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Baby-leaf NFT production and water management in aquaponic system

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Index

Abstract

Introduction: As the global demand for seafood is rising rapidly with a growing population consuming larger amounts of fish on their diets, most of the additional demand is now met by aquaculture. Aquaculture has the potential to feed millions of people, but wastes accumulate and will degrade the production system and the landscapes by adding excess nitrogen, phosphorus and organic matter. The incorporation of recirculation systems with fish and plants i of great importance in terms of maximizing the production of plants and recovery nutrients, for exchanging or minimizing water losses, and in terms of environmental impacts due to the accumulation of nutrients for releasing the medium. Aquaponics is one of the few techniques available that can remove at low concentrations dissolved N and P generated via aquaculture. **Purpose:** The main aim was to study the capability to raise a marketable and healthy baby-leaf yield in an AP system using fish water and to investigate a further utilization of the water from the AP system once it becomes limiting for the plant growth (usually depleted of P and K) and the N accumulates in the water. **Methods:** Total nine hydroponic systems were installed side by side in a foliar greenhouse. Three were operated with water from an existing aquaponic (fish water, FW), three with FW, supplemented with nutrients (corrected fish water, CFW), and three with conventional hydroponic solution (HC) as control. The systems were monitored for the pH, EC and nutrient concentrations in water. The NFT channels of all systems were planted with Mizuna (*Brassica rapa* L. spp. *Nipposinica* - M) and rocket salad (*Eruca vesicaria* – R), which were monitored for growth and harvested at the end of experiment for analyses of biomass composition. The experimental set up was repeated for two consecutive growing cycles (24/2-29/3/2017; 30/3-30/4/2017) . **Results:** SPAD and plant height highlighted the inability of FW to meet the nutritional needs of both species. Yield in FW was 0.3 kg m^{-2}, followed with higher in HC (1.59 kg m^{-2}) and CFW (1.51 kg m^{-2}). Water consumption and water quality parameters were significantly influenced by the experimental treatments. The starting N-NO_3 concentration, similar in all systems, decreased by 31.2% in FW, 72.0% in HC and 82.7% in CFW. Higher content of nitrates, sulphates, ammonium, magnesium and calcium in FW corresponded to an abnormal absorption of these nutrients compared to HC and CFW, due to the absence of P and K. **Discussion and conclusion:** FW must be supplemented with macro- and meso-nutrients in order to ensure a suitable production. The use of CFW for short-cycle vegetable cultivation is an effective solution to manage the fish water at the end of an AP cycle, achieving significant production, reducing nitrogen load and thus further reducing the environmental impact of the system. The high production potential of CFW is worth further consideration as it could be linked to the presence of organic compounds with bio-stimulant activity.

Keywords: mizuna, rocket salad, yield, quality, sustainability

Riassunto

Introduzione: Poiché la domanda globale di prodotti ittici sta aumentando rapidamente con una popolazione in crescita che consuma quantità maggiori di pesce, la maggior parte della domanda aggiuntiva viene ora soddisfatta dall'acquacoltura. L'acquacoltura ha il potenziale di nutrire milioni di consumatori, ma i rifiuti si accumulano e degradano il sistema di produzione e i paesaggi a causa di eccesso di azoto, fosforo e sostanza organica. L'incorporazione di sistemi di ricircolo con pesci e piante è di grande importanza in termini di massimizzazione della produzione di piante e nutrienti di recupero, per lo scambio o la riduzione delle perdite d'acqua, e in termini di impatti ambientali dovuti all'accumulo di sostanze nutritive per il rilascio del mezzo. L'acquaponica è una delle poche tecniche disponibili che può rimuovere N e P disciolti in basse concentrazioni, derivanti dall'acquacoltura. **Scopo**: Lo studio rientra in questo contesto e si pone come obiettivo principale quello di studiare la capacità di aumentare la produzione di ortaggi di IV gamma (*baby-leaf vegetables*) commercializzabili e sani in un sistema AP tramite il riciclo dell' acqua di pesce e di indagare su un ulteriore utilizzo del sistema AP una volta che esso diventa limitante per la crescita della pianta (di solito esaurito di P e K) e l'azoto si accumula nell'acqua. **Metodi**: Tre replicati del sistema AP sono stati installati fianco a fianco in una serra fogliare, ciascuno costituito da trattamento con acqua di pesce (FW) messo a confronto con un trattamento di controllo idroponico (HC) e un trattamento di acqua di pesce corretta (CFW). Mizuna (*Brassica rapa* L. spp. *Nipposinica* - M) e rucola (*Eruca vesicaria* - R) sono state utilizzate contemporaneamente per i due cicli di crescita consecutivi (24/2-29/3/2017; 30/3-30/4/2017) in un sistema NFT. **Risultati**: SPAD e altezza della pianta hanno evidenziato l'incapacità di FW di soddisfare i bisogni nutrizionali di entrambe le specie. La produzione in FW è stata di 0,3 kg m^{-2}, seguita da HC (1,59 kg m^{-2}) e CFW (1,51 kg m^{-2}). Il consumo di acqua e i parametri di qualità dell'acqua sono stati significativamente influenzati dai trattamenti sperimentali. La concentrazione iniziale di $N\text{-}NO_3$, simile in tutti i sistemi, è diminuita del 31,2% in FW, del 72,0% in HC e dell'82,7% in CFW. Un contenuto più elevato di nitrati, solfati, ammonio, magnesio e calcio in FW corrisponde a un assorbimento anormale di questi nutrienti rispetto a HC e CFW, a causa dell'assenza di P e K. **Discussione e conclusione**: FW deve essere integrato con macro e meso-nutrienti per garantire una produzione adeguata. L'uso di CFW per la coltivazione di ortaggi a ciclo breve è una soluzione efficace per gestire l'acqua di pesce alla fine di un ciclo AP, ottenendo una produzione significativa, riducendo il carico di azoto e riducendo ulteriormente l'impatto ambientale del sistema. L'alto potenziale di produzione di CFW merita un'ulteriore considerazione in quanto potrebbe essere collegato alla presenza di composti organici con attività di biostimolante.

Parole chiave: mizuna, rucola, produzione, qualità, sostenibilità

1 Introduction

1.1 Aquaculture – a brief general overview

As the global demand for seafood is rapidly rising with a growing population consuming larger amounts of fish on their diets, most of the additional demand is now met by aquaculture, that is the cultivation of freshwater and marine plants and animals (Adler *et al.*, 2000). It is estimated that at least an additional 40 million tonnes of aquatic food will be required by 2030 to maintain the current per capita consummation (FAO, 2013). Moreover, growth in the global supply of fish for human consumption has outpaced population growth in the past five decades, increasing at an average annual rate of 3.2% in the period 1961– 2013, double that of population growth, resulting in increasing average per capita availability (FAO, 2016).

Despite rapid increase in recent decades, growth in global aquaculture production may be slowing (FAO, 2011). Continued growth in aquaculture production is likely to come from intensification of fish, shellfish and algae production, as natural resource limitations and negative environmental impacts are two of the most significant impediments to continued growth (Bostock, 2011).

1.1.1 Problems and solutions of aquaculture

Aquaculture has the potential to feed millions of people, but as with any agricultural enterprise where animals are concentrated, wastes accumulate and will degrade the production system and the landscapes unless handled properly (Buzby and Lin, 2014). Some types of aquaculture production may severely degrade aquatic ecosystems, pose health risks to consumers, reduce incomes and employment in the capture fisheries sector, and diminish food resources for poor populations (Klinger and Naylor, 2012).

Wastewater from aquaculture can pollute streams by adding excess nitrogen, phosphorus, and organic matter (Adler *et al.*, 2000). About 36% of the feed is excreted as a form of organic waste (Brune *et al.*, 2003). Around 75% of the feed nitrogen and phosphorus is unutilized and remain as waste in water (Piedrahita, 2003; Gutierrez-Wing and Malone, 2006). Depending on the species and culture technique, up to 85% of phosphorus, 80–88% of carbon and 52–95% of nitrogen input into a fish culture system may be lost to the environment through feed wastage, fish excretion, fecal production and respiration (Cripps and Kumar, 2003). Moreover, aquaculture wastes are challenging as they are either suspended or dissolved in water (Buzby and Lin, 2014). Thus, the removal of these nutrients from wastewater is an important operation because these compounds play a critical role in eutrophication (Adler *et al.*, 2000).

The solutions to the resource and environmental problems of aquaculture systems include changes to culturing system, feed strategies, and species selection (Klinger and Naylor, 2012). Considering the culture systems, possible improvements aim to (*a*) reduce land and freshwater use by recycling water, intensifying production, or moving into the ocean; and (*b*) reduce nutrient and chemical pollution by treating, converting, or diluting waste. Not all of these solutions can be considered to be equally sustainable.

Recently, the incorporation of recirculation systems with fish and plants has become an interesting model for scientists, for the aquaculture industry, and for the environmentalists. These recirculating aquaponic systems are of great importance in terms of maximizing the production of plants and nutrient recovery, for minimizing water losses, and in terms of environmental impacts due to the accumulation of nutrients from releasing the medium (Endut *et al*., 2009). Although the practices of fish farming and hydroponics have been traced to ancient times, the combination of the two is quite new (Al-Hafedh *et al*., 2008). Aquaponics is one of the few techniques available that can remove dissolved N and P at low concentrations generated via aquaculture (Buzby and Lin, 2014). Thus, it is a promising solution for the negative environmental impacts typically associated with intensive fish and crop production (Maucieri *et al*., 2017).

1.2 Aquaponic system as a sustainable solution

Aquaponics is an integrated food production system that links recirculating aquaculture with hydroponic vegetable, flower, and/or herb production (Diver, 2006). An aquaponic system can benefit the aquaculture operation by improving the quality of recirculated water (Rakocy *et al*., 2006) or by reducing costs associated with treating effluent from flow-through raceways (Buzby *et al*., 2016). The hydroponic operation benefits through the reduction of fertilizers inputs and labor or facilities needed to maintain adequate moisture levels. The linking of fish culture with plant culture allows both operations to reduce inputs and has thus the potential to make the enterprise more sustainable (Tyson *et al*., 2011).

The essential elements of an aquaponic system are a fish rearing tank, a suspended solid removal component, a biofilter, a hydroponic component, and a sump (Rakocy and Hargreaves, 1993). It is a very productive and ecologically sound food production system, where nutrients generated by the fish, either by direct excretion or microbial breakdown of organic wastes, are absorbed by plants cultured in hydroponic. As the aquaculture effluent flows through the hydroponic component of the recirculating system, fish waste metabolites are removed by nitrification and direct uptake by plants, thereby treating the water, which flows back to the fish rearing component for reuse (Endut *et al*., 2009).

Different types of hydroponic media have been used for growing crops in freshwater aquaponic systems, including gravel bed ebb and flow systems, aeroponics, nutrient film technique (NFT), rock wool culture, and sand beds (Gonzales, 2002). According to Rakocy (1994), raft hydroponics, which consists of floating sheets of polystyrene for plant support, can provide sufficient biofiltration if the plant production area is sized properly. Therefore, the hydroponic component performs as a biofilter, purifies effluents, reuses water , and eliminates partly or wholly the need for a separate biofilter.

Typical aquaponic systems culture fish that require warm temperatures (25–27 °C) such as tilapia (Al-Hafedh *et al*., 2008; Rakocy *et al*., 2006; Graber and Junge, 2008; Castillo-Castellanos *et al*., 2015), Murray cod (Lennard and Leonard, 2006), and African catfish (Endut *et al*., 2009). A wide variety of crops have been grown in aquaponics including basil (Adler *et al*., 2003), lettuce and tomato (Rakocy *et al*., 1993), cucumber and herbs (Savidov *et al*., 2007), aubergine (Graber and Junge 2009).

One of the main technical obstacles to expanding aquaponic production is the difficulty of creating a system that offers optimal growth environments for fish, nitrifying bacteria, and plants in terms of temperature and pH for optimal crop development (Tyson *et al*., 2011). In order to better understand the aquaponic system, hydroponics and its role in the treatment of aquaculture wastewater are described more in detailed.

1.3 Hydroponics

Predominant thinking regarding the use of food crops to treat aquaculture effluents has been that plants cannot remove nutrients in water to low levels without a reduction in productivity and quality. Because greenhouse space is expensive, maintaining maximum productivity is critical to sustain a profitable cultivation (Adler *et al*., 2000). However, the reuse of treated wastewater for crop irrigation has been widely recommended, especially in those areas with problems of water shortage (Pereira *et al*., 2002; Qadir *et al*., 2007).

Integrating fish farming with plants has been tested in hydroponic systems where effluent was used as nutrient solution. These systems were designed for lettuce, tomatoes and other crops

(Parker *et al*., 1990; McMurtry *et al*., 1993; Rakocy *et al*., 1993; Ghaly and Snow, 2008). Previous studies showed different types of hydroponic systems that have been used for growing crops in aquaponic systems (Table 1).

Hydroponic plants can efficiently absorb dissolved compounds in wastewater as nutrients for plant growth (Rakocy *et al*., 1989; Adler *et al*., 2003; Dushenkov *et al*., 1995). However, physical and chemical properties of the effluent (temperature, nutrient concentration, etc.) are dependent on the type and quality of fish being grown and may not be suitable for all crops (Buzby *et al*., 2016).

Table 1 Different types of hydroponic systems in aquaponic systems.

Type of hydroponic	Fish	Crop	Reference
Drip irrigation	Palaemonid shrimp	Lettuce and watercress	Castellani *et al*., 2009
Floating raft	Murray cod	Green Oak lettuce	Lennard and Leonard, 2006
Floating raft	Tilapia	Basil	Rakocy *et al*., 2004
Floating raft	Tilapia	Leaf lettuce	Al-Hafedh *et al*., 2008
Gravel bed	Murray cod	Green Oak lettuce	Lennard and Leonard, 2006
Gravel bed	African catfish	Spinach, Green mustard	Endut *et al*., 2011
Gravel bed	Tilapia	Aubergine	Graber and Junge, 2009
Gravel bed	Eurasian perch	Tomato, cucumber	Graber and Junge, 2009
NFT*	Murray cod	Green Oak lettuce	Lennard and Leonard, 2006
NFT*	Rainbow trout	Lettuce and basil	Adler, 1998

**nutrient film technique*

1.3.1 Plant uptake and fish output – nutrient removal

Plants require 17 essential nutrient elements (Table 2) without which they are unable to complete a normal life cycle (Epstein and Bloom, 2005; Trejo-Tellez and Gomez-Merino, 2012; Bittsanszky *et al*., 2016). In contrast to plants, fish nutrition is very different. Typically, fish feed contains an energy source (carbohydrates and/or lipids), essential amino acids, vitamins, and altogether 21 different macro- and micro-minerals (Table 3) (Davis, 2015). Nitrogen is associated with protein, which is the major source of nitrogen for fish cultivation, representing 50-70% of fish production costs (Valente *et al*., 2011). Only 30% of nitrogen added through feed is removed through fish harvest in an intensive fish farming (Brune *et al*., 2003), while the remaining dissolved nitrogen is released in the environment. It is estimated that between 30 and 65% of feed N in form of ammonia and up to 40% of feed P is excreted into the surrounding environment (Schneider *et al.*, 2005).

