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Nutrient Cycling with Duckweed for the Fertilization of Root, Fruit, Leaf, and Grain Crops: Impacts on Plant–Soil–Leachate Systems

Carlos R. Fernandez Pulido [†,‡], Pandara Valappil Femeena and Rachel A. Brennan *

Department of Civil and Environmental Engineering, The Pennsylvania State University, 212 Sackett Building, University Park, PA 16802, USA; crfp@agro.au.dk (C.R.F.P.); fpp5057@psu.edu (P.V.F.)
* Correspondence: rab44@psu.edu
† This work is based in part on the M.S. thesis of first author Carlos Rolando Fernandez Pulido in the Environmental Engineering program at The Pennsylvania State University.
‡ Current address: Department of Agroecology, Aarhus University, 8830 Tjele, Denmark.

Abstract: The increasing energy required to synthesize inorganic fertilizers warrants more sustainable soil amendments that produce comparable crop yields with less environmental damage. Duckweed, a prolific aquatic plant, can not only sequester carbon dioxide through photosynthesis, but also hyperaccumulate nutrients from its environment and upcycle them into valuable bioproducts. In this study, dried duckweed, grown on treated wastewater treatment plant effluent, was utilized as a fertilizer for a variety of crops (beet, tomato, kale, and sorghum). Comparative experiments examined the effect of duckweed, inorganic fertilizer, and a 40–60 mix of both on crop yield and nutrient fate in the plants, soil, and leachate. Comparable yields of beet, tomato, and sorghum were generated with duckweed and inorganic fertilizer. Duckweed significantly enhanced phosphorus (P) uptake in sorghum, exhibiting a P use efficiency level of 18.48%, while the mix treatment resulted in the highest P use efficiencies in beet and tomato. Duckweed-amended beet and kale systems also increased residual soil N (0.9% and 11.1%, respectively) and carbon (4.5% and 16.6%, respectively). Linear regression models developed using the data collected from all crops confirmed that duckweed can be used as a substitute for inorganic fertilizer without negative effects to food yield or nutritional quality.

Keywords: duckweed; organic fertilizer; nutrient cycling; soil fertility; fertilization; nutrient leaching

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1. Introduction

By 2050, the global demand for food production is expected to have increased by more than 70% of its 2009 levels, primarily driven by the dramatic rise in population [1]. With escalating food demand, the overall requirement for three major fertilizers—nitrogen (N), phosphorous (as phosphate, P_2O_5), and potassium (as potash, K_2O)—was predicted to rise from 185.1 million tons in 2016 to 200.9 million tons in 2022 [2]. The Haber–Bosch process for the production of synthetic ammonia (NH_3)—an expensive and energy-intensive procedure—consumes almost 1% of the total energy produced worldwide and accounts for 1.4% of global carbon dioxide emissions [3].

In addition to the high cost, a notable aspect of fertilizer use is the range of environmental impacts arising from its mismanagement and excessive application. The majority of inorganic fertilizers are applied to fields in a mineral form so that they are readily available to plants, a characteristic that also makes them more susceptible to being transported in solution to adjacent water bodies after heavy rain events. The application of inorganic fertilizers typically only results in a conversion of 50% of the fertilizer N into plant tissue, while the remaining 50% ends up in the water as either superficial or groundwater runoff, or in the atmosphere as nitrous oxide (N_2O) or N_2 [4]. Excessive N and phosphorus (P) released from agricultural fields feeds algal blooms and is one of the primary causes of eutrophication, ultimately leading to detrimental outcomes on aquatic and human life [5]. Microbial breakdown of synthetic fertilizers further fuels global warming via excessive N_2O emissions, with over 20% of worldwide agricultural greenhouse gases coming from fertilizers alone [6,7]. Solutions to overcome these challenges include the following: (a) devising improved fertilization techniques that favor higher nutrient use efficiency; and (b) finding alternative plant-based fertilizers that have minimal impacts on water resources and the environment as a whole.

The application of organic fertilizers such as compost and animal manure are regaining popularity as a more sustainable approach to mitigate the environmental impacts of using conventional synthetic fertilizers [8]. Contrary to fertilizers applied in mineral form, they provide a slow release of nutrients, converting organic N into ammonium (NH_4^+) and nitrate (NO_3^-) over time. In addition, they also contribute to increasing organic matter and micronutrients such as copper (Cu), zinc (Zn), calcium (Ca), and magnesium (Mg), among others, which improve the soil's structure, microbial diversity, and water and nutrient retention capacity [9,10]. Although an environmentally friendly option, these organic amendments are typically only used to complement traditional synthetic fertilizers due to the higher costs associated with manure management and their lower nutrient availability compared to inorganic alternatives [11].

Another environmental challenge aggravated by population growth is wastewater discharges. Of the 380 billion m^3 of municipal wastewater generated annually, over 80% is discharged into the environment without adequate treatment [12]. This, along with the agricultural runoff described previously, creates a dual risk to health and economic development, especially in low-income countries with limited wastewater treatment technologies. Existing urban and agricultural waste management systems currently follow a linear economy whereby waste nutrients are lost (or released into the environment) at the end of the cycle [13]. With the increased push towards sustainable agricultural production, there is a need to transition toward a circular bioeconomy, which offers an eco-friendly pathway for the beneficial reuse and recycling of nutrients and resources with co-generation of value-added products. One way to sustain this effort is to treat wastewater while generating a viable by-product capable of providing or supplementing the nutrients required for agricultural crops [14,15]. Considering that communities in developing countries with limited infrastructure contribute disproportionately to municipal and agricultural wastewater discharges, it is necessary to find broadly applicable and economically viable solutions to this problem.

Duckweed, a small aquatic plant, has been demonstrated to grow prolifically under a wide range of environmental conditions (pH from 3 to 7, and temperatures between 6 °C and 33 °C) [16,17]. Widely known for its phytoremediation capability, duckweed has been extensively studied for its ability to capture nutrients from different kinds of wastewater. Its rapid doubling time (between 2 to 4 days) and ease of harvesting make this hyperaccumulator an appealing candidate for use in sustainable wastewater treatment and nutrient recovery [16,18]. Lately, increasing attention has been drawn towards duckweed's varied applications as a fertilizer [19,20], animal feed [21], biofuel feedstock [22,23], and human food [24].

In the farming sector, utilizing wastewater-grown duckweed as a fertilizer creates an opportunity for implementing circularity in agriculture. However, limited studies have been conducted to assess the effect of duckweed fertilization on different crops, with work generally focusing on a single crop such as rice or sorghum. For example, studies have shown that cultivating duckweed in floodwaters of paddy fields can effectively decrease NH_3 volatilization, enhance N bioavailability, and consequently boost crop yields while reducing ammonia emissions by up to 55% compared to conventional fertilizers or wastewater alone [25–28]. A few field studies have examined duckweed as an alternative amendment for growing sorghum [19,20]. Duckweed-amended sorghum plots were able to retain 30% more total mineral N compared to plots receiving diammonium phosphate (DAP), while leaching 22% less phosphate than DAP and still achieving a comparable sorghum yield [20]. The overarching goal of this study is therefore to directly compare synthetic fertilizers and duckweed amendments to understand the similarities and differences in their respective impacts on the yields of different types of crops (root, leaf, fruit, and grain), and the resulting crop nutrient (N and P) characteristics, soil health (as residual soil nutrients), and nutrient loss to the environment. We also examine the corresponding effects of using a mixed fertilization option, combining duckweed and inorganic fertilizers (in a 40–60% proportion). By conducting and modeling experiments on different types of crops, this work performs the following: (a) informs the wider research community on the potential impacts of using duckweed as a plant-based fertilizer; and (b) potentially aids farmers interested in adopting new and sustainable plant-based fertilization strategies which support nutrient cycling.

