (AG + SS) was well correlated to HI.

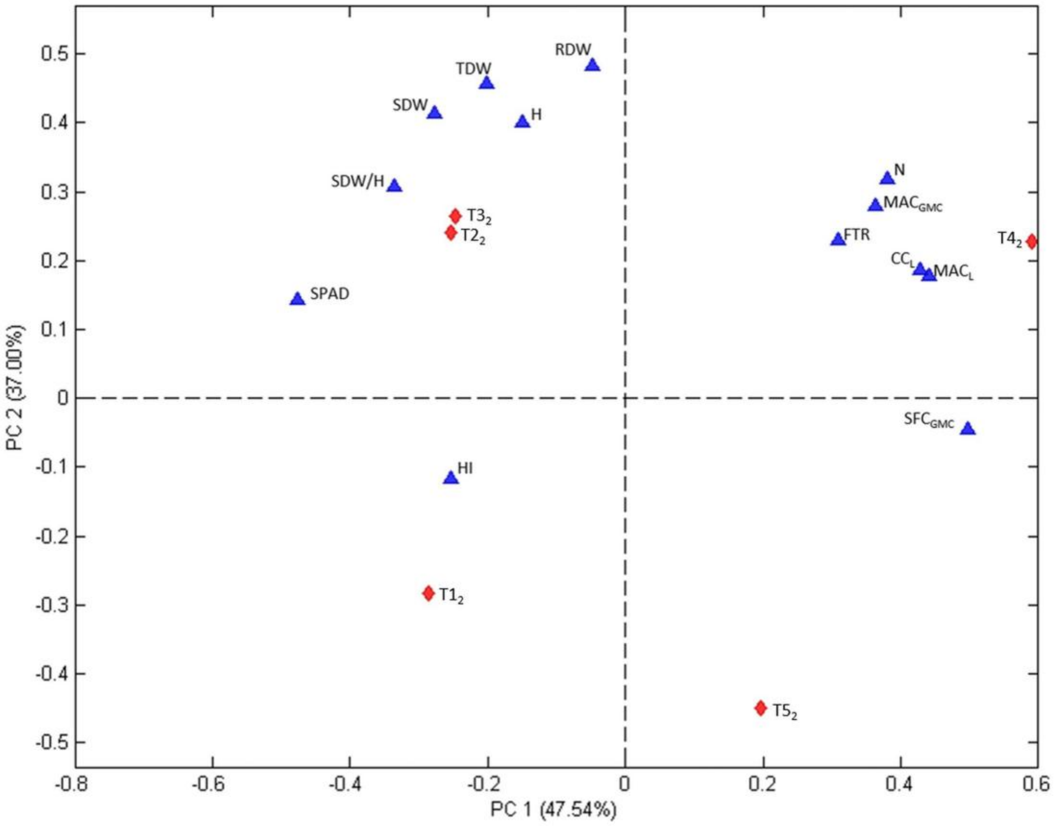

Figure 2. Ordination biplot of principal component analysis of the second crop cycle (27 ± 2 °C). Labels in the graph represent the investigated parameters: CC_L = clostridium charge of baby leaf lettuce; SDW= shoot dry weight; RDW= root dry weight; TDW = total dry weight; H= plant height; HI = harvest index; SDW/H = shoot dry weight-height ratio; FTR = biomass fraction of dry weight to root; MAC_{GMC} = mesophilic aerobic charge of the growing media combinations; MA_{CL} = mesophilic aerobic charge of baby leaf lettuce; SFC_{GMC} = spore-forming charge of the growing media combinations; SPAD; N = baby leaf lettuce nitrate content; T1-T5 = the different hydroponic systems: T1 = AG + SS; T2 = AG + LD; T3 = SD + SS; T4 = SD + LD; T5 = S + SS. AG = agriperlite; LD = liquid digestate; S = soil; SD = solid digestate; SS = standard solution. Number 2 following the hydroponic systems investigated indicate the corresponding crop cycle (the second one).

Summarizing across the two first crop cycles investigated, SDW, RDW, TDW, H and SDW/H were the ~~trait~~parameters most consistently associated to the hydroponic systems AG + LD and SD + SS. In the third crop cycle, PM *vs* PD were investigated as growing media. As reported in Figure 3, PC1 accounted for 66.31% of the variance, and PC2 accounted for 21.75%, and their sum explained

88.06% of the total variance. The hydroponic system $T8_3$ (PD + SS) was high correlated and mainly influenced by descriptive ~~trait~~parameters regarding biomass (shoot and total dry weight, shoot dry weight-height ratio, and SPAD index), while the hydroponic system $T9_3$ (PD + LD) was closely associated with the microbiological parameters relates to the initial charges contained in the growing media and nutrient solution investigated (MAC_{GMC}, and SFC_{GMC}). Finally, the hydroponic system $T6_3$ (PM + SS) was correlated to HI and CC_L.

