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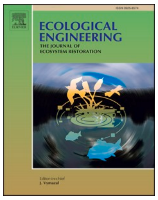

Recovery of waste nutrients by duckweed for reuse in sustainable agriculture: Second-year results of a field pilot study with sorghum

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ABSTRACT

Between 62% and 92% of industrial and municipal wastewater in upper-middle, low-middle, and low income countries is discharged to the environment untreated, releasing valuable nutrients such as nitrogen (N) and phosphorus (P) into rivers, lakes, and oceans (Lipponen and Nikiforova, 2017). This, in addition to excess nutrients often present in agricultural runoff due to overuse and misuse of fertilizers, can lead to eutrophication, often causing irreparable damage to aquatic ecosystems. For these reasons, new techniques must be found to effectively recover waste nutrients and upcycle them into natural soil amendments that can be used to enrich soil quality and grow food for future generations while minimizing agricultural runoff. Duckweed is a small floating aquatic plant that can hyperaccumulate nutrients present in wastewater and agricultural runoff and then be harvested and reused to replace or supplement commercial soil fertilizers. As part of a two-year field trial, duckweed was tested for the second consecutive year in this study as a soil amendment in comparison to, and in combination with, commercial fertilizer for the growth of sorghum, a drought-resistant grain. Relative to fertilizer in all cases, soils amended with duckweed generated less ammonia and nitrate in surficial runoff. No differences in P in cumulative runoff were found among the different treatments ($p = 0.509$). Additionally, duckweed application produced sorghum grains with greater N and P content than other treatments (1.63 ± 0.03% N ($p = 0.001$) and 0.35 ± 0.0% P ($p = 0.016$)). Duckweed treatments also showed increased soil residue carbon and P after harvesting the crop. When normalized by germination rate, sorghum yield was similar across treatments. In agreement with first-year findings, the results indicated that duckweed may be a viable alternative to commercial fertilizer from an environmental and agricultural perspective, providing acceptable yields and contributing to the buildup of beneficial nutrients in the soil profile. Additional testing is needed to further evaluate potential germination inhibitors, greenhouse gas emissions (ex., N_2O), and efficacy when applied to different crops and soil types.

1. Introduction

The global human population is projected to reach 10.9 billion by 2050, spurring the need to increase food production by 70% (United Nations, 2019). To help meet this additional food demand, more fertilizers will be produced and utilized. By 2022, global fertilizer demand (N-P-K) is expected to reach 200.9 million tons (FAO, 2019).

After inorganic fertilizers are applied to agricultural fields, only approximately 50% of the N is converted into plant tissue, while the remaining 50% is lost either as: superficial runoff; groundwater leachate; or atmospheric nitrous oxide (N_2O) or N_2 emissions (Cassman et al., 2016; Smil, 1999). Meeting increased food demand is anticipated to put extra pressure on the environment, compromising the integrity of water resources and aquatic environments. The excessive release of nutrients from agricultural fields, like N and P, into the environment often leads to eutrophication and the formation of “dead zones” (Paerl, 2014). When coupled with soil acidification (Bouwman et al., 2002) and nitrous oxide (N_2O) emissions (Haider et al., 2020), irreparable damage may be inflicted on a variety of ecosystems.

Duckweed, an aquatic plant from the family Araceae and the subfamily Lemnoidae, is present worldwide and has the potential to help solve the aforementioned problems. It can grow and tolerate a wide pH range from 3 to 7.5, temperatures between 6 °C and 33 °C (Culley et al., 1981), and possesses a doubling time of 2 to 4 days under optimal

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growing conditions (Culley et al., 1981; Skillicorn et al., 1993). In addition, its floatability makes it easy to harvest, making this hyperaccumulator attractive for recovering nutrients from water. Duckweed has been studied and evaluated for many years to determine its performance in different applications, such as the treatment of municipal and industrial wastewaters (Ozengin and Elmaci, 2007) and animal wastewater (Cheng and Stomp, 2009; Sońta et al., 2018). For example, *Lemna minor* growing in domestic wastewater treatment systems exhibited an N uptake of 350–1200 kg ha^{-1} yr^{-1} and a P uptake of 116–400 kg ha^{-1} yr^{-1} (Reddy and DeBusk, 1987). Similarly, *Lemna minor* L. grown on municipal and industrial wastewater at laboratory scale was able to remove 83–87% of total nitrogen (TN) and 70–85% total phosphorus (TP) (Ozengin and Elmaci, 2007). Duckweed grown in a 6% swine water lagoon with a concentration of 1123 mg L^{-1} of TN and 296.5 mg L^{-1} of TP was able to reduce these concentrations by 83.7% and 89.4%, respectively (Xu and Shen, 2011). A co-culture of *Lemna japonica/minor* and *Wolffia columbiana* grown on partially treated municipal wastewater was shown to produce 5–10 times higher protein yields than conventional land grown crops, such as soybeans, maize, and oats (Roman and Brennan, 2019). When compared to commercial compost, *Lemna obscura* collected from a natural pond receiving effluent from a municipal wastewater treatment plant had almost 2× the N content and 20× more P than compost, in addition to higher amounts of organic matter, potassium (K), and magnesium (Mg) (Calicioglu and Brennan, 2018; Kreider et al., 2019). When applied to agricultural soils, the accumulated nutrients in duckweed can be mineralized and assimilated by growing crops, allowing a better utilization of nutrients over time, and avoiding excess nutrient availability when the plants are not able to utilize them. In a series of experiments, Kreider et al. (2019) demonstrated that duckweed: 1) achieved a higher degree of N mineralization than compost in a microcosm experiment; 2) generated less N leachate than diammonium phosphate in a column experiment; and 3) produced comparable yields of sorghum compared to those of commercial fertilizer in a pilot-scale field experiment. Beyond these experiments, the relative scarcity of data about duckweed as a nutrient source for crops presents a good opportunity to explore duckweed utilization as a sustainable soil amendment, quantifying its contribution to nutrient runoff, crop yield, and soil nutrient residue.