2. Materials and Methods

A controlled greenhouse experiment was conducted to test duckweed as a sustainable soil amendment, evaluating its contribution to crop yield and nutrient leaching in comparison to commercial inorganic fertilizer. To study impacts on different crops, we selected beet, tomato, kale, and sorghum as representative root, fruit, leaf, and grain vegetables. Three types of fertilization options were applied to each crop: (1) dried duckweed; (2) inorganic commercial fertilizer; and (3) mix (40% duckweed and 60% inorganic fertilizer). A control (no fertilizer) was also simultaneously evaluated to assess background contributions from the soil. Results from the experiments were analyzed to determine any statistically significant differences between the four different nutrient treatments. Data from all four crops were subsequently used in a regression model to generalize trends and ascertain the dominant variables and/or amendment types impacting crop yield and nutrient fate in crops, soil, and leachate.

2.1. Experimental Methods

The experiment was performed in a greenhouse in the College of Agricultural Sciences at The Pennsylvania State University (Penn State, University Park, PA, USA). Hagerstown silty clay loam (USDA-NRCS) collected from the Sustainability Experience Center at Penn State was used in these experiments. Polyvinyl chloride (PVC) pots with different diameters were selected based on the distance between plants that is typically utilized in the field (diameters of 0.15 m for beet, 0.18 m for kale, 0.25 m for tomato, and 0.28 m for sorghum), as recommended by the Greenhouse Manager for the Department of Plant Science in the College of Agricultural Sciences at Penn State. Secondary containment trays of 40 cm $\times$ 31.8 cm $\times$ 15.2 cm with a volume capacity of 11.4 L (Sterilite, Townsend, MA, USA) were also utilized to collect leachate. Different soil masses were used for the crops based on their recommended pot dimensions: 2 kg for beet and kale; 7 kg for tomato; and 9 kg for sorghum. Triplicate pots were prepared for each crop with four different treatment types: Control (C); Duckweed (D); Inorganic Fertilizer (F); and Mix (M). In other words, it was a two-factor experiment with four crops and four fertilization options (including a control), resulting in 16 treatments, each conducted in triplicate (i.e., 48 pots in total). Since the soil had been treated with similar amendments in experiments conducted the previous year, initial composite soil samples from the triplicate pots were vigorously homogenized by hand by stirring for two minutes, and sent for soil fertility analysis (Agricultural Analytical Services Laboratory, Penn State) prior to the application of each amendment. The initial soil composition results include pH, total carbon (TC), total nitrogen (TN), phosphorus (P), potassium (K), magnesium (Mg), calcium (Ca), and cation exchange capacity (CEC) (Table S1, Supplementary Information).

The following seed varieties were utilized in these experiments: Detroit Supreme beets (Burpee Seeds, Philadelphia, PA, USA); Lacinato kale (Burpee Seeds); Rutgers tomato (American Seed Company, Spring Grove, PA, USA); and sorghum variety AF7202 Medium-Early Brachytic Dwarf (Alta Seeds, Amarillo, TX, USA). The growing periods for the different plants were as follows: 80 days for beet; 75 days for kale; 120 days for tomato;

and 122 days for sorghum. Based on the results of soil fertility testing (Table S1), nutrient recommendations from the Agricultural Analytical Services Laboratory (Penn State) were followed for beet, sorghum, and kale (Table 1). However, for the Rutgers tomato variety (which typically has high nutrient requirements), the recommendation from the University of California Extension Services was applied after consulting with the Penn State greenhouse facility manager.

Table 1. Plant nutrient requirement recommendations based on soil fertility testing for beet, kale, tomato, and sorghum in the greenhouse experiment. Highlighted cells indicate the limiting nutrient for each plant.

	kg/ha				
Plant	**Nitrogen (N)**	**Phosphorus (P)**	**Phosphorus Pentoxide (P_2O_5)**	**Potassium (K)**	**Potassium Oxide (K_2O)**
Beet	151	100	230	176	211
Kale	402	97	224	121	145
Tomato	302	42	97	225	269
Sorghum	157	47	109	191	230

Seeds were initially planted in small seedling pots using potting mix as the substrate, and then, after a period of germination of 10–14 days, the seedlings were transplanted to large, nutrient-amended pots. Duckweed used for the experiments was collected from The Living Filter at Penn State—a research facility where effluent from the university's wastewater treatment plant is sprayed on open fields and woodlands to provide additional polishing treatment and to simultaneously recharge the local groundwater supply. The duckweed had been previously identified as a monoculture of *Lemna obscura* [22]. Although utilizing plants grown in wastewater may pose risks if they accumulate harmful metals, the duckweed harvested from this system was found to be safe under the requirements for land application [29]. Post harvesting, the duckweed was dried (at 60 °C for 18 to 22 h) and stored until the application day. The oven-dried duckweed contained 3.1% N, 0.8% P, and 4.2% K. The complete analysis of duckweed's nutritional composition is provided in the Supplementary Information (Table S2). For the inorganic fertilizer treatment, a pasture blend fertilizer with a composition of 16% N (2.4% ammoniacal nitrogen and 13.6% urea nitrogen), 6% P_2O_5 (2.62% P), 16% K_2O (13.28% K), and 16% chloride (Cl) was used. The amendments were mixed in the topsoil of the pots: the upper 2 kg (6 cm) of soil for sorghum and tomato, and top 1 kg (4 cm) for beet and kale. The initial nutrients in the soil were determined by multiplying the soil nutrient concentrations from laboratory analysis with the mass of soil in each pot and converting it into kg of nutrient per hectare utilizing the surface area of each pot. The amounts of amendments added were calculated based on their composition and the plant requirements (Tables 1 and 2). With the exception of beet, which has P as the limiting nutrient, the fertilization rates for the remaining three crops (kale, tomato, and sorghum) were determined on an N basis (Table 1). As seen in Table 2, the plant requirements for P and K were provided in excess, so P or K deficiencies were not anticipated for any of the crops. To be consistent with a similar prior experiment, the N applied for kale was 47% above the recommended value.

Table 2. Mass of amendments applied and the resulting nitrogen (N), phosphorus (P), and potassium (K) concentrations in the soil for beet, kale, tomato, and sorghum in the greenhouse experiment.

Plant	Treatment	Mass of Amendment (g/pot)	N (kg/ha)	P (kg/ha)	K (kg/ha)
Beet	Control	0	0	0	0
	Fertilizer (F)	2.73	247	40	205
	Duckweed (D)	21	366	95	499
	Mix (D, F)	8.4, 1.656	295	62	323
Kale	Control	0	0	0	0
	Fertilizer (F)	8.7	547	89	454
	Duckweed (D)	45.35	549	143	748
	Mix (D, F)	18.14, 5.22	548	111	572
Tomato	Control	0	0	0	0
	Fertilizer (F)	9.67	316	52	262
	Duckweed (D)	50.5	317	82	432
	Mix (D, F)	20.2, 5.8	316	64	330
Sorghum	Control	0	0	0	0
	Fertilizer (F)	5.95	155	25	128
	Duckweed (D)	31	155	40	211
	Mix (D, F)	12.4, 3.55	155	31	162

After transplanting, the pots were irrigated approximately every two days with tap water as needed to maintain the health of each plant type (irrespective of fertilizer treatment), but at a low enough rate to avoid leachate generation. Water was added to the center of the pots, avoiding the edges, to prevent short-circuiting. The volumes added were approximately 200 mL per pot for kale and beet, 600 mL per pot for tomato, and 900 mL per pot for sorghum. The volume of water was increased by 20% after fruit or grain production for tomato and sorghum. Periodically, artificial "rain events" of 30–40 mm (where mm = L/m^2) each were created to mimic average rain events in the local region to obtain leachate, from which nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), and ammonium (NH_4^+) ion concentrations were analyzed. The leachate generation process involved saturating the soil with tap water and then adding additional water in 100 mL increments until the amount of water that the rain event would have discharged over the pot area for each plant type was reached. Incremental water addition was performed every 15–30 min to avoid over-generation of leachate. As a result of this process, total volumes of leachate ranging from 500 to 700 mL were collected. The first leaching event was 2–4 days after transplanting, followed by every 15–30 days. At the end of the growing period, the biomass of the different crops was collected, weighed on a wet (fresh) basis, and subsequently dried at 60 °C to quantify dry biomass yield and the nutrient composition of plant tissues.