Table 2 Essential elementary nutrient requirements of the three basic compartments of an aquaponic system (Bittsanszky *et al.*, 2016). [a] (Bittsanszky *et al.*, 2016); [b] (Epstein and Bloom, 2005) (Trejo-Téllez and Gomez-Merino, 2012); [c] (Kantartzi *et al.*, 2006); M: macroelements; μ: microelements; -: not present.

Aquaponic compartment	Element																							
	Boron	Calcium	Carbon	Chlorine	Cobalt	Copper	Chromium	Fluorine	Hydrogen	Iodine	Iron	Magnesium	Manganese	Molybdenum	Nitrogen	Nickel	Oxygen	Phosphorous	Potassium	Sodium	Sulfur	Zinc	Selenium	Silicone
Fish [a]	-	M	M	M	μ	μ	μ	μ	M	μ	μ	M	μ	μ	M		M	M	M	M	M	μ	μ	-
Plant [b]	μ	M	M	M	μ	μ	-	-	M	-	μ	M	μ	μ	M	μ	M	M	M	-	M	μ	μ	μ
Biofilter [c]	-	M	M	μ	μ	μ	-	-	M	-	μ	μ	μ	μ	M		M	M	M	μ	M	μ	μ	-

Buzby and Lin (2014) reported a nitrate and phosphate removal from the aquaculture effluent by *Nasturtium* reducing the concentration from 0.30 to 0.11 mg L^{-1} and from 0.14 to 0.05 mg L^{-1}, respectively, whereas lettuce was ineffective at their removal at these concentrations. Endut *et al.* (2016) studied a system of African catfish (*Clarias gariepinus*) and water spinach and mustard greens, and has shown that using crop vegetables can be one of the ways to mitigate the toxicity effect of ammonia. He observed significant decreases in nitrite-N, nitrate-N and orthophosphate in aquaculture effluent . Ghaly *et al.* (2005) investigated the use of hydroponically grown barley for treatment of wastewater from recirculating aquaculture system stocked with tilapia and reported NO_2-N reductions of 98.1% after 21 days of growth. Adler *et al*. (2000) reported removing P from an aquaculture effluent with hydroponic production of lettuce and basil using Nutrient Film Technique (NFT).

Factors regulating plant nutrient uptake include light intensity, root zone temperature, air temperature, nutrient availability, growth stage and growth rate (Buzby and Lin, 2014). Aquaculture effluent can provide most of the nutrients required by plants if the optimum ratio between daily feed input and plant growing area is maintained (Rakocy *et al*., 2004). As plants grow and biomass increases, nutrient removal from the effluent stream will improve. Therefore, to be most effective, the aquaponic system must be sized correctly with the optimum balance between nutrient production from fish culture and nutrient uptake by the plant component (Buzby and Lin, 2014). Waste generation by fish is directly related to the quantity and quality of feed applied (Lam *et al*., 2015).

When the system is in balance, high production of fish and plant crops at high stocking densities can be obtained without the use of chemical fertilizers, herbicides, or pesticides (Nelson, 2008). Diver (2006), Rakocy *et al*. (2006) and Seawright *et al*. (1998) reported that with appropriate fish stocking rates the levels of NO_3, P, B, and Cu in aquaculture effluents are sufficient for good plant growth, while levels of K, Ca, and Fe are generally insufficient for maximum plant growth. The question thus arises whether it is necessary and effective to add nutrients to aquaponic systems. In such cases, HydroBuddy is available as free software (Fernandez, 2016) to calculate the amount of required mineral nutrient supplements.

Bittsanszky *et al.* (2016) suggested that supplying the aquaponic system with additional organic nutrients, instead of mineral, could be a positive effect on both plants and microbial population. Special care has to be taken through the continuous monitoring of the chemical composition of the recirculating water for adequate concentrations and ratios of nutrients and of the potentially toxic component, ammonium. However, a perfect formulation of nutritional requirements for a particular crop does not exist, as the nutritional requirements might vary with variety, life cycle stage, day length, and weather conditions (Bittsanszky *et al*., 2016).

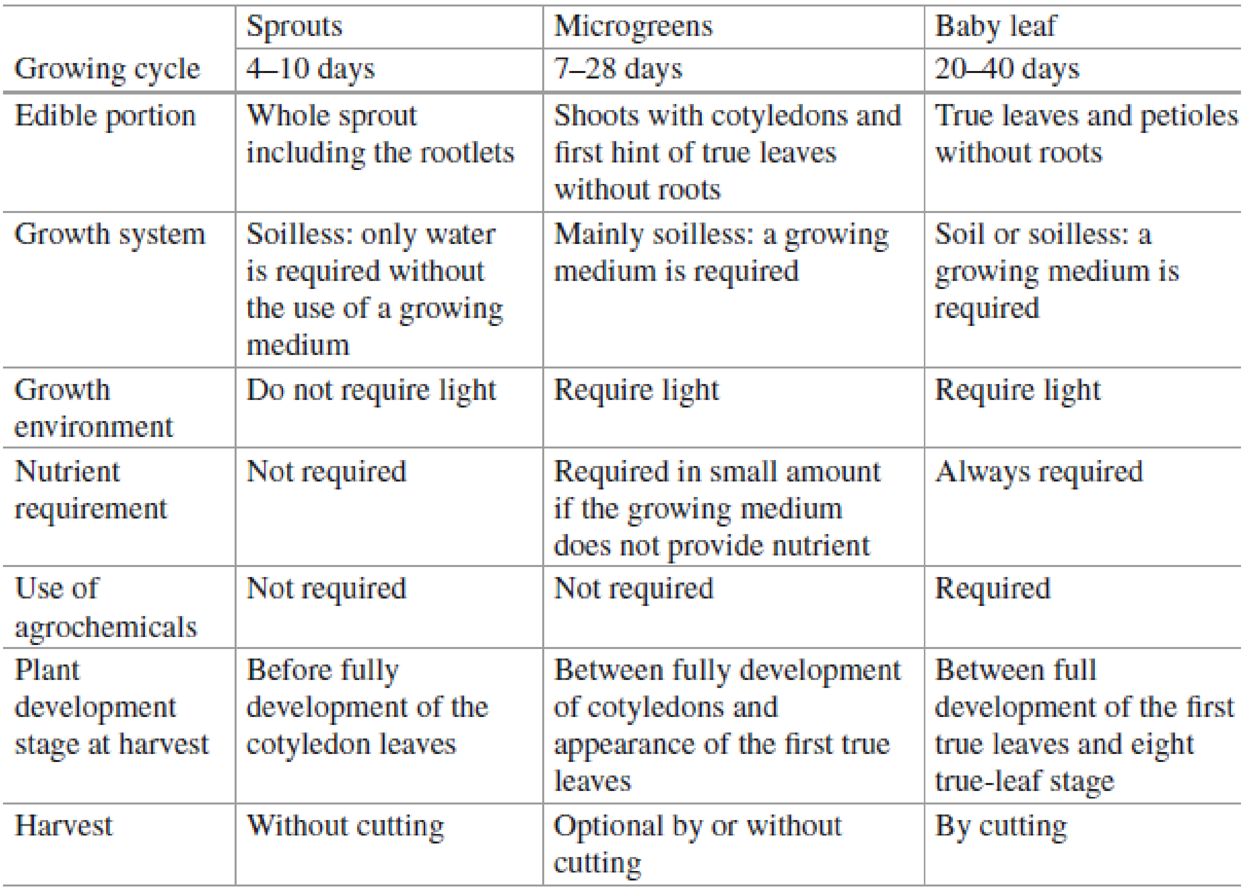
1.4 AP systems and possible horticultural uses: sprouts, microgreens, baby-leaf production

In recent years, people have developed a substantial interest for the consumption of fruits and vegetables characterized by a high content of bioactive substances. It is known that these are beneficial because besides providing essential nutrients for the human body, they have positive effects on human health (Galaverna *et al*., 2008). Sprouts, microgreens and ready-to-eat “baby leaf” vegetables constitute a growing market segment within the sector of the vegetable products (Di Gioia *et al*., 2017).

According to the Commission Implementing Regulation (EU) 208/2013, the term “sprouts” indicates “the product obtained from the germination of seeds and their development in water or another medium, harvested before the development of true leaves and which is intended to be eaten whole, including the seed" (European Union, 2013). According to the Commission Regulation (EU) 752/2014, the term "baby leaf" indicates "the young leaves and petioles of any crops (including *Brassica*) harvested up to 8 true leaf stage. Instead, "microgreens" is a marketing term used to describe a category of product that has no legal definition, yet (Treadwell *et al.*, 2010). To better understand the distinctive traits of these three types of product, Table 3 reported the main differences among sprouts, microgreens and baby leaf vegetables.

Different species have been investigated in AP systems, mainly lettuce, water spinach, basil and tomato as listed above, but very few studies have been carried out to evaluate sprouts, microgreen and baby-leaf production. It is interesting though to have closer a look into these minimally processed vegetables and their nutritional traits and production, as the growth of sprouts, microgreens and baby-leaf is highly promoted in a future aquaponics system.

Table 3 Differences among sprouts, microgreens and "baby leaf" vegetables (Di Gioia *et al.*, 2017). Translated from Di Gioia *et al.* (2015a).



	Sprouts	Microgreens	Baby leaf
Growing cycle	4–10 days	7–28 days	20–40 days
Edible portion	Whole sprout including the rootlets	Shoots with cotyledons and first hint of true leaves without roots	True leaves and petioles without roots
Growth system	Soilless: only water is required without the use of a growing medium	Mainly soilless: a growing medium is required	Soil or soilless: a growing medium is required
Growth environment	Do not require light	Require light	Require light
Nutrient requirement	Not required	Required in small amount if the growing medium does not provide nutrient	Always required
Use of agrochemicals	Not required	Not required	Required
Plant development stage at harvest	Before fully development of the cotyledon leaves	Between fully development of cotyledons and appearance of the first true leaves	Between full development of the first true leaves and eight true-leaf stage
Harvest	Without cutting	Optional by or without cutting	By cutting

1.4.1 Sprouts

Sprouts are what is called “biogenic food”, which means they are a living food (Helweg, 2011), characterized by high levels of vitamins, minerals, protein and/or other healthy compounds. Crop groups used for sprouting include legumes, cereals, pseudo-cereals, as well as vegetables (i.e. broccoli, cabbage, carrot, celery, clover, fennel, kale, leek, lettuce, mustard, parsley, radish, arugula, snow and garden peas, spinach, spring onion, turnip, watercress) (Ebert, 2012).

Sprouts are commonly considered highly nutritious and are sometimes called “miracle food” (Meyerowitz, 2010). In fact, they are outstanding source of proteins, vitamins and minerals and have high content of beneficial compounds such as glucosinolates, phenolic and selenium-containing components in the *Brassica* species or isoflavones in the soybean (Meyerowitz, 2010). Therefore, sprouts can be considered as new foodstuffs rich in nutrients and phytonutrients beneficial for human health. Sprouts have been used to reduce inflammation, cure rheumatism and produce a laxative effect (Helweg, 2011).

Given their short growing cycle, sprouts are usually grown in the dark, without a growing medium and without external inputs such as fertilizers and agrochemicals, and are characterized by a very short cycle: after 3–5 days, the sprouts may be grown 5–8 cm in length and may be ready for consumption. The sprout production takes a few days and can either be done at home manually, or as a semi-automated process, or industrially on a large scale (Di Gioia *et al*., 2017).

1.4.2 Microgreens

Microgreens are an emerging class of specialty fresh produce, which have gained increasing popularity with chefs and consumers in recent years (Xiao *et al*., 2016). They are young seedlings of vegetables and herbs, harvested when cotyledons are fully developed and the first pair of true leaves are emerging or partially expanded (Figure 1). Mostly exploited are species belonging to the families *Brassicaceae*, *Asteraceae*, *Chenopodiaceae*, *Lamiaceae*, *Apiaceae*, *Amarillydaceae*, *Amaranthceae* and *Cucurbitaceae* (Kyriacou *et al*., 2016).

Figure 1 Ready to harvest microgreens of (A) red beet (*Beta vulgaris* L.), (B) cilantro (*Coriandrum sativum* L.), (C) radish (*Raphanus sativus* L.), and (D) brassica raab (*Brassica rapa* L., Broccoletto group), grown in trays on a peat mix (A, B and C), or in hydroponic growing channels on a fibrous mat (D). Photos courtesy of Francesco Di Gioia. (Kyriacou *et al*., 2016)

Previous studies have shown that microgreens are good source of vitamins and other phytonutrients, such as carotenoids and polyphenols (Sun *et al*., 2013; Xiao *et al*., 2012). Recent reports demonstrated that microgreens contain higher amounts of phytonutrients (ascorbic acid, *β*-carotene, *α*-tocopherol and phylloquinone) and minerals (Ca, Mg, Fe, Mn, Zn, Se and Mo) and lower nitrate content than their mature leaf counterparts (Pinto *et al*., 2015; Xiao *et al.*, 2012). The consumption of microgreens could be a health-promoting strategy to meet the requirement of element dietary reference intakes, particularly for children (Xiao *et al.*, 2016).

Microgreens are produced in a variety of environments (open air, protected environment, indoor) and growing systems (soil, soilless), depending on the scale of production (Kyriacou *et al*., 2016). The commercial production of microgreens can be performed in "containers" constituted by plastic trays, on "channels" or on benches (made of plastic, aluminium, galvanized iron, wood) of different sizes, or in "floating system" on polystyrene plug trays of different sizes float on the nutrient solution contained in a basin or a bench (Di Gioia *et al*., 2017).

One of the most critical aspects involved in the production of microgreens is the selection of the growing media as it plays a fundamental role in determining the productivity and quality of microgreens, as well as the sustainability of the production process (Di Gioia *et al*., 2015b). It is also of fundamental importance that the growing media is not microbiologically contaminated.

1.4.3 Baby-leaf

In today's modern society, a busy lifestyle does not allow much time to purchase, process and prepare the meals. Thus, the demand for nutritionally rich convenient food products is gradually increasing in both developed and developing countries. Minimally processed baby-leaf vegetables (BLVs), sold as the ready-to-eat salad, is one such convenient food, rich in vitamins, minerals, phenolic compounds and dietary fibers (Saini *et al*., 2017), shown to have higher nutrient content compared to commonly used vegetables. However, the biosynthesis, composition and concentration of health-promoting compounds vary widely among leafy vegetables and carry the influence of genetic and environmental factors, growing conditions, harvest practices, postharvest handling conditions (Rouphael *et al*. 2012) and maturity stage (Di Gioia *et al*., 2017).