In this study, a field experiment was performed to evaluate the effectiveness of duckweed as a soil amendment in comparison with commercial fertilizer for a second consecutive year (Year 1: Kreider et al., 2019). Duckweed, commercial fertilizer, and a combination of the two ("mix" = 40% duckweed and 60% fertilizer w/w), were utilized as treatments. The hybrid mix option was evaluated to supply rapid nutrient availability for early plant development (provided by commercial fertilizer) coupled with a slow release nutrients for later growth (provided by duckweed). Complementing inorganic fertilization with organic amendments (such as duckweed) has been suggested as a method to reduce fertilization costs while enhancing plant yield (Admas et al., 2015). Sorghum, a drought-resistant grain, was selected as the test crop for the field trials. Surficial runoff was collected after rain events and analyzed to quantify ammonium (NH_4^+), nitrate (NO_3^-), phosphate (PO_4^{3-}), pH, electrical conductivity (EC), and oxidation-reduction potential (ORP) to determine how the different amendments contributed to pollution of the local environment. After harvesting, the mass of wet and dry plants, nutrients in the plant tissue, crop yield, and residual soil nutrients were quantified to evaluate the performance of each amendment. It was expected that, compared to commercial fertilizer, duckweed could provide comparable crop yields, reduce N and P runoff, and generate beneficial nutrient residue in agricultural soils.

2. Materials and methods

2.1. Location and soil characteristics

The experiment was located on a gently sloping hillside at the Sustainability Experience Center at The Pennsylvania State University (University Park, PA, USA, 40.811198 N, −77.846992 W) in 2015. The soil is cataloged as Hagerstown silt loam, Typic Hapludalf (USDA Soil Survey). Fifteen plots, each 3 m wide by 9 m long (upslope to downslope), oriented parallel to each other, were utilized for the experiment. A composite soil sample was prepared from each plot before seeding and adding the amendments by combining 15 soil cores from the surface to 20 cm depth. Soil fertility tests were performed on composite samples by the Penn State Agricultural Analytical Services Laboratory. Total carbon (TC) and TN were measured using the combustion method (Bremmer, 1996), (Nelson and Sommers, 1996); pH was measured utilizing 1:1 soil water dilution (Eckert and Sims, 1995); and Cation Exchange Capacity (CEC) was measured by summation (Ross, 1995). Extractable Ca, K, Mg, and P were measured by the Mehlich 3-ICP method (Wolf and Beegle, 1995), while Cu and Zn (used for plant tissue analysis) were quantified using EPA Method 3050B/3051 + 6010 (USEPA, 1986). Results of the soil analysis are provided in the supplemental information (**Table S1**).

2.2. Crop, nutrient application, and soil treatments

Forage sorghum AF7202 Medium-Early Brachytic Dwarf (Alta Seeds) was utilized. Based on the crop type and soil quality, the Penn State Agricultural Analytical Services Laboratory recommended a nutrient application of: 146 kg-N/ha, 45 kg-P_2O_5/ha, and 90 kg-K_2O/ha. The nutrient treatments utilized in this experiment included: control (no amendment); dried duckweed; commercial inorganic fertilizer; and mix (40% duckweed and 60% fertilizer www/w). The amount of amendment needed was calculated based on the soil analysis N recommendation, meaning that the same amount of N (146 kg ha^{-1}) was provided by all treatments. The N, P, and K added per plot per treatment are presented in.Table 1. Duckweed (identified as *Lemna obscura* (Calicioglu and Brennan, 2018) was collected from the Living Filter at The Pennsylvania State University (University Park, PA) in pond C7. Water quality in this pond was measured to be: 7.8 ± 0.8 mg L^{-1} nitrate, 2.3 ± 0.9 mg L^{-1} ammonia, and 2.2 ± 0.4 mg L^{-1} phosphate (n = 3). The Living Filter consists of approximately 600 acres of agricultural fields and forested land where treated effluent from the University's activated sludge wastewater treatment plant is spray irrigated and allowed to recharge the underlying aquifer. Duckweed grows naturally in ponds that are irrigated with the above-mentioned water. Since the University wastewater treatment plant complies with discharge standards under the Clean Water Act, the duckweed in the Spray Fields is grown in relatively low nutrient water. If grown under higher nutrient conditions, greater accumulation of nutrients in the plant tissue could be obtained, as we have previously demonstrated with higher strength wastewater (Roman and Brennan, 2019). Duckweed for this work was harvested every 5 days between April and July 2015, collecting half of the biomass and leaving

Table 1
Amount of amendment applied per treatment based on sorghum requirements. Quantities per plot and their agricultural equivalence in kg ha^{-1} show that the amount of N is equal for all the treatments.