2.2. Analytical Methods

Soil and plant tissue analyses were conducted by the Agricultural Analytical Services Laboratory (Penn State). For the leachate samples, NO_2^-, NO_3^-, and PO_4^{3-} analyses were carried out with a Dionex IC-1100 instrument (Dionex Corporation, Sunnyvale, CA, USA), and NH_4^+ was measured using an Orion portable probe (Thermo Scientific, Waltham, MA, USA, model 951201).

2.3. Derived Parameters

Several parameters were derived from the collected data to support our interpretation of the experimental results. Nutrient use efficiency (N use efficiency (NUE) or P use efficiency (PUE)) is a measure of how effective the amendments were in delivering nutrients and supporting crop yield (Equation (1)). This measurement provides insight into how much of the applied N or P is accumulated in the plant tissue relative to the control [30].Typically, the higher the nutrient use efficiency, the better the treatment option, as it can result in crops with enhanced nutritional content.

$$\%\ nutrient\ use\ efficiency = \left(\frac{Nutrient\ uptake_{treatment} - Nutrient\ uptake_{control}}{Nutrient\ applied_{treatment}}\right) \times 100 \quad (1)$$

where 'Nutrient uptake' refers to N or P in plant tissues (kg/ha) and 'Nutrient applied' is the N or P amended (kg/ha) for the specific treatment. Here, the surface area of the pots was used to convert from pot to kg/ha.

The loss of total inorganic nitrogen (TIN = $NH_4^+ - N + NO_3^- - N$) was estimated using Equation (2). This parameter accounts for the total TIN lost with respect to N applied, and enables comparisons across the different treatments by accounting for any background effect of the control soil.

$$\%TIN\ loss_{treatment} = \left(\frac{TIN\ loss_{treatment} - TIN\ loss_{control}}{TIN\ applied_{treatment}}\right) \times 100 \quad (2)$$

At the end of the experiment, a composite soil sample of the triplicates of each treatment was analyzed to quantify pH, TC, TN, P, K, Mg, Ca, CEC, Zn, and Cu. The change in soil TN between the pre-treatment and post-harvest soils was calculated using Equation (3).

$$\%TN\ change = \left(\frac{Final\ Soil\ TN - Initial\ Soil\ TN}{Initial\ Soil\ TN}\right) \times 100 \quad (3)$$

And finally, a mass balance approach was utilized to estimate N lost to the environment (Equation (4)), which indirectly provides a better understanding of how different crop-amendment combinations contribute to N emissions into the atmosphere.

$$\%N\ loss = \frac{\left[(Initial\ Soil\ TN + N_{added}) - N_{planttissue} - TIN_{leached} - Final\ Soil\ TN\right] \times 100}{Initial\ Soil\ TN + N_{added}} \quad (4)$$

2.4. Statistical Analysis and Regression Modeling

Data was statistically analyzed using the software Minitab® 21.0. One-way ANOVA was used to quantify the differences between treatment means, and a Tukey test (95% confidence) was conducted to understand whether or not the treatment responses were statistically different from each other.

Linear regression modeling was conducted using IBM® (Armonk, NY, USA) SPSS® Statistical Tool (version 28.0.1.1) to synthesize data from all crops and to determine general trends in crop and leachate responses to different amendments. A linear fit was chosen after running the 'Curve estimation' feature in SPSS, which concluded that a linear regression was the best type of model fit. Three parameters were considered in the model: (1) Initial soil N (kg/ha); (2) Initial soil P (kg/ha); and (3) Treatment type (Fertilizer, Duckweed, and Mix). The treatment type was converted into three separate categorical variables (with 0 and 1 values). Equation (5) describes the model predictors (left) and the associated independent variables (right) used in their regression models. The SPSS linear regression tool automatically generates results representing the model accuracy (coefficient of determination, R^2) and significance of the independent variables studied (at the 95% confidence interval). Standardized beta coefficients generated by the models were used to compare the relative significance of variables (the higher the coefficient value, the higher its effect on the predictor variable).

$$\left.\begin{array}{c} Plant\ N(\mathrm{kg/ha}) \\ Plant\ P(\mathrm{kg/ha}) \\ NUE(\%) \\ PUE(\%) \\ TIN\ Leached(\mathrm{kg/ha}) \\ Final\ SoilN(\mathrm{kg/ha}) \\ Wet\ Biomass(\mathrm{kg/ha}) \end{array}\right\} = f(Initial\ Soil\ N, Initial\ Soil\ P, Treatment\ type) \tag{5}$$

3. Results

3.1. Experimental Results and ANOVA

3.1.1. Variation in Leaching of Ions

The nutrient loss from soils growing beet and kale was analyzed following four leaching events, while five events were studied for tomato and sorghum due to their longer growing periods. No PO_4^{3-}-P was found in the leachate of any of the plants irrespective of the treatment applied, which is not unexpected considering that P leaching through soils is typically minimal compared to its dissolution in surface runoff [31]. Nitrite (NO_2^-) was also not detected in any of the experimental trials. The highest NH_4^+-N loss was observed in the first leaching event, with its concentration subsequently reducing to <1 mg/L in the last event (Figure 1). The elevated concentration in the first event implies that the young plants did not have adequate time to absorb the available NH_4^+-N before the first leaching event took place, and/or that the microorganisms could not completely nitrify the NH_4^+-N. A similar response of excess nutrient leaching is often observed in full-scale agriculture when rain events hit the cropland in the early stages of plant growth. Efforts are therefore being made in the scientific community to address this widespread problem by developing new approaches that optimize fertilizer application timing based on meteorological data [32,33].

Regarding the variations in NH_4^+-N leached, inorganic fertilizer contributed significantly more than the other treatments in the first leaching due to having N in a mineralized form (Figure 1). Duckweed leached less than the other treatments, while the control with no amendment showed the smallest ammonium leaching. Other studies have also demonstrated that substituting conventional synthetic fertilizers with organic forms can significantly reduce nutrient leaching, but this also depends on factors like type of organic material, crop category, soil texture, and land management practices [34,35]. On a crop basis, ammonium leaching in the first event was the highest for kale compared to the other plants, potentially due to N applied in excess of the recommended value (Fertilizer: 132.5 ± 23.2 mg/L; Duckweed: 104.0 ± 24.1 mg/L; Mix: 112.4 ± 26.3 mg/L). This was followed by beet (17.3–32.7 mg NH_4^+-N/L across the three different treatments), which also received excess N to be able to supply the P required according to plant nutrient requirements. One-way ANOVA with a Tukey test (Figure 2) indicated no significant differences between the means of total NH_4^+-N leached by the sorghum pots ($p = 0.535$). For the other three crops, inorganic fertilizer resulted in relatively high NH_4^+-N in the leachate compared to the duckweed and mix treatments, with tomato and kale plants showing significantly more leaching with the commercial fertilizer ($p < 0.05$). With beet, we found that NH_4^+-N leaching with the duckweed treatment was very low and even statistically comparable to that of the control.

