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Duckweed as an Agricultural Amendment: Nitrogen Mineralization, Leaching, and Sorghum Uptake

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Abstract

Excessive N and P in surface waters can promote eutrophication (algae-dominated, low-O_2 waters), which decreases water quality and aquatic life. Duckweed (Lemnaceae), a floating aquatic plant, rapidly absorbs N and P from water and its composition shows strong potential as a soil amendment. Therefore, it may be used to transfer N and P from eutrophic water bodies to agricultural fields. In this work, dried duckweed was incorporated into agricultural soil in microcosm, column, and field tests to evaluate biological N cycling, nutrient retention, and crop yield compared with compost, diammonium phosphate (DAP), and an amendment-free control. In microcosm tests, 25 ± 13% of duckweed N was mineralized, providing on average less mineral N than DAP (107 ± 21%), but more than compost (11 ± 12%). In columns, duckweed treatments leached only 2% of the N added, significantly less than DAP, which leached 60% of its N. Compared with the control, DAP leached significantly more phosphate (78%), whereas duckweed and compost treatments leached less (56 and 27%, respectively). Crop yield, as well as runoff N and P, were measured in field tests growing forage sorghum [*Sorghum bicolor* (L.) Moench.]. Although less total N was applied to duckweed plots than to DAP plots (75 vs. 130 kg ha^{-1}, respectively), duckweed was found to retain 30% more total mineral N in a tilled agricultural field than DAP, while supporting a comparable yield. These tests indicate that duckweed may provide a sustainable source of N and P for agriculture.

Core Ideas

- In microcosm tests, 25% of organic N in duckweed was mineralized within 5 d.
- In 22-d column tests, duckweed leached only 2% of the N applied from its biomass.
- In 22-d column tests, duckweed leached 56% less phosphate than the control.
- In field tests, duckweed reduced inorganic N runoff by 30% compared with mineral fertilizer.
- In field tests, sorghum yield was comparable for duckweed and mineral fertilizer treatments.

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Reducing eutrophication-inducing N and P inputs to surface water requires investment in wastewater treatment, changes to farming practices, and responsible land management. In addition to reducing nutrient inputs, a process to remove nutrients from eutrophic water may be necessary. Duckweed (Lemnaceae), a family of 38 species of simple aquatic plants, is capable of hyperaccumulating N and P at rates rivaling those of algae (Oron et al., 1987). Duckweed grows on the water surface and reproduces rapidly, forming a mat of small plants with 1- to 5-mm fronds (Farrell, 2012). Duckweed may be harvested using nets or large-scale equipment, providing a relatively easy means to remove nutrients from surface waters. Furthermore, harvested duckweed can supply nutrients for a variety of beneficial applications. For example, duckweed has been successfully used as animal fodder (Azim and Wahab, 2003) and as a feedstock for bioethanol production (Cheng and Stomp, 2009; Calicioglu and Brennan, 2018). Duckweed contains comparable concentrations of N, P, and K to most manure-based fertilizers (Penn State College of Agricultural Sciences, 2013), and it has been speculated that duckweed would perform well as a soil amendment to replace synthetic fertilizers (Lam et al., 2014), which have been fluctuating in cost by >100% since 2004 (USDA, 2013). Furthermore, using duckweed as a replacement to chemical fertilizers should reduce agricultural runoff, since organic N bound within the biomass must be mineralized to NH_4^+ and then to NO_3^- before being used by crops, making it a slow-release supplement. Despite these advantages, experimental data on using duckweed as a soil amendment are lacking.

The objectives of this study were to determine if duckweed applied to agricultural soils can effectively supply N and P to crops, and if it can reduce nutrient pollution in leachate and runoff relative to synthetic fertilizers. To test this hypothesis, three controlled experiments were performed. First, laboratory microcosms were used to measure N cycling in agricultural soil amended with either duckweed or conventional fertilizer. Second, a column study was conducted to measure leachate from amended soils under simulated rain events. Finally, a field study was performed to measure the yield of forage sorghum [*Sorghum bicolor* (L.) Moench.], as well as runoff of nutrients from plots amended with duckweed or conventional fertilizer.