Treatment	N (kg ha^{-1})[a]	P (kg ha^{-1})[b]	K (kg ha^{-1})[b]	Mass of amendment (kg ha^{-1})
Control (no amendment)	0	0	0	0
Duckweed (dry)	146	38	205	4866[*]
Fertilizer	146	55	145	1073
Mix (40% duckweed, 60% fertilizer)	146	48	169	1946, 644

[a] Target amount.
[b] Derived from amendment composition after aiming for N requirement.
[*] Mass of duckweed required with 3% N content. If grown in higher nutrient water, duckweed biomass with 6% N can be achieved and correspondingly the mass of amendment could be reduced by half.

the remaining portion as seed. At each harvesting event, the pond was covered with duckweed and never depleted. Duckweed was skimmed from the top of the pond using nets, and immediately after collection, placed into a 45-L cooler. It was oven dried at 60 °C for 18 to 22 h in an air flow oven, and stored in dark plastic hermetic bags to avoid moisture or light degradation until the field application day. Three composite samples of duckweed were sent to the Penn State Agricultural Analytical Services Laboratory for a Plant Tissue Analysis to quantify its nutrient composition, and to determine the mass needed to supply the recommended nutrients for sorghum. Primary macronutrients such as N, K, and P, and secondary macronutrients such as Ca, Mg, and sulfur (S) were analyzed, as well as micronutrients and minerals such as aluminum (Al), boron (B), Cu, iron (Fe), manganese (Mn), sodium (Na), and Zn (Table 2). Pasture inorganic blend fertilizer (Tractor Supply Company) was utilized, with a percent composition of 16% N (2.4% ammoniacal nitrogen and 13.6% urea nitrogen), 6% P_2O_5, 16% soluble potash (K_2O), and 16% chloride (Cl).

2.3. Field set up, sampling, and analysis

Three no-amendment control plots (C), and four replicate plots of each amendment (duckweed (D), fertilizer (F), and mix (M)), were utilized. The M treatment was applied to plots which had been used as controls in the previous year (Kreider et al., 2019), while D and F treatments were maintained in their original plots. Amendments were distributed manually on the top of the plots, and the soil was immediately tilled three times by a cultipacker to incorporate the amendments to plow depth (18 cm). After incorporation of amendments, sorghum was sown by a grain drill (John Deere) the same day at a rate of 247,100 seeds per hectare with a 38 cm row separation. Plots were divided using HDPE corrugated half-pipes (10 cm diameter, Corex) to prevent surficial runoff from different treatments mixing. Simultaneously, runoff collection canals were dug at the down gradient edge of each plot (30 cm deep and 30 cm wide), lined with 6 mm black polyethylene (Husky), and 7.5 cm diameter HDPE corrugated pipes were installed to transport the collected runoff to 150 l plastic tanks.

Nine rain events were measured during the growing season. Immediately after each rain event, the collected runoff volume and some water quality parameters were recorded on site, including oxidation-reduction potential (ORP), electrical conductivity (EC), and temperature, utilizing a multiparameter probe (556 Handheld YSI a Xylem brand). Ammonium concentration was measured using an NH_4^+ portable probe (Orion 951,201, Thermo Scientific). A sub-sample was collected from each runoff collection tank into a 50 ml centrifuge tube (no headspace), put on ice, and transported to the laboratory to be analyzed the same day of collection by Ion Chromatography (Dionex IC-1100, Sunnyvale, CA) for ions including: chloride (Cl^-), NO_3^-, phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}). Analysis was carried out using an AS-22 column (Dionex, Sunnyvale, CA) with an eluent solution of 4.5 mM sodium carbonate and 1.4 mM sodium bicarbonate at a temperature of 30 °C with a flow rate of 0.9 ml/min.

Table 2
Plant tissue nutrient content in composite samples of dried duckweed utilized for these experiments. Values are averages (n = 3); error represents one standard deviation.

Elements	Dry weight basis
C (%)	36.5 ± 0.4
N (%)	3.1 ± 0.2
P (%)	0.8 ± 0.0
K (%)	4.2 ± 0.2
Ca (%)	2.6 ± 0.2
Mg (%)	0.6 ± 0.0
Mn (ppm)	1666 ± 215
Fe (ppm)	1009 ± 146
Cu (ppm)	7.1 ± 0.5
B (ppm)	409 ± 11
Al (ppm)	550 ± 112
Zn (ppm)	64 ± 9
Na (ppm)	9321 ± 721

After 122 days, the sorghum plants were ready to be harvested. To estimate the yield, sorghum plants were harvested over an area of 1.5 m by 9 m in each plot by a forage crop-harvesting machine (Champion C-1200, Kemper), biomass weight was determined using an internal scale mounted in the harvester, and the number of plants present in the harvested area were counted by hand to estimate the total mass of sorghum per hectare. The composite samples (whole plants) were oven dried at 60 °C for 48 h. To quantify tissue nutrients in the sorghum grains, six sorghum heads were randomly harvested from each plot, dried in an air flow oven at 60 °C for 48 h, and a composite sample sent for Plant Tissue Analysis to quantify plant nutrient uptake (Agricultural Analytical Services Laboratory, Penn State).

2.4. Statistical analysis

The data was statistically analyzed using Minitab 17.0 software (State College, Pennsylvania, USA). A one-way analysis of variance (ANOVA) was used to analyze the differences between treatment means, and a Tukey test (95% confidence) was used to understand which treatments were statistically different.