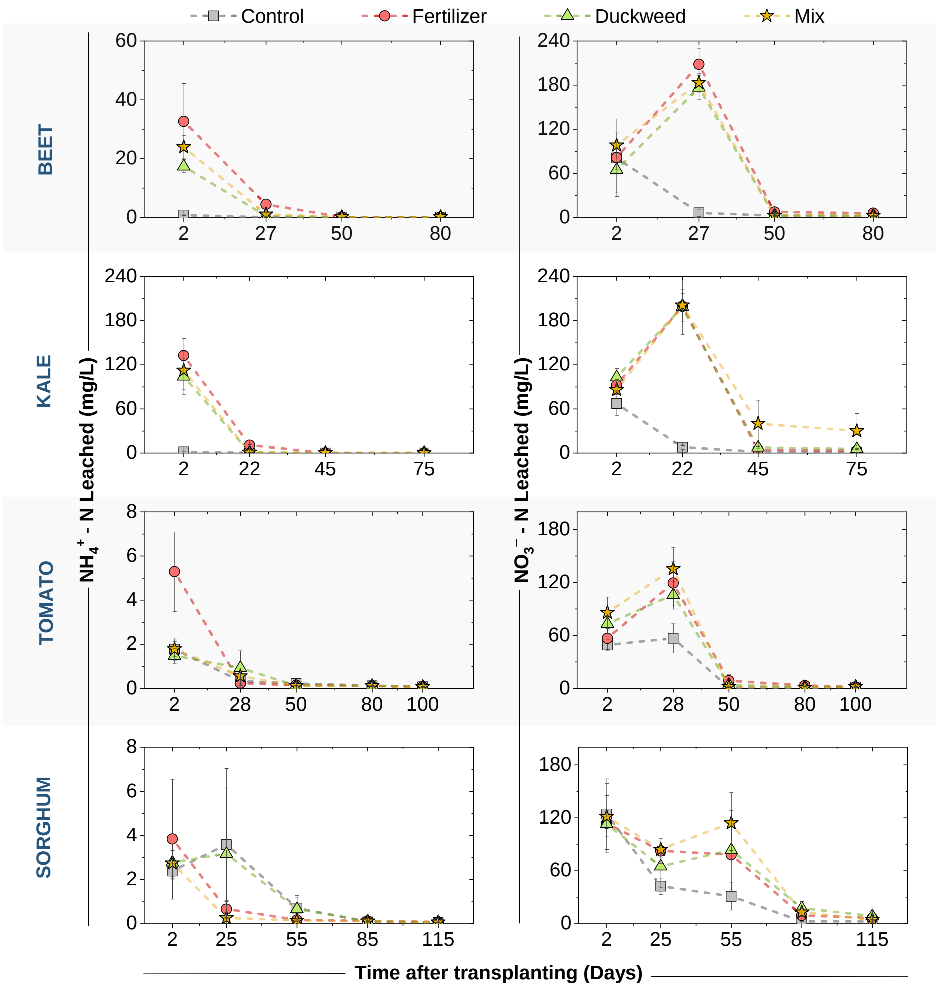

Figure 1. Concentrations of NH_4^+-N and NO_3^--N leached from soil growing beet, kale, tomato, and sorghum treated with either control (no amendment), inorganic fertilizer, duckweed, or mix (40% duckweed and 60% fertilizer). Data points represent triplicate averages; error bars represent one standard deviation.

Based on the concentration of NO_3^--N in the collected leachate, it was the largest contributor of N loss in the aqueous form (Figure 2). This is not surprising given the affinity of NH_4^+-N to be adsorbed by soil particles [36]. Unlike NH_4^+-N, the highest amount of NO_3-N loss was generally observed during the second leaching event (Beet: 162.9–208.3 mg/L; Kale: 179.2–200.9 mg/L; Tomato: 105.8–135.3 mg/L), except for sorghum, which showed similar NO_3^--N loss in the first three events. This can be attributed to the lag time between the first and second events, which provided an opportunity for NH_4^+-N and some of the organic N present in the duckweed to become nitrified, increasing the amount of NO_3^--N in the soil as well as in solution. The control leachate, as expected, showed the lowest concentrations of NO_3^--N, but none of the three treatment options showed significant differences. Hence, no clear pattern could be derived to conclude which treatment contributed more to NO_3^--N leaching. Since nitrate was the major contributor to total inorganic nitrogen, a similar trend was reflected in the TIN concentrations (Figure 2).

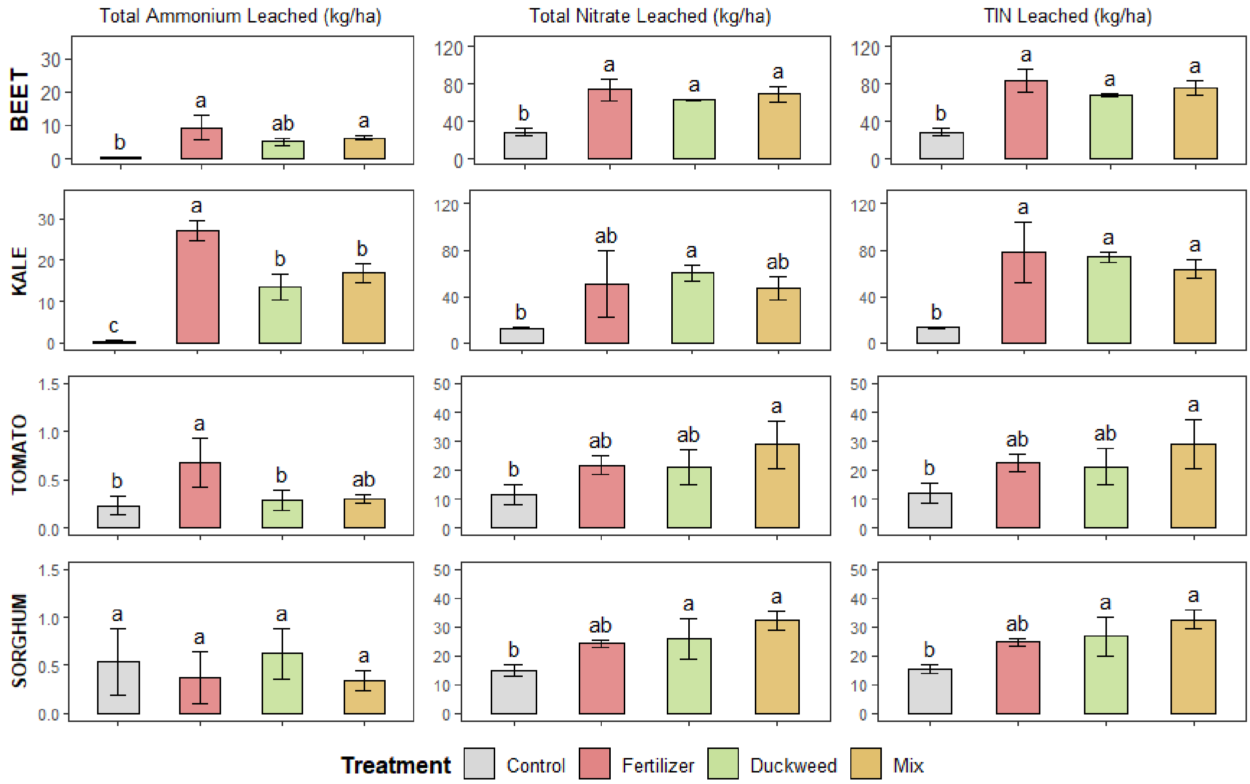

Figure 2. Results of one-way ANOVA showing differences in NH_4^+-N, NO_3^--N, and total inorganic nitrogen (TIN) leached from soil containing the different treatments. Data points represent triplicate averages; error bars represent one standard deviation. Different letters indicate significant differences among the treatments ($p < 0.05$). Treatment types that do not share a letter are statistically significantly different from each other.

Statistical analysis of the %TIN lost from all the treatments showed no significant differences for kale, tomato, and sorghum ($p = 0.538$, $p = 0.218$, $p = 0.470$, respectively), conveying that the new treatment methods studied here (duckweed and mix) did not generate more TIN leachate than conventional inorganic fertilizer (Table S3, Supplementary Information). For beet, however, significant differences were found among the treatments according to ANOVA results ($p = 0.046$), and Tukey analysis confirmed that duckweed amendments with beet led to significantly less TIN loss than inorganic fertilizer: compared to the control, duckweed only leached 10% more TIN, whereas inorganic fertilizer leached 20% more TIN. The complete set of ANOVA results is provided in the Supplementary Information.

One important conclusion from this analysis is that the lack of statistically significant differences between the leachate generated by the treatment options for most crops can be viewed as positive, particularly when the goal is to substitute existing conventional inorganic fertilizers with a novel amendment material such as duckweed. A previous field experiment growing sorghum with similar treatment options (duckweed, fertilizer, and mix) also showed no significant differences in cumulative NO_3^--N, NH_4^+-N, and TIN leached ($p > 0.05$) [19]. The present study therefore further strengthens the hypothesis that duckweed can be used as an alternative to conventional inorganic fertilizers without the risk of increasing nutrient runoff from agricultural fields. In future experiments, providing an initial acclimatization period for plants and adding more frequent, but smaller, leaching events, might prove useful in studying finer temporal variations in nutrient leaching.

