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Intermittent Flow Alleviated Root Zone Stress and Increased Organic Lettuce Yield in Nutrient Film Technique

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Abstract. **Organic lettuce production often yields less than conventional hydroponics. A previous study from our laboratory showed that an intermittent organic nutrient solution flow scheme with a microbial root inoculant (TerraBella; AquaBella Organics LLC, Sebastopol, CA, USA) in a nutrient film technique (NFT) system resulted in lettuce yield comparable to that of conventional hydroponics. In this study, we investigated the effects of intermittent flow frequencies with or without a root inoculant (TerraBella) on the growth, yield, and mineral nutrition of 'Casey' lettuce in organic NFT systems. Two intermittent flow schemes were tested: 15 minutes of flow/45 minutes of no flow and 15 minutes of flow/105 minutes of no flow with or without TerraBella inoculation. Continuous flow of nutrient solution (0 minutes of no flow) was used as control, and the inorganic NFT was used as the inorganic reference. The results showed that organic lettuce fresh weight increased by 71% when the no-flow period increased from 0 to 105 minutes. Inoculating TerraBella to organic NFT systems with intermittent flow decreased lettuce yield to a level similar to that of continuous flow, which was unexpected. In organic nutrient solutions, regardless of the flow scheme or TerraBella inoculation, high ammonium and nitrite concentrations were observed, which likely contributed to the lower yield compared with that of conventional production. These results indicate that intermittent nutrient solution flow likely improved oxygen availability to roots and reduced the exposure of roots to ammonium and nitrite toxicity. Additionally, the intermittent flow scheme in organic NFT systems not only improved lettuce yield but also led to energy savings.**

The organic food market in the United States has grown rapidly from approximately $10 billion in 2001 to nearly $56 billion in 2020 according to the Economic Research Service (ERS) of the US Department of Agriculture (USDA). On the supply side, despite the 347% increase in certified organic farmland acreage from 2002 to 2019 according to the ERS report (US Organic Production, Markets, Consumers, and Policy 2000 to 2021), imports of organic food to the United States increased from $0.8 billion in 2011 to $2.7 billion in 2021 (https://www.ers.usda.gov/topics/natural-resources-environment/organic-agriculture; accessed 26 Mar 2025). The growing gap between demand and domestic production in the United States suggested the need for research to tackle the challenges of organic production and increase the domestic supply.

In the United States, the USDA does not explicitly prohibit hydroponics in organic production, and hydroponic productions can acquire organic certification from certifiers (Di Gioia and Rosskopf 2021). Organic production systems often have lower yields than inorganic systems (Burnett et al. 2016; Heeb et al. 2006; Kniss et al. 2016; Mäder et al. 2002; Seufert et al. 2012; Timsina 2018), and organic hydroponic systems are no exception (Burnett et al. 2016; Williams and Nelson 2014; Zhai et al. 2009). Common challenges of organic hydroponics include supplying sufficient nutrients during key growth stages, excessive or unwanted compounds in organic nutrient sources such as high nitrite concentrations or heavy metals, dramatic pH fluctuations, high electrical conductivity (EC), and biofilm formation (Ahmed et al. 2021; Bergstrand et al. 2020; Hooks et al. 2022; Williams and Nelson 2014; Zhai et al. 2009; Zikeli et al. 2017).

Commonly used organic nutrient sources are manure, green manure, compost, and byproducts of agricultural activities such as slaughterhouse byproducts, fish scraps, and molasses (Bergstrand 2022; Chatzistathis et al. 2021). These organic nutrient sources often have imbalanced nutrient composition, and the release of organic nutrients is often slow and difficult to control (Bergstrand 2022; Bergstrand et al. 2020; Burnett et al. 2016; Pang and Letey 2000; Timsina 2018; Williams and Nelson 2014; Zhai et al. 2009; Zikeli et al. 2017). Synthetic fertilizers offer more available nutrients and can be tailored to meet the specific needs of any crop. Organic fertilizers also may contain excessive or unwanted compounds such as high nitrite concentrations or heavy metals, leading to salinity stress and/or toxicity (Burnett et al. 2016; He et al. 2022; Shinohara et al. 2011). Regarding organic hydroponics, a few studies have attempted to increase fertilizer rates to meet crop growth demands, but these attempts were often detrimental because of toxicity caused by high ammonium and nitrite concentrations (Carballo et al. 2009; Krishnasamy et al. 2012; Mupambwa et al. 2019; Szekely and Jijakli 2022).

Biofilm is a greater problem in organic hydroponics than in organic field or substrate production. Biofilm is an aggregated community of microorganisms that adheres to any surface and can occur with a wide range of microorganisms (Hall-Stoodley et al. 2004; O'Toole and Kolter 1998). Biofilm formation and buildup in hydroponic systems can clog pumps, air stones, and tubing, require more labor to clean and maintain, and reduce the lifespan of hydroponic systems (Lee et al. 2015). Biofilm formed by plant and human pathogens often causes root diseases in hydroponics (Sato et al. 2023) and pose food safety risks to humans once introduced (Li et al. 2019; Tham et al. 2024). Cells living in a biofilm become more resistant to environmental stressors and water treatments such as antibiotics and ultraviolet light (Elasri and Miller 1999; Lee et al. 2015; Li et al. 2019; O'Toole and Kolter 1998), thus creating challenges in biofilm control. Research has been conducted to develop efficient treatments to suppress biofilms in hydroponic systems, especially those with higher resistance (Lee et al. 2015; Tham et al. 2024). In some cases, biofilms formed by the beneficial rhizobacteria can increase crop salinity tolerance, promote plant growth, and suppress diseases (Sato et al. 2023). Commercial products comprising beneficial rhizobacteria, such as TerraBella (AquaBella Organics LLC, Sebastopol, CA, USA), are available on the market. TerraBella contains beneficial soil microbes and is marketed as a root inoculant for soil, soilless media, and hydroponics to increase crop yield and quality, promote nutrient availability and uptake, and enhance stress tolerance of crops.

For hydroponics with organic nutrient sources, however, the formation of biofilm by microbes plays a critical role in nutrient release (Garland et al. 1997). As mentioned, organic nutrients rely on these microbes for mineralization before becoming available to crops.

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Organic compounds inhibit the growth of bacteria involved in nitrogen mineralization such as *Nitrosomonas* spp. and *Nitrospira* spp. in organic hydroponics (Shinohara et al. 2011). For example, in hydroponic cultivation of tomato, fish-based organic fertilizer contained high ammonium concentrations that killed all tomato plants (Shinohara et al. 2011). With microbial inoculation, nitrate concentrations increased while ammonium was undetected, and tomato plants exhibited normal growth (Shinohara et al. 2011). Therefore, in organic hydroponics, microbes and biofilm are important components in crop production.

A previous study by our laboratory reported potential lettuce root asphyxiation caused by biofilm inside of propagation plugs in organic nutrient film technique (NFT) systems (Hooks et al. 2022). With continuous flow of the organic nutrient solution, lettuce showed stunted root development and poor overall growth. While supplying organic nutrient solutions 15 min per hour, crop performance significantly improved (Hooks et al. 2022). Low oxygen stress at the root zone is not typically a concern in an NFT system because only a thin film of nutrient solutions is presented at the bottom of the propagation plugs. It has been hypothesized that in organic NFT systems, the formation of biofilm in the propagation plugs competed with crop roots for oxygen and created low oxygen stress within the plugs. Intermittent flow schemes gave the roots a "dry" period, allowing air to enter the plugs and alleviating low oxygen stress (Hooks et al. 2022). Applying TerraBella inoculant to nutrient solution further promoted root growth by increasing nutrient mineralization and reducing phytotoxicity (Hooks et al. 2022). The previous study only observed improvement after implementing intermittent flow for 15 min per hour. We hypothesized that longer no-flow periods of organic nutrient solution flow would improve lettuce growth by reducing root zone stress, and that TerraBella inoculation would further enhance growth by promoting nutrient availability. The objective of this study was to investigate the effect of different regimens of intermittent flow with or without TerraBella inoculant on lettuce growth, mineral nutrition of plant tissue, and nutrient solutions in organic hydroponics.