2.5. Germination test

As different sorghum germination rates were observed between Year 1 (Kreider et al., 2019) and Year 2 (this work), a follow-up experiment was performed in greenhouse pot tests to evaluate the potential effects of duckweed processing treatments and soil conditions on the germination of sorghum seeds. Duckweed for the germination test was freshly collected from the same location as for the field study. Drying procedures utilized for the field experiments in Year 1 and 2 were used to prepare two batches of duckweed: one by pre-drying by sun/air in a greenhouse for 3 days prior to oven drying at 60 °C for 48 h (mimicking the conditions of Year 1); and the other by direct oven drying at 60 °C for 22 h (identical to the conditions in Year 2). After drying, triplicate duckweed samples from each batch were sent for plant tissue analysis (Agricultural Analytical Services Laboratory, Penn State) to determine its nutrient composition (**Table S2**, supplemental information), making it possible to calculate the amount of duckweed needed to reach the nutrient fertilization goal for forage sorghum, 146 kg-N/ha. The same variety and batch of forage sorghum seeds was used in the germination tests as was used for the field experiment in Year 2 (Alta Seeds, Advanta US, AF7202 Medium-Early Brachytic Dwarf). Polyvinyl chloride (PVC) pots of 0.28 m in diameter containing a total of 7 kg of soil were utilized for all treatments. Pasture inorganic blend fertilizer was utilized, with a percent composition of 16% N (2.4% ammoniacal nitrogen and 13.6% urea nitrogen), 6% P_2O_5, 16% soluble potash (K_2O), and 16% chloride (Cl).

Six treatments were utilized: 1) Control (no amendment); 2) inorganic fertilizer; 3) direct oven-dried duckweed; 4) Mix (40% direct oven-dried duckweed, 60% fertilizer); 5) greenhouse-dried duckweed (50% of N requirement for sorghum); and 6) greenhouse-dried duckweed (100% of N requirement for sorghum). To mimic the duckweed and soil conditions from each year of the study, control soil that had been air dried and saved in closed containers prior to Year 1 testing was utilized for treatments 1, 5, and 6, whereas soil collected before the initiation of the field experiments in Year 2 was utilized for treatments 2, 3, and 4. The experiment was carried out in triplicate in a greenhouse where the temperature was between 22 °C to 26 °C. The amendments were incorporated into the pots in the greenhouse by mixing them in the upper 2 kg (6 cm) of soil. After the amendments were incorporated, 20 sorghum seeds were planted in the pots, evenly distributed with a 2 cm separation between each one. The soil was slightly moisturized after seed planting following agronomic recommendations. Pots were watered periodically with tap water to prevent the soil from drying out and potentially inhibiting seed germination. The germination rate was calculated after emergence ceased (14 days) using eq. 1:

$$\%Germination = \left(\frac{Seedling_{emerged} \times 100}{Seeds_{planted}}\right) \tag{1}$$

3. Results

3.1. Runoff

After sorghum was sown in Year 2, runoff from a series of 9 rain events were collected and analyzed (**Fig. 1**). Rainfall was measured on site with two rain gauges. After the 9th rain event (35 days after planting), the concentration of NH_4^+-N in the runoff water was negligible and the majority of the nutrients were apparently either absorbed by the plants, leached in the previous runoff events, or released to the atmosphere.

Detailed information about runoff volume and water quality parameters in Year 2, including pH, ORP, EC, chloride, phosphate, and sulfate concentrations are provided in **Figs. S1** to **S8** (supplemental information). In brief, over all the rain events, surficial runoff pH oscillated between 6.4 and 7.5, which is suitable for N mineralization and nitrification, and does not promote appreciable NH_3 losses by volatilization. Soil pH across treatments was between 6 and 6.2 before sowing ($p = 0.759$), and between 6.15 and 6.67 after harvesting ($p = 0.056$), without exhibiting significant differences between treatments. The change in soil pH is not believed to be enough to have caused severe changes in microbial activity. Over time, ORP and conductivity decreased, indicating that fewer nutrients were present in the runoff. Phosphate (PO_4^{3-}) did not exhibit obvious differences between control, duckweed, and fertilizer treatments; however, more PO_4^{3-}-P was leached by plots amended with the mix for the majority of the rain events ($p = 0.509$), indicating that the presence of inorganic fertilizer may have induced more mineralization of P compounds from duckweed. Plots treated with duckweed generated less cumulative NH_4^+-N runoff than those treated with commercial fertilizer or the mix (**Fig. 2a**), but the difference was not statistically significant ($p = 0.17$). Duckweed appeared to leach less cumulative NO_3^--N than fertilizer and mix (**Fig. 2b**), but the difference was not statistically significant ($p = 0.32$). Differences in collected runoff volume (**Fig. S1**, supplemental information) contributed to large standard deviations within the replicates of the same treatment, which likely explains these results. A comparison of total inorganic N (TIN) (sum of NH_4^+-N and NO_3^--N) and PO_4^{3-}-P contributions shows no significant differences among the treatments (Table 3).

3.2. Sorghum yield

Large differences in germination rate were observed across the different treatments in Year 2, but not in Year 1. Table 4 shows that where duckweed was incorporated as an amendment in Year 2, germination was heavily reduced by 40% for duckweed treatment and 52% for mix (in which duckweed accounted for 40% of the total amendment mass added). By comparison, germination for control and fertilizer plots was 92% and 90% respectively, which is close to the optimal expected range for normal agricultural operation (95%). This evidence suggests that something associated with the duckweed amendment may have interfered or affected the seed germination mechanism in Year 2. A germination test replicating the nutrient application and using the same seeds and soil was performed to investigate this hypothesis (Section 3.4).