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Abbreviations: AASL, Agricultural Analytical Services Laboratory; CEC, cation exchange capacity; DAP, diammonium phosphate; DO, dissolved oxygen; NSF, National Science Foundation; ORP, oxidation reduction potential.

Materials and Methods

Amendment Materials

The soil for the laboratory experiments was collected from the Sustainability Experience Center at The Pennsylvania State University (University Park, PA) to a depth of 20 cm. This soil is classified as Hagerstown silt loam (fine, mixed, semiactive, mesic Typic Hapludalfs) in the USDA Soil Survey. Collected soil was air dried, passed through a No. 4 mesh (4.75 mm) sieve, and then analyzed by the Agricultural Analytical Services Laboratory at The Pennsylvania State University (AASL) by Mehlich-3 inductively coupled plasma (Wolf and Beegle, 1995). Organic matter was measured by loss on ignition (Schulte and Hoskins, 2011). Total N was measured by combustion (Bremner, 1996). pH was measured using a 1:1 soil water dilution (Eckert and Sims, 1995). Cation exchange capacity (CEC) was measured by summation (Ross, 2011). Zinc and Cu were measured using USEPA Method 3050B/3051 + 6010 (https://www.epa.gov/sites/production/files/2015-06/documents/epa-3050b.pdf).

Duckweed used in the experiments originated from two different sources. Duckweed used in the microcosms and columns was collected from the third open aerobic tank in Penn State's Eco-Machine (a pilot-scale ecological wastewater treatment system) and was previously identified as a co-culture of *Lemna japonica/minor* Landolt. and *Wolffia columbiana* Karst. (Calicioglu and Brennan, 2018). Duckweed used in the field tests was collected from a pond in Pennsylvania State Game Land no. 176, which is spray irrigated with treated effluent from the Penn State Wastewater Treatment Plant, and consisted of a monoculture of *Lemna obscura* (Austin) Daubs (Calicioglu and Brennan, 2018). A pool skimmer was used to collect duckweed and deposit it into coolers, where it was allowed to drain, rinsed with tap water, and drained again. Eco-Machine duckweed was dried in a 60°C convection oven for 3 d, and then stored in plastic bags in the dark at 3°C. Spray field duckweed was air dried in a greenhouse for 3 d and then directly applied to the field. Duckweed was analyzed by AASL according to Test Methods for the Examination of Composting and Compost (Thompson et al., 2002). Spray field duckweed had 2.5% N, whereas the Eco-Machine duckweed contained 5.6% N (Table 1), likely due to higher wastewater strengths in the Eco-Machine. After drying, the spray field duckweed was digested in HNO_3 and analyzed for metals by inductively coupled plasma emission spectrometry (ICP–AES, detection limit = 0.01 mg kg^{-1}) by the Materials Characterization Laboratory (The Pennsylvania State University).

Finished compost was obtained from the Penn State Composting Center (University Park, PA), with starting materials composed of food wastes, manure, and leaves. Compost composition was determined by the AASL (Table 1). Dried duckweed and compost were ground with a mortar and pestle and passed through a 0.5-mm screen before use in the laboratory experiments. For the field experiments, duckweed was not ground or sieved. Diammonium phosphate (DAP) fertilizer used in the microcosm test and column test was laboratory grade (J.T. Baker).

Nitrogen Cycling in Microcosms

A sacrificial microcosm experiment was performed to measure N cycling in soil between organic matter, NH_4^+, and NO_3^- after one of four different treatments: compost, duckweed, DAP, or no amendment (negative control). Amendments were added at 75 mg N kg^{-1} soil. Assuming a soil bulk density of 1.2 g cm^{-3}, this is equivalent to ~140 kg N ha^{-1} which is a typical amount applied to corn (*Zea mays* L.), sorghum, and other crops with a high demand for N. The target application rates of N, P, and K are provided in Table 2. The soil and amendments for each treatment were mixed in closed containers and vigorously shaken by hand for 10 min and then distributed in 40.0-g aliquots to 30 replicate 125-mL flasks.

Table 1. Composition of duckweed, compost, and soil used in mineralization and leaching experiments. The average composition of forage sorghum grown in these tests is also reported.