3.1.2. Variation in Crop Yield

Crop responses to fertilizer treatments were analyzed based on plant biomass yield and plant nutrient compositions. Distinct parts of the plant were used in this analysis depending upon their most common food usage: roots of beet; leaves of kale; fruits of tomato;

and grain head of sorghum. On average, beet and kale generated slightly more fresh biomass with synthetic fertilizer (11 and 15 ton_{fresh}/ha higher than duckweed and mix), while tomato performed best with the mix treatment (143 $\pm$ 30.15 ton_{fresh}/ha) (Figure 3). The fewest variations in biomass within 1 ton_{dry}/ha were seen with sorghum. It is noted that the sorghum yield observed here was different from an earlier study that reported significant differences in sorghum yields (6.7 and 9.89 ton_{dry}/ha with duckweed and fertilizer, respectively) which was attributed to an unexplainable lower seed germination rate with duckweed in that experiment (40% compared to 90% using inorganic fertilizer) [19]. In the current study, Tukey tests showed no significant differences (at alpha = 0.05) between the means of the fresh or dry masses of harvested beet, tomato, and sorghum for the different soil amendments (Table 3). It was only in the case of kale that conventional inorganic fertilizer produced a significantly higher yield (45.79 $\pm$ 4.43 ton_{fresh}/ha; $p < 0.001$), but biomass production was similar whether duckweed was applied on its own or mixed with inorganic fertilizer.

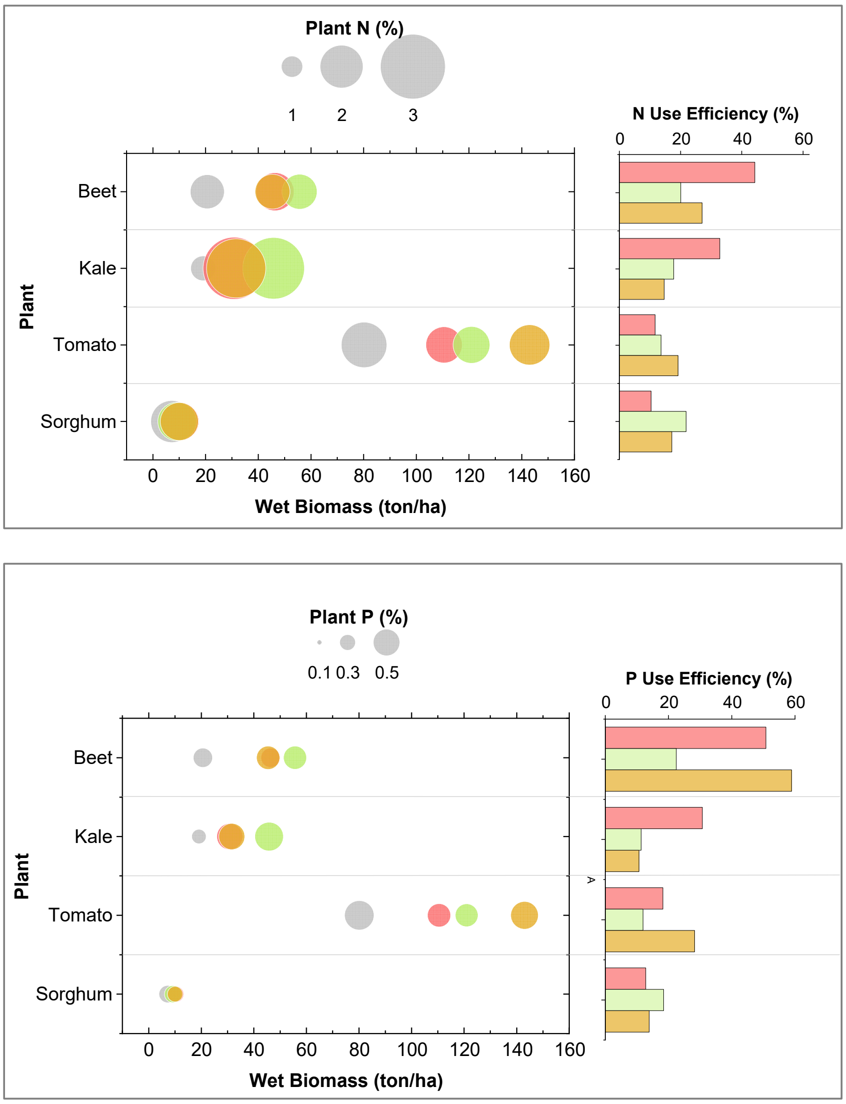

Figure 3. Nutrient compositions of harvested crops presented along with their wet (fresh) biomass values. Bubble sizes represent N/P content in plant tissue and the color corresponds to the treatment type: control (grey); fertilizer (pink); duckweed (green); mix (orange). Bar plots on the right show fertilization efficiencies of different treatments. Data points represent singlet measurements of composite samples (n = 3).

Table 3. Plant biomass yield (fresh and dry) of beet, kale, tomato, and sorghum grown in a greenhouse experiment treated with either control (no amendment), inorganic fertilizer, duckweed, or mix (40% duckweed and 60% fertilizer). Data are triplicate averages with one standard deviation from replicate pot tests. Different letters indicate significant differences among the treatments ($p < 0.05$).

Crop	Treatment	Fresh Mass (ton/ha)	Dry Mass (ton/ha)
Beet (root)	Control	20.62 ± 3.3 (b)	3.7 ± 0.25 (b)
	Fertilizer	55.68 ± 11.19 (a)	9.55 ± 2.86 (a)
	Duckweed	46.33 ± 7.59 (ab)	8.13 ± 1.42 (ab)
	Mix	45.44 ± 9.46 (ab)	8.62 ± 1.34 (ab)
	p-value	0.016	0.040
Kale (leaves)	Control	19.11 ± 0.15 (c)	3.75 ± 0.26 (c)
	Fertilizer	45.79 ± 4.43 (a)	7.77 ± 0.26 (a)
	Duckweed	30.85 ± 3.7 (b)	4.96 ± 0.21 (b)
	Mix	31.62 ± 1.69 (b)	4.55 ± 0.1 (b)
	p-value	<0.001	<0.001
Tomato (fruit)	Control	80.13 ± 14.44 (a)	4.49 ± 1.06 (a)
	Fertilizer	120.95 ± 31.74 (a)	7.83 ± 1.85 (a)
	Duckweed	110.48 ± 28.28 (a)	8.11 ± 1.95 (a)
	Mix	143 ± 30.15 (a)	8.3 ± 1.48 (a)
	p-value	0.213	0.141
Sorghum (grain head)	Control	7.2 ± 0.47 (b)	5.1 ± 0.4 (b)
	Fertilizer	9.23 ± 0.13 (a)	6.56 ± 0.04 (a)
	Duckweed	10.28 ± 0.64 (a)	7.49 ± 0.52 (a)
	Mix	10.03 ± 0.64 (a)	7.15 ± 0.5 (a)
	p-value	0.001	0.002

3.1.3. Variation in Crop Nutrients and Nutrient Use Efficiency

A preliminary crop nutritional analysis performed using composite samples (combining triplicates) indicated that beet and kale supplied with any of the three treatments contained more N and P (in their roots and leaves, respectively) than the control plants (Figure 3 and Table S4, Supplementary Information). Tomato (fruit) and sorghum (grains) plants, on the other hand, accumulated higher nutrients when exposed to control conditions without any fertilizers. Looking at the average values across the three treatment types, N and P compositions showed few to no differences (±0.1%) in all four crops studied here.