Most of the baby-leaf vegetables used in commercial production belongs to family *Asteraceae* and *Brassicaceae* (Saini *et al*., 2016). Different types of lettuce belongs to family *Asteraceae*, whereas, kale (*Brassica oleracea* var *acephala*), rocket salad (*Eruca sativa*, syn. *E. vesicaria* subsp. *sativa* Mill.) and wild rocket (*Diplotaxis tenuifolia* (L.) DC) are the most common baby-leaf vegetables of *Brassicaceae* family. Today baby leafs are mainly grown under protected environment, where it is possible to obtain a clean product as well as good management of the pest control and fertilization (Di Gioia *et al*., 2017). Having a longer growth cycle, baby leaves require the use of fertilizers and agrochemicals and are harvested after the development of true leaves at a very early stage of maturation and prepared with minimal processing methods such as cutting, washing, rinsing and packaging with polymeric films under chilling temperatures.

A valid alternative is to produce baby leaf vegetables in hydroponic systems that allow a direct control of the nutrient supply. An instant modification of the composition and concentration of the nutrient solution could fix qualitative standard as regards the dry matter (or crunchiness), nitrate content or other organoleptic and aesthetic features of products (Santamaria *et al*., 2001). Among hydroponic methods to produce baby leaf vegetables, the floating system is the easiest and cheapest because of its low installation and manpower costs. This system shows high water and fertilizer efficiency and a very low environmental impact (Gonnella *et al*., 2002).

2 Purpose

The purpose of this experiment was to evaluate the efficiency of the AP system for short-cycle baby-leaf production in a NFT growing system. The main aim was to study the capability to raise a marketable and healthy baby-leaf yield in an AP system using fish water and to investigate a further utilization of the AP system water once it becomes limiting for the plant growth (usually depleted of P and K), and the N accumulated in the water. The second objective was to compare the water quality parameters, the crop yield characteristics and the assimilation of nutrients from the fish water (FW) with the same water supplemented with macro-nutrients (CFW), and with a hydroponic control (HC).

The information gained will enable better maximization of plant production, a complete re-use of nitrogen and a reduction of the environmental impact of the system.

HYPOTESES:

1. the final production of baby-leaf vegetables differ among the three treatments (HC, CFW and FW);
2. the water taken from the operating aquaponic system (fish water, FW) is enriched with N and depleted of P and K, therefore increasing the mismatch between the nutrients provided by the fish compartment and the requirements of the plants. FW itself is not able to provide a balanced mix of nutrients for the plants and, thus, it is not possible to obtain a healthy and marketable crop yield;
3. pH and EC and nitric nitrogen parameters are expect to increase and decrease, respectively, over the growing cycle.

3 Materials and Methods

The experiment consisted on two identical growing cycles, from 14th February 2017 to 29th March 2017 and from 30th March 2017 to 30th April 2017, respectively.

Nine mecocosm AP systems were installed side by side in a foliar greenhouse at the Campus Grüental of Zurich University of Applied Sciences (ZHAW) in Wädenswil, Switzerland. The area of the greenhouse was 270 m^2 and the volume was 1500 m^3 (17.0 m x 16.0 m x 5.5 m) in total (Figure 2).

Figure 2 AP system at the foliar greenhouse at Campus Grüental, Wädenswil (Foto: Nicoletto, C.)

3.1 Experimental setup

The AP system consisted of NFT channels, a water holding tank (275 L), a pump and pipelines as seen in Figure 3. Each treatment block was composed by four 2.5 m long parallel channels holding 1.5 m NFT (Nutrient Film Technique) and two narrow tubes for irrigation.

Pipelines were installed to connect each component to the system for the purpose of water recirculation. Time regulated pump (Oase, Aquarius Universal ECO 4000) was pumping water to the system three times a day for one hour each time (first irrigation from 10 am to 11 am, second irrigation from 12 pm to 1 pm, third irrigation from 2 pm to 3 pm).

The water (1.65 m^3) from an already existing recirculating AP system (150 Pangasius fish with an average weight of 300 g each in a tank of 3 m^3 and 7.6 m^2 planted area with a complete AP water volume of 7 m^3) was diverted to operate the mesocosm AP systems. This so called "fish water" (FW), which contained almost exclusively nitric nitrogen, was compared with 1) the same water supplemented with P and K plus meso- and micro nutrients (CFW); 2) with a hydroponic control characterized by the same content of nitric nitrogen present in fish water and added with the same nutrients of correct fish water (HC).

To ensure a constant water level (275 L) in the fish tank, fresh tap water was pumped daily into the system, recording the litres added. The heating element (Newa Therm VTX 300 W) in the water of each holding tank ensured constant water temperature (28°C). Nutrients present in the water effluent were absorbed by plants in a grow bed and the water was recirculated between tank and grow bed in aquaponics; no water exchange was conducted during the study period except for replenishing evapotranspiration.

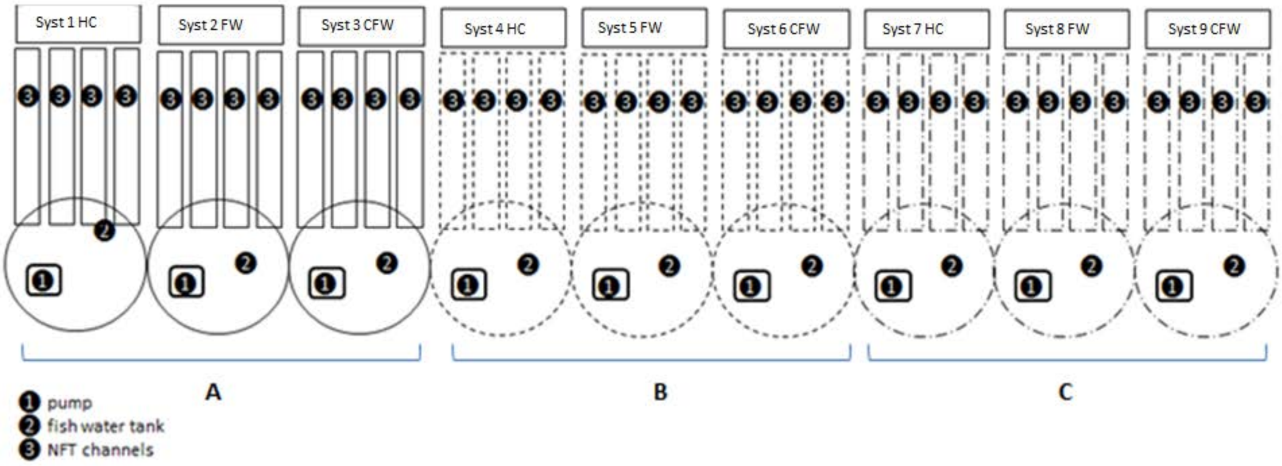


Figure 3 Plan of the AP system with three replicates (A, B, and C) at the Zurich University of Applied Sciences in Wädenswil.

Two leafy plant species, mizuna (*Brassica rapa* L. spp. *Nipposinica* - M) and rocket salad (*Eruca vesicaria* - R), were planted for the growing cycles.

The NFT channels were used for high density sowing (3000 plants m^{-2}). Seeds were set to germinate on the NFT channel as a hydroponic system and kept moist with tap water only. After the germination period, healthy plants seedlings were added to the growing bed and being supplied with three different water qualities for the experimental period.

3.2 Determination of nutrient supplementation for the Hydroponic and Corrected Fish Water part of the system

Further nutrient supplementation, such as phosphorus, potassium and meso- and micro nutrients, was added to the CFW in order to compensate the lack of nutrients in the FW, essential for the plants. As regards to the HC treatment, a balanced hydroponic nutrient solution that contains the same content of nitric nitrogen present in fish water and the same nutrients of correct fish water was applied.

The nutrient solution was prepared according to the recipe of Resh (2012) for leafy vegetables and Hydrobuddy free software (Fernandez, 2013) was used to calculate the amount of each nutrient supplementation needed to reach target values for the Hydroponic and Correct Fish Water part of the system. The water characteristics are summarized in Table 4.

Table 4 Target values Chemical composition of the nutrient solutions according to the recipe of Resh (2012) for leafy vegetables and Hydrobuddy free software.

	$N\text{-}NH_4$	$N\text{-}NO_2$	$N\text{-}NO_3$	K	$P\text{-}PO_4^{3}$	Ca	SO_4^{2-}	Mg	pH	EC
Water					**(mg L^{-1})**					**(µS/cm)**
HC	0	0	65	120	25	66	70	20	8.78	1658
FW	0.075	0.023	63.5	0.078	1.55	66	82.8	21	8.43	893
CFW	0.075	0.023	63.5	120	25	66	70	20	8.16	1679

3.3 Water sampling and analytical methods

Electrical conductivity (EC) and pH were measured *in situ* using a handheld multi-electrode meter HACH HQ40d Portable Multi-Parameter Meter. Water samples were taken in chemically clean 50 mL plastic tubes from each water holding tank three times a week for chemical analysis and were analysed immediately for nitrate-nitrogen (NO_3-N) concentration using DR 3800 VIS Spectrophotometer (HACH Lange) and HACH Lange LCK tests (LCK 339). Ionic chromatography analysis was conducted to measure anion (nitrate, phosphate and sulphate) and cation (kalium, calcium, magnesium, ammonium) concentrations using ICS-900 system (Dionex Corp.,Milan).

3.3.1 Quantitative Determination of Anions and Cations by Ion Chromatography (IC)

IC was performed using an ICS-900 system (Dionex Corp.,Milan). Chromeleon 6.5 Chromatography Management software was used for system control and data processing. A Dionex IonPac AS23 analytical column (4 x 250 mm) and guard column (4 x 50 mm) were used for anion separation, whereas a Dionex IonPac CS12A analytical column (4 x 250 mm) and guard column (4 x 50 mm) were used for cation separation. The eluent consisted of 4.5 mmol L^{-1} sodium carbonate and 0.8 mmol L^{-1} sodium bicarbonate at a flow rate of 1 mL min^{-1} for anions and of 20 mmol L^{-1} methanesulfonic acid for cations at the same flow rate. For the calibration, Dionex solutions containing seven anions at different concentrations and five cations were taken as standards and the calibration curves were generated with concentrations ranging respectively from 0.4 to 20 mg L^{-1} and 0.5 to 50 mg L^{-1}.

3.3.2 Hach Lange LCK tests – method

Three times per week, the levels of ammonium nitrogen ($NH4^+$–N) and nitrite nitrogen (NO_2 $^-$–N) were determined photometrically (cuvette tests, Hach Lange GmbH, Germany).

3.4 Plant sampling and qualitative analyses

The plant height and chlorophyll content were measured from randomly chosen samples (15 samples for every NFT channel) of mizuna and rocket salad three times a week (handheld meter Konica Minolta Chlorophyll Meter SPAD-502).

Samples of mizuna and rocket salad plants at microgreen and baby-leaf stages of both cycles were dried in order to compare the fresh and dry weight of the aerial part and roots (only for microgreen stage).

At the end of both cycles, all remaining samples of mizuna and rocket salad were transported to the laboratories of the University of Padua, where vitamin C (ISO 6557), antioxidant content (FRAP method), total phenols content (Folin-Ciocalteau method), cations and anions (UNI EN12014-2) was carried out.

3.4.1 Determination of fresh and dry matter content

3.4.1.1 Microgreen stage

Starting with one treatment at a time, an average of 30 samples of mizuna and rocket salad (15+15) of each case were carefully uprooted manually from the NFT channel, placed between wet paper to keep their freshness, and returned immediately to the laboratory. Fresh aerial and radical parts of each species were separately weighted (roots were first cleaned of the remaining substrate) on Sartorius CPA64 analytical balance (64 g x 0.1 mg). Samples were then oven-dried at 65°C for at least 24 hours, after which their dry weights were recorded as described above.

3.4.1.2 Baby-leaf stage

The determination of fresh and dry content of baby-leaf stage affected only the aerial part of the two species, as it was impossible to remove the entire root system due to its significant growth. Samples were removed from the NFT channel using garden scissors. The whole process was analog to the one described above for the microgreen stage.

3.4.2 Extraction and Analysis of Phenols by the Folin–Ciocalteau (FC) method

Plant tissues (0.2 g) were homogenized in methanol (20 mL) with an Ultra Turrax T25, until reaching uniform consistency, at 13,500 rpm. Samples were filtered (filter paper, 589 Schleicher) and appropriate aliquots of extracts were assayed by the Folin–Ciocalteau (FC) assay for total phenol content. For the HPLC analysis, extracts were further filtered through cellulose acetate syringe filters (0.45 µm). For each sample, triplicate extractions and analyses were carried out. Results are reported on a dry matter basis.

The content of total phenols was determined by the FC assay (Singleton *et al*., 1999), with gallic acid as the calibration standard, using a Shimadzu UV-1800 spectrophotometer (Columbia, MD, USA). The FC assay was carried out by pipetting 200 µL of plant tissue extract into a 10 mL PP tube. This was followed by the addition of 1 mL FC reagent (Labochimica s.r.l.). The mixture was vortexed for 20–30 s and 800 µL of filtered 20% sodium carbonate solution was added 8 minutes after the addition of the FC reagent. This was recorded as time zero; the mixture was then vortexed for 20–30 s. After 2 h at room temperature, the absorbance of the colored reaction product was measured at 765 nm. Total phenol content in the extracts was calculated from a standard calibration curve, built with different concentrations of gallic acid, ranging from 0 to 400 µg mL^{-1} (correlation coefficient: R^2 = 0.9988). Results were expressed as milligrams of gallic acid equivalent per kg (mg GAE kg^{-1}) of dry matter.

Phenolic acids were separated and quantified by an HPLC diode array detection using a Jasco X-LC system, consisting of a model PU-2080 pump, a multi-wavelength detector (model MD-2015), autosampler (model AS-2055) and column oven (model CO-2060). ChromNAV Chromatography Data System software was used for the analysis of results. The separation of phenolic acids was achieved on a Tracer Extrasil OSD2 column (5 µm, 250 x 4.6 mm), operating at 35°C, at a flow rate of 1 mL min^{-1}. The mobile phase consisted of two solvents: 0.1% formic acid (A) and methanol (B). Gradient elution was as follows: 0–100% B over 50 min and held at 100% B for an additional 10 min to clean up the column. Two wavelengths (310 and 325 nm), were used to detect eluent composition. HPLC analysis at 325 nm was used for the quantification of chlorogenic acid, caffeic acid, and ferulic acid. Quantification of p-coumaric acid was performed at 310 nm. Phenolic acids were quantified following a calibration method. Four standards ranging from 0.3 to 30 mg L^{-1} of chlorogenic acid hemihydrate, p-coumaric acid, caffeic acid, and ferulic acid were used.

3.4.3 Determination of Antioxidant Activity by Ferric Reducing Antioxidant Power (FRAP)

The assay was based on the methodology of Benzie and Strain (Benzie and Strain, 1996). The FRAP reagent was prepared fresh so that it contained 1 mm 2,4,6-tripyridyl-2-triazine (TPTZ) and 2 mm ferric chloride in 0.25 m sodium acetate at pH 3.6. A 100 µL aliquot of the methanol extract prepared as above was added to 1900 µL FRAP reagent and thoroughly mixed. After leaving the mixture at 20 °C for 4 min, the absorbance at 593 nm was determined. Data were determined by using a calibration curve (0–6000 µg mL^{-1} ferrous ion), produced by the addition of freshly prepared ammonium ferrous sulfate. FRAP values were calculated as µg mL^{-1} ferrous ion (ferric reducing power) from three determinations and are presented as mg kg^{-1} $Fe^{2+}E$ (ferrous ion equivalent).