Property	Material			
	Duckweed	Compost	Soil	Forage sorghum
Wet mass basis				
pH	6.76	7.65	6.5	NA
Acidity, cmol kg^{-1}	NA†	NA	2.2	NA
Soluble salts, m℧ cm^{-1}	3.18	8.11	NA	NA
Solids, %	4.48	52.3	NA	23
Moisture, %	95.5	47.7	NA	77
Dry mass basis				
Organic matter, %	78.8	58.2	2.6	NA
Total N, %	5.63	3.35	0.27	1.09
Organic N, %	5.11	3.25	NA	NA
NH_4^+–N, mg kg^{-1}	5221	820	4.9	NA
NO_3^-, mg kg^{-1}	NA	NA	7.8	NA
C, %	39.1	35.0	NA	NA
C/N	7.04	10.5	NA	NA
P, % as P_2O_5	2.59	1.07	0.0069	0.575
K, % as K_2O	4.12	1.11	0.0232	1.91
Ca, %	1.49	NA	0.126	0.29
Mg, %	0.436	NA	0.0218	0.31
S, %	0.448	NA	6.80×10^{-4}	0.11
Na, mg kg^{-1}	6763	NA	NA	14.6
Al, mg kg^{-1}	273	NA	NA	14.1
Fe, mg kg^{-1}	548	NA	NA	76.0
Mn, mg kg^{-1}	238	NA	NA	50.6
Cu, mg kg^{-1}	35.0	NA	1.70	<5
Zn, mg kg^{-1}	59.2	NA	2.70	33.2
CEC‡, cmol kg^{-1}	NA	NA	10.8	NA
Cr, mg kg^{-1}	ND§	NA	NA	NA
Pb, mg kg^{-1}	ND	NA	NA	NA
Ni, mg kg^{-1}	ND	NA	NA	NA
Zn, mg kg^{-1}	20–100	NA	NA	NA

† NA, not analyzed.

‡ CEC, cation exchange capacity.

§ ND, not detected.

Table 2. Target concentrations of nutrients added to soil for each of the three experiments.

Treatment	Nutrient added			
	N	P	K	C
	mg kg^{-1}			
Microcosms & columns				
Compost	75	10	20	772
Duckweed	75	25	42	522
Diammonium phosphate	75	82	0	0
Field plots				
Duckweed	25	18	35	
Fertilizer†	58	22	58	0

† Composed of 2.4% diammonium phosphate (DAP)-N, 13.6% urea-N, 2.6% DAP-P, and 13% KCl-K.

After adding deionized water to reach 60% water-filled pore space (12.5 mL), the flasks were capped with Parafilm to prevent moisture loss and stored in the dark at room temperature (19–21°C). Microcosms were weighed weekly and replenished with distilled water as necessary. Microcosms were sacrificed in triplicate periodically over 2 mo. The original mixture ($t = 0$) was extracted in singlet.

Soil samples were extracted into 2.0 M KCl solution following the method of Robertson et al. (1999) and then analyzed on a Lachat QuikChem FIA +8000 machine for NH_4^+ and NO_3^-.

Column Leaching during Simulated Rain Events

The four soil treatments and loading rates used in the microcosm experiment (Table 2) were also tested in a column leaching experiment. After mixing the soil amendments, 400 g of each treatment was dry-packed to a density of 1.08 g cm^{-3} in triplicate clear polyvinyl chloride (PVC) columns that were 50 mm in diameter and 300 mm high. Glass beads (4-mm diam.) were added as a 20-mm lift to the bottom of the column to prevent clogging, and to the top of the column to help disperse applied "rain events" evenly. Distilled water was compared with rainwater in a preliminary leaching experiment, and no differences in downgradient water chemistry were detected; therefore, distilled water was used in all column tests. The flow rate through the columns was equivalent to a 100-yr storm in the local area (125 mm d^{-1} in Centre County, Pennsylvania), which corresponds to 0.18 mL min^{-1} within the 50-mm-diam. column over a simulated rain event of 9 h. Rain events were simulated every 2 to 4 d for over 3 wk.

In a follow-up experiment, similar conditions were repeated, except the influent distilled water was sparged with air to ensure O_2 saturation prior to being applied continuously (instead of intermittently).