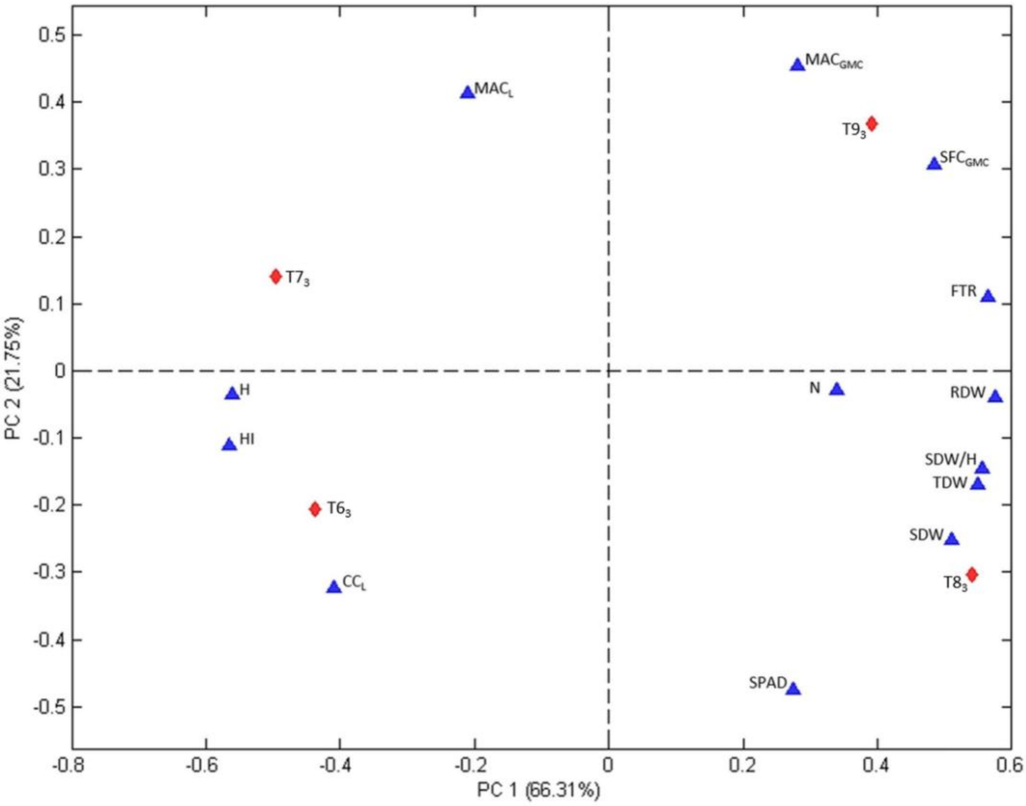

Figure 3. Ordination biplot of principal component analysis of the third crop cycle (24 ± 2 °C). Labels in the graph represent the investigated parameters: CC_L = clostridium charge of baby leaf lettuce; SDW= shoot dry weight; RDW= root dry weight; TDW = total dry weight; H= plant height; HI = harvest index; SDW/H = shoot dry weight-height ratio; FTR = biomass fraction of dry weight to root; MAC_{GMC} = mesophilic aerobic charge of the growing media combinations; MA_{CL} = mesophilic aerobic charge of baby leaf lettuce; SFC_{GMC} = spore-forming charge of the growing media combinations; SPAD; N = baby leaf lettuce nitrate content;T6-T9 = the different hydroponic systems: T6 = PM + SS; T7 = PM + LD; T8 = PD + SS; T9 = PD + LD; LD = liquid digestate; PM = peat moss; PD = pelletized digestate; SD = solid digestate; SS = standard solution. Number 3 following the hydroponic systems investigated indicate the corresponding crop cycle (the third one).

4. Discussion

In literature there are works whose main aim were testing the use of digestate as fertilizer, for the cultivation in open field (Lukehurst et al., 2010; Makádi et al., 2012). Despites the fact that some agronomic studies on aerobic and anaerobic digestion have been performed (Goddek et al., 2016; Stoknes et al., 2016), the impact of both SD and LD on plant growth is not completely clear. In addition, as far as the present state of art on this topic, the information regarding the use of digestate as fertilizer in greenhouse is limited, especially for soilless systems (Liedl et al., 2006). Hence, the present study aimed to analyse the effects of digestates on yield and other agronomical and microbiological as alternative and sustainable growing media and nutrient solution for the cultivation of baby leaf lettuce using hydroponics.

Baby leaf vegetables are fresh foods that are frequently linked with food borne outbreaks (Nicola et al., 2009). In fact, contaminations with pathogenic microorganisms might have occurred during crop cycle due to the contact with soil and irrigation water (Tournas, 2005). In order to adopt strategies that could minimize the risk of microbiological contamination within agricultural system, it is important to understand the charge of pathogens, in growing media and soils and how their might influence the contamination (Nicola et al., 2009). In the present study, SD and LD reported the highest charge of MAC and SFC, respectively (Table 1). Moreover, the hydroponic system SD + LD and PD + LD highlighted the highest charge of MAC_{GMC} and SFC_{GMC} (Table 2). The presence of *Clostridium spp*. bacteria observed in the digestate was already reported in earlier studies (Bagge et al., 2005, Bonetta et al., 2011). In general, anaerobic digestion does not reduce *Clostridium spp*. content (Bagge et al., 2005). The genus *Clostridium* survived in the anaerobic digestion process (Schnurer and Jarvis, 2009) because only vegetative cells are susceptible to temperatures above 50 °C, while the elimination of spores requires further and more intense heat-treatments (Watcharasukarn et al., 2009). Bagge et al. (2005) reported that pathogen regrowth during storage was probably due to non-hygienic conditions of the storage tanks, as showed for pelleted digested in the present work versus the data reported by Pulvirenti et al. (2015).