3.4.4 Determination and extraction of ascorbic acid

Samples were frozen, freeze dried and stored at −80 ◦C before proceeding with the analysis. Samples (0.5 g) were homogenized until uniform consistency in a meta-phosphoric acid and acetic acid solution. Ascorbic acid was determined following the ISO 6557 method.

3.5 Statistical Analysis

Statistical analysis was performed according to a randomized block design with three treatments in triplicate. Data were normally distributed (Shapiro–Wilk'sWtest), and so they were analyzed by analysis of variance (ANOVA). In the case of a significant F-value, the means were compared using Tukey's Honest Significant Difference (HSD) test.

3.6 Observations during the process

Starting from the first week, all mizuna and rocket salad seedlings of the FW treatment were prone to a nutrient deficit. Most of FW mizuna leaves turned yellowish and it was recorded a high plant mortality (Figg. 4, 5). Leaves and root system of both species never had the possibility to propagate over study period (Figure 5) as it was observed in HC and CFW treatments (Figure 6).

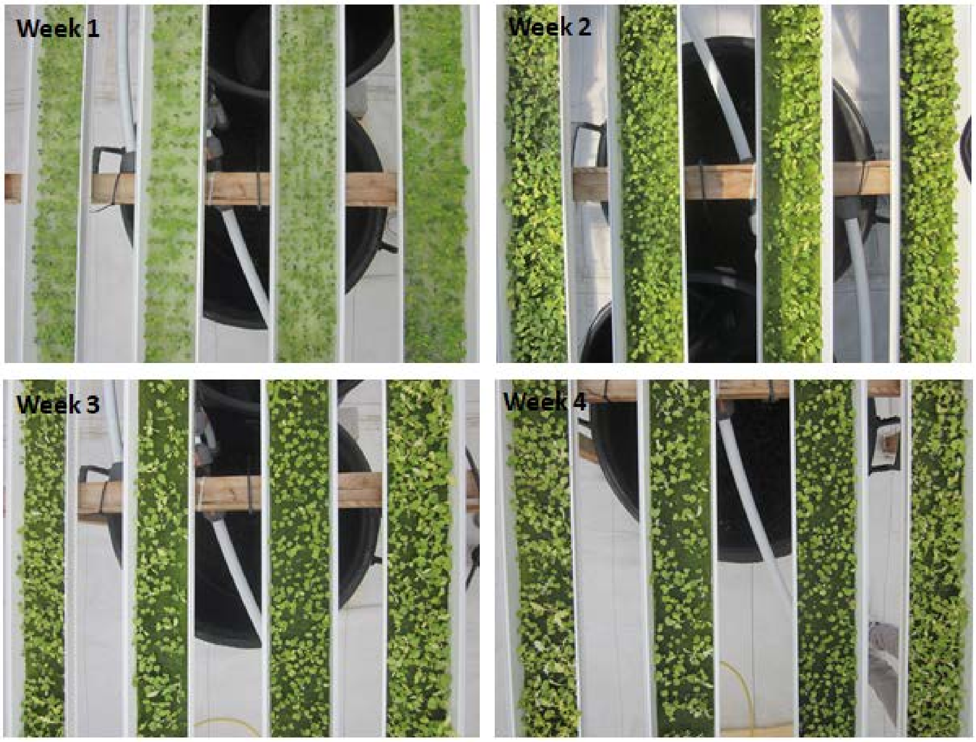


Figure 4 Growth trend of the FW treatment over the study period in the aquaponic system.

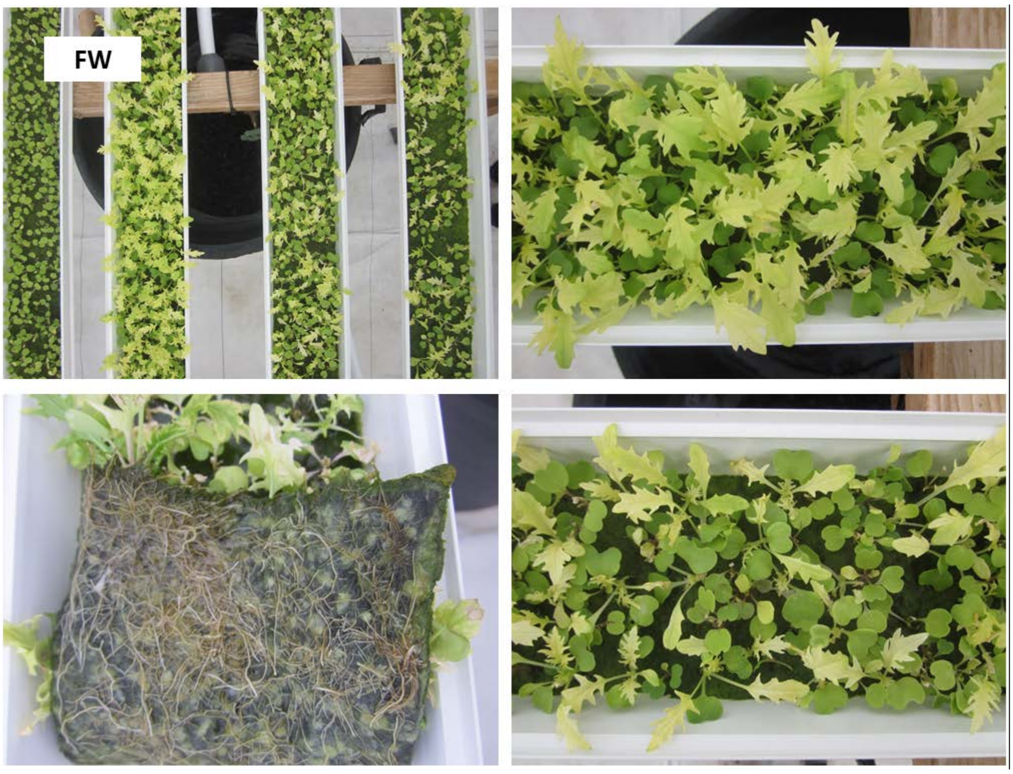


Figure 5 Second week observations on FW treatments: mizuna leaves all yellow and difficult growth for the root system.

Figure 6 Baby-leaf vegetable crop in HC and CFW treatments in the aquaponic system: plants look green and healthy and the root system is well developed on the NFT growing bed.

The first days of the Week 1 in both cycles a thin layer of oily patina was observed on the surface of the water of the HC treatment (Figure 7). It was probably due to the water quality used for the hydroponic nutrient solution prepared for the plants, which disappeared at the end of the week.

Figure 7 First week observation of a thin oily patina on the water surface of the HC treatment.

Since the second week, when the plant growth and the root system were successfully establishing, colonies of green and brown algae started colonizing the NFT channel. It was probably due to the small amount of water hold by the NFT growing bed and roots of the plants which established an *optimum* for their growth. Nevertheless, the algae colony did not have any influence on the AP system over the study period.

4 Results

The results presented in this study are the average of two growing cycles since all applied treatments provided the same responses in both cycles.

4.1 Water evaluation

4.1.1 Water consumption and water quality parameters

Water consumption and water quality parameters were significantly influenced by the experimental treatments. Cumulative water consumption did not differ between CFW and HC with 218 and 214 L respectively, whereas FW showed significant ($P<0.05$) lower water consumption (182 L) as a result of the lower crop growth. In relation to EC, pH and nitric nitrogen, the responses were conditioned by the nutrient solution type. EC significantly decreased in systems where plants grew well (HC and CFW), but any significant results were recorded for FW, as seen in Figure 8. EC decreased over the cultivation cycle in HC and CFW, from 1954 to 1434 µS/cm and from 1900 to 1505 µS/cm respectively, but not in FW (from 930 to 835 µS/cm).

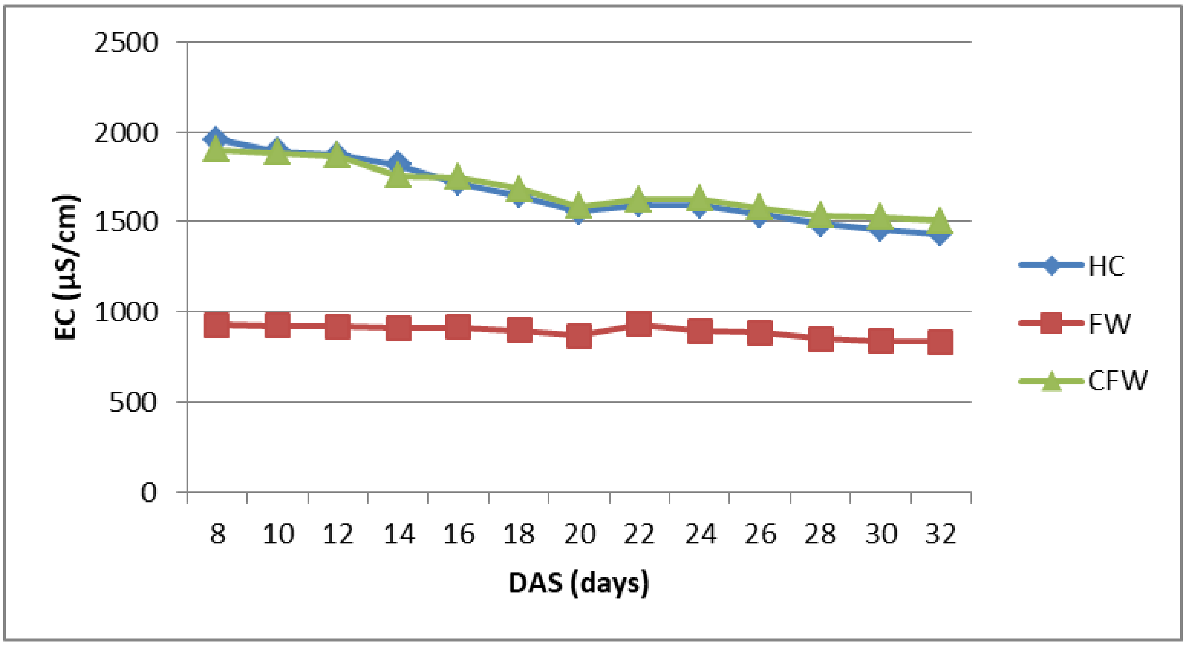


Figure 8 The change in EC values over the time of the experiment. The values shown are average of three replicates (CFW: corrected fish water; FW: fish water; HC: hydroponic control).

In general, the pH increased during the crop cycle reaching high values close to 9 at the end of cultivation. This result is mainly linked to the continuous refilling with the freshwater, which is characterized by high carbonate and bicarbonate contents. pH values were highest (9.4) in the HC treatment and lowest in the FW and CFW treatments (9) (Figure 9).

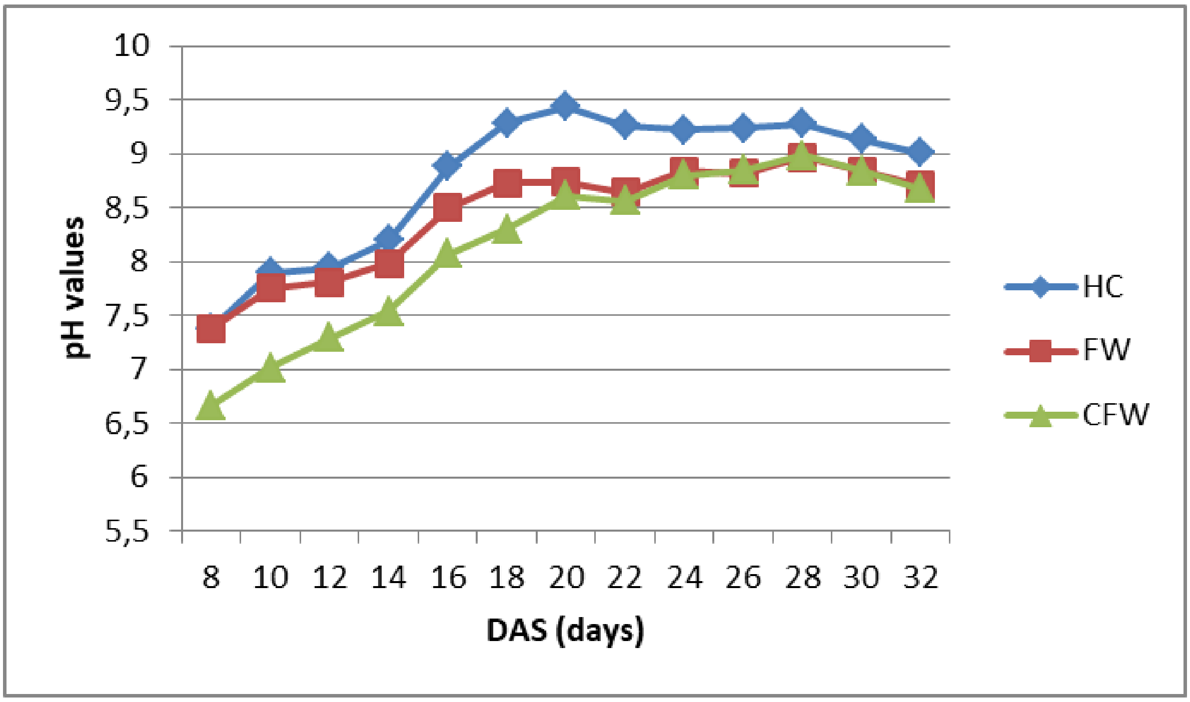


Figure 9 The change in pH values over the time of the experiment. The values shown are average of three replicates (CFW: corrected fish water; FW: fish water; HC: hydroponic control).

As regards to the concentration of nitrate-N, initially there was a similar concentration in CFW and FW and slightly higher in HC. As shown in Figure 10, the amount of N-NO_3 changed differently among the treatments over the growing cycle: the concentration of N-NO_3 gradually decreased from 86.62 to 29.21 mg L^{-1} in HC, from 69.45 to 46.34 mg L^{-1} in FW and from 67.4 to 12.72 mg L^{-1} in CFW.