Leachate collected from the bottom of the columns was measured using standard electrodes for NH_3, pH, dissolved O_2 (DO), and oxidation reduction potential (ORP). Ammonia was measured with a Thermo Scientific Orion 951201 probe. pH was measured with a Thermo Scientific GS9106BNWP probe. Dissolved O_2 was measured using a VWR SympHony 11388-374 probe. Oxidation reduction potential was measured with an Oakton ORP Testr 10 field probe. Anions were measured on an ion chromatograph (Dionex IC-1100) equipped with an AS-22 column (Dionex). Cumulative plots (Fig. 1) were generated by summing the mean for each treatment and adding the SD in quadrature.

Runoff and Sorghum Yield Field Study

A pilot-scale field test was performed to measure the yield of forage sorghum and nutrient concentrations in runoff from an agricultural field subjected to different treatments. Three treatments were evaluated using five replicates in a randomized block design, which controlled for inherent soil variations across subplots. The treatments were duckweed, a commercial-blend 16–6–16 (N–P–K) fertilizer, and a control with no amendment. The fertilizer was composed of 2.4% DAP-N, 13.6% urea-N, 2.6% DAP-P, and 13% KCl-K. Total N, P, and K applied to the various plots is provided in Table 2. The experiment was performed on a hillside (8–15% grade) at the Sustainability Experience Center divided into 15 subplots, each 4 by 10 m. The composite average starting soil conditions across the whole field site (n = 45) were initially 2700 mg kg^{-1} total N, 30 mg kg^{-1} total P (Mehlich-3, optimum range), 193 mg kg^{-1} K (Mehlich-3, optimum range), 218 mg kg^{-1} Mg, 1262 mg kg^{-1} Ca, 2.6% organic matter, pH 6.5, 2.2 cmol kg^{-1} acidity, and 10.8 cmol kg^{-1} CEC.

To reduce residual N in the test plots and minimize competition with sorghum, grass in the field was removed by mowing, raking, and applying a paraquat-based herbicide (Gramoxone SL, Syngenta, 2.47 kg ha^{-1}). One week later, the field was tilled with a moldboard plow. Five weeks after herbicide application, the amendments were evenly applied by hand and then lightly incorporated using a cultipacker. Within hours, forage sorghum (Alta Seeds AF7202) was planted in rows spaced 38 cm apart using a seed drill. Forage sorghum was selected as a demonstration crop because it has a comparable N requirement to corn, which has the highest N demand among commodity crops (Penn State College of Agricultural Sciences, 2013), thereby testing the ability of duckweed to supply N. Sorghum is also more drought tolerant than corn, making it appropriate for warm environments where duckweed is likely to be cultivated.

Berms were dug between each subplot to hydrologically isolate rainfall in each area. Plastic sheeting (0.15 mm thick) was laid in troughs at the downgradient edge of each subplot to collect runoff and direct it into a high-density polyethylene (HDPE) corrugated pipe (75 mm) that drained into a covered 150-L collection tank. Immediately after natural rain events, runoff volume in each tank was measured and water samples collected for analysis. Conductivity and pH were measured with an Oakton Multiparameter PCS Testr 35. Ammonia, ORP, and anions were measured as in the column experiment.

During harvesting with a rotary corn head forage harvester (117 d after planting), the total mass of chopped forage (including grain, stalk, and foliage) collected from each plot was recorded. After harvesting, samples of chopped forage sorghum were measured for moisture content and were analyzed by AASL using a dry ash method (Table 1; Miller, 1998). Total N was measured by combustion (Horneck and Miller, 1998). The average mass and percentage moisture of six grain heads, as well as the dry mass of 1000 grains, were determined in each plot at consistent locations.

Statistical analyses were performed using SAS 9.4 (SAS Institute, 2013). A two-way ANOVA was used to control for baseline variation across subplots. Treatment differences were evaluated using Tukey's test to compare treatment means and were considered to be significantly different when $p \leq 0.05$.