Materials and Methods

Plant material and growth condition

Lettuce (cv. Casey; Osborne Quality Seeds, Mount Vernon, WA, USA) seeds were sown on two types of propagation plugs (described in the Propagation plugs section) that were placed in nursery trays and covered with humidity domes. Seedlings were propagated in an indoor vertical grow rack under white light-emitting diode lights (Arize® Life[2] 48-inch BRI; Current Lighting Solutions LLC, Greenville, SC, USA) at approximately 100 $\mu mol \cdot m^{-2} \cdot s^{-1}$ under a 24-h photoperiod. Four days after sowing, when most of the seeds had germinated, humidity domes were removed. Seedlings began receiving half-strength Hoagland solution (50 $mg \cdot L^{-1}$ N). Light intensity was increased to 200 $\mu mol \cdot m^{-2} \cdot s^{-1}$ at 5 d after sowing. At 14 d after sowing, seedlings were transplanted into six NFT systems (NFT 8-4 System; CropKing Inc., Lodi, OH, USA), where the treatments were applied. Each NFT system had a 94.6-L (25-gal) reservoir tank and four channels, with each channel holding 12 plants, resulting in a total of 48 plants per system.

The first replicate was transplanted to the NFT system on 29 May 2024, and it was harvested on 17 Jun 2024. The second replicate was transplanted on 10 Jul 2024, and it was harvested on 30 Jul 2024. Average day/night air temperatures in the greenhouse were 26.2 °C [standard deviation (*SD*), ±2.3 °C]/20.7 °C (*SD*, ±0.8 °C) for the first replication and 26.5 °C (*SD*, ±2.2 °C)/20.6 °C (*SD*, ±0.3 °C) for the second replication. The daily light integral (DLI) values were 17.4 $mol \cdot m^{-2} \cdot d^{-1}$ (*SD*, ±7.5 $mol \cdot m^{-2} \cdot d^{-1}$) and 18.3 $mol \cdot m^{-2} \cdot d^{-1}$ (*SD*, ± 5.6 $mol \cdot m^{-2} \cdot d^{-1}$) for the first and second replications, respectively.

Treatments

Propagation plugs. Two propagation plugs, Grodan Rockwool A-OK Starter Plugs (referred to as Grodan plugs; B.V., Roermond, The Netherlands) and closed bottom organic plugs (CBOPs; RIOCOCO®/Ceyhinz Link Inc., Irving, TX, USA), were used. Grodan plugs are made from rockwool. The CBOPs are compressed coconut coir disks that expand upon rehydration. Twenty-four plants of each plug type were transplanted to each NFT system. The physical properties of the two propagation plugs are presented in Table 1. The dry weight of propagation plugs was measured using a digital scale. Air volume in plugs was measured using the water placing method. The water holding capacity was measured by subtracting the plug dry weight from the weight of water-saturated plugs.

Table 1. Dry weight, volume when fully expanded, volume of air in fully expanded plugs, and water holding capacity per plug of Grodan plugs and closed bottom organic plugs (CBOPs).

Plug type	Grodan	CBOP
Dry weight (g)	1.5	2.7
Fully expanded volume (cm^3)	21.7	18.5
Air volume (cm^3)	3.6	3.5
Water holding capacity (g)	17.3	13.9

Nutrient flow schemes

One of the six NFT systems was used as the inorganic reference with Hoagland nutrient solution at 200 $mg \cdot L^{-1}$ N. The Hoagland solution contained the following (in $mg \cdot L^{-1}$): 29 phosphorus (P), 223 potassium (K), 123 calcium (Ca), 34 magnesium (Mg), 44 sulfur (S), 3.38 iron (Fe), 0.52 boron (B), 0.34 manganese (Mn), 0.19 zinc (Zn), 0.08 copper (Cu), and 0.06 molybdenum (Mo). The organic nutrient solution was prepared using Pre-Empt (Coastal Fertilizer & Supply Inc., Labele, FL, USA) at a rate of 15.9 $mL \cdot L^{-1}$ (2 oz/gal according to manufacturer recommendation). Based on the Pre-Empt fertilizer label, organic solutions contained (in $mg \cdot L^{-1}$) approximately 3.18 Ca, 3.18 Mg, 1.59 Fe, 1.59 Mn, 1.59 Zn, and 0.32 B. No N, P, and K contents were provided by the Pre-Empt label. Two of the five organic NFT systems were inoculated with TerraBella. The label of TerraBella indicates that it contains 80 million colony-forming units (CFUs; units/mL) of aerobic bacteria and 170 CFUs of anaerobic bacteria; however, no information regarding specific species of microorganisms was provided. Then, nutrient solutions were left uncovered for at least 24 h to release chlorine from tap water before TerraBella inoculation. TerraBella was inoculated to nutrient solution tanks at a rate of 3.13 $mL \cdot L^{-1}$ (0.4 oz/gal). The nutrient solution pH of all NFT systems was adjusted to 6.2 before transplanting, and it was adjusted to 6.5 weekly with pH Up and pH Down (General Hydroponics, Santa Rosa, CA, USA). The pH and EC of the nutrient solutions were measured daily on weekdays throughout the growth cycle using an EC/pH probe (HI98129; Hanna Instruments, Woonsocket, RI, USA).

The experiment was designed to test the effects of intermittent organic nutrient solution flow schemes on lettuce growth. One organic NFT system received a continuous flow of fertilizer solution (CF). As the inorganic reference, the NFT system with Hoagland fertilizer solution was supplied with continuous nutrient solution flow (inorganic). Two organic NFT systems were provided with intermittent flow: one received 15 min of flow/45 min of no flow (15/45), and the other received 15 min of flow/105 min of no flow (15/105). Organic nutrient solutions of the last two NFT systems were inoculated with TerraBella and had the same intermittent flow patterns (TB15/45 and TB15/105). These treatments are summarized in Fig. 1. This experiment followed a completely randomized design, and the six treatments were randomly assigned to the six NFT systems and repeated twice.

Changes in water of the propagation plugs were also quantified in response to the intermittent nutrient solution flow. Five days after transplanting, six plugs from the intermittent flow treatments (15/45, 15/105, TB15/45, and TB15/105) were weighed at the end of the flow and no-flow periods. Water loss during the no-flow cycle per plug was calculated by dividing the difference in weight by six. We assumed that plugs of continuous flow and inorganic treatments did not experience water loss because of the continuous solution flow.

Later in the growing cycle, the flow of 15/105 and TB15/105 were adjusted to meet the higher water demand of larger plants. In both replications, at 14 d after transplanting, the intermittent flow of 15/105 and TB15/105 were set to 15 min of flow/85 min of no flow.

Data collection

Top-view photos of plant canopies and side-view photos of roots in the channels in

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Treatment	2-hr cycle			
Inorg	120 min			
CF	120 min			
15/45	15 min	45 min	15 min	45 min
15/105	15 min	105 min*		
TB15/45	15 min	45 min	15 min	45 min
TB15/105	15 min	105 min*		

Fig. 1. Flow schemes of all treatments. The inorganic Hoagland nutrient solution (inorg) is shown in blue. The white background represents no flow during the time interval. Organic nutrient solution (Pre-Empt; Coastal Fertilizer & Supply Inc.) is shown in green. The organic nutrient solution of Pre-Empt inoculated with TerraBella (AquaBella Organics LLC) biostimulant is shown as pale pink.