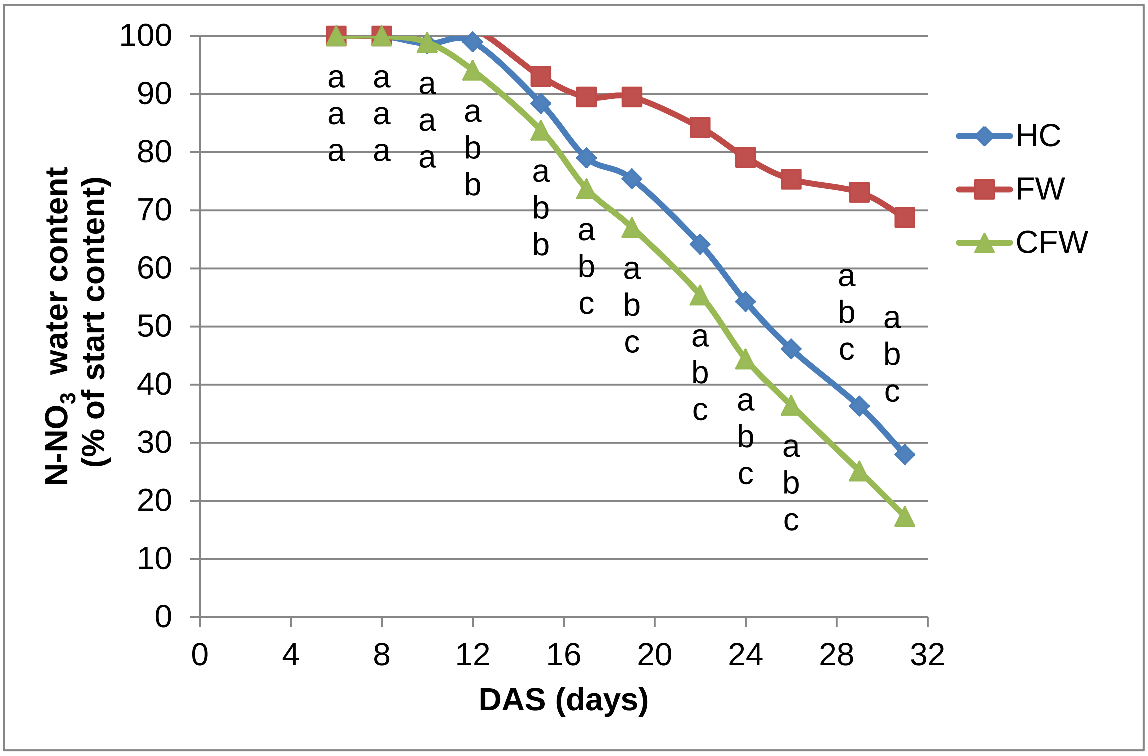


Figure 10 Effect of different water treatments on N-NO_3 starting concentration. The values shown are average of three replicates (CFW: corrected fish water; FW: fish water; HC: hydroponic control).

4.1.2 Plants nutrient uptake: anions and cations

This section presents the comparison between the average content of anions and cation in the AP system (Cl^-, NO_2^-, NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}). There are analyzed three different situations: the main effect of the 3 types of nutrient solution used; the main effect of the species (mizuna – M and rocket salad – R); the significant interactions between type of nutrient solution and species.

The nutrient solution had a significant effect of nutrient absorption by the plants among the treatments (Figure 11). In general, the difference in the nutrient absorption between HC and CFW was negligible and there was no significant difference among the types of nutrient solution ($P>0.05$), with the exception for the NO_2^-, K^+ and Mg^{2+} contents, which were higher in HC, and the nitrites content higher in CFW. In contrast, plants growing with FW showed different effects of nutrient uptake than HC and CFW. There was a significantly ($P<0.05$) higher content of Cl^-, NO_3^-, SO_4^{2-}, Na^+, NH_4^+, Mg^{2+} and Ca^{2+} in the biomass, probably due to the nutritional imbalance present in FW.

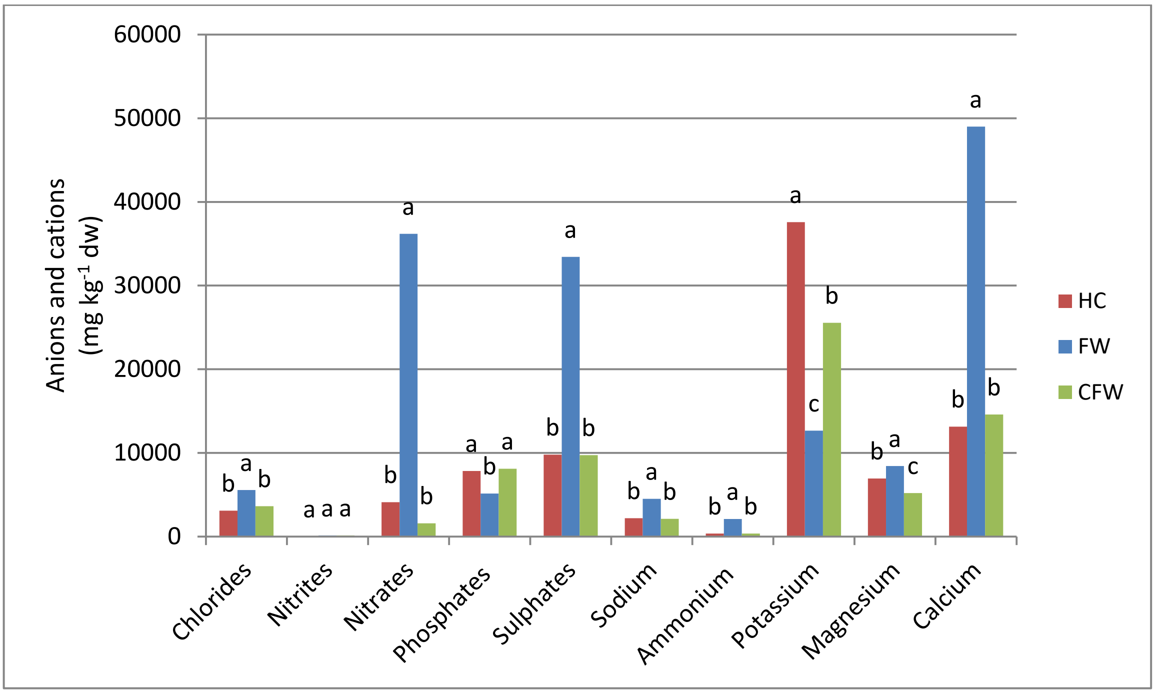


Figure 11 Water treatments main effect on plant anions and cations content (CFW: corrected fish water; FW: fish water; HC: hydroponic control). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

Figure 12 is showing the main effects of the species in relation to the absorption of nutrients. Mizuna and rocket salad showed a different assimilation of the nutrients which can be related to a different adaptability of the species to the implemented cultivation system. The absorption of nitrates, sulphates, sodium, ammonium and potassium were significantly (P<0.05) higher in M, whereas nitrite content was significantly (P<0.05) higher in R (127 mg kg^{-1} dw). Finally, the concentration of chlorides, phosphates, magnesium and calcium did not differ between the species (P>0.05).

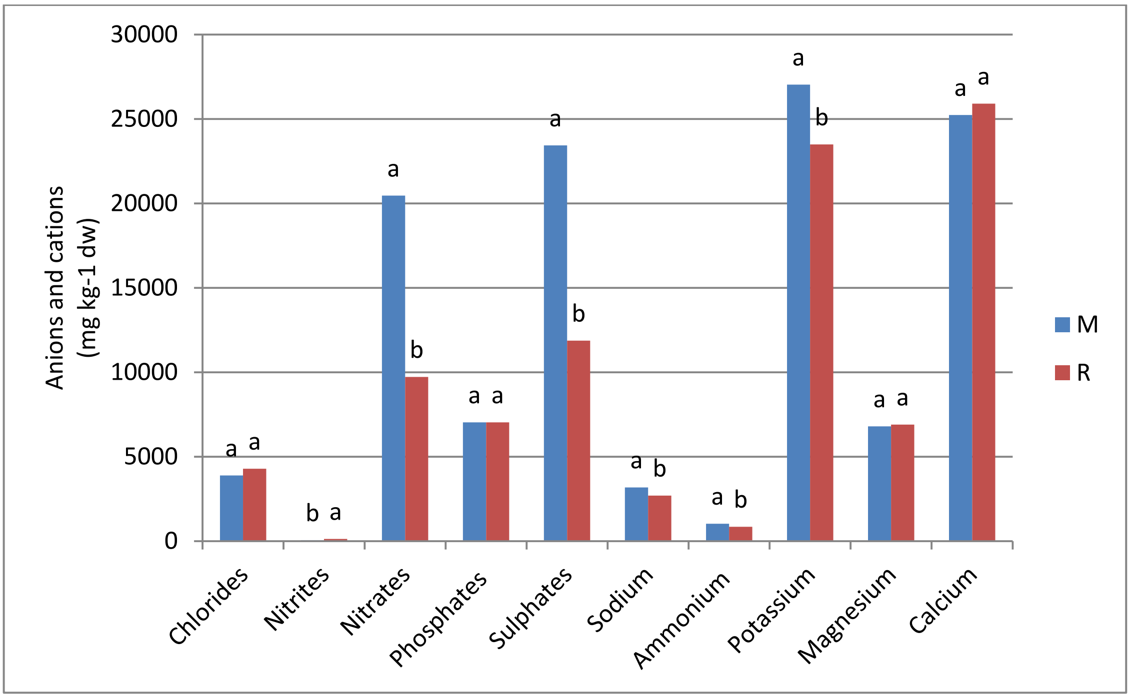


Figure 12 Species main effect (M: mizuna; R: rocket salad) on plant anions and cations content. Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

The interaction between type of water and species can be seen in Figure 13, where the assimilation of NO_3^-, PO_4^{3-}, SO_4^{2-}, Na^+ and NH_4^+ presents different behaviors between the species. Firstly, nitrate and sulphate absorption were higher in FW, but the species presented a different absorption capacity: nitrate and sulphate content was higher in M (52.49 and 42.02 g kg^{-1} dw, respectively). The M PO_4^{3-} uptake was higher in CFW, whereas the R PO_4^{3-} uptake was higher in HC. As expected, ammonium and sodium content was significantly higher in FW for both species.

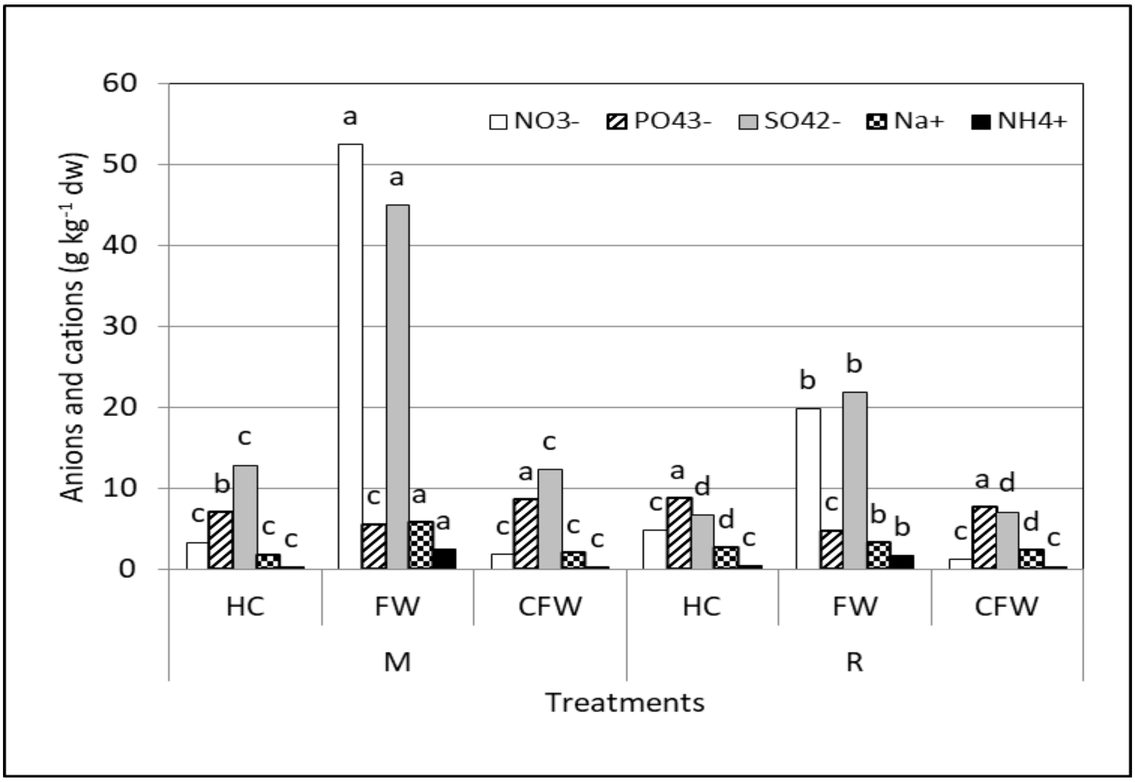


Figure 13 Significant interactions between water treatment and species on plant anions and cations content. (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

4.2 Baby-leaf production

4.2.1 Plant growth

After approximately 5-7 days, the radicles of mizuna (*Brassica rapa* L. spp. *Nipposinica* - M) and rocket salad (*Eruca vesicaria* - R) had broken through the seed coat and were visible on 70-80% of the seed. During the germination period, the plant seedlings in all growing NFT beds grew rapidly and fairly uniform and appeared healthy with green in color. At the end of the germination period, some differences in plant growth among the different treatments were observed. While HC and CFW plants seemed to grow quickly and healthy, the FW treatment showed some signs of nutrient deficiency syndromes or toxic effect during the entire growth period. The leaves of both species appeared chlorotic and weak through the entire cycle and the root system was not developed enough to strongly anchor the plants to the NFT channel.

4.2.2 Plant vitality parameters

Visible differences among the treatments were observed through the plant height and SPAD values (index of relative chlorophyll content). In Figure 14, it can be observed that HC and CFW present an exponential plant growth with the maximum pick on the last days of the plant production, reaching a maximum of 128.98 mm and 123.53 mm, respectively, whereas plant height in FW is drastically different: the maximum height is 26.2 mm (plants were non-marketable and chlorotic) while the remaining heights are constant around 20 mm, with some maximum picks due to plant mortality which acts like a temporary nutrient addition to the rest of the growing production.