Crop yield was quantified on a mass basis by weighing the harvested plant biomass and grains, and from a nutritional perspective by analyzing the nutrient composition of the plant tissues. The commercial fertilizer plots exhibited the highest average sorghum biomass yield, 9896 ± 681 kg ha^{-1}, followed by the mix with 7513 ± 386 kg ha^{-1}, then the control with 7084 ± 684 kg ha^{-1}, and last, duckweed with 6746 ± 853 kg ha^{-1}. The average dry sorghum yield with duckweed was 32% less than that for fertilizer. Considering that duckweed plots had a germination rate 50% smaller than the fertilizer plots, this indicates that duckweed is capable of generating good commercial yields if 90–96% germination can be achieved. Fig. 3 provides a comparison of sorghum yields between Years 1 and 2 with duckweed versus conventional fertilizer as soil amendments. To compare potential yields under similar seed emergence, the low germination rate observed in plots amended with duckweed in Year 2 was normalized by the germination rate observed in the fertilizer plots in Year 2 (90%), illustrating that duckweed has the potential to generate comparable, if not higher biomass, if regular agronomical germination rates can be obtained.

There were significant differences between the treatments when comparing wet and dry grain mass (Table 5). Dry matter content is important, as some moisture will be lost during the silage process (when crops are prepared for animal feed); therefore, crops with higher dry matter content are more attractive for farmers. Neglecting the control, no significant differences were observed between duckweed, fertilizer, and mix for either wet or dry grain mass. However, differences were observed between the control and mix, and control and duckweed on a wet grain mass basis, and between the control and the mix on a dry mass basis. One interpretation that can be made out of this result is that duckweed performed similar to commercial fertilizer and better than the

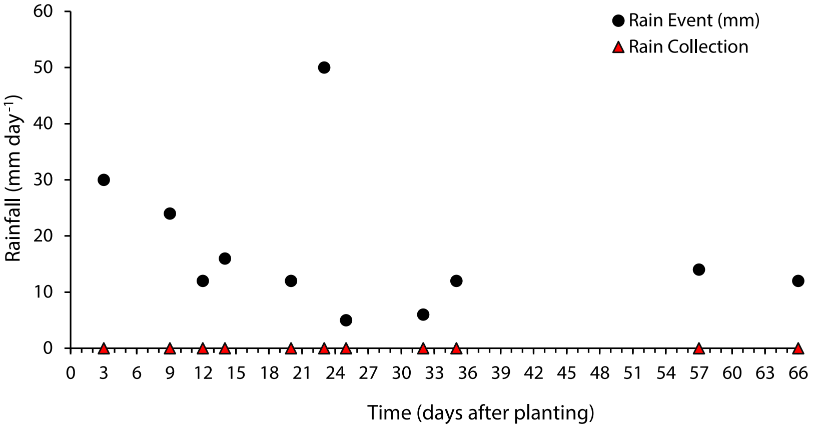

Fig. 1. Rainfall and runoff collection events at the field site. Black dots illustrate the magnitude of each rain event. Red triangles represent runoff collection events, showing that runoff was collected for at least 90% of all rainfall events during this period. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

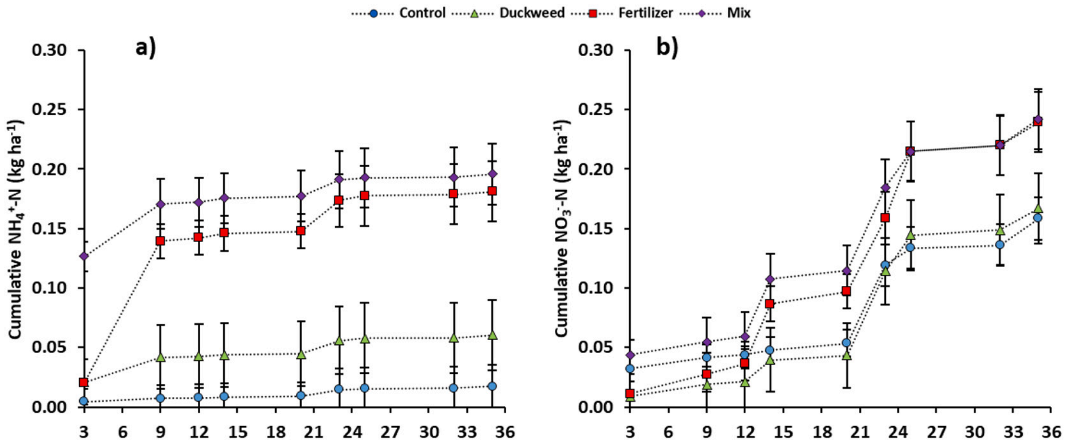

Fig. 2. Cumulative NH$_4^+$-N (**a**) and NO$_3$-N (**b**) in runoff water from sorghum plots treated with control (no amendment), duckweed, fertilizer, and a duckweed-fertilizer mix. Data points represent averages of four replicate plots for each treatment; error bars represent one standard deviation.

Table 3
Average cumulative NH$_4^+$-N, NO$_3^-$-N, TIN, and PO$_4^{3-}$P leached in runoff from sorghum plots in Year 2 treated with control (no amendment), duckweed, fertilizer, or mix. Data are averages of four replicate plots for each treatment; error represents one standard deviation.