Soil growing media might influence both the germination and the emergence of seedlings. In the present study, the germination assays indicated that there were no phytotoxicity issues in the analysed growing media, nutrient solutions and their combinations. In fact, they showed values of the germination index greater than 50% (Table S1), which may be considered as a threshold value for phytotoxicity (Zucconi et al. 1981). In particular, GI% proved greater values for H_2O + LD and H_2O + SD, thus they showed a biostimulant effect might due to digestate content. In fact, Yu et al. (1995) confirmed that the germination power and percentage should increase in seeds previously soaked in LD. Moreover, Gell et al. (2011) and Sánchez et al. (2008) obtained similar results evaluating the digestate phytotoxicity on lettuce, radish, wheat, and garden cress. On the other hand, the combined use of digestate as GM and NS slightly decreased the germination index values of the other investigated treatments, probably due their pH values. Hence, although depending on species growth and yield adaptation, this constitutes a limit for the use of digestate without pH correction as already reported by Endo et al. (2016). Moreover the phytotoxicity of digestate could be related to the presence of NH_4^+-NNH^{4+} -N and organic acids (Möller and Müller, 2012).

As far as the agronomical investigated traitparameters, our results were in agreement with Vimolmangkang et al. (2010) who showed how “deep flow” technique increased mint growth. Baby leaf total dry weight was improved by ca. two-fold using hydroponic systems respect to soil (Table 3 and 4). In addition, taking into account the most important traitparameters such as shoot dry weight in the first and the second crop cycles (Table 1 and 2), the hydroponic systems AG + LD and SD + SS reported the highest values, which were probably due to the presence in SD of unknown compounds/molecules either acting as, or mimicing plant growth promoters. In fact, previous studies have shown that digestates contain phytohormones – above all, gibberellins, indole acetic acid, auxin-like and auxin-active molecules – dissolved in the organic matter, and other bioactive compounds that have the potential to promote plant growth and to increase the tolerance to biotic and abiotic stresses (Liu et al., 2009; Scaglia et al., 2017; Yu et al., 2010).

Comparing the two investigated temperatures, RDW and TDW showed higher values, while HI lower, at 24 °C rather than 27 °C. Moreover, in the present study soilless cropping reduces harvest index compared to soil. However, this reduction was ascribed to a higher increase of root growth than of shoot growth in hydroponics, as already reported by Olympios (1999). This growth acceleration, especially in root, was due to a more and constant availability of nutrients as showed in processing tomato (Ronga et al., 2017). Moreover, several studies compared the crop cultivation in soil *vs* soilless systems highlighting that soilless reduces the crop cycle and increase crop yield (Fontana and Nicola, 2009; Incrocci et al., 2001), and the latter was shown in the present study.

Regarding the nutritional status, SPAD index values recorded in the second and third experiments were in accordance with Chrysargyris et al. (2017) who showed that nitrogen levels affected plant growth and chlorophyll. On the other hand, the same trend was not recorded in the first experiment probably due to the low temperature that did not allow the same availability of the nutrients, bioactive compounds and microorganism contained in the solid digestate. However, further studies are needed to confirm these hypotheses.

Food safety management in the fresh-cut chain is expected before processing, thus the food safety risks depend on genotypes, management, environment and their interactions (Kirezieva et al., 2013). The microbial contamination of lettuce irrigated using the furrow system was much lower than lettuce irrigated using sprinklers (Fonseca, 2006). Moreover, processing operations might contaminate fresh vegetables if the edible portions were in direct contact with water or soil containing pathogens (Solomon et al., 2003). The microbiological analysis of the baby leaf lettuce demonstrates low level of aerobic mesophilic contamination as shown in the Tables 3 to 5. In fact, no sample had a level above 5.0E+05·CFU g^{-1} of product. This is the safety threshold for selling fresh vegetables (HPA, 2009). The *Coliform* analysis showed a very low charge under the selling threshold (1.0E+03 CFU g^{-1}), except for the theses S + SS, SD + SS, SD + LD and PM + SS that have a *Coliform* charge higher than the threshold and reach a maximum of 1.0E+03 CFU g^{-1} with the thesis SD + LD (Tables 3 and 4) (HPA, 2009). In general, vegetables cropped in the open filed reach a total bacterial count of 1.0E+06 to 1.0E+09 CFU g^{-1}, which might be reduced by 2-3 log CFU g^{-1} after washing practices (Nicola and Fontana, 2014; Selma et al., 2012) confirming the results obtained in the present study, where the leaves were microbiological analysed without washing. However, in baby leaf vegetables washing operations are crucial to make the product ready-to-eat and will able to reduce the microbiological charges recorded in the present study. Finally, baby leaf vegetables should be clean, free of soil residue, insects, metals and weeds.

Analysing the relationships between recorded parameters and hydroponic systems, from the PCA analysis of the first and second crop cycle emerged that the hydroponic systems T2 and T3 are associated with SDW, TDW, RDW and FTR, while the T4 is related with CC_L, MAC_L and MAC_{GMC}. Regarding to the PCA analysis of the third crop cycle, the T8 was related with SDW, TDW and SPAD and the T9 is connected with MAC_{GMC} and SFC_{GMC}. Finally, the T6 was associated with CC_L, HI and H. In general, the digestates used as growing media (solid and pelleted) performed better using standard solution as nutrient solution in all three experiments, probably due to a better balance of organic and mineral nutrient availability. However, further researches are needed to corroborate this hypothesis. In vegetables, quality ~~trait~~parameters such as firmness, dry matter percentage and soluble sugar content are negatively correlated with nitrogen content. An excess of nitrogen availability might increase crop susceptibility to biotic and biotic stress, however, neither were recorded in the present study (data not shown). Moreover, the hydroponic combination that performed better in each experiment (T2, T3 and T8) were negatively correlated with the nitrate content in baby leaf samples (Figure 1-3). In the EU, there is a specific regulation (EU Reg. 1258/2011, amending EU Reg. 1881/2006 that amended EU-Reg. N. 563/2002) that sets the threshold levels of nitrate content (below 2500 mg kg^{-1} f.w.) in the edible part of vegetables such as lettuce (*Lactuca sativa* L.), and in the present study all samples were below the threshold ranging between 338 and 1640 mg kg^{-1} f.w. (Table S1~~data not shown~~).