NFT systems were obtained using a Pixel 4 cellphone (Google LLC, Mountain View, CA, USA). Top-view photos were obtained at 1, 5, 8, and 15 d after transplanting, while root photos were obtained at 16 d after transplanting. The canopy area was measured using ImageJ (National Institutes of Health, Bethesda, MD, USA). The soil plant analysis development (SPAD) index of four plants per plug type per NFT system was obtained using a SPAD 502 meter (Minolta Co. Ltd., Osaka, Japan).

Nineteen days after transplanting, lettuce shoots were harvested, and their fresh weight was measured using a digital scale. Leaf area was measured by a leaf area meter (LI-3100C; LI-COR Biosciences, Lincoln, NE, USA). Then, shoots were dried in a drying oven at 80 °C for at least 72 h before dry weight was measured. Plugs and roots were collected and stored at 4 °C in sealed plastic bags before root wash. Root wash was completed within 1 week of harvest. Root tissues were similarly dried at 80 °C in the drying oven for at least 72 h before root dry weight was measured. The root-to-shoot ratio was calculated as the root dry weight divided by shoot dry weight.

Nutrient solution samples were collected immediately before transplanting and after harvesting. Concentrations of nitrate-nitrogen (NO_3^--N), nitrite (NO_2^--N), ammonium (NH_4^+-N), and phosphorous (P) nutrient solution samples were measured using a benchtop spectrophotometer (model number: HI83200; Hanna Instruments).

Statistical analysis

An analysis of variance (ANOVA) was conducted using SAS OnDemand for Academics (SAS Institute Inc., Cary, NC, USA). Mean separations were performed using Student's *t* test at $\alpha = 0.05$.

Stepwise regression analyses were performed to test the effects of independent variables on shoot fresh weight and root dry weight using SAS OnDemand after addressing the multicollinearity among these independent variables. We found high multicollinearity among independent variables; therefore, we assessed the variance inflation for all variables and removed the variable if variance inflation was >10, which indicated multicollinearity. The phosphorus concentration in freshly made nutrient solutions before transplanting and nitrate-N and ammonium-N concentrations in nutrient solutions at harvest were not included in the stepwise regression analysis because of their high variance inflation. The following were used to construct models in stepwise regression analyses: plug type; length of the no-flow period; TerraBella inoculation; change in water weight in plugs during the no-flow period; nitrate-N, nitrite-N, and ammonium-N concentrations in freshly made nutrient solutions before transplanting; and nitrite-N and phosphorus concentrations in nutrient solutions at harvesting.

Results

Nutrient solution dynamics at the root zone

Water loss from plugs was affected by flow schemes and plug type (Fig. 2). There was no significant difference in water weight in plugs among different flow schemes after the 15-min flow period ($P = 0.1247$ for CBOPs and $P = 0.4532$ for Grodan plugs), indicating that 15 min of nutrient solution flow was sufficient to fully hydrate propagation plugs. TerraBella inoculation did not significantly affect the water weight ($P = 0.63$). Plugs subjected to a 105-min no-flow period experienced greater water loss than those subjected to a 45-min no-flow period ($P = 0.02$) (Fig. 2A). The CBOPs lost water faster than Grodan plugs during the no-flow period ($P = 0.03$) (Fig. 2B).

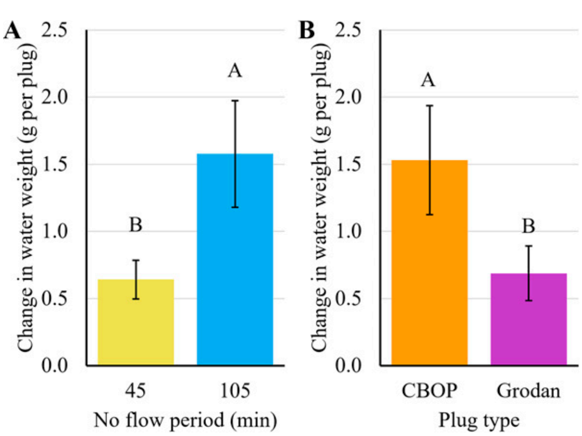

Fig. 2. Change of water weight in plugs was affected by the length of the no-flow period in intermittent flow (**A**) and the propagation plug type (**B**). No interaction was found between flow schemes and plug types. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$; n = 2). Error bars represent standard deviations. CBOP = closed bottom organic plug.

The pH of organic nutrient solutions fluctuated greatly at the beginning of the growth cycle; then, they fluctuated less dramatically later in the growth cycle (Supplemental Fig. 1A). However, pH of the inorganic nutrient solutions increased during the first week but decreased during the second week (Supplemental Fig. 1A). The EC of organic nutrient solutions increased throughout the growth cycle (Supplemental Fig. 1B). For the inorganic nutrient solutions, the EC began to decrease by 2 weeks after transplanting (Supplemental Fig. 1B).

At harvest at 23 d after transplanting, the nitrate-N concentration was highest with the continuous flow and 15/45 treatments and lowest with the inorganic, 15105, and TB15105 treatments, while it was intermediate with the TB15/45 treatment (Fig. 3A). We noticed that plants in the inorganic nutrient solutions (inorganic treatment) used more water than those in organic nutrient solutions (15/45, 15/105, TB15/45, and TB15/105), likely because of higher transpiration rates with larger sizes. We had to top-off the reservoir tank of the inorganic treatment with water during the second experimental repetition, although we did not quantify the exact amount of water removed from reservoir tanks. The water consumption and water use efficiency were not quantified, but the minimal solution level of 10 gal (37.9 L) was maintained throughout this study. The nitrite-N concentration was extremely low in the inorganic nutrient solution (1.0 ± 0.0 mg·L^{-1} N) (Fig. 3B). Among the organic nutrient solutions, the nitrite-N concentration was highest with the 15/45 treatment, followed by that with the continuous flow and TB15/45 treatments, and it was lowest with the 15/105 and TB15105 treatments (Fig. 3B). The ammonium-N concentration was also lowest with the inorganic nutrient solution (1.0 ± 1.2 mg·L^{-1} N). Among the organic nutrient solutions, the ammonium-N concentration was highest with the TB15/105 treatment, followed by that with the 15/45, 15/105, and TB15/45 treatments, and it was lowest with the continuous flow treatment (Fig. 3C). The phosphorus concentration was highest with intermittent flow schemes (15/45, TB15/45, 15/105, and TB15/105) at 463 ± 122 mg·L^{-1} (Fig. 3D), and it was lowest with the inorganic reference (12 ± 7 mg·L^{-1}) (Fig. 3D); however, it was intermediate with the continuous flow treatment (197 ± 81 mg·L^{-1}) (Fig. 3D). The ion concentration of freshly made nutrient solutions (immediately before transplanting) for each of the treatments is presented in Supplemental Fig. 2.