Results and Discussion

Nitrogen Cycling in Microcosms

Nitrogen mineralization appeared to reach steady state in the microcosms after 5 d (Fig. 2). The ammonification rate of the compost and duckweed treatments were positive and nearly indistinguishable from each other, indicating that organic N was continuously converted to NH_4^+ throughout the experiment. Nitrification rates were noticeably different between the treatments after 28 d: at that time, DAP-N was most rapidly converted to NO_3^- (5.0 ± 1.0 mg N kg^{-1} d^{-1}), followed by duckweed (2.4 ± 0.2 mg N kg^{-1} d^{-1}), compost (1.6 ± 0.3 mg N kg^{-1} d^{-1}), and the control (1.7 ± 0.2 mg N kg^{-1} d^{-1}). Excessive nitrification rates, such as exhibited by DAP, are not desirable to maintain mineral N in soil since NO_3^- is soluble and easily lost from soil, whereas NH_4^+ is retained on soil cation

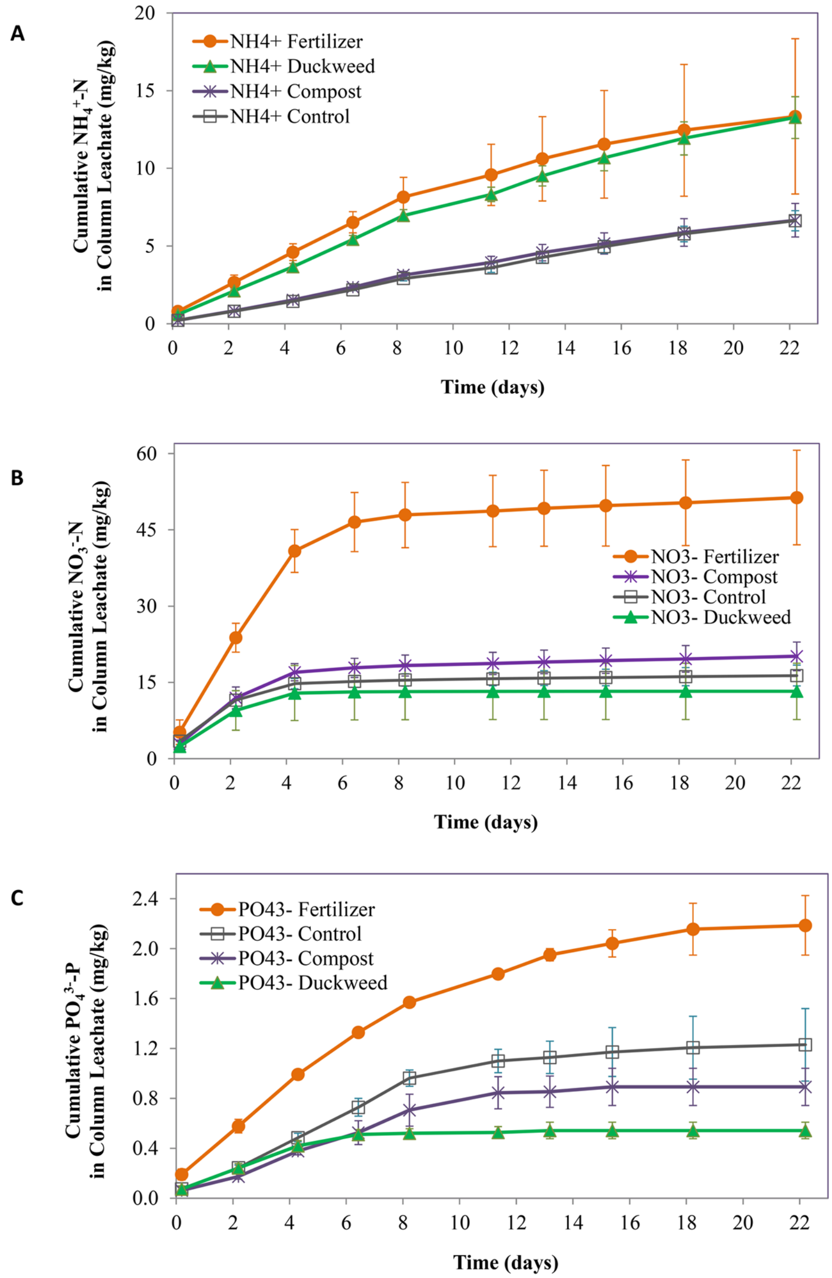

Fig. 1. Cumulative masses of ions leached from soil columns under simulated intermittent rainfall: (A) NH_4^+, (B) NO_3^-, and (C) PO_4^{3-}. The extended presence of NH_4^+ in duckweed leachate indicates that enough N was mineralized to saturate the soil cation exchange capacity. The minimal NO_3^- and PO_4^{3-} leached by duckweed treatments suggests that pollution may be minimized with this amendment.

exchange sites. Since the nitrification of organic N occurs more slowly than DAP-N, it may be desirable for certain agricultural operations.