The most striking differences between the agriperlite and the digestate were recorded for the combination of AG + LD and SD + SS that improved the shoot dry weight of baby leaf lettuce both in the first and in the second experiment (Figure 4), while the combination of SD + LD did not perform as well.

Figure 4. The five representative pots of baby leaf lettuce cultivated in the first and second cycle. AG + SS = agriperlite + standard solution; AG + LD = agriperlite + liquid digestate; SD + SS = solid digestate + standard solution; SD + LD = solid digestate + liquid digeste; S + SS = soil + standard solution.

The similar trend was highlighted during the third experiment where PD + SS and PD + LD performed better than the other investigated hydroponic systems (Figure 5).

Figure 5. The four representative pots of baby leaf lettuce cultivated in the third cycle. PM + SS = peat moss + standard solution; PM + LD = peat moss + liquid digestate; PD + SS = pelleted digestate + standard solution; PD + LD = pelleted digestate + liquid digestate.

The lower performance showed by hydroponic system SD + LD and PD + LD could be caused by inorganic nitrogen content in the digestate was provided as a high concentration of NH_4^+ and high pH value. In fact, Endo et al. (2016) reported inhibition when cucumber seedlings were grown hydroponically with digestate as nutrient solution. However, the feasibility of nitrification could be tested also on digestates. In fact, Cáceres et al. (2015) using the nitrified leachates formed during composting of cattle and pig manure, as liquid fertilizer, obtained similar lettuce productivities in comparison to the standard nutritive solution.

Finally, pellet digestate could be an interesting alternative growing media. In fact, digestate transport from farms located farther than 20 km from the AD plants is convenient in terms of associated costs and carbon footprint; therefore, this alternative way that reduces the overall environmental impacts of AD plants might improve the economic value of digestate and the agricultural sustainability.

5. Conclusions

~~The present study underlined how digestates might improve the sustainability of baby leaf lettuce in hydroponics. Nowadays, there are just a few studies about the use of digestate in soilless systems; so, the availability of data to compare the results obtained is scarce.~~ Considerable effort has been made in the search of improved sustainability of hydroponics, and microbiological control for fresh-cut vegetables. Nowadays, there are just a few studies about the use of digestate in soilless systems; so, the availability of data to compare the results obtained is scarce. The present study underlined how digestates might improve the sustainability of baby leaf lettuce in hydroponics. The combination of agriperlite + liquid digestate, solid digestate + standard solution and pelleted digestate + standard solution recorded higher values of root, shoot and total dry weights and SPAD, compared to the average value of the all assessed treatments, in the all investigated experiments. This study highlighted the possible use of solid and liquid digestates as growing media and~~or~~ nutrient solution, respectively to grow baby leaf lettuce with high yield and low microbiological contaminations. ~~Solid and liquid~~ D~~d~~igestates for hydroponic lettuce cultivation show a great potential in the future of hydroponic greenhouse due to its low cost, environment sustainability, and interesting agronomical and microbiological ~~trait~~parameters. However, further studies are needed to improve the combined use of solid and liquid digestates, despite this, the baby leaf lettuce produced in this way showed a great potential for the scale-up.

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Tables

Medium and nutrient solutions[a]	MAC (CFU g^{-1})	SFC (CFU g^{-1})
SD	**8.3E+06 a**	5.5E+05 b
PM	6.4E+05 b	0 c
S	5.2E+05 c	1.3E+04 c
AG	5.2E+03 d	1.1E+03 c
LD	7.6E+03 d	**7.3E+05 a**
PD	1.5E+03 d	1.1E+03 c
SS	0 d	0 c
VE	1.1E+03 d	2.3E+02 c
Average	**1.2E+06**	**1.6E+05**

Table 1. Microbiological quality of the growing media and nutrient solutions. Means followed by the same letter do not significantly differ at $P<0.05$. AG = agriperlite; LD = liquid digestate; PD = pelletized digestate; PM = peat moss; S = soil; SD = solid digestate; SS = standard solution; VE = vermiculite; MAC = mesophilic aerobic charge; SFC = spore forming charge. See text for details.