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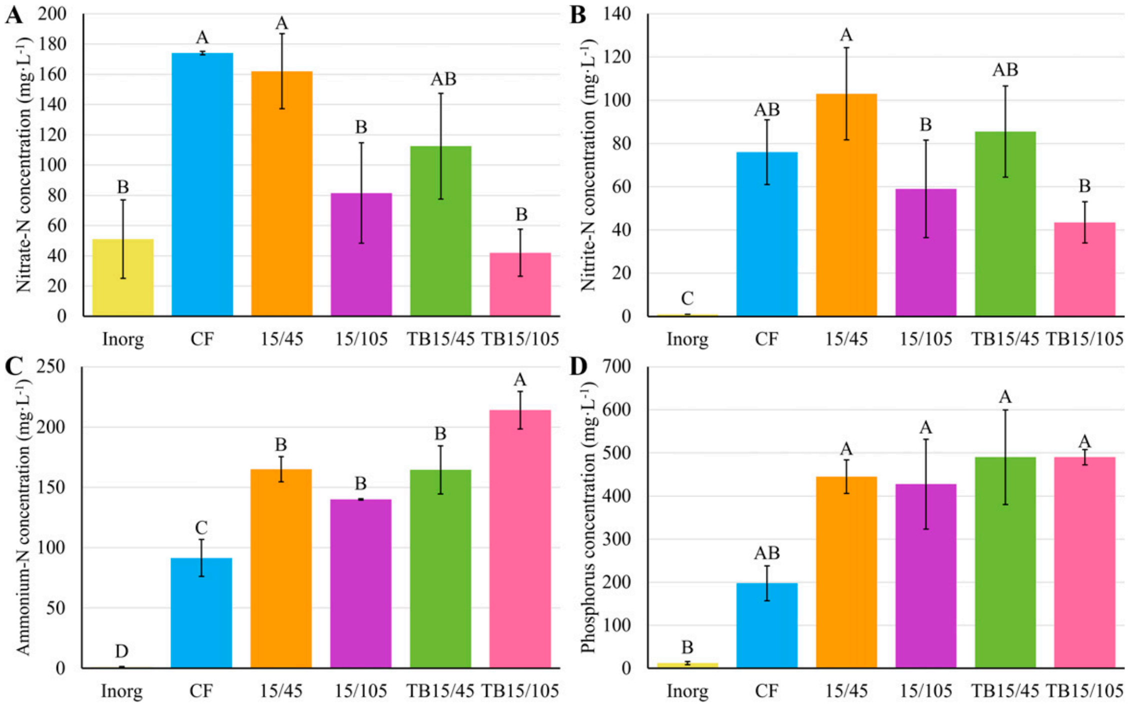

Fig. 3. Nitrate-N (**A**), nitrite-N (**B**), ammonium-N (**C**), and phosphorus (**D**) concentrations in nutrient solutions at harvest (23 d after transplant). Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$; n = 2). Error bars represent standard deviations. Treatment details can be found in Fig. 1.

Shoot growth and development

One day after transplanting, the average canopy area was 6.18 ± 0.33 cm^2 per plant and did not significantly differ among NFT systems (P = 0.78) or between plug types (P = 0.75). This indicated that uniform seedlings were selected for this study. The difference in canopy size appeared on 5 d after transplanting, with lettuce grown in the inorganic treatment having the largest canopy (17.8 ± 2.5 cm^2 per plant). Inorganic fertilizer resulted in the largest canopies throughout this study. Fifteen days after transplanting, among plants in organic nutrient solutions, continuous flow resulted in the smallest canopy, which was only 42.8% of Inorg (Figs. 4 and 5A). Intermittent flow increased the canopy size, with a longer no-flow period resulting in a larger canopy (Fig. 5A). However, TerraBella inoculation decreased canopy size compared with noninoculated treatments and resulted in canopy sizes similar to those of the continuous flow treatment (37.5% and 39.2% of Inorg) (Fig. 5A). Lettuce grown with CBOPs (52.5% of Inorg) had larger canopies than that grown with Grodan plugs (35.7%) (Fig. 5B).

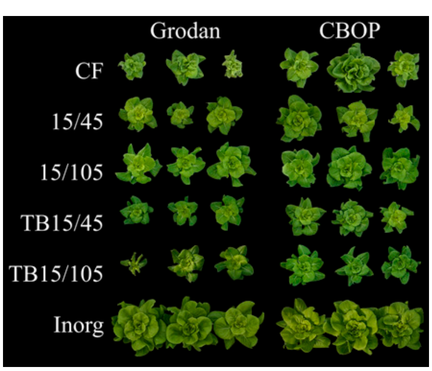

Fig. 4. Example top-view canopy photos of lettuce (cv. Casey) plants grown on inorganic nutrient film technique (NFT) hydroponic systems and on organic NFT systems with consistent or intermittent nutrient solution flow. Treatment details can be found in Fig. 1. CBOP = closed bottom organic plug.

Nineteen days after transplanting, the leaf area was unaffected by plug types (P = 0.07). With inorganic fertilizer, the lettuce leaf area was 1761.4 ± 692.8 cm^2 per plant. The leaf area was affected by flow schemes. The 15/105 treatment resulted in higher leaf area (44.0% of Inorg) than that with continuous flow treatment (25.8%), while 15/45 treatment (36.5%) resulted in intermediate leaf area (Fig. 6A). TerraBella inoculation resulted in leaf area similar to that resulting from continuous flow treatment (Fig. 6A). Shoot fresh weight showed a trend similar to that of leaf area and was highest with inorganic fertilizer (86.2 ± 38.5 g per plant). When grown in organic nutrient solutions, lettuce had the highest shoot fresh weight with 15/105 treatment (40.3% of Inorg), followed by 15/45 treatment (32.0%), while continuous flow treatment resulted in the lowest fresh weight (23.5%) (Fig. 6B). TerraBella inoculation decreased shoot fresh weight to a level similar to that of continuous flow treatment, regardless of the length of the no-flow period (both 24.0% of Inorg) (Fig. 6B). Shoot fresh weight was unaffected by plug type (P = 0.17). Shoot dry weight was highest in the 15/105 treatment (58.3% of Inorg) and lowest with TerraBella inoculation (40.8% and 41.8% for TB15/45 and TB15/105, respectively) (Fig. 6C). Lettuce grown in the continuous flow and 15/45 treatments had intermediate shoot dry weight (Fig. 6C). Shoot dry weight was also affected by plug type, with CBOPs resulting in higher shoot dry weight (54.4% of Inorg) than that associated with Grodan plugs (39.4%) (Fig. 6D).

A stepwise regression analysis showed that shoot fresh weight in organic NFT systems was positively correlated with the length of the no-flow period and average pH of the nutrient solution, and it was negatively correlated with TerraBella inoculation and Grodan plugs (R^2 = 0.58; P = 0.0079). Stepwise regression also determined that shoot fresh weight was unaffected by the change in water weight in plugs during flow schemes (P = 0.72), nitrite-N (P = 0.83), ammonium-N (P = 0.66), and phosphorus concentrations (P = 0.65) in nutrient solutions at harvest.

Lettuce grown in the inorganic nutrient solutions had a lower SPAD index (25.3 ± 1.3) than that of lettuce grown in the organic nutrient solutions (40.0 ± 4.7) (Fig. 7), which implied that organic nutrient solutions resulted in greener plants compared to inorganic nutrients.