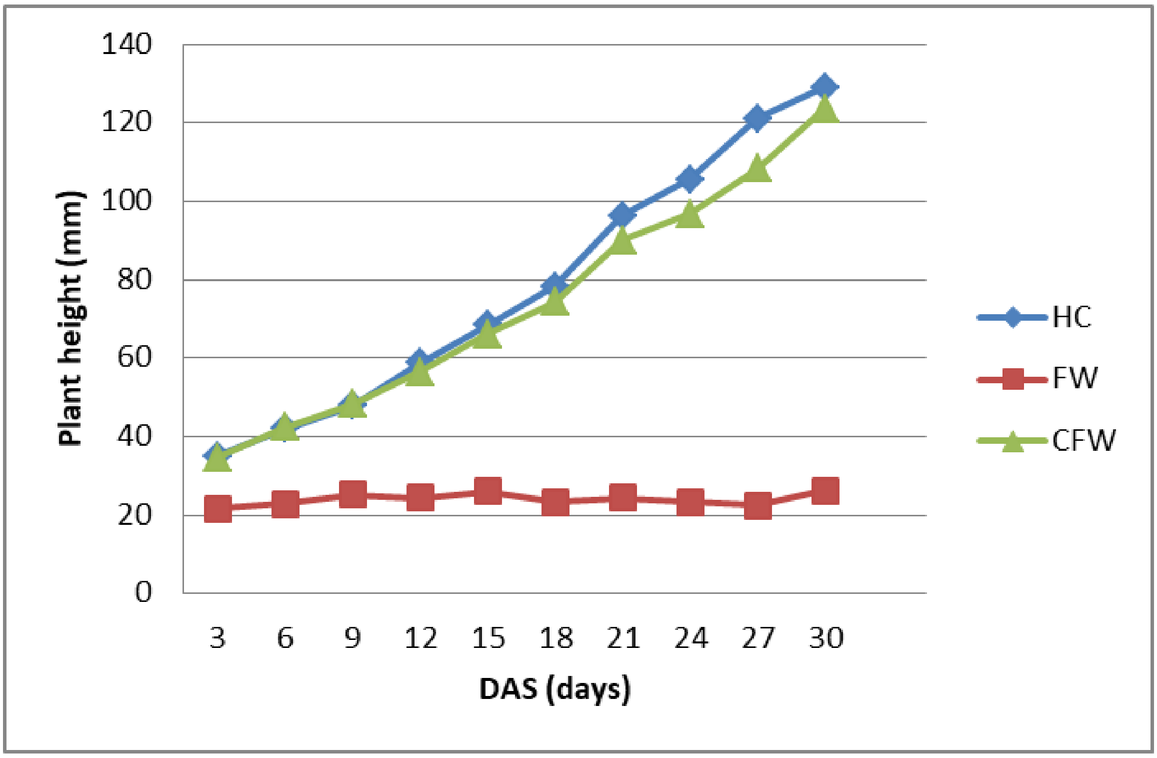


Figure 14 Average of plant height values in different treatments of the aquaponic system (CFW: corrected fish water; FW: fish water; HC: hydroponic control).

Mizuna reached higher height than rocket salad in all treatments (Figure 15a). Although this aspect can be clearly seen in HC-CFW treatments, such difference is slightly observed in FW, where mizuna and rocket salad had maximum values of 25 mm and 20.4 mm, respectively, as both species could not develop the baby-leaf stage. As regards to the mizuna height for HC-CFW treatments, the trend appeared to be similar through the entire study, showing a maximum of

160.76 mm and 152.41 mm, respectively. Similar height trend can be observed for the rocket salad, with a maximum of 86.31 mm for CFW and 68.43 mm for HC.

Considering the general height growth of mizuna and rocket salad, Figure 15b clearly shows the different adaptability of the species to the implemented cultivation system. Known the mizuna to be more vigorous and faster grower than the rocket salad, the competition between the two species brought the rocket salad to reach higher height values than usually would in its first growth stages, presenting longer internodes instead of more developed leaves.

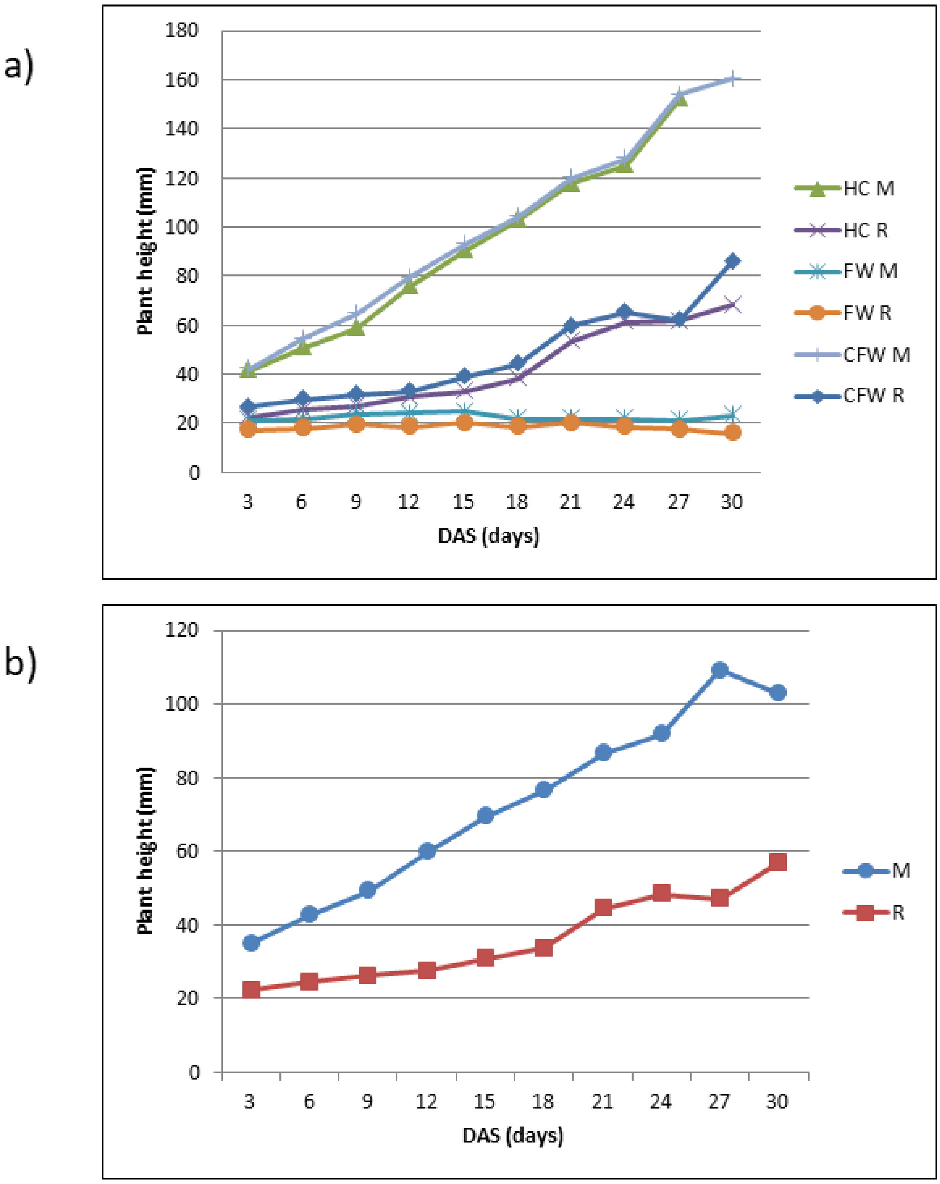


Figure 15 Height values for mizuna (M) and rocket salad (R) in different treatments of the aquaponic system: a) within each treatment and b) general trend of the species (CFW: corrected fish water; FW: fish water; HC: hydroponic control).

As regards to the SPAD sampling, SPAD values of plants in FW were between 5 and 10, in contrast the plants in HC and CFW showed on average values between 25 and 33 (Figure 16). Moreover, in HC and CFW SPAD values increased during the production, whereas in FW the SPAD values decreased.

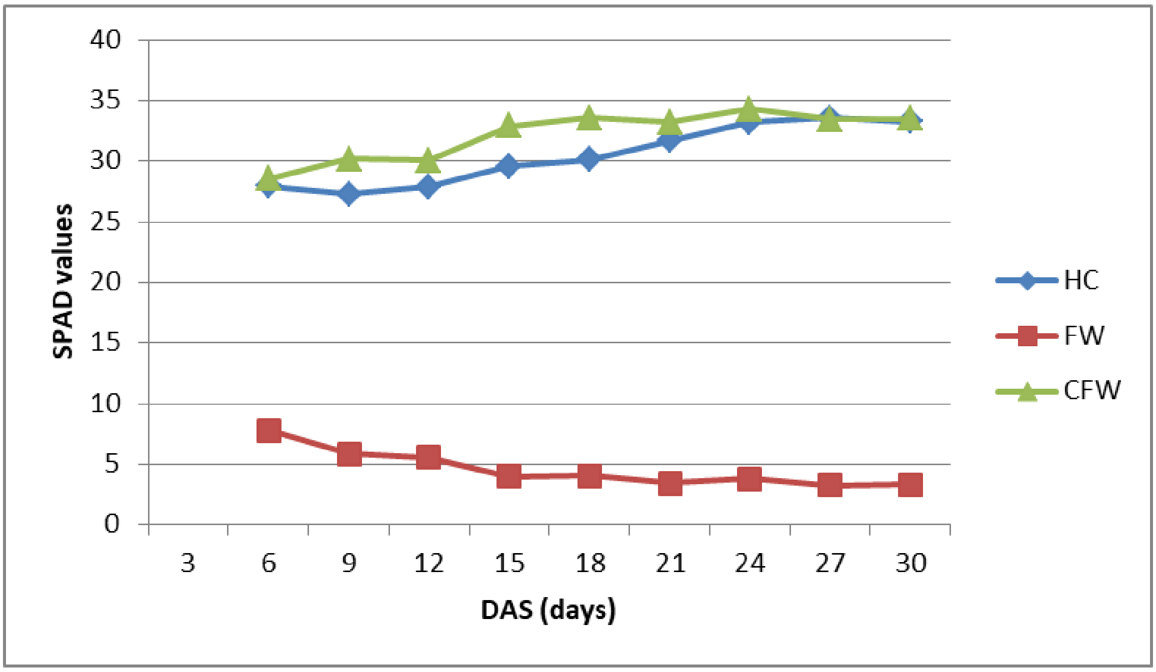


Figure 16 Effect of different water treatments on SPAD values. The values shown are average of three replicates (CFW: corrected fish water; FW: fish water; HC: hydroponic control).

Observing the results in Figures 17a and 17b, in HC treatment, SPAD values for mizuna and rocket salad are similar and both species are increasing their chlorophyll content, and thus the leaf nitrogen, during the production cycle. This trend can be explained by the sufficient nutrients available for both species. In CFW treatment there are some differences between the two plants: SPAD values for mizuna are increasing, whereas for rocket salad they are decreasing, as the majority of nutrients are taken by the mizuna yield. Similar trend can be observed in the FW treatment: while SPAD values are increasing for rocket salad, the ones for mizuna are decreasing. As a matter of fact, the mizuna yield suffered more the nutrient deficiency as it grew and died faster than the rocket salad, which then had more nutrient availability.

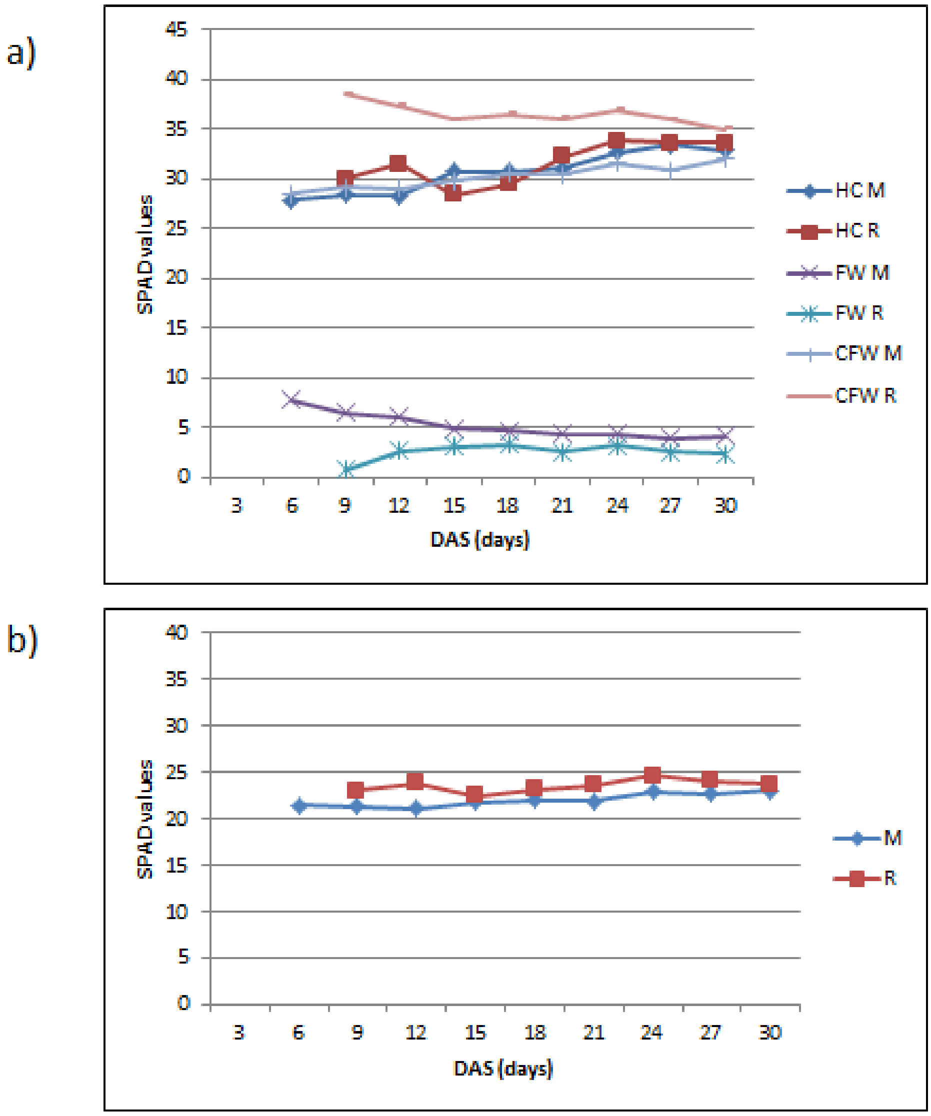


Figure 17 Effect of different water treatments and species on SPAD values: a) within each treatment and b) general trend of the species (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad).

4.2.3 Determination of yield production and dry matter

At the end of the microgreens and baby-leaf growing cycle (plant height equal to 50-60 mm and 100-120 mm, respectively) total yield and dry matter (65°C) were determined through destructive measurements.

4.2.3.1 Microgreens cycle

As it can be seen in Figure 18, there is no significant difference between HC and CFW treatment, as both treatments had similar yield results compared to FW which had very low yield values (0.3 kg m^{-2}). M displayed yield results equal to 0.8 kg m^{-2}, while R had drastically lower values (0.2 kg m^{-2}). As regards to the dry matter, CFW treatment showed the highest dry matter value, equal to 13,9 %, followed by HC (12%) and FW treatments (9%). In this case, dry matter was significantly ($P<0.05$) higher in R and lower in M (Figure 18).