Hydroponic system[a]	MAC_{GMC} (CFU g^{-1})	SFC_{GMC} (CFU g^{-1})
A. 1st and 2nd crop cycles		
AG + SS	7.4E+05 c	3.0E+03 c
AG + LD	1.3E+06 b	2.5E+03 c
SD + SS	8.6+05 c	1.5E+03 c
SD + LD	**3.6E+06 a**	**1.3E+05 a**
S + SS	4.0E+05 c	8.0E+04 b
Average	**1.4E+06**	**4.3E+04**
B. 3rd crop cycle		
PM + SS	4.5E+05 c	7.7E+02 b
PM + LD	3.9E+05 c	8.0E+02 b
PD + SS	8.7E+07 b	2.7E+02 b
PD + LD	**1.4E+08 a**	**9.1E+04 a**
Average	**5.7E+07**	**2.3E+04**

Table 2. Microbiological quality of the growing media and nutrient solution combinations. Means followed by the same letter do not significantly differ at $P<0.05$. AG = agriperlite; LD = liquid digestate; S = soil; SD = solid digestate; SS = standard solution; PM = peat moss; PD = pelletized digestate; MAC_{GMC} = mesophilic aerobic charge of the growing media and nutrient solution combinations; SFC_{GMC} = spore forming charge of the growing media and nutrient solution combinations. See text for details.

Hydroponic system[a]	**H (cm)**	**SDW (g pot^{-1})**	**RDW (g pot^{-1})**	**TDW (g pot^{-1})**	**HI**	**SDW/H (g cm^{-1})**	**SPAD**	**MAC$_L$ (UFC g^{-1})**	**CC$_L$ (UFC g^{-1})**
AG + SS	**9.00 a**	0.64 ab	0.87 ab	1.52 ab	0.43 bc	0.07 n.s.	11.33 n.s.	**0 c**	**0 d**
AG + LD	6.67 b	**0.82 a**	**1.30 a**	**2.12 a**	0.39 c	0.13 n.s.	13.80 n.s.	**9.65E+01 c**	**0 d**
SD + SS	**9.17 a**	**0.85 a**	0.83 ab	1.68 ab	0.53 ab	0.10 n.s.	13.03 n.s.	3.00E+02 b	8.50E+02 c
SD + LD	7.07 b	0.45 b c	0.37 bc	0.82 bc	0.55 ab	0.06 n.s.	13.93 n.s.	1.60E+03 a	6.10E+03 a
S + SS	4.83 c	0.22 c	0.12 c	0.34 c	**0.64 a**	0.04 n.s.	12.17 n.s.	5.00E+01 c	3.10E+03 b
Average	**7.35**	**0.60**	**0.70**	**1.36**	**0.51**	**0.08**	**12.85**	**4.09E+02**	**2.01E+03**

Table 3. Production, nutrition status and microbial charge of baby lettuce grown on different substrates and nutrient solutions in the first crop cycle (24 ± 2 °C). Means followed by the same letter do not significantly differ at $P<0.05$; n.s = not significantly different; AG = agriperlite; SS = standard solution; LD = liquid digestate; SD = solid digestate; S = soil; MAC$_L$ = mesophilic aerobic charge of lettuce; CC$_L$ = Coliform charge of lettuce; H = plant height; SDW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; HI = harvest index; SPAD. See text for details.

Hydroponic system[a]	**H (cm)**	**SDW (g pot^{-1})**	**RDW (g pot^{-1})**	**TDW (g pot^{-1})**	**HI**	**SDW/H (g cm^{-1})**	**SPAD**	**MAC$_L$ (UFC g^{-1})**	**CC$_L$ (UFC g^{-1})**
AG + SS	9.67 b	0.54 ab	0.13 b	0.67 bc	0.82 n.s.	0.06 n.s.	**12.90 a**	0 c	1.10E+01 c
AG + LD	9.67 b	**0.72 a**	**0.31 a**	1.03 ab	0.71 n.s.	0.07 n.s.	**13.77 a**	3.50E+02 b	2.60E+01 b
SD + SS	**11.17 a**	**0.74 a**	**0.34 a**	**1.08 a**	0.69 n.s.	0.07 n.s.	**13.20 a**	**0 c**	**0 d**
SD + LD	10.00 b	0.53 ab	0.27 ab	0.80 abc	0.68 n.s.	0.05 n.s.	9.17 b	4.95E+03 a	4.00E+03 a
S + SS	5.83 c	0.31 b	0.13 b	0.43 c	0.66 n.s.	0.05 n.s.	9.83 b	4.00E+02 b	1.00E+01 cd
Average	**9.27**	**0.57**	**0.23**	**0.80**	**0.71**	**0.06**	**11.77**	**1.14E+03**	**8.09E+02**

Table 4. Production, nutrition status and microbial charge of baby lettuce grown on different substrates in the second crop cycle (27 ± 2 °C). Means followed by same letter do not significantly differ at $P<0.05$; n.s. = not significantly different; AG = agriperlite; SS = standard solution; LD = liquid digestate; SD = solid digestate; S = soil; MAC$_L$ = mesophilic aerobic charge of lettuce; CC$_L$ = Coliform charge of lettuce; H = plant height; SDW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; HI = harvest index; SPAD. See text for details.