Root growth

Root growth was highest with inorganic nutrient solutions (1.35 ± 1.02 g per plant) (Fig. 8). Among the organic treatments, the 15/105 treatment resulted in the highest root dry weight (45.5% of Inorg) (Fig. 9). Both more frequent nutrient solution flow (continuous flow and 15/45 treatments) and TerraBella inoculation (TB15/45 and TB15/105) resulted in lower root dry weights compared

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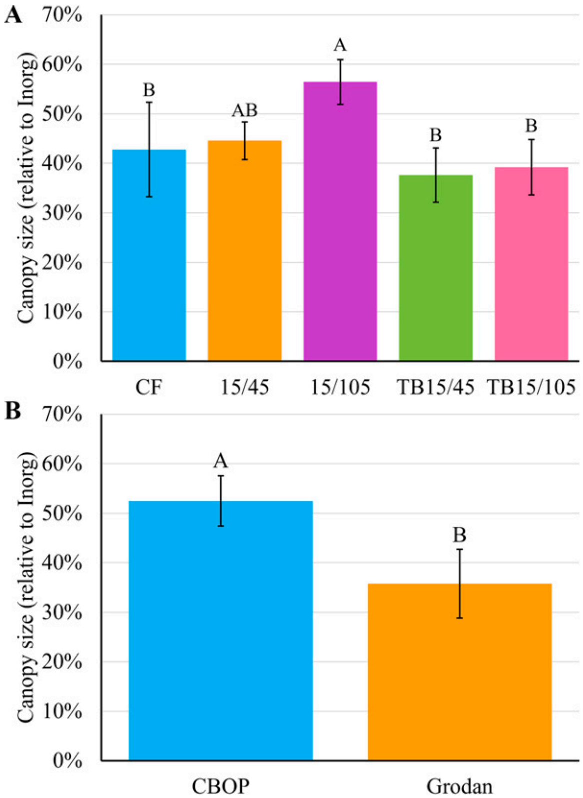

Fig. 5. Projected canopy size of 'Casey' lettuce grown in nutrient film technique (NFT) systems on day 15 was affected by organic nutrient solution flow schemes (**A**) and plug types (**B**). No interaction was found between flow schemes and plug types. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$; n = 2). Error bars represent standard deviations. Treatment details can be found in Fig. 1. CBOP = closed bottom organic plug.

with that associated with the 15/105 treatment (Fig. 9). Interestingly, root growth was unaffected by plug type ($P = 0.2803$).

A stepwise regression analysis suggested that the root dry weight was positively correlated with the length of the no-flow period and negatively correlated with TerraBella inoculation ($R^2 = 0.51$), and it was not correlated with plug types ($P = 0.76$), pH ($P = 0.13$), the weight of water change in plugs ($P = 0.23$), or the concentrations of nitrite-N ($P = 0.82$), ammonium-N ($P = 0.32$), and phosphorus ($P = 0.21$) in nutrient solutions at harvest.

Shoot mineral concentration

The shoot nitrogen concentration was higher with Grodan plugs (6.4% ± 0.6%) than with CBOPs (5.8 ± 0.6%), but it was unaffected by the flow scheme or TerraBella inoculation. The shoot phosphorus concentration was highest with the TB15/105 (1.7% ± 0.6%) and 15/105 (1.3% ± 0.5%) treatments, lowest with inorganic (0.7% ± 0.0%) and continuous flow (0.7% ± 0.1%) treatments, and intermediate with TB15/45 (1.2% ± 0.4%) and 15/45 (1.1% ± 0.3%) treatments (Fig. 10A). Shoot phosphorus was also lower when lettuce plants were grown with Grodan plugs (1.0% ± 0.3%) rather than with CBOPs (1.3% ± 0.6%) (Fig. 10B). The shoot potassium concentration was higher with inorganic nutrient solutions (8.4% ± 2.6%) than with organic nutrient solutions (5.5% ± 0.7%), regardless of the flow scheme or TerraBella inoculation. Lower calcium and magnesium concentrations in shoot tissues were observed with organic nutrient solutions than with the inorganic reference (Fig. 10C and 10D). No effects of plug type on shoot calcium and magnesium concentrations were detected ($P = 0.29$ and $P = 0.54$, respectively).

Discussion

In this study, the yield of 'Casey' lettuce was significantly lower in organic nutrient solutions compared with that in inorganic nutrient solutions (Figs. 5 and 6B). Intermittent flow of organic nutrient solutions increased both shoot and root growth (Figs. 6 and 9), with the longest no-flow period (105 min of no flow over 120 min) resulting in the greatest growth.

Lower yield in organic agriculture systems compared with those in inorganic systems have been observed among many food crops (Heeb et al. 2005, 2006; Mäder et al. 2002; Pang and Letey 2000; Ponisio et al. 2015; Seufert et al. 2012; Zandvakili et al. 2019). In hydroponics production, similar yield reduction has been reported in organic hydroponics and in inorganic hydroponics (El-Shinawy et al. 1997; Kechasov et al. 2021; Tikasz et al. 2019; Williams and Nelson 2014). Several factors have potentially contributed to the lower yield with organic NFT systems observed in this study.

Root asphyxiation

In hydroponics, sufficient oxygen supply to the roots is important to support root respiration for nutrient uptake and assimilation, plant growth, and disease resistance (Herzog et al. 2016; Roosta 2024). In NFT systems, the root zone oxygen supply is typically not a limiting factor because part of the root zone is exposed to air (Fig. 8). However, a previous study conducted in our laboratory suggested that the formation of biofilm and consequently, a low oxygen level inside CBOPs, resulted in poor root development and reduced overall growth of lettuce 'Red Mist' in organic NFT systems (Hooks et al. 2022).

Labile organic matter supplies easily available energy source for microbes, promoting microbial growth and biofilm formation (Burford and Bremner, 1975; Szekely and Jijakli 2022). In this study, organic NFTs exhibited nitrite-N production during the growth cycle and formation of biofilm in reservoir tank and root channels (Figs. 3 and 8, Supplemental Fig. 3), indicating significant microbial activity. Biofilm could coat the roots and proliferate within the air spaces inside the propagation plugs (Hooks et al. 2022), and it competes with the root for oxygen both through occupying air space physically and possibly through high biological activity. Thick biofilm can cover tomato roots grown in organic fertilizer derived from fish broth (Sato et al. 2023). Biofilm coating on lettuce roots was similarly observed in this study, particularly when grown with continuous organic nutrient flow (Fig. 8B). The growth and biological activity of these microbes create high oxygen demand and compete with crop roots (Carballo et al. 2009). It is possible that biofilm actively decomposes labile organic compounds and consumes oxygen; therefore, it reduces oxygen availability to the roots in saturated CBOPs.

Intermittent organic nutrient flow potentially improved crop performance by alleviating low oxygen stress in the root zone (Hooks et al. 2022). During the no-flow period, water drained out of plugs, allowing air to enter and providing more oxygen to roots. Therefore, intermittent flow, especially with a longer no-flow period (15/105), increased lettuce canopy size, yield, shoot dry weight, and root dry weight (Figs. 4–6, 8, and 9). Also, CBOPs lost more water than Grodan plugs during the no-flow period (Fig. 2B) and provided more air to lettuce roots for respiration under intermittent flow schemes. As a result, greater canopy size (Figs. 4 and 5B) and shoot dry weight (Fig. 6D) were observed in lettuce grown in CBOPs. However, TerraBella inoculation of organic nutrient solutions potentially intensified the oxygen competition between microorganisms and roots and had a negative impact on crop growth (Figs. 6 and 9).