For N use efficiency, beet and kale performed more positively with conventional inorganic fertilizer (for beet: fertilizer = 44.3%, duckweed = 20.5%, mix = 27.0%; and for kale: fertilizer = 32.8%, duckweed = 17.8%, mix = 14.7%). A similar trend was seen with P use efficiency in beet and kale, for which the duckweed treatment resulted in a lower PUE (22.5% in beet and 11.4% in kale) when compared to inorganic treatment (50.8% in beet and 30.7% in kale). For tomato, as with crop yield, N use efficiency was the highest with the mix treatment, followed by duckweed and conventional inorganic fertilizer (for NUE: fertilizer = 11.7%, duckweed = 13.7%, and mix = 19.2%; for PUE: fertilizer = 18.2%, duckweed = 11.9%, and mix = 28.3%). Duckweed demonstrated superior performance in terms of nutrient accumulation in sorghum plants, exhibiting considerably higher nutrient use efficiency (21.8% NUE and 18.5% PUE) compared to inorganic fertilizer (10.4% NUE and 12.8% PUE). Overall, duckweed treatments were able to successfully amass a similar or higher percentage of P in plant tissues when compared to other treatments.

The duckweed–fertilizer mix treatment had an advantage over other treatments for beet and tomato plants, which is analogous to an earlier study that reported higher PUE in broccoli when treated with a mixture of compost and conventional fertilizer [37]. It is also important to consider that P remains in the soil for an extended time and its residual or legacy effect makes it accessible to plants in the long term [38]. Since organic soil amendments such as duckweed are more capable of retaining soil nutrients (discussed later in Section 3.1.4), it is reasonable to assume that duckweed may promote higher P uptake and PUE over a longer period than inorganic fertilizers.

The NUE and PUE parameters calculated here consider the total nutrient content (in kg/ha) and not the % N and P composition. Since conventional inorganic fertilizer tends to increase the crop yield in most cases, fertilization efficiencies reported here may not provide the complete picture of nutrient conversion per mass of plant. This is especially noteworthy in kale, for which duckweed proved to be beneficial in accumulating reasonable amounts of % N and P in plants, but due to its lower total yield, NUE and PUE were found to be lower with the duckweed treatment (17.8% NUE, 11.4% PUE) compared to that of inorganic fertilizer (32.8% NUE, 30.7% PUE). The findings suggest that duckweed used as a soil amendment can provide adequate nutrition to plants comparable to that of conventional inorganic fertilizers, resulting in higher nutrient content in crops compared to a control, and in some cases higher than that obtained with commercial inorganic fertilizer.

3.1.4. Variation in Soil Nutrient Residue

Changes in soil N before and at the end of the growing season are a good measure of whether a specific fertilization treatment is capable of retaining soil nutrients and improving soil fertility. Enhanced accumulation of N and P in the soil could be viewed as beneficial for more sustainable land management by reducing the need for fertilization in subsequent growing seasons. Comparing the soil nutrients pre-treatment and post-harvest, it is evident that duckweed plays a crucial role in increasing residual soil N (Table 4 and Figure 4). For beet and kale, while the other treatments reduced the soil TN, duckweed was able to boost the N content in the soil (0.9% and 11.1% increase, respectively). Tomato pots showed the highest residual nitrogen with the mix treatment (7.0% change from control), followed by duckweed (−3.5% change). Although final soil TN was lower than its initial values for all treatments in sorghum pots, duckweed still had a relatively low reduction in N (−1.9% change from control). Previous sorghum field trials have also estimated an increase in residual soil N with duckweed application [19], even when lower amounts of N were applied compared to diammonium phosphate [20].

Table 4. Mass balance of system N for beet, kale, tomato, and sorghum grown in a greenhouse experiment treated with control (no amendment), inorganic fertilizer, duckweed, or mix (40% duckweed and 60% fertilizer). Data are singlet measurements of composite samples (n = 3).

				(kg/ha)			(%)	
Plant	**Treatment**	**Initial Soil TN (Before Planting)**	**N Added**	**N Plant Tissue**	**TIN Leached**	**Final Soil TN (After Harvest)**	**TN Change**	**N Loss**
Beet	Control	3395.3	0.0	56.6	28.5	3276.5	−3.5	1.0
	Fertilizer	3621.7	247.2	169.8	83.3	3440.6	−5.0	4.5
	Duckweed	3734.8	366.0	130.2	68.1	3768.8	0.9	3.3
	Mix	3621.7	294.7	135.8	75.4	3406.6	−5.9	7.6
Kale	Control	2436.4	0.0	43.2	12.9	2326.4	−4.5	2.2
	Fertilizer	2515.0	547.0	224.0	61.2	2436.4	−3.1	11.1
	Duckweed	2750.8	548.9	141.5	73.8	3057.3	11.1	0.8
	Mix	2515.0	547.8	121.8	63.6	2153.5	−14.4	23.6
Tomato	Control	4420.7	0.0	93.7	11.9	4227.2	−4.4	2.0
	Fertilizer	4563.3	316.2	130.4	22.6	4386.1	−3.9	7.0
	Duckweed	4563.3	316.9	136.5	21.3	4402.4	−3.5	6.6
	Mix	4563.3	316.4	154.8	29.2	4881.1	7.0	−3.8
Sorghum	Control	3637.8	0.0	99.1	15.2	3363.4	−7.5	4.4
	Fertilizer	3637.8	154.6	115.3	24.7	3337.4	−8.3	8.3
	Duckweed	3637.8	155.1	133.2	26.4	3569.6	−1.9	1.7
	Mix	3637.8	154.8	125.1	32.5	3423.5	−5.9	5.6

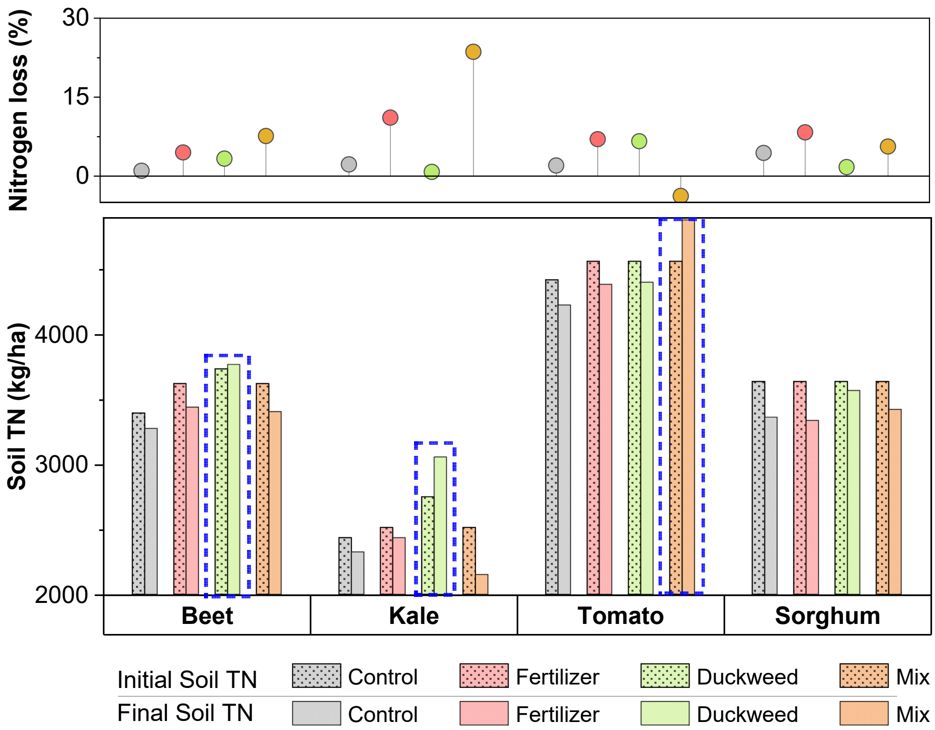

Figure 4. Initial and residual soil nitrogen before treatment and after harvesting for the different treatment types, with highlighted blue boxes showing samples where residual N > initial N. Corresponding percentage nitrogen loss (input–output N) is shown at the top as drop lines, with colors corresponding to the treatments as shown in the key: control (grey); fertilizer (pink); duckweed (green); mix (orange). Data points represent singlet measurements of composite samples (n = 3).