Treatment	NH$_4^+$-N Leached (kg ha^{-1})	NO$_3^-$-N Leached (kg ha^{-1})	TIN Leached (kg ha^{-1})	PO$_4^{3-}$-P Leached (kg ha^{-1})
Control	0.02 ± 0.00	0.15 ± 0.05	0.17 ± 0.05	0.13 ± 0.00
Duckweed	0.06 ± 0.06	0.32 ± 0.16	0.37 ± 0.17	0.13 ± 0.03
Fertilizer	0.18 ± 0.09	0.50 ± 0.30	0.69 ± 0.31	0.14 ± 0.03
Mix	0.16 ± 0.15	0.47 ± 0.26	0.64 ± 0.30	0.17 ± 0.02
p-value	0.170	0.320	0.222	0.509

Table 4
Germination rate, sorghum plants per hectare, and kg of dry sorghum per hectare in Year 2 plots treated with control (no amendment), duckweed, fertilizer, or mix. Data are averages of four replicate plots for each treatment; error represents one standard deviation. Treatments with different letters, A or B, are considered significantly different.

Treatment	% Germination	Sorghum plants ha^{-1}	Sorghum kg ha^{-1}
Control	92	226,282 ± 18,214 (A)	7084 ± 684 (B)
Duckweed	40	99,386 ± 16,986 (B)	6746 ± 853 (B)
Fertilizer	90	222,096 ± 20,999 (A)	9896 ± 681 (A)
Mix	52	128,089 ± 11,841 (B)	7513 ± 386 (B)
p-value	0.000	0.000	0.001

control.

The same sorghum heads used for yield analysis were sent for tissue analysis to the Analytical Services Laboratory. Significantly higher nutrient (N and P) content was found in sorghum grown with duckweed than conventional fertilizer (**Table**). An increase in N and P in animal feed could provide both better nutrition and a reduction in costs if these elements do not need to be added as supplements. Plant uptake of N and P per plot were similar for duckweed and fertilizer treatments ($p = 0.6$ and $p = 0.602$, respectively). Considering that the plant density was lower in the duckweed-amended plots, this indicates that duckweed facilitates better nutrient uptake.

3.3. *Soil nutrients*

Soil nutrients were compared in samples that had been collected before planting the seeds and after the sorghum was harvested (Eq. 2) for the different treatments. After two years of treating the same plots with the same amendments, we observed differences for nutrients

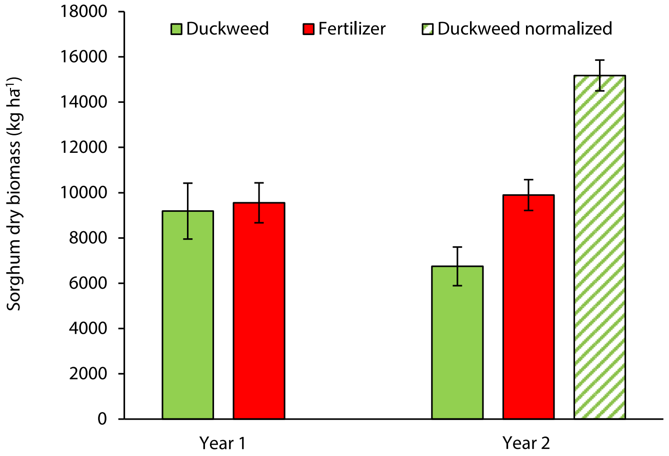

Fig. 3. Sorghum yield comparison in Year 1 and Year 2 with duckweed vs. conventional fertilizer as soil amendments. The low germination rate observed in plots amended with duckweed in Year 2 have been normalized in the bar at the far right to estimate the yield that could have been achieved had the germination rate been equivalent to that of fertilizer in Year 2. Data are averages (Year 1, $n = 5$; Year 2, $n = 4$); error bars represent one standard deviation.

Table 5
Sorghum grain mass, N and P grain content, and N and P uptake in Year 2 plots treated with control (no amendment), duckweed, fertilizer, or mix. Data are averages of four replicate plots for each treatment with one standard deviation. Treatments with different letters, A or B, are considered statistically significantly different.

	Wet mass of sorghum (g/head)	Dry mass of sorghum (g/head)	N in grain tissue (%)	P in grain tissue (%)	N uptake (kg ha^{-1})	P uptake (kg ha^{-1})
Control	28.6 ± 1.3 (B)	18.8 ± 0.7 (B)	1.40 ± 0.09 (B)	0.31 ± 0.02 (AB)	62 ± 11	14 ± 1
Duckweed	54.4 ± 5.5 (A)	34.4 ± 5.9 (AB)	1.63 ± 0.03 (A)	0.35 ± 0.00 (A)	93 ± 30	19 ± 6
Fertilizer	52.9 ± 7.7 (AB)	35.1 ± 5.2 (AB)	1.48 ± 0.02 (B)	0.30 ± 0.01 (B)	75 ± 26	16 ± 5
Mix	61.0 ± 15.4 (A)	39.4 ± 10.1 (A)	1.63 ± 0.06 (A)	0.35 ± 0.02 (A)	101 ± 55	21 ± 12
p-values	0.015	0.019	0.001	0.016	0.600	0.602

present in the soil (Table 6): the duckweed treatment increased the C and K content in the soil more (on average) than control and fertilizer treatments, lost less Ca and TN than fertilizer and mix treatments, and increased the P content in a range comparable to the fertilizer treatment. This is due to the heterogeneous composition of duckweed, which is more carbonaceous than the inorganic fertilizer used in the study. However, statistical analysis indicates that only P and Ca showed significant differences between treatments ($p = 0.02$ and $p = 0.026$, respectively).

$$Nutrient\ change = \left(\frac{Nutrient_{Pre-sowing,Year\ 2} - Nutrient_{Post-harvest,Year\ 2}}{Nutrient_{Pre-sowing,Year2}}\right) \times 100 \tag{2}$$

The soil was also analyzed to determine the soil residue of NH_4^+, NO_3^-, and total inorganic N (TIN). To calculate the soil residue per hectare, the soil depth was assumed to be 20 cm (plow depth) and the soil bulk density was 1.4 g cm^3, giving 2,241,701 kg of soil per hectare. The TIN residue in the soil was significantly higher for duckweed treatment than for the other treatments except for the mix (Table 7), indicating that duckweed enhanced the soil N content.