Accumulation of phytotoxic compounds

High concentrations of nitrite and ammonium were observed in organic nutrient solutions at harvest (Fig. 3) and were associated with the low oxygen condition discussed

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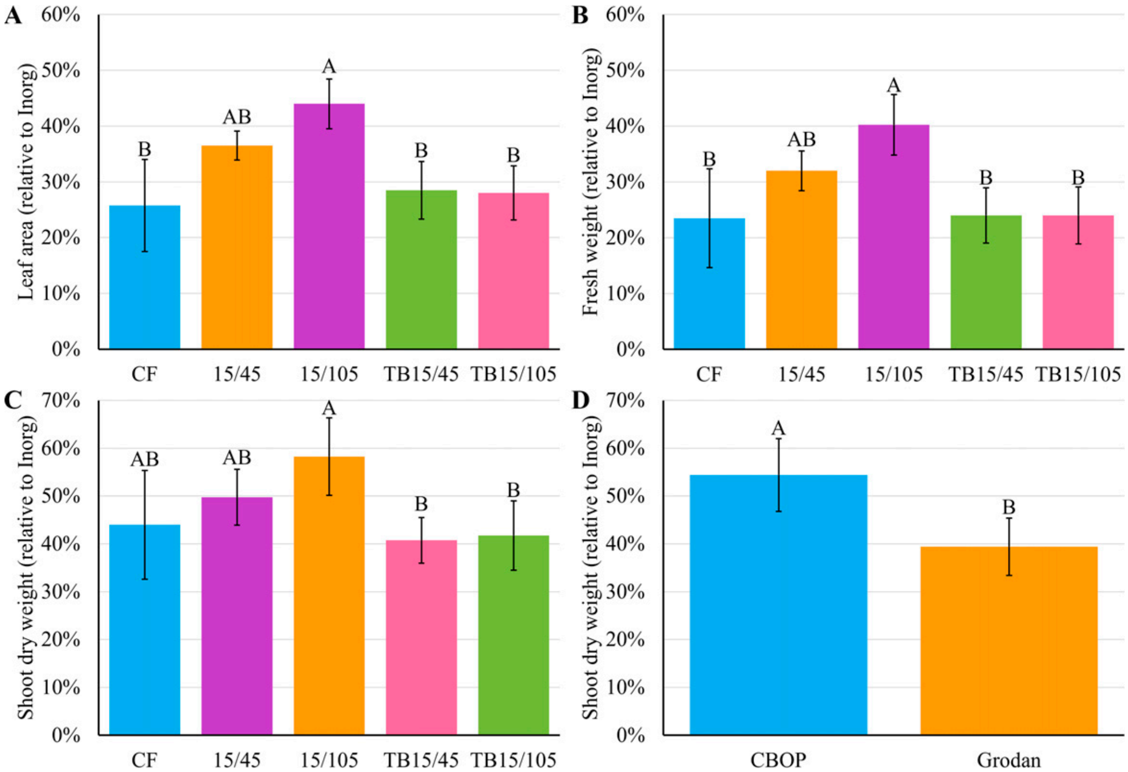

Fig. 6. Effects of organic nutrient solution flow schemes on leaf area (**A**), shoot fresh weight (**B**), and shoot dry weight (**C**) of 'Casey' lettuce and effects of plug type on shoot dry weight (**D**). No interaction was found between flow schemes and plug types. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$, n = 2). Error bars represent standard deviations. Treatment details can be found in Fig. 1. CBOP = closed bottom organic plug.

previously. Nitrite accumulation often occurs with the presence of organic matter under low oxygen and/or high pH conditions in both fields (Hamilton and Lowe 1981; Oke 1966) and hydroponics (Szekely and Jijakli 2022). Plants are highly sensitive to nitrite. A nitrite concentration as low as 5 $mg \cdot L^{-1}$ N can negatively affect lettuce shoot and root growth in hydroponic systems (Hoque et al. 2007). Plant height, leaf number, and total dry matter of both romaine and iceberg lettuce linearly decreased with nitrite concentrations up to 40 $mg \cdot L^{-1}$ N (Hoque et al. 2007). Chlorosis induced by nitrite toxicity was observed in both leaf and roots (Hoque et al. 2007). In this study, smaller shoot and root sizes and root discoloration were similarly observed in organic NFT (Figs. 5, 6, and 8). Nitrite concentrations in organic nutrient solutions ranged from 43 to 103 $mg \cdot L^{-1}$ N (Fig. 3B); this range was well above the levels that cause toxicity to lettuce roots.

High ammonium-N concentrations were also observed in our study at harvest (Fig. 3C). Ammonium at a high concentration causes leaf chlorosis and stunted growth, inhibits nutrient uptake, and causes yield reduction (Britto and Kronzucker 2002; Esteban et al. 2016; Roosta and Schjoerring 2007; Xiao et al. 2023). The exact mechanism of ammonium toxicity is not well understood (Britto and Kronzucker 2002; Esteban et al. 2016; Xiao et al. 2023). Many studies have shown that a high concentration of organic fertilizers is detrimental to crop yield in hydroponics, which was attributed to a high ammonium concentration (Carballo et al. 2009; Krishnasamy et al. 2012; Liedl et al. 2004; Mupambwa et al. 2019; Phibunwatthanawong and Riddech 2019; Szekely and Jijakli 2022). For example, the organic nutrient solution (made with cow manure extract) with 285.1 $mg \cdot L^{-1}$ ammonium-N (366.6 $mg \cdot L^{-1}$ ammonium) resulted in death of all hydroponic lettuce and kale (Tikasz et al. 2019). Both lettuce and kale were able to survive at 172.6 $mg \cdot L^{-1}$ N ammonium, but they experienced stunted growth, with lettuce being more sensitive than kale (Tikasz et al. 2019). In our study, the highest ammonium-N concentration at harvest was found with the TB15/105 treatment (214 $mg \cdot L^{-1}$ N), followed by other intermittent flow treatments (15/45, 15/105, and TB15/45: 156 $mg \cdot L^{-1}$ N), while continuous flow treatment had the lowest ammonium concentration (91 $mg \cdot L^{-1}$ N) (Fig. 3C). It is not clear why different flow schemes resulted in different ammonium concentrations. Based on the existing literature, the ammonium concentration range is very likely to induce ammonium toxicity (Tikasz et al. 2019).

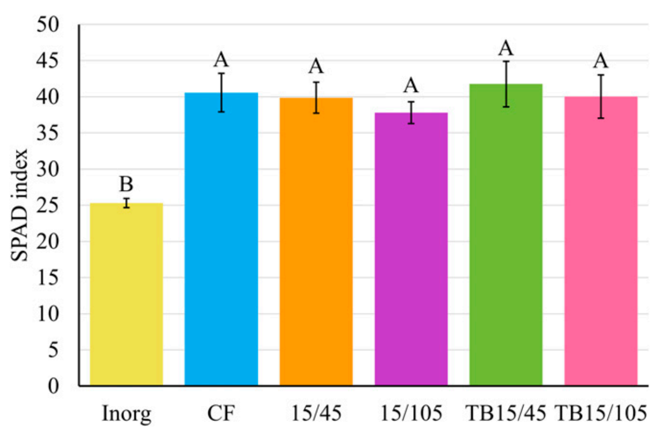

Fig. 7. Soil plant analysis development (SPAD) index of 'Casey' was affected by nutrient solution type (inorganic vs. organic) but not by flow scheme or TerraBella inoculation. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$; n = 2). Error bars represent standard deviations. Treatment details can be found in Fig. 1.

The high concentrations of both nitrite and ammonium ions in organic nutrient solutions likely imposed stress on lettuce roots. Roots with continuous nutrient flow mostly