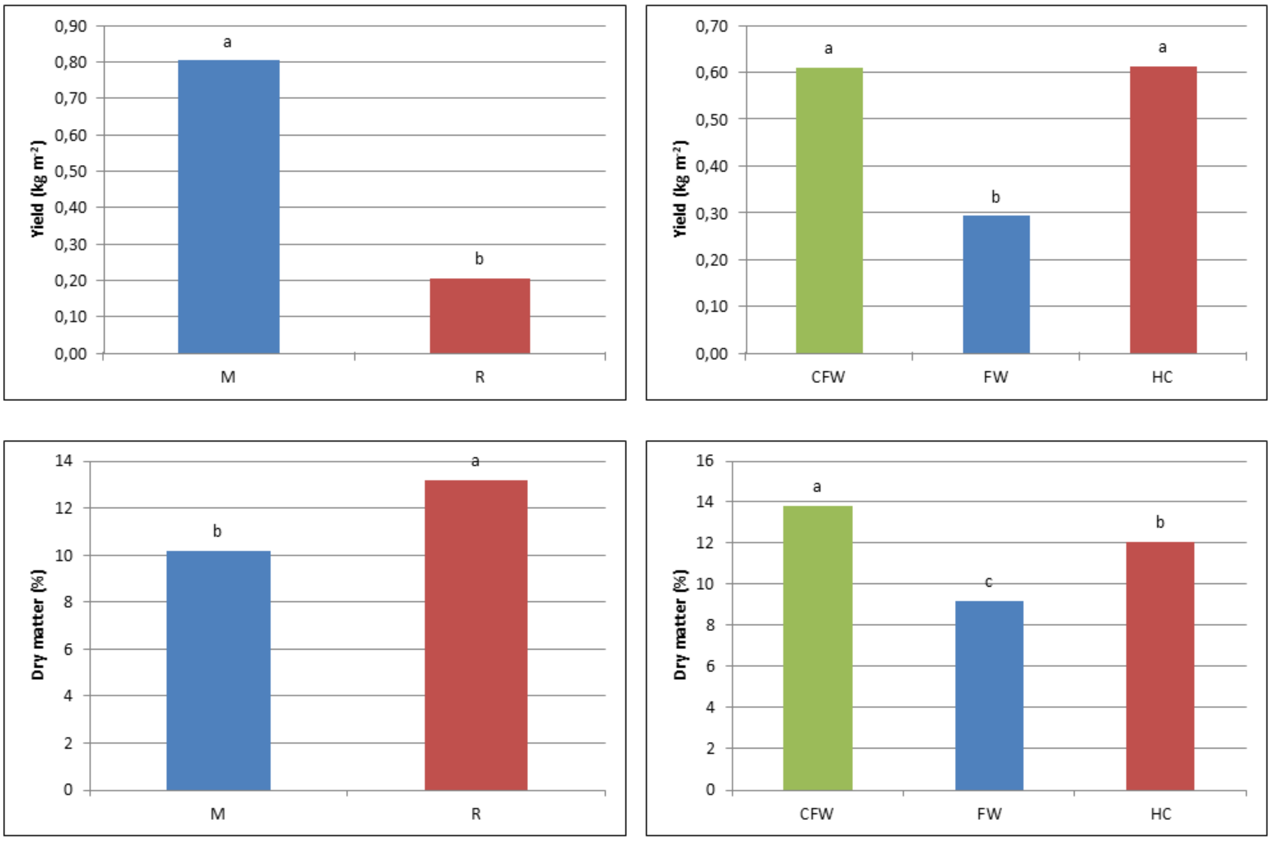


Figure 18 Effect of different water treatments and species on crop yield (above) and dry matter (below) for microgreens. (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

There was a visible difference between M and R in the crop yield recorded among the treatments, but in both cases the crop yield of FW treatment was the lowest, whereas HC and CFW treatments had similar results (Figure 19). Dry matter was higher for both species in CFW treatment (11.5%

and 16% for M and R, respectively) and lower in FW treatment (8% and 10% for M and R, respectively).

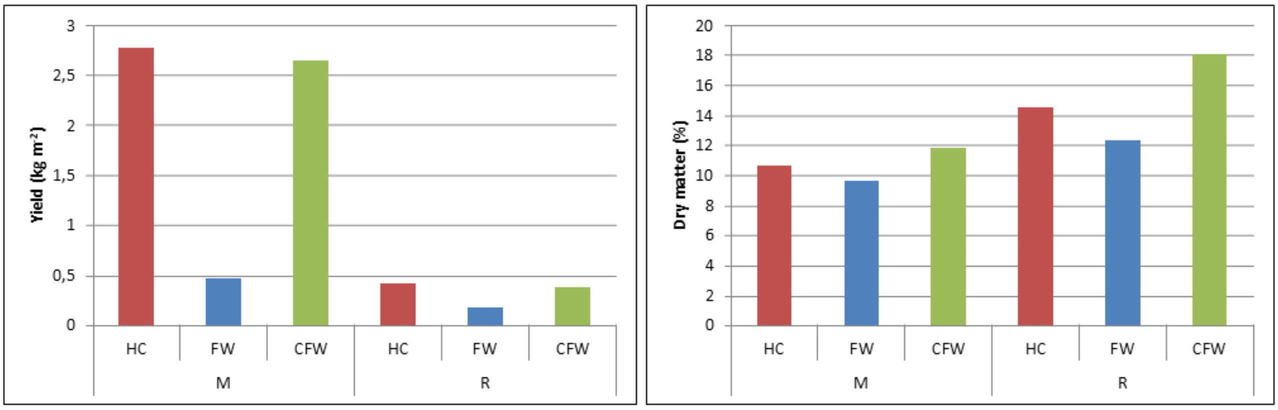


Figure 19 Different effect of species at the end of the microgreens cycle in relation to nutrient solution on crop yield (on the left) and dry matter (on the right) (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad).

4.2.3.2 Baby-leaf cycle

HC and CFW treatment had similar crop yield production, equal to 1.6 and 1.5 kg m^{-2} respectively, whereas FW treatment had the lowest yield production. A significant difference ($P<0.05$) was recorded for M and R crop yield: M displayed yield results equal to 1.93 kg m^{-2}, whereas R showed poor production with considerable low value (0.3 kg m^{-2}) (Figure 20).

Dry matter was significantly higher for R (15%) compared to M (10.7%). Among the treatments, CFW showed the highest value of dry matter, whereas FW the lowest (Figure 20).

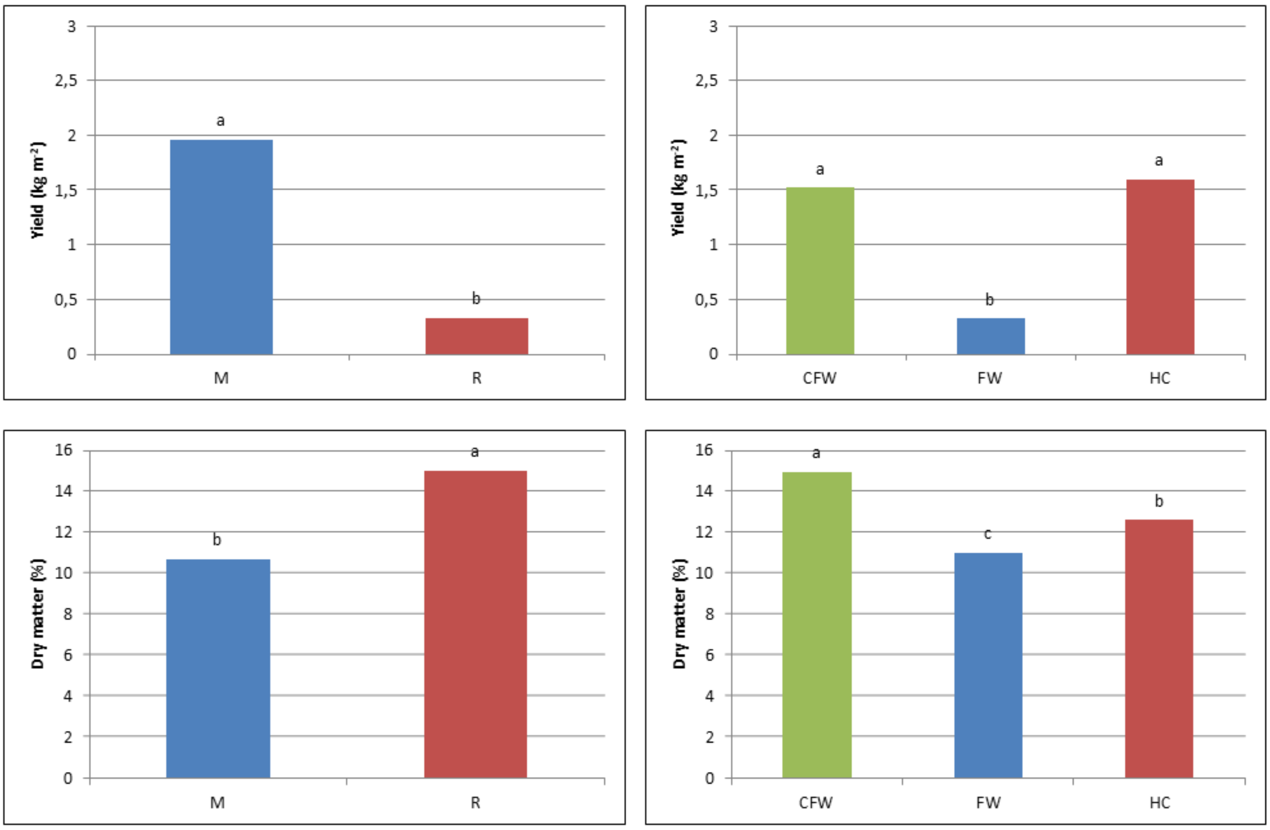


Figure 20 Effect of different water treatments and species on crop yield (above) and dry matter (below) for baby-leaf production (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

As it was recorded at the end of the microgreens cycle, the differences between M and R in the crop yield among the treatments increased the previous results. M and R yield crop was 2.6 and 0.3 kg m^{-2} for CFW, 0.45 and 0.2 kg m^{-2} for FW, 2.8 and 0.4 kg m^{-2} for HC, respectively (Figure 21).

There was a visible difference of M and R dry matter among the treatments, showing higher values in CFW and lower values in FW treatments for both species.

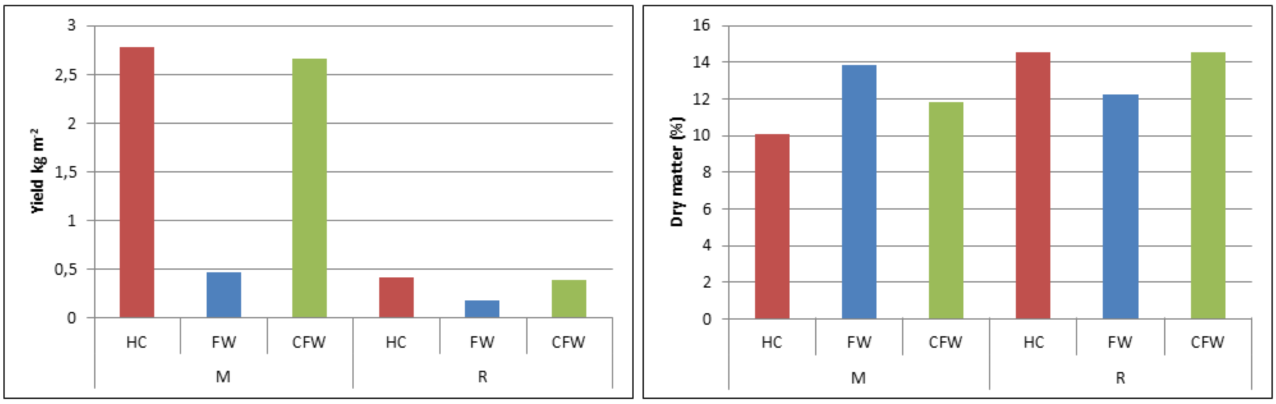


Figure 21 Different effect of species at the end of the baby-leaf cycle in relation to nutrient solution on crop yield (on the left) and dry matter (on the right) (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad).

4.2.4 Antioxidant, total phenols and vitamin C content

The different type of nutrient solution affected the content of antioxidants, phenolic compounds and vitamin C of the AP system. The results are comparing the qualitative parameters of the baby-leaf vegetables referred to the dry matter obtained of the nutrient solutions and of the two species. The study showed a significant ($P<0.05$) increase of antioxidants and phenolic compounds in R compared to M, as shown in Figures 22 and 23. The results obtained for the nutrient solutions showed significant differences for both antioxidant and phenol content, with higher values in FW treatment and lower values in CFW treatment probably due to the less stressful conditions.

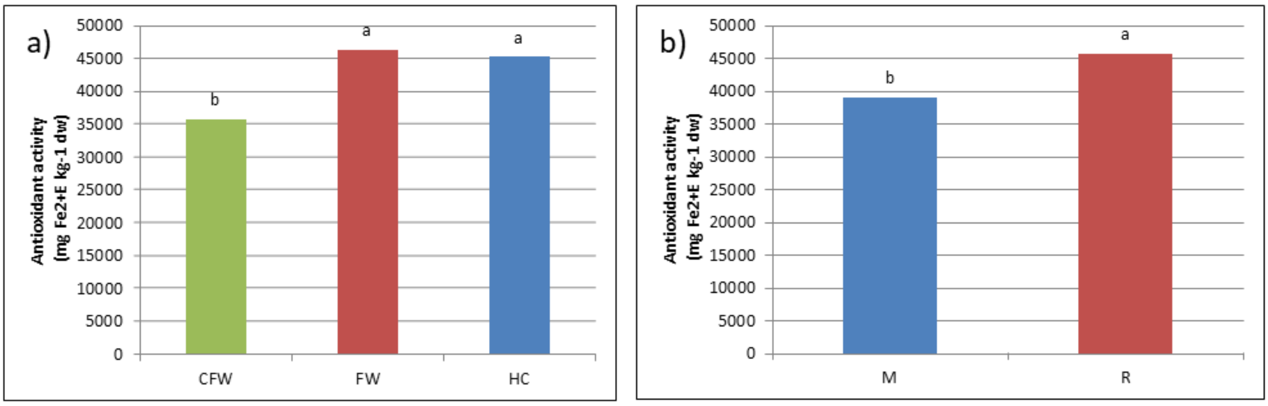


Figure 22 Effect of a) different water treatments and b) species on plant antioxidant activity of the aquaponic system (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

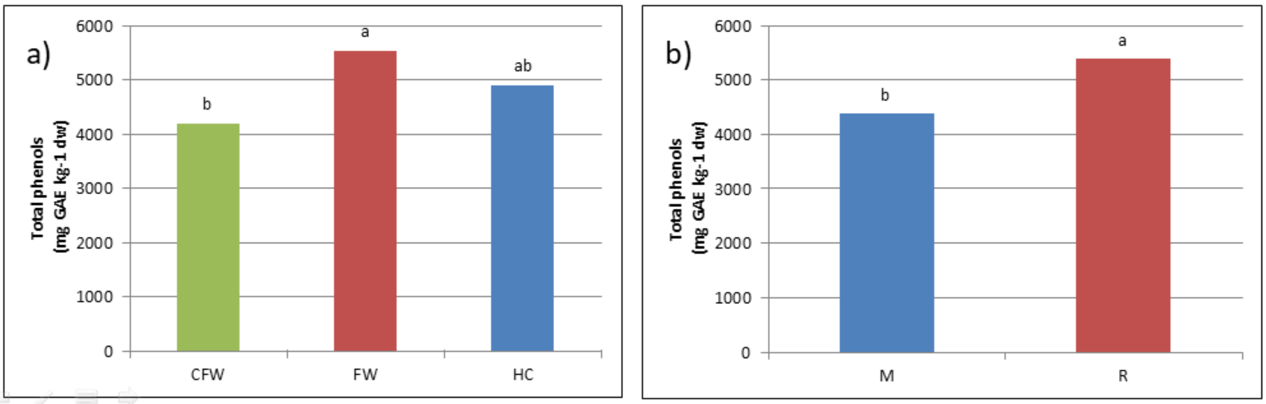


Figure 23 Effect of a) different water treatments and b) species on plant total phenols content of the aquaponic system (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

The levels of Vitamin C in the biomass were not significantly influenced by the treatment (Figure 24a)(P>0.05). R had significantly higher vitamin C values compared to M, 3860 and 2955 mg kg^{-1} respectively (Figure 24b).