Mass balances of N and P in the different treatment plots of the Year 2 study are provided in **Table S3** and **Table S4**, respectively (supplemental information). These mass balances were done considering N and P in the soil, grain tissue,and runoff, but did not include atmospheric losses of N such as nitrogen gas (N_2) or nitrous oxide (N_2O). The duckweed treatment provided on average better TIN residue to the soil, and provided better N and P nutrition to grain tissue than fertilizer. Losses of N and P were smaller in plots treated with duckweed than in those treated with commercial fertilizer.

3.4. *Germination test*

In a controlled greenhouse germination test, sorghum seedlings started to emerge 6 days after transplanting, and germination stopped after 14 days. No statistically significant differences were found in the germination rate between treatments. Treatments containing conventional fertilizer (fertilizer and mix) both exhibited the smallest average germination rates (75%). Oven-dried duckweed, greenhouse-dried duckweed (50% N requirement), and greenhouse-dried duckweed (100% N requirement), showed the highest germination rates, at 85%, 90%, and 90%, respectively (**Table S5**). Interestingly, the germination rates for the control (82%) and conventional fertilizer (75%) treatments were below the rates observed in the field for the same two treatments.

4. Discussion

The cumulative mass of NH_4^+-N and NO_3^--N leached during each rain event was smaller for duckweed than for fertilizer or mix. This was determined not to be statistically significant; however, the results obtained suggest that duckweed is likely to generate less N runoff that conventional inorganic fertilizer. As expected, differences in PO_4^{3-}-P were not observed due to the fact that just a small portion of the P in soil is soluble and available for plants (see supplemental information). No P deficiencies were observed in sorghum plants. Mix plots contributed to higher leaching of NH_4^+ and NO_3^- possibly related to the combination of C from the duckweed and the easily available N from inorganic fertilizers. This, along with the water saturation in the soil after rain events, could facilitate N turnover contributing to higher ion mobility and potential gas emissions. Although microbial analysis and gas measurements were beyond the scope of this study, these considerations should be studied to draw definitive conclusions with respect to nutrient cycling in duckweed amended soils. Incubation studies in between rain events may be used to understand the mineralization of N and other N-transformations in future work.

Table 6
Change in soil nutrients before and after Year 2 trials for sorghum plots treated with control (no amendment), duckweed, fertilizer, or mix. Data are averages of four replicate plots for each treatment; error represents one standard deviation. Treatments with different letters, A or B, are considered statistically different.

	Nutrient % change before and after Year 2 field trials					
Treatment	% C	% TN	% P	% K	% Mg	% Ca
Control	−7.4 ± 6.8	−15.4 ± 4.4	−27.6 ± 7.7 (B)	−31.8 ± 0.58	−8.6 ± 3.90	13.3 ± 18.15 (A)
Duckweed	2.6 ± 11.5	−1.6 ± 9.2	21.7 ± 3.6 (A)	6.3 ± 25.15	−14.9 ± 7.19	−2.6 ± 7.71 (AB)
Fertilizer	−4.4 ± 9.6	−11.9 ± 5.1	28.0 ± 27.5(A)	−9.2 ± 9.94	−22.9 ± 14.84	−18.0 ± 3.13 (B)
Mix	3.7 ± 3.9	−7.3 ± 2.4	11.4 ± 17.6 (AB)	−4.1 ± 15.54	−14.0 ± 14.96	−14.7 ± 6.85 (AB)
p-value	0.471	0.085	0.020	0.091	0.75	0.026

Table 7
Soil residue of NH_4^+, NO_3^-, and TIN remaining in sorghum plots after Year 2 field trials with control (no amendment), duckweed, fertilizer, or mix. Data are averages of four replicate plots for each treatment; error represents one standard deviation. Treatments with different letters, A or B, are considered significantly different.

	Soil residue after Year 2 field trials (kg ha^{-1})		
Treatment	NH_4^+	NO_3^-	TIN
Control	3.78 ± 0.22	21.63 ± 1.44 (B)	25.41 ± 1.48 (B)
Duckweed	4.74 ± 0.93	29.22 ± 1.56 (A)	33.96 ± 1.81 (A)
Fertilizer	4.30 ± 0.52	23.41 ± 3.37 (B)	27.7 ± 3.41 (B)
Mix	4.04 ± 0.41	24.74 ± 2.04 (AB)	28.78 ± 2.07 (AB)
p-value	0.35	0.015	0.015

Significant differences were observed in crop yield due to the low germination rates observed with duckweed and mix: 50% and 40% below the expected germination rate, respectively. Nevertheless, the nutritional value of sorghum grains grown with duckweed and mix was significantly higher than grains grown with fertilizer treatment. In addition, this difference makes it clear that despite the smaller number of plants per plot with duckweed amendments, the plant uptake of N and P was higher with duckweed treatments than with conventional fertilizer. After two consecutive years of utilizing duckweed fertilization in the same plots, the average concentrations of nutrients like C and P in the soil were greater, and the losses of TN, Mg, and Ca were smaller, than in the other treatments. As a general conclusion, duckweed performed statistically similar to commercial fertilizer, but provided better plant nutrition in terms of N uptake.