Hydroponic system[a]	H (cm)	SDW (g pot^{-1})	RDW (g pot^{-1})	TDW (g pot^{-1})	HI	SDW/H (g cm^{-1})	SPAD	MAC_L (UFC g^{-1})	CC_L (UFC g^{-1})
PM + SS	9.50 a	0.66 bc	0.10 b	0.76 c	0.87 a	0.07 c	15.95 a	7.00E+02 n.s.	1.20E+02 n.s.
PM + LD	8.66 b	0.54 c	0.08 b	0.62 c	0.86 a	0.06 c	11.43 b	6.00E+02 n.s.	5.00E+01 n.s.
PD + SS	5.60 d	**0.85 a**	**0.26 a**	**1.11 a**	0.77 b	**0.15 a**	17.63 a	3.00E+02 n.s.	3.50E+01 n.s.
PD + LD	6.33 c	0.72 ab	0.22 a	0.94 b	0.76 b	0.11 b	13.33 b	8.00E+02 n.s.	1.00E+01 n.s.
Average	**7.52**	**0.69**	**0.17**	**0.86**	**0.81**	**0.08**	**14.59**	**6.00E+02**	**5.38E+01**

Table 5. Production, nutrition status and microbial charge of baby lettuce grown on different substrates in the third crop cycle (24 ± 2 °C). Means followed by same letter do not significantly differ at $P<0.05$; n.s. = not significantly different; PM = peat moss; SS = standard solution; LD = liquid digestate; PD = pelletized digestate; MAC_L = mesophilic aerobic charge of lettuce; CC_L = Coliform charge of lettuce; H = plant height; SDW = shoot dry weight; RDW = root dry weight; TDW = total dry weight; HI = harvest index; SPAD. See text for details.

Figures

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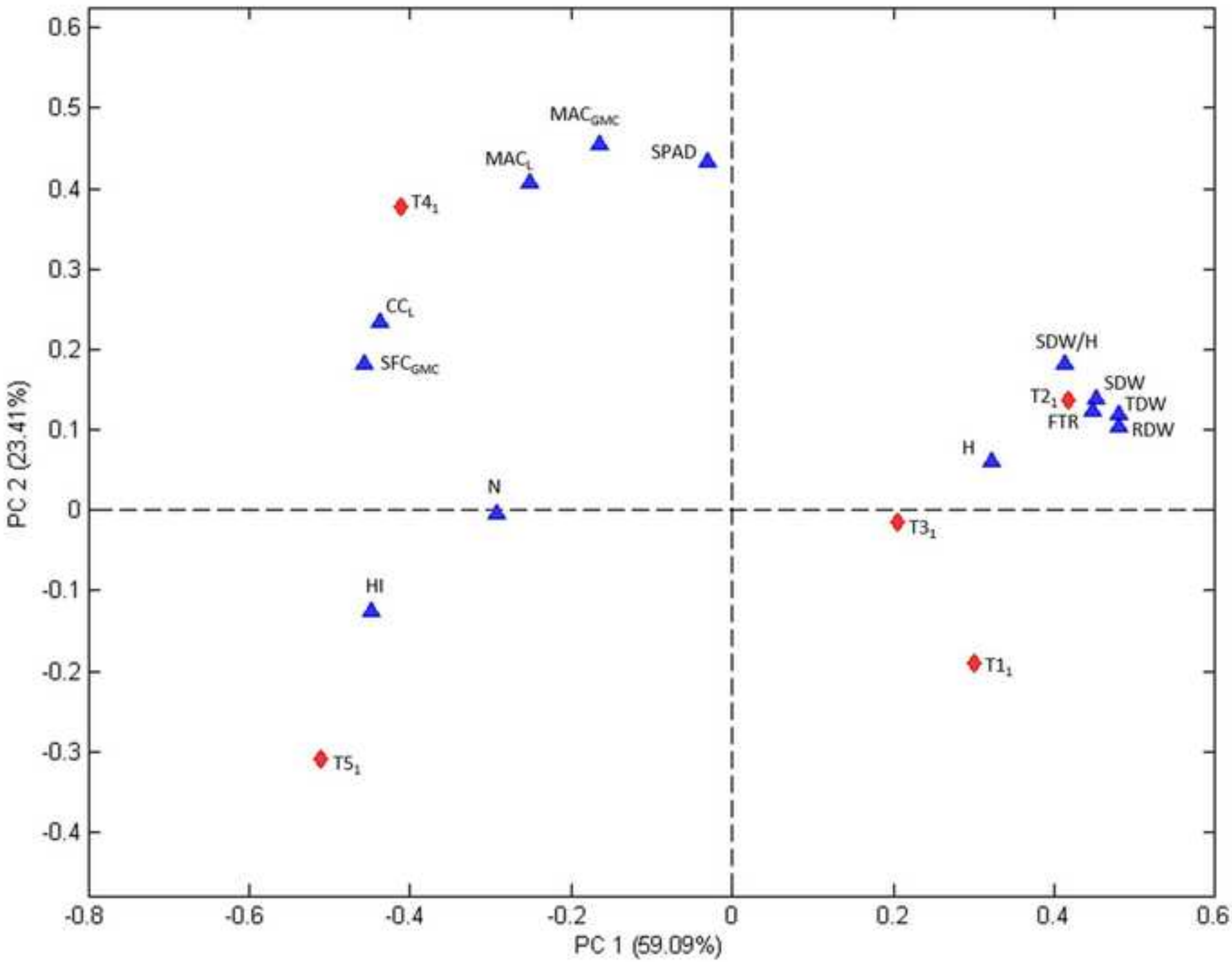