Synthetic inorganic fertilizers are considered to be one of the primary drivers contributing to anthropogenic nitrous oxide (N_2O) emissions from agricultural land [39,40]. Considering the potent nature of N_2O as a greenhouse gas (~300 times that of carbon dioxide), there is value in understanding the complete N mass balance of our crop–soil–leachate system [41]. The TN loss parameter (Table 4 and Figure 4) represents the percentage difference in input N (initial soil N + added N) and output N (leached N + residual N), which indirectly provides an insight into the amount of N that may be lost to the environment in different forms, including as N_2O. Among the three fertilization treatments, the lowest N losses were observed with duckweed for three crops (3.3% in beet, 0.8% in kale, and 1.7% in sorghum), generally yielding values closer to that of control samples. As with most other parameters discussed above, tomato was the exception, with the mix treatment displaying a negative value (output > input N). Adding plant residues to inorganic N-based fertilizers is typically known to increase N_2O emissions [42,43], but the plant's chemical composition (C:N ratio, lignin content, etc.) plays a key role in regulating these emissions [44]. In this context, the above findings potentially point to a promising aspect of duckweed-based fertilizers to reduce atmospheric N release. It is worth clarifying that the N cycle in agricultural fields may include processes like atmospheric N deposition and N fixation, which are not considered within the scope of this study. Hence, the N loss values presented here are not entirely indicative of N_2O emissions, and may require further examination to arrive at an accurate conclusion.

Regarding soil carbon (C) content, the average changes before and after the growing period showed that duckweed consistently generated beneficial effects in C accumulation, performing better than treatments with conventional inorganic fertilizer for beet, kale, and sorghum (4.5, 16.6, and −0.5 points change from initial % C content, respectively) (Table 5). Mixed fertilizer was the only treatment which generated positive changes in C residue in the tomato pot soil. Duckweed was also the most effective in enhancing P and K buildup in the soil in comparison to conventional inorganic fertilizer for all the crops except beet, which showed higher accumulation with the mix treatment. For Ca and Mg, the duckweed treatment led to higher residual concentrations in the soil of kale and tomato pots, whereas the mix treatment was slightly more advantageous in the case of beet. These outcomes further validated our earlier research that showed an increase in residual soil C and K when sorghum plants were treated with duckweed [19].

Table 5. Change in soil nutrients for beet, kale, tomato, and sorghum grown in a greenhouse experiment treated with either control (no amendment), inorganic fertilizer, duckweed, or mix (40% duckweed and 60% fertilizer). Data are singlet measurements of composite samples (n = 3). The color range of minimum to maximum values is determined for each crop–treatment combination. The green color represents higher soil nutrient retention compared to initial conditions.

		Change between Pre-Treatment and Post-Harvest Soils					
		Min. Value			Max. Value		
Plant	**Treatment**	**% C**	**% TN**	**% P**	**% K**	**% Mg**	**% Ca**
Beet	Control	−1.6	−3.6	13	−25	21	39
	Fertilizer	−5.7	−5	104	67	23	37
	Duckweed	4.5	0.9	98	59	20	48
	Mix	−4.4	−5.9	134	90	28	54
Kale	Control	−0.5	−4.5	5	14	23	48
	Fertilizer	0.1	−3.1	19	197	25	42
	Duckweed	16.6	11.1	164	346	30	84
	Mix	−12.5	−14.3	33	160	38	36
Tomato	Control	−2.4	−4.4	−26	−46	5	26
	Fertilizer	−2.7	−3.9	15	−3	18	27
	Duckweed	−5.3	−3.5	31	38	31	47
	Mix	8.5	7	15	−8	17	27
Sorghum	Control	−7	−7.5	16	−25	29	52
	Fertilizer	−6.1	−8.3	17	−9	16	38
	Duckweed	−0.5	−1.9	30	56	15	41
	Mix	−2.3	−5.9	25	57	16	41

Overall, at the end of the growing season, soil nutritional quality was best maintained with duckweed or mix treatments—a finding that emphasizes duckweed's potential as a sustainable and slow-releasing soil amendment. It can be concluded that using duckweed as a primary soil amendment or as a complement to another treatment (inorganic fertilizer in this case) improves the nutrient residue in the soil, which is typically not achieved by application of commercial inorganic fertilizers alone. As reported in many studies, mixing organic and inorganic fertilizers can improve soil fertility and alleviate soil acidification and degradation without having a negative effect on productivity, and in some cases increase the overall crop yield [45–47]. Therefore, even in situations where crop yields are not enhanced with duckweed application alone, there may still be value in using it as a supplement to conventional inorganic fertilizer. Furthermore, supplying the amount of N needed by plants with duckweed concomitantly provides the addition of important macronutrients such as P, K, Mg, and Ca, as well as micronutrients including Cl, Cu, Fe, Mn, and Zn [20] at no additional cost.

3.2. Regression Modeling

Simple linear regression of dependent predictor (Y) variables (including plant N, plant P, nutrient use efficiencies (NUE and PUE), TIN leached, final soil N, and fresh biomass) was carried out using relevant independent (X) variables (including initial soil N, initial soil P, and treatment type) (Figure 5). Data from all crops were combined for this modeling exercise in order to derive a generalized regression equation that would demonstrate the broad significance of different variables in predicting the 'Y' parameters. Standardized beta coefficients from the SPSS tool were used as a proxy to study the relative importance of the variables in model prediction. All the models had an R^2 value > 0.5, indicating reasonable predictive power. A higher R^2 was not targeted because the primary goal of this task was not to propose a model that could be used for predicting these parameters, but to identify the effect of key variables and treatment types on biomass yield and nutrient content in crop, soil, and leachate.