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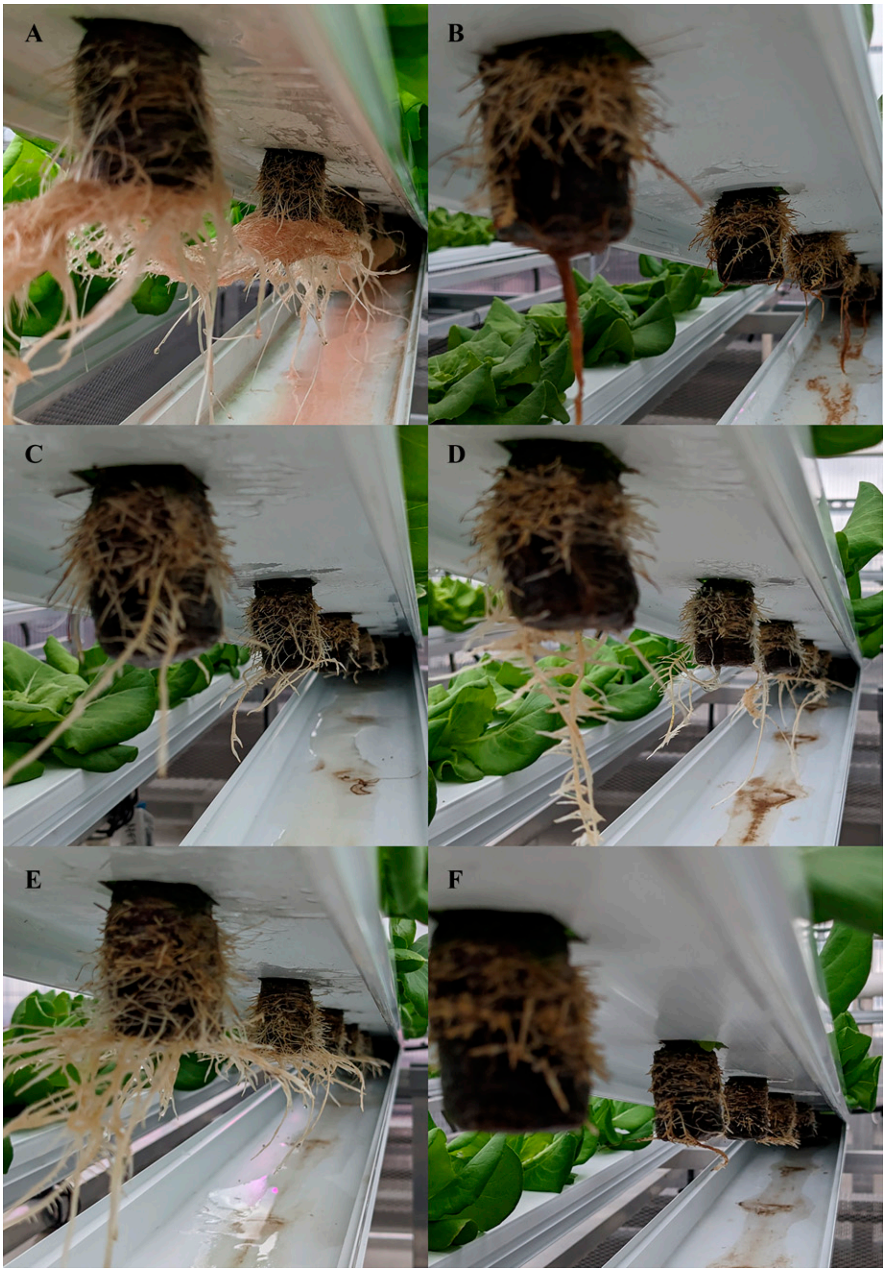

Fig. 8. Representative side-view root photos of lettuce (cv. Casey) grown in closed bottom organic plugs (CBOPs) in nutrient film technique (NFT) hydroponic systems 16 d after transplanting. Roots shown were subjected to inorganic (**A**), continuous flow (CF) (**B**), 15/45 (**C**), TB15/45 (**C**), 15/105 (**E**), and TB15/105 (**F**) treatments. Treatment details can be found in Fig. 1.

clustered on the upper portion of propagation plugs and avoided the portion that was in constant contact with nutrient solutions (Fig. 8B). Stepwise regression analyses suggested that both shoot and root growth with organic nutrient solutions were unaffected by nitrite-N and ammonium-N concentrations, but they were positively correlated with the length of the no-flow period. The no-flow period might temporarily remove nitrite and ammonium toxicity in the root zone, thus promoting the root growth, especially at the lower portion of propagation plugs (Fig. 8C and 8E). With a longer no-flow period, roots experienced lower nitrite and ammonium toxicity overall, thus exhibiting better growth (Figs. 8 and 9), particularly when concentrations of nitrite-N and ammonium-N were high in all organic solutions (Fig. 3B and 3C). However, nitrite and ammonium toxicity alone could not explain the lower shoot and root growth of TB15/45 and TB15/105 compared with that of 15/105 in our study (Figs. 4–6, 8, and 9). Root asphyxiation likely remains an important factor.

Unbalanced nutrient availability

A major challenge of organic fertilizers is providing sufficient nutrients to support crop growth without inducing toxicity and stress (Alneyadi et al. 2024; Bergstrand et al. 2020; Liu et al. 2024; Szekely and Jijakli 2022). Organic fertilizers, depending on their sources and process methods, contain a wide range of nutrient concentrations and forms of nutrients. The release of nutrients from the organic form often requires microbial activity, thus potentially causing a low nutrient supply that does not meet the crop's demand (Burnett et al. 2016; Liu et al. 2024; Pang and Letey 2000; Timsina 2018; Zikeli et al. 2017).

With intermittent nutrient solution schemes, it is intuitive to hypothesize that with longer no-flow periods, roots had less time to stay in contact with nutrients in solutions and take up lesser amounts. However, with our study, the longer no-flow period (15/105) resulted in better shoot and root growth as well as a higher shoot phosphorus concentration compared with the continuous flow scheme (Figs. 4, 5A, 6B, 6C, 8, 9, and 10A). It is possible that the better oxygen supply at the root zone in the 15/105 treatment improved root respiration and, thus, nutrient uptake. Nevertheless, shoot phosphorus concentrations in all treatments were above 0.7%, which is considered "high" for Boston lettuce (Hochmuth et al. 2012). Shoot nitrogen and potassium concentrations were unaffected by flow schemes and were both within or above the "adequate" range for Boston lettuce (Hochmuth et al. 2012). With Pre-Empt, the organic fertilizer used in our study, nitrate and phosphorus in nutrient solutions were above 40 and 190 $mg{\cdot}L^{-1}$, even at harvest (Fig. 3A and 3D). However, some evidence suggested that microbial biofilm on the root surface can create a strong nitrogen sink that competes with crops through denitrification (Burford and Bremner 1975; Finger and Strayer 1994; Szekely and Jijakli 2022). Denitrification is particularly strong under anaerobic conditions and is more likely to occur in the root zone of the continuous flow treatment in this study. Therefore, it is possible that lettuce in the continuous flow treatment experienced localized nitrogen deficiency in the root zone, resulting in lower growth compared with the intermittent flow schemes, despite the relatively high nitrate-N concentration in nutrient solutions in reservoir tanks. It would be interesting to collect nutrient samples from root channels and quantify nutrient concentration as well. We plan to incorporate this measurement in a future study.

Shoot calcium (0.29%) and magnesium (0.17%) concentrations of lettuce with organic nutrient solutions were significantly lower than those with inorganic nutrient solutions (1.23% and 0.35%, respectively) (Fig. 10C and 10D); all of these values were lower than the "adequate" calcium and magnesium ranges for Boston lettuce (2.0% and 0.6%, respectively), as proposed by Hochmuth et al. (2012). Calcium is important in cell wall synthesis, cell division, and signal transduction, while magnesium is an essential element in photosynthesis and enzyme activation. Low calcium and magnesium supplies likely limit crop growth and development. Organic nutrient solutions in our study had significantly lower calcium and magnesium concentrations (both were 3.18 $mg{\cdot}L^{-1}$ based on fertilizer label) than the recommended ranges of 200 to 300 $mg{\cdot}L^{-1}$ for calcium and 30 to 50 $mg{\cdot}L^{-1}$ for magnesium

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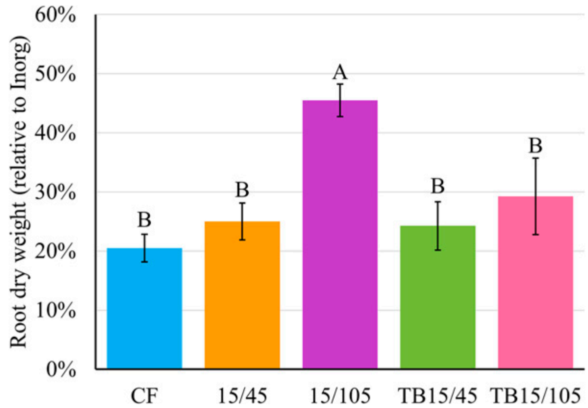

Fig. 9. Root dry weight of 'Casey' lettuce as affected by the organic nutrient flow scheme and TerraBella inoculation. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$; n = 2). Error bars represent standard deviations. Treatment details can be found in Fig. 1.