In order to facilitate a comparison of nutrient losses from the field during Years 1 and 2, Table 8 illustrates the total masses of NH_4^+-N, NO_3^--N, and PO_4^{3-}-P leached by control, duckweed, and fertilizer plots. Less NH_4^+-N and NO_3^--N were leached in the second year overall, whereas more PO_4^{3-}-P was discharged. A possible explanation for lower NH_4^+-N and NO_3^--N masses in the second year could be due to the lower volumes of water that were collected during the rain events in Year 2 (see **Fig. S1** and **S2**, supplemental information). This is likely an indication that the trench that was dug on the upper part of the field before the Year 2 trial successfully prevented surficial runoff generated on the up-gradient grassed hill from infiltrating the collection system (this was not done during the first run of the experiment in Year 1). A plausible explanation for the higher leaching of PO_4^{3-}-P is continuous fertilization (in Year 1 and 2) with P. This element accumulates in agricultural soils over time, since not all the P applied to the soil is absorbed by the plants for physical or chemical reasons. Application limitations, such as when P is limited to the upper layer of the soil without reaching root zone, or P forming compounds with other elements present in the soil such as Fe or Al, make some of the P unavailable for plant uptake. After the P is fixed in the soil, time and changing environmental conditions may help the P

Table 8

Longitudinal comparison between Year 1 and Year 2 of NH_4^+-N, NO_3^--N, TIN, and PO_4^{3-}-P leached from sorghum plots treated with control (no amendment), duckweed, or fertilizer. Data are averages of four replicate plots for each treatment; error represents one standard deviation.

	kg ha^{-1}							
	NH_4^+-N leached		NO_3^--N leached		TIN leached		PO_4^{3-}-P leached	
Treatment / year	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Control	0.98 ± 0.10	0.05 ± 0.01	1.78 ± 0.58	0.41 ± 0.13	2.76 ± 0.66	0.46 ± 0.13	0.20 ± 0.06	0.36 ± 0.01
Duckweed	0.86 ± 0.12	0.15 ± 0.16	1.73 ± 0.45	0.86 ± 0.43	2.62 ± 0.52	1.00 ± 0.46	0.23 ± 0.06	0.35 ± 0.07
Fertilizer	1.31 ± 0.30	0.49 ± 0.24	2.29 ± 0.68	1.36 ± 0.82	3.60 ± 0.80	1.85 ± 0.85	0.23 ± 0.08	0.38 ± 0.09

to become soluble and available for plants.

The germination inhibition that was observed for duckweed amended field plots in Year 2 could not be replicated in the controlled greenhouse germination test, suggesting that other factors might have had an effect on seed germination in the field. The presence of high salt concentrations that could damage the seeds, excessive moisture held by the upper part of the soil due to the presence of duckweed, the effect of large rain events that occurred during the germination phase, or fungi colonizing some of the seeds could all be factors that prevented germination from being optimal. Of these, the presence of salts carried on the duckweed appeared to be the most likely explanation, since some facilities on campus were known to have utilized an excessive amount of salt to regenerate ion exchange water softeners during the period in which duckweed was collected for the Year 2 field and greenhouse experiments. For a more in-depth analysis of the Na content in dry duckweed utilized in the different field trials and in the germination test see **Table S6** (supplemental information). The duckweed utilized during Year 1 was higher in Na than in Year 2, but due to the differences in the application rate (mass of duckweed applied per area), the final amount of Na added to the plots was smaller during Year 1. The amount of Na applied during Year 2, 44 ± 2 kg ha^{-1}, was significantly higher than in Year 1, 27 ± 2 kg ha^{-1} ($p = 0.000$), which may support the observation of low germination rates in Year 2. On the other hand, the Tukey test did not find significant differences between the amount of Na that was applied to: (i) mix plots in Year 2 (51.8 ± 4.8% germination); (ii) direct oven-dried mix treatment in the germination test (75.0 ± 10.8% germination); and (iii) duckweed with 50% N requirement treatment in the germination test (90.0 ± 4.1% germination). This is evidence that when low amounts of Na are applied ($<$ 17 kg ha^{-1}), germination is affected in different ways for different treatments, indicating that there must be another factor (besides Na) that is affecting germination. It is therefore necessary to continue exploring germination under different conditions to discover possible factors, other than Na, associated with the presence of duckweed that may have inhibited seed germination during Year 2 of the field experiment.

5. Conclusions

In this study, soils amended with duckweed generated less ammonia and nitrate in surficial runoff than commercial fertilizer while enriching the soil and producing sorghum grains with greater N and P content. When normalized by germination rate, duckweed produced comparable sorghum yields, indicating the need to further evaluate the cause of potential germination inhibitors. The mix treatment (containing a combination of duckweed and commercial fertilizer) may have contributed to higher N losses than inorganic fertilizer or duckweed alone. Overall, these results indicate that duckweed may be a viable alternative to commercial fertilizer; however, potential greenhouse gas emissions (ex., N_2O) and groundwater leachate should be quantified in order to link this promising amendment with nitrogen use efficiency in crops. Additionally, its efficacy when applied to different crops should be demonstrated, which will be explored in future work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2021.106273.

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