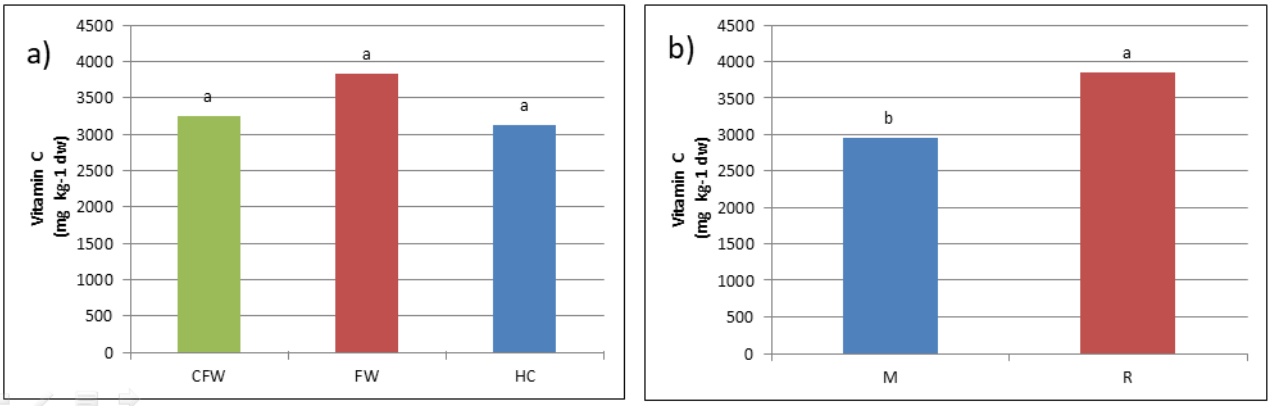


Figure 24 Effect of a) different water treatments and b) species on plant vitamin C content of the aquaponic system (CFW: corrected fish water; FW: fish water; HC: hydroponic control; M: mizuna; R: rocket salad). Within each parameter, different letters indicate significant differences according to the Tukey's HSD test at P<0.05.

5 Discussion

5.1 Water evaluation

5.1.1 Water consumption and water quality parameters

Water consumption and water quality parameters were significantly influenced by the experimental treatments. Cumulative water consumption did not differ between CFW and HC with 218 L and 214 L respectively as both treatment had similar plant production, whereas FW showed significant ($p<0.05$) lower water consumption (182 L) as a result of high plant mortality and general low crop growth.

In general, the pH increased during the crop cycle reaching high values close to 9 at the end of cultivation. This result is mainly linked to the continuous daily refilling with the freshwater, which is characterized by high carbonate and bicarbonate contents. The ideal EC is specific for each crop and dependent on environmental conditions (Sonneveld and Voogt, 2009); however, the EC values for hydroponic systems range from 1.5 to 2.5 dS m^{-1}. Higher EC hinders nutrient uptake by increasing osmotic pressure, whereas lower EC may severely affect plant health and yield (Samarakoon *et al.*, 2006). In this specific case, EC greatly decreased within range values in systems where plants grew well (HC and CFW), whereas EC decreased only 10.2% in FW, below range values.

As regards to the concentration of nitrate-N, there were significant changes over the growing cycle: $N\text{-}NO_3$ decreased by 31.2% in FW, 72.0% in HC and 82.7% in CFW. The nitrate-N removal can be explained by analysing the growth of the plants which was significantly different among the treatments: plants grown in FW could not propagate and ended to be chlorotic and non-marketable (thus, explained the low nitrate-N reduction); plants in HC and CFW grew fast and healthy, showing a positive response to the $N\text{-}NO_3$ concentration, which was higher in CFW due to relatively higher root surface area and better yield production. Similar results as HC and CFW were found by Endut *et al.* (2011) where nitrate-N was gradually reduced by water spinach with efficiencies of 79.17-87.10% in an aquaponics recirculation system. Ghaly and Snow (2008) investigated the possibility of using hydroponically grown barley for the treatment of aquaculture wastewater and reported $NO_3\text{-}N$ reductions in the effluent of 68.8-76.7% after 21 days of plant growth. Clarkson and Lane (1991) evaluated the feasibility of utilizing a nutrient-film technique to reduce the mineral content of wastewater from an aquarium stocked with common carp (*C. carpio*) and rainbow trout (*O. mykiss*). During a 4-week period, nitrate- N concentrations in the effluent were reduced from 33.03 to 3.03 mg/L using barley. In agreement with previous findings, therefore it can be assumed that the decrease in nitrate-N concentration could be due to plant absorption, assimilation of water microorganisms and association of biofilms with roots mats of vegetables (Endut *et al.*, 2011).

5.1.2 Plants nutrient uptake: anions and cations

According to Buzby and Lin (2014), AP system should be sized correctly to balance nutrient production from fish culture and nutrient uptake by plants in order to be effective at nutrient removal. This specific study describes a method where the plant component was isolated from the fish rearing operation so that nutrient removal could be evaluated independently. In relation to the anions and cations content in the plant, significant differences ($p<0.05$) were recorded between both species and nutrient solutions. M was able to assimilate more nutrients, probably because of the good adaptability to the NFT growing system and a more developed root system, compared to R. The only parameter in which R showed significantly ($p<0.05$) higher values was NO_2^- content; the concentration of Cl^-, PO_4^{3-}, Mg^{2+} and Ca^{2+} did not differ between species. In line with the World Health Organization (WHO), the nitrate commercial threshold is fixed by Commission Regulation (EU) 1258/2011 for lettuce, spinach and rocket (European Union 2011). The NO_3^- accumulation in both species was within the limits proposed by the EU Regulation N.1258/2011, as an important quality feature for leafy vegetables, and especially for minimally processed products, is a low nitrate content (Di Gioia *et al.*, 2017). Thus, nitrate content should be more carefully controlled because of the particular environmental conditions that occur in the packaging (low oxygen level, high humidity and presence of cut portions of tissues) enhancing nitrate reduction to nitrite, which could be dangerous in the case of elevated dietary intake. (Di Gioia *et al.*, 2017). In general, there was no significant difference between HC and CFW, with the exception for the NO_2^-, K^+ and Mg^{2+} contents higher in HC, and the nitrites content higher in CFW. In contrast, plants treated with FW showed different effects of nutrient uptake than HC and CFW. There was a higher content of Cl^-, NO_3^-, SO_4^{2-}, Na^+, NH_4^+, Mg^{2+} and Ca^{2+}, due to the nutritional imbalance present in FW. Moreover, higher content of nitrates, sulphates, ammonium, magnesium and calcium in FW corresponded to an abnormal absorption of these nutrients compared to HC and CFW, due to the absence of P and K. According to Al-Hafedh *et al.*, (2008) nutrient concentrations must be continuously monitored, and nutrient supplementation and water replacement must be used to correct for nutrient deficiencies and salt accumulation, respectively. As a result, FW cannot be used to cultivate short-cycle vegetable cultivation because it was unable to provide a balanced mix of nutrients for plants and, thus, missing nutrients must to be integrated.

5.2 Baby-leaf production

5.2.1 Plant vitality and yield production

Aquaponic systems are considered to possess great potential in the food production industry in economics terms (Savidov, 2005) as well as in being an organic production method for aquatic organisms and vegetables (Mateus, 2009).

In terms of plant growth, this experiment demonstrated that plants HC and CFW treatments seemed to grow quickly and healthy, whereas FW treatment showed some signs of nutrient deficiency syndromes over the entire growth period. The SPAD and plant height have clearly revealed the inability of FW to meet the nutritional needs of both species. The SPAD values of plants in FW were between 5 and 10, in contrast the plants in HC and CFW showed on average values between 25 and 33. The plant height was also drastically different between FW and HC-CFW treatments: in the first case the maximum height was 26.2 mm (plants were non-marketable and chlorotic), in the second one plants were higher than 120 mm.

Concerning production, the first yield production values obtained at the end of the microgreens cycle already showed visible differences among the treatments and the species, which increased further over the short-cycle vegetable cultivation. M and R yields showed different adaptability to the AP system that can be noticeable by the yield production. M displayed yield results comparable with those reported in literature (Di Gioia *et al.*, 2017) and equal to 1.96 kg m^{-2}, whereas R showed poor production (0.32 kg m^{-2}) with considerably lower values than normally obtained (Nicola *et al.*, 2016). The observed lower dry matter of the M compared to the R could be stemming from the fact that M yield was greater and healthier than R yield, as well as for CFW and HC treatments compared to FW, whose dry matter content was lower (10.5%). In general, it was observed lower production in FW treatment (0.32 kg m^{-2}) than in HC treatment (1.59 kg m^{-2}), where nutrients were supplied in ideal amounts. The lower production in FW can be attributed to poor nutrients, in particular lack of K and P, because it was observed that growth and yield were lower compared to CFW (1.52 kg m^{-2}), where the same fish water was supplemented with P and K plus meso- and micro nutrients. The high production potential of CFW is worth further consideration as it could be linked to the presence of organic compounds with bio-stimulant activity and could be able to guarantee better/similar cultivation conditions than/as HC treatment. Thus, further research is needed in order to better investigate the possible presence of bio-stimulant organic compounds.

5.2.2 Antioxidant, total phenols and vitamin C content

Baby-leaf vegetables have shown the antioxidant potential in reducing metal ions (FRAP) or radical cations (ABTS), and in scavenging free radicals (DPPH) (Saini *et al*., 2016). The antioxidant potential of plant-based food is primarily attributed to the presence of phenolic compounds (Soobrattee *et al*., 2005). The phenolic content of a plant is affected by several factors like plant species, cultivar, environmental conditions, water availability, light exposure, germination, maturity, processing and storage (Alarcón-Flores *et al*., 2013; Herrero *et al*., 2013). Moreover, plants typically respond to environmental stresses by inducing antioxidants as a defense mechanism (Oh *et al*., 2013).

This study conducted the quantification of the antioxidant content (FRAP method), total phenols content (Folin-Ciocalteau method) and vitamin C (ISO 6557) in the baby-leaf vegetables in order to better understand the general conditions of the short-growing cycle.

The highest antioxidant activity by FRAP method was detected in R (45686 mg Fe^2+E kg^{-1} dw). This fact can be explained by a higher stress condition experienced by R over the growing cycle as there was a strong competition with M. Among the nutrient solutions, CFW treatment had lower antioxidant activity (35638 mg Fe^2+E kg^{-1} dw) probably due to less stressful conditions. In contrast, FW treatment showed considerable higher antioxidant activity (46180 mg Fe^2+E kg^{-1} dw) which could confirm the inability to provide a healthy and marketable production due to stressful conditions for the plants.

As Heimler *et al*. (2006) found in their study of the antiradical activity and polyphenol composition of broccoli, cabbage, and other green *Brassicaceae*, there is a correlation between antioxidant activity and total polyphenol content. As expected, total phenols content was higher for FW (5546 mg GAE kg^{-1} dw) and lower for CFW (4204 mg GAE kg^{-1} dw). As regards to the species, R recorded the highest phenolic values (5390 mg GAE kg^{-1} dw).

The vitamin C content of the baby-leaf vegetables was 2955 and 3860 mg kg^{-1} dw for mizuna and rocket salad, respectively. The highest content of vitamin C was observed in the FW treatment, followed by CFW and HC treatments. In Table 5 there are shown some studies conducted in baby-leaf vegetables cultivated in fields for the quantification of these bioactive compounds: all previous values seem to have no correlation with the values (expressed in mg kg^{-1} fw) obtained in this specific study, as many different factors were involved, including production systems, nutrient solution, environmental conditions, growing period among others, since they are known to influence the antioxidant activity of these vegetables, thus, further research is needed.

Table 5 Comparison between Martinez-Sanchez *et al*. (2008) study conducted in baby-leaf vegetables for the quantification of important bioactive compounds and this specific study.

		Phenolic compounds by Folin-Ciocalteau [mg GAE kg^{1} fw]	**Antioxidant Activity by FRAP [mg Fe^{2+}E kg^{-1} fw]**	**Vitamin C by ISO 6557 [mg kg^{-1} fw]**
This study; Rocket salad	HC	581	4484	401
	CFW	699	5538	539
	FW	752	5866	532
Martinez-Sanchez *et al*. (2008)	Rocket salad	1323 ± 171	880	800
This study; Mizuna	HC	524	4533	303
	CFW	519	4956	425
	FW	678	6027	449
Martinez-Sanchez *et al*. (2008)	Mizuna	994 ± 11 *	1230	640

** Phenolic compounds were measured by the PLE Method.*

6 Conclusions

Aquaponics is one of the few techniques available that can remove at low concentrations dissolved N and P generated via aquaculture (Buzby and Lin, 2014). Thus, it is a promising solution for the negative environmental impacts typically associated with intensive fish and crop production (Maucieri *et al.*, 2017). This study investigated the possibility of cultivating baby-leaf vegetables using waste water coming from semi-open AP system and came with the conclusion that FW cannot be used as it is because it was unable to provide a balanced mix of nutrients for plants, which lead to an abnormal absorption of nitrates, sulphates, ammonium, magnesium and calcium when depleted of potassium and phosphorus. HC and CFW treatments recorded a higher yield production than FW, as plants grew quickly and healthy. The SPAD and plant height have clearly revealed the inability of FW to meet the nutritional needs of both species, because plants were chlorotic and non-marketable, as the antioxidant activity, total phenolic compound and vitamin C showed how stressful the growing conditions were for the crop. The starting N-NO3 concentration, similar in all systems, decreased by 31.2% in FW, 72.0% in HC and 82.7% in CFW. As a result, the efficient nitrate-N removal in CFW is correlated to the yield production. Additionally, it is supposed that the high production potential of CFW could be linked to the presence of organic compounds with bio-stimulant activity. Thus, further research is needed in order to better investigate the possible presence of bio-stimulant organic compounds.

In conclusion, the use of CFW is an effective solution to manage the fish water at the end of an AP cycle and can be successfully used for short-cycle vegetable cultivation, achieving significant production, reducing nitrogen load and further reducing the environmental impact of the system.

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