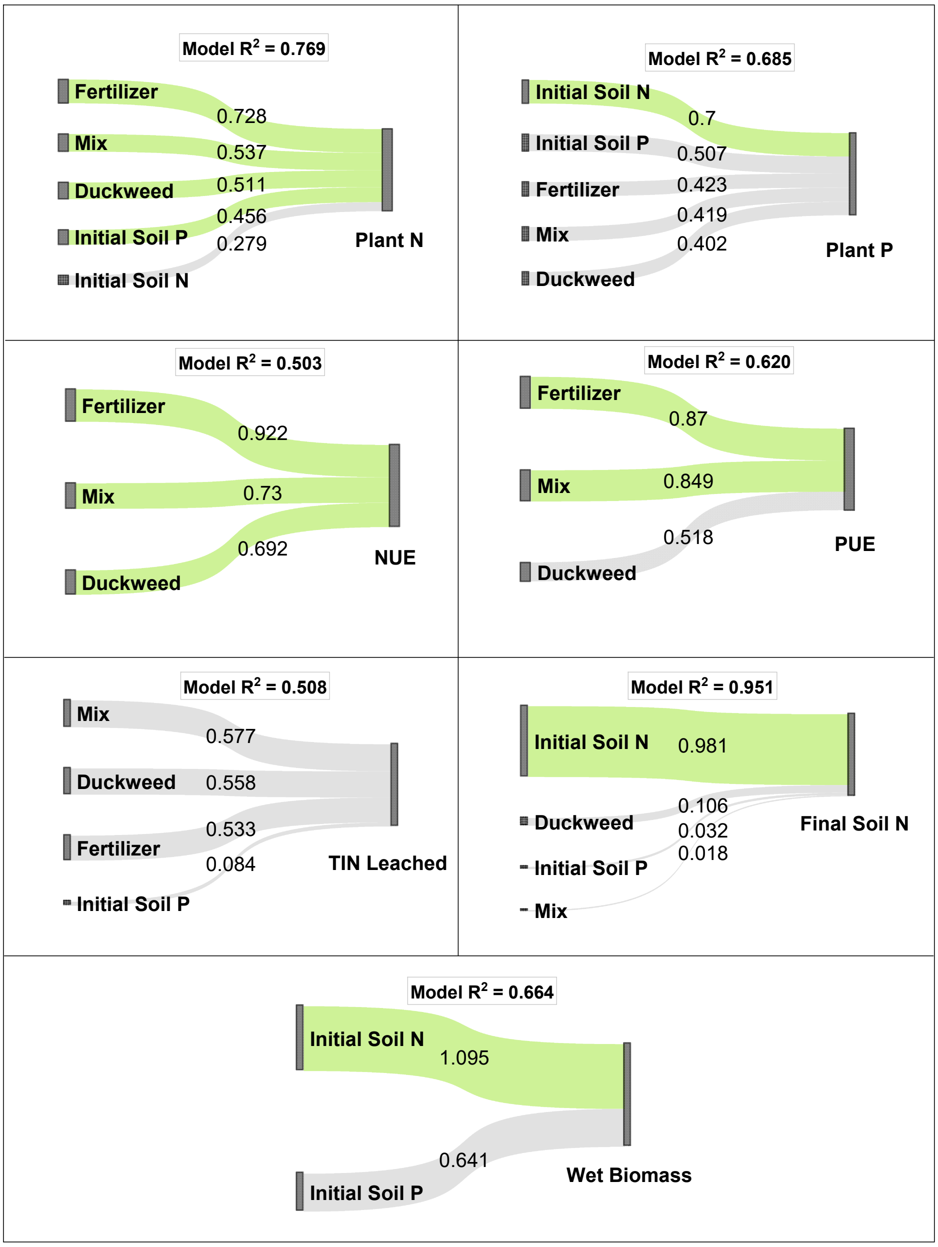

Figure 5. Regression modeling of plant N, plant P, NUE, PUE, TIN leached, final soil N, and wet (fresh) biomass presented as Sankey charts. Line thickness corresponds to the standardized beta coefficients of independent variables. The green color represents statistically significant variables and the grey color indicates variables with $p > 0.05$.

The plant N model ($R^2 = 0.77$) revealed that all three treatment types and initial soil P were significant variables (within a 95% confidence interval), but initial soil N did not play a prominent role in influencing N in plant tissues (Figure 5). Beta coefficients indicate that inorganic fertilizer (0.728) was better for N accumulation in plant tissues, followed by the mix (0.537) and duckweed (0.511) treatments. A similar model attempted for plant P returned only one significant variable—initial soil N—with no effect seen for the different treatment types. The interesting finding here is the inverse impact of soil nutrients on plant tissue nutrients (soil N affecting plant P, and soil P affecting plant N). Although seemingly counterintuitive, synergistic growth responses to N and P availability have been documented in the literature, suggesting that organisms tend to trade P for N or vice versa, by adjusting their nutrient acquisition rates [48]. Soil N absorption and N use efficiency have reportedly been seen to rise when exposed to higher P levels in the soil [49,50]. Possible explanations for increased P uptake with higher soil N are as follows: (a) excess N salts may encourage plant P sorption by changing the solubility of P in the soil; or (b) N may promote root growth and increase the plant's foraging capacity for P [51]. In addition, work done in the past has verified elevated enzymatic activity under higher N conditions, which increase the plant-available P through mineralization [52–54]. Another factor to consider while examining the change in uptake rates is the role of microorganisms in driving plant nutrient accumulation. Given the differences in initial soil conditions used for the different crops, it is possible that uptake was influenced by the biological composition of the soil as well [55].

Both NUE and PUE models showed significant effects of the three soil amendment types (R^2 = 0.5–0.6 in both cases). From the relative values of beta coefficients, it is clear that conventional synthetic fertilization led to higher NUE and PUE. The comparable coefficient values in the TIN-leached model imply that amendment type did not substantially affect the total N leached, but since the p-values were <0.05 for these variables, it is unreasonable to make a conclusive remark. It is possible that other factors like soil texture and flow rate of artificial rainfall events may have additionally influenced the leachate nutrient concentrations. A solute uptake model developed by [56] showed that rate of nutrient movement, which can vary depending on the soil structure, can highly influence plant N or P uptake. Since the soil samples used in our study came from a previous experiment, the inherent differences in soil profiles, or its change after incorporating the treatments, may also have affected the NUE and/or PUE of the plants.

Final soil N was strongly correlated to initial N in the soil (R^2 = 0.95), causing the amendment-type variables to be insignificant. This is in agreement with past research which suggests that rate of fertilization, rather than the source of N, characterizes the amount of residual soil N [57,58]. The slight advantage of duckweed in increasing soil N is signified by the relatively high coefficient value of duckweed (0.106) when compared to other treatments (0.032 and 0.018). In Section 3.1.4, crop-specific analyses demonstrated that duckweed is highly capable of enriching the soil with nutrients. However, in regression modeling, when combining data from all crops (including tomato, which did not show a positive effect from duckweed), the overall impact of duckweed amendment diminishes, rendering it insignificant. In summary, while ANOVA helped in understanding crop-specific responses, regression modeling takes a more general approach, allowing us to decipher whether these effects are consistent and applicable to a variety of different crops.

The fresh biomass of crops was also more influenced by the initial N in the soil than by the amendments, highlighting that duckweed produces similar yield responses to those of conventional inorganic fertilizer. It was also observed that at a 95% confidence interval, initial soil P was moderately important in enhancing crop yield (p = 0.055). Although, in Figure 3, we found that inorganic fertilizer increased the yield slightly for kale and beet, the differences are not large enough to generate a significant effect across all crops. Even though several studies have shown that organic soil amendments can perform similarly to inorganic fertilizers in enhancing yields of crops like rice, maize, and sorghum [20,59], numerous others have shown that mixing organic and inorganic fertilizers offers the best alternative, with simultaneous increase in productivity and improvement of soil health [60,61]. A moderate to high yield response to the mixed treatment of duckweed and inorganic fertilizer obtained in our trials (Table 3) further corroborates this statement.

In summary, this study establishes duckweed's potential to be used as a substitute or complementary fertilizer to boost soil fertility, while maintaining good-quality crop yields.

Application of wastewater-grown duckweed to agricultural crops could prove to be a sustainable and eco-friendly choice in undertaking a waste-to-resource recovery approach, while promoting a circular bioeconomy. Since this is still an emerging field of research, there is a growing opportunity to investigate the environmental and socio-economic impacts of large-scale implementation of duckweed-based fertilizers. A major drawback of the modeling work discussed here is the limited number of data points used to interpret the models. Therefore, it is important to emphasize that gathering additional data from future experiments would be essential in increasing confidence in the developed models and further reinforcing the conclusions derived from this study. In addition, expanding our study to field trials of duckweed application on farms with real-world environmental and weather conditions would certainly broaden our understanding of nutrient dynamics, productivity, and impacts on natural resources.

4. Conclusions

The experiments conducted here were designed to identify the impacts of substituting or complementing duckweed as a soil amendment to agricultural crops and to examine the nutrient-cycling dynamics of the respective crop–soil–leachate systems. Overall, it was observed that duckweed amendments released similar or lower inorganic N than conventional fertilizer, demonstrating that the slow-release and organic nature of duckweed helps retain nutrients in the soil, gradually making them available for plant uptake. Nutrient compositions of the plants were found to be adequate for all of the treatments and all the crops tested, which underscores the conclusion that fertilization carried out by duckweed (alone) or as a complement (mix) is a viable option in obtaining good-quality crops. Total N losses (potentially as emissions into the atmosphere) were reduced with duckweed treatments in comparison to commercial fertilizer for beet, kale, and sorghum. Regression modeling further strengthened our experimental findings revealing that the duckweed treatments performed favorably with respect to all the parameters (biomass generation, plant nutrients, leached N, and residual soil N), but were not statistically different from inorganic fertilizer across all crop types. For many crop types, however, duckweed can reduce nutrient loss to the environment and increase residual soil nutrients without sacrificing crop yield or nutritional quality. Incorporating other crop/treatment options and accounting for additional nutrient flow processes and greenhouse gas emissions could offer a more complete picture of how well duckweed nutrients are distributed into the soil, air, and plant biomass.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture14020188/s1, Table S1: Initial average composite results of fertility tests performed on the soil used for each of the crops in the greenhouse experiment before adding the soil amendments; Table S2: Nutrient content of duckweed after collection from the Living Filter at Penn State University; Table S3: Leached masses of NH_4^+-N, NO_3^--N, TIN, and average percentage of TIN lost from pots containing beet, kale, tomato, and sorghum grown in a greenhouse experiment treated with control (no amendment), duckweed, fertilizer, and mix; Table S4: Nutrient use efficiency and N and P present in the plant tissues of beet, kale, tomato, and sorghum grown in a greenhouse experiment treated with control (no amendment), duckweed, fertilizer, or mix.

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