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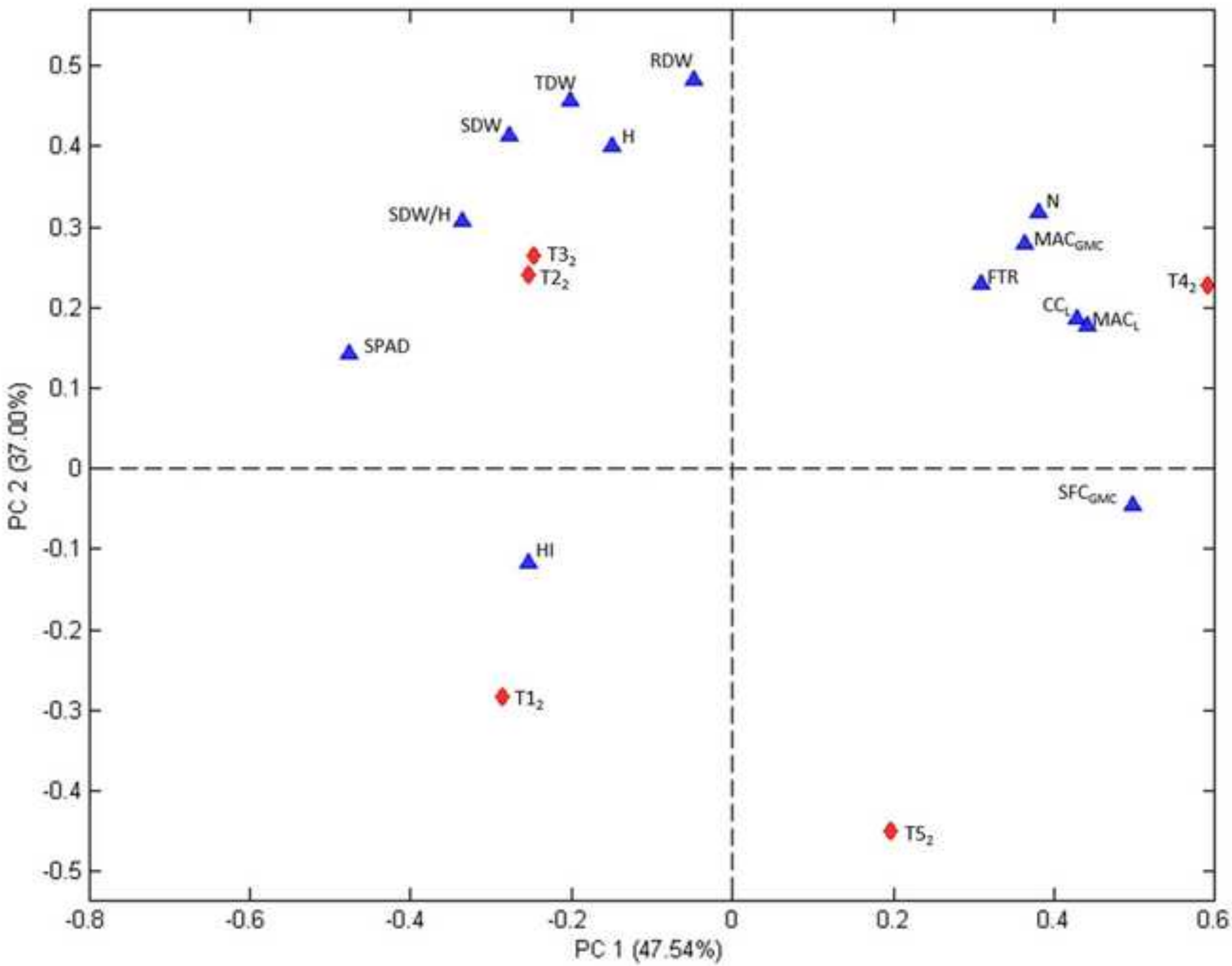

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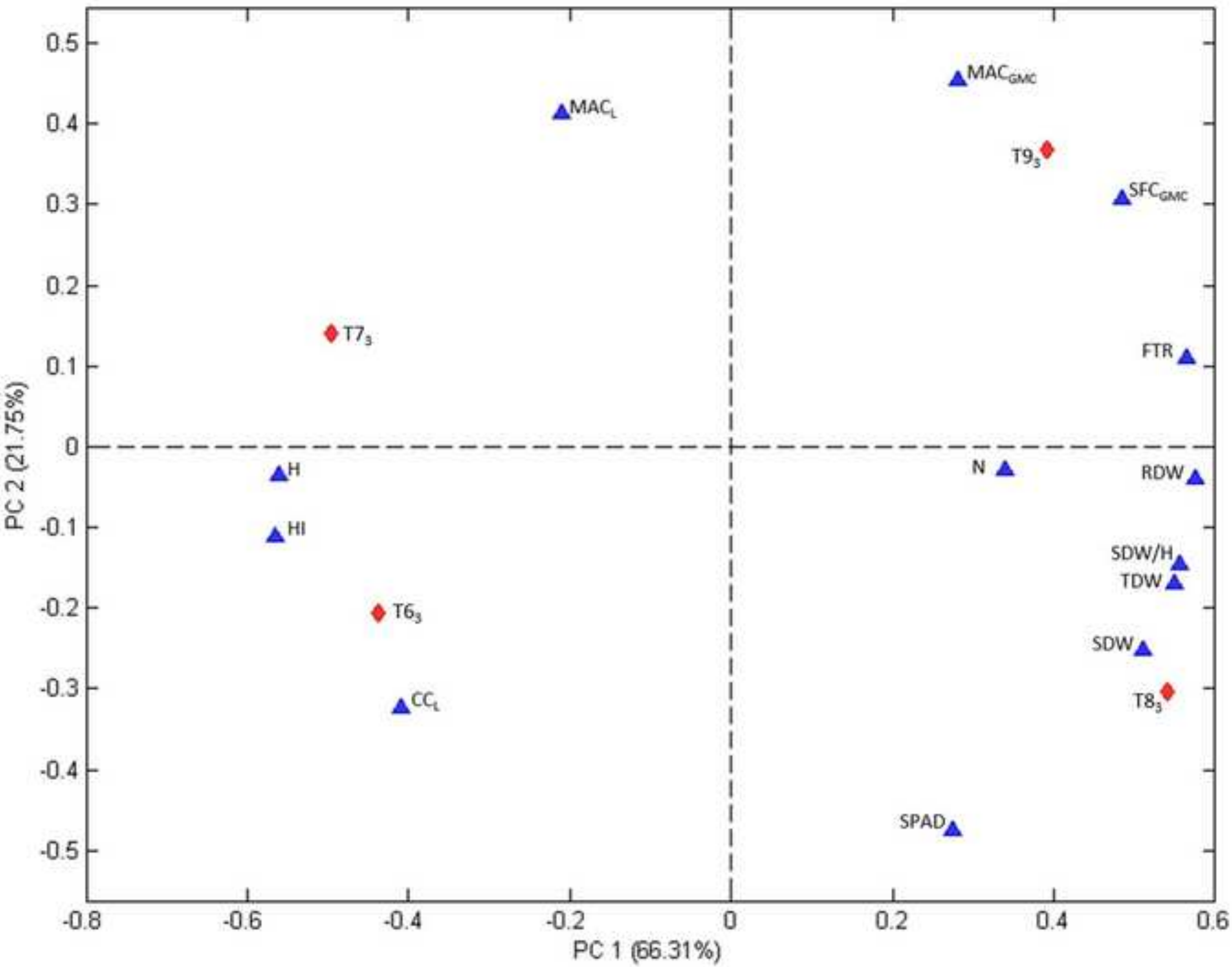