(Jones 2014). Furthermore, a high ammonium concentration was shown to inhibit calcium and magnesium uptake by plants (Bonomelli et al. 2021; Hernández-Gómez et al. 2015; Roosta and Schjoerring 2007). Although there were trends of higher shoot calcium and magnesium concentrations with a longer no-flow period, these trends were not statistically significant (Fig. 10C and 10D). A better oxygen supply at the root zone with intermittent flow did not compensate for the extreme low calcium and magnesium supplies. Low calcium and magnesium supplies from organic fertilizers were previously reported (Alneyadi et al. 2024; Liedl et al. 2004; Wang et al. 2019). These results call for calcium and magnesium fortification of organic hydroponics with Pre-Empt fertilizer.

Comparison with previous research

In this study, organic hydroponics produced lower lettuce yield compared with inorganic hydroponics (Figs. 5 and 6B). Although lettuce grown in organic nutrient solutions had high SPAD values (Fig. 7), which indicate darker green color and better appearance, fresh weight was less than half that of lettuce grown with inorganic nutrient solutions (Fig. 6B). The following several factors potentially contributed to the yield reduction with organic nutrient solutions: root asphyxiation, accumulation of phytotoxic compounds, and unbalanced nutrient availability. The intermittent flow scheme of organic nutrient solutions is likely to alleviate root zone stresses imposed by a low oxygen supply and ammonium and nitrite toxicity, but it could not address calcium and magnesium deficiency in organic nutrient solutions. However, a previous study reported comparable lettuce yields between organic solutions with intermittent flow and TerraBella inoculation and inorganic nutrient solutions (Hooks et al. 2022). The different results in this study are potentially attributed to the following three reasons: higher organic fertilizer rates in our study; different lettuce cultivars; and variations in TerraBella application rates.

In this study, we used Pre-Empt as the organic nutrient source, and nutrient solutions were created at a rate of 15.9 $mL \cdot L^{-1}$. In the previous study, Pre-Empt was used at a rate of 10 $mL \cdot L^{-1}$ (Hooks et al. 2022). As a result, the organic nutrient solutions in this study experienced great fluctuations in pH compared with those in the previous study (Supplemental Fig. 1) (Hooks et al. 2022). It is also likely that organic nutrient solutions in this study had greater microbial growth and activity as well as higher ammonium and nitrite production, which imposed greater stress on lettuce and significantly reduced lettuce yield. Also, we used Boston-type lettuce 'Casey' in this study, which possibly has low stress resistance compared with that of 'Red Mist' that was used in the previous study (Hooks et al. 2022). TerraBella is a root inoculant that improves nutrient uptake and disease resistance of crops in soil, soilless medium, and hydroponics. TerraBella contains a blend of beneficial aerobic and anaerobic microbes. The TerraBella inoculation rate in this study was 3.13 $mL \cdot L^{-1}$ (approximately 3.34 $g \cdot L^{-1}$), which was significantly higher than the rate of 50 $mg \cdot L^{-1}$ used by Hooks et al. (2022). The high TerraBella inoculation rate in our study

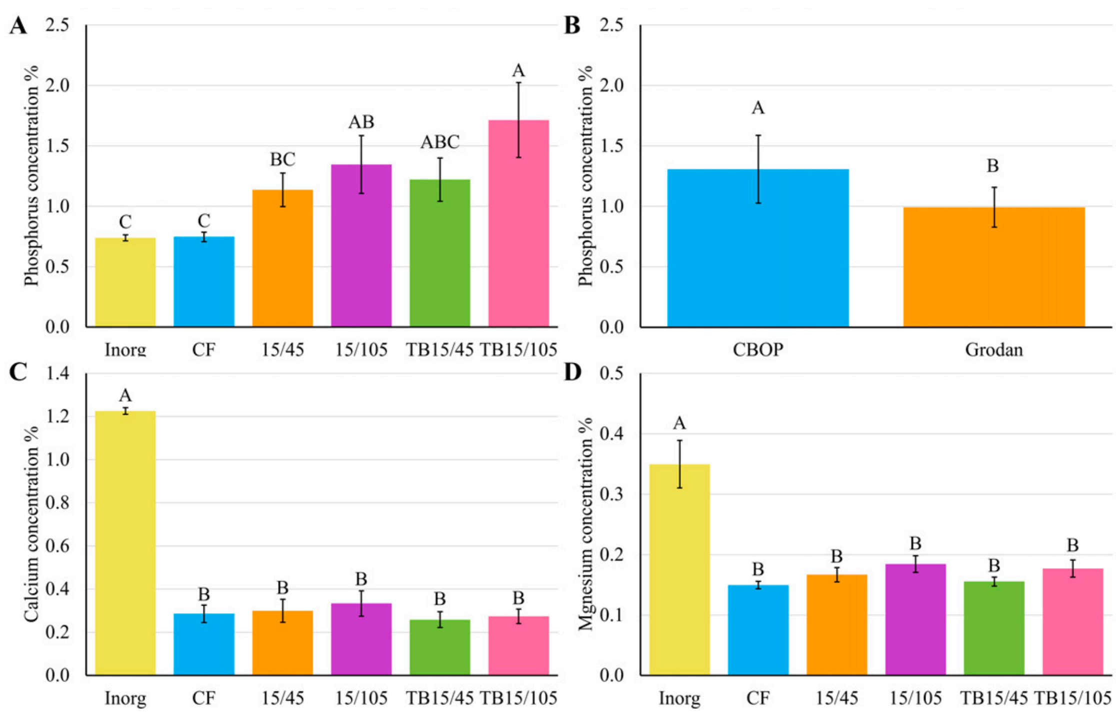

Fig. 10. Shoot phosphorus (**A** and **B**), calcium (**C**), and magnesium (**D**) concentrations were affected by flow schemes (**A, C, and D**) or plug type (**B**). No interaction was found between flow schemes and plug types. Bars labeled with different letters indicate significant differences between treatments ($P < 0.05$; n = 2). Error bars represent standard deviations. Treatment details can be found in Fig. 1. CBOP = closed bottom organic plug.

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may have contributed to the low oxygen stress discussed in the Root asphyxiation section, thus potentially explaining the different results between the two studies. It is also possible that the variations in microbial species or activity existed in TerraBella used between the two studies.

Throughout this study, we found parallels between organic hydroponics and aquaponics as well as digestion of organic waste. All three systems face challenges such as ammonium and nitrite production and slow nutrient release from organic forms. Drawing upon established practices in aquaponics and digestion of organic waste, we propose that potential methods to improve the efficiency of organic fertilizers in hydroponics should include aerating nutrient solutions similar to aerated digestion of organic waste and incorporating biofilters similar to those in aquaponics.

Conclusion

This study demonstrated that intermittent nutrient flow in organic hydroponics can reduce root zone stress, such as toxicity caused by nitrite and ammonium and low oxygen, and increase both root and shoot growth in lettuce. This practice also provides opportunities for energy-saving in hydroponics. However, challenges in managing organic nutrient solutions, such as low calcium and magnesium contents and insufficient nitrogen mineralization, remain. More research is needed to overcome these challenges and improve productivity of organic hydroponic systems.

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