Figures

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Figure 1. Ordination biplot of principal component analysis of the first crop cycle (24 ± 2 °C). Labels in the graph represent the investigated parameters: CC_L = clostridium charge of baby leaf lettuce; SDW= shoot dry weight; RDW= root dry weight; TDW = total dry weight; H= plant height; HI = harvest index; SDW/H = shoot dry weight-height ratio; FTR = biomass fraction of dry weight to root; MAC_{GMC} = mesophilic aerobic charge of the growing media combinations; MA_{CL} = mesophilic aerobic charge of baby leaf lettuce; SFC_{GMC} = spore-forming charge of the growing media combinations; SPAD; N = baby leaf lettuce nitrate content; T1-T5 = the different hydroponic systems: T1 = AG + SS; T2 = AG + LD; T3 = SD + SS; T4 = SD + LD; T5 = S+SS. AG = agriperlite; LD = liquid digestate; S = soil; SD = solid digestate; SS = standard solution. Number 1 following the hydroponic systems investigated indicate the corresponding crop cycle (the first one).

Figure 2. Ordination biplot of principal component analysis of the second crop cycle (27 ± 2 °C). Labels in the graph represent the investigated parameters: CC_L = clostridium charge of baby leaf lettuce; SDW= shoot dry weight; RDW= root dry weight; TDW = total dry weight; H= plant height; HI = harvest index; SDW/H = shoot dry weight-height ratio; FTR = biomass fraction of dry weight to root; MAC_{GMC} = mesophilic aerobic charge of the growing media combinations; MA_{CL} = mesophilic aerobic charge of baby leaf lettuce; SFC_{GMC} = spore-forming charge of the growing media combinations; SPAD; N = baby leaf lettuce nitrate content; T1-T5 = the different hydroponic systems: T1 = AG + SS; T2 = AG + LD; T3 = SD + SS; T4 = SD + LD; T5 = S + SS. AG = agriperlite; LD = liquid digestate; S = soil; SD = solid digestate; SS = standard solution. Number 2 following the hydroponic systems investigated indicate the corresponding crop cycle (the second one).

Figure 3. Ordination biplot of principal component analysis of the third crop cycle (24 ± 2 °C). Labels in the graph represent the investigated parameters: CC_L = clostridium charge of baby leaf lettuce; SDW= shoot dry weight; RDW= root dry weight; TDW = total dry weight; H= plant height; HI = harvest index; SDW/H = shoot dry weight-height ratio; FTR = biomass fraction of dry weight

to root; MAC_{GMC} = mesophilic aerobic charge of the growing media combinations; MA_{CL} = mesophilic aerobic charge of baby leaf lettuce; SFC_{GMC} = spore-forming charge of the growing media combinations; SPAD; N = baby leaf lettuce nitrate content;T6-T9 = the different hydroponic systems: T6 = PM + SS; T7 = PM + LD; T8 = PD + SS; T9 = PD + LD; LD = liquid digestate; PM = peat moss; PD = pelletized digestate; SD = solid digestate; SS = standard solution. Number 3 following the hydroponic systems investigated indicate the corresponding crop cycle (the third one).

Figure 4. The five representative pots of baby leaf lettuce cultivated in the first and second cycles. AG + SS = agriperlite + standard solution; AG + LD = agriperlite + liquid digestate; SD + SS = solid digestate + standard solution; SD + LD = solid digestate + liquid digeste; S + SS = soil + standard solution.

Figure 5. The four representative pots of baby leaf lettuce cultivated in the third cycle. PM + SS = peat moss digestate + standard solution; PM + LD = peat moss + liquid digestate; PD + SS = pelleted digestate + standard solution; PD + LD = pelleted digestate + liquid digestate.

Supplementary Material

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