In total, 25 ± 13% of the organic N in the duckweed treatment was mineralized (which was calculated as the sum of NH_4^+, NO_3^-, and NO_2^-, whereas compost mineralized only 11 ± 12%. When accounting for both the higher initial fraction of N and higher fraction of mineralization, duckweed provides approximately twice as much inorganic N per pound of organic material than compost.

The DAP treatment, already in mineral form, showed consistent inorganic N throughout the experiment (107 ± 22%), providing significantly more inorganic N than organic treatments ($p < 0.0001$). Relatively constant N concentrations with DAP suggest that microbial uptake of N with this amendment was minimal.

Column Leaching during Simulated Rain Events

Intermittent Simulated Rainfall Column Tests

Measured total ammonia N was assumed to be predominantly in the NH_4^+ form because the leachate pH was 7.5 ± 0.2 for all samples, below the pK_a for NH_3 (9.3). Leachate NH_4^+ concentrations from the control and compost treatments remained fairly constant over time (Fig. 1A), whereas those of duckweed and DAP treatments steadily decreased, indicating that NH_4^+ was buffered into solution by the soil CEC. After 22 d of intermittent rainfall (achieving an average cumulative liquid/solid ratio = 2.4 mL g^{-1}), the average cumulative NH_4^+–N leached was 13.3 ± 50 mg kg^{-1} in DAP, 13.3 ± 1.3 mg kg^{-1} in duckweed, 6.7 ± 1.1 mg kg^{-1} in compost, and 6.6 ± 0.7 mg kg^{-1} in control treatments (Fig. 1A). The similarity between duckweed and DAP indicates that the soil

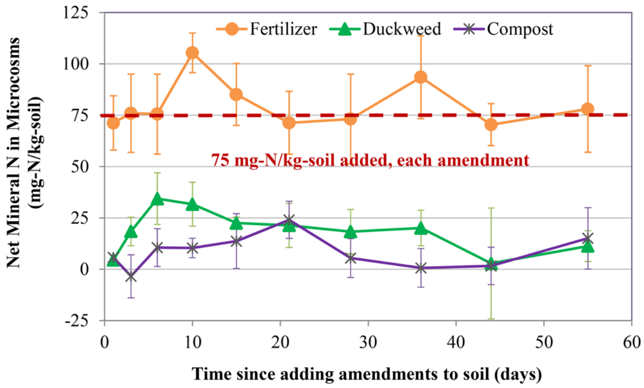

Fig. 2. Net mineral N concentrations over 55 d in sacrificial soil microcosms (*n* = 3) treated with different amendments. Net mineral N for treatments was calculated by subtracting the control. Data points are triplicate averages; error bars represent 1 SD.

CEC was saturated with NH_4^+ for both treatments. The difference between duckweed and compost treatments indicates that duckweed was mineralized to a greater extent. Assuming that duckweed and compost both have labile C, it is unlikely that microbial uptake scavenged NH_4^+ in the compost treatment.

Over 90% of the NO_3^-–N leached from the columns was lost within 6 d, indicating that NO_3^- was not well retained (Fig. 1B). As expected, the majority of the 75 mg kg^{-1} of DAP-N added was leached as NO_3^- and was significantly higher than all other treatments ($p < 0.0001$). The average cumulative NO_3^-–N leached after 22 d was 51.4 ± 9.3 mg kg^{-1} in DAP, 20.1 ± 2.8 mg kg^{-1} in compost, 16.3 ± 2.1 mg kg^{-1} in the control, and 13.2 ± 5.5 mg kg^{-1} in duckweed treatments (Fig. 1B). Both duckweed and compost treatments leached similar quantities of NO_3^- as the control, indicating that N added in these organic forms as applied here will not result in increased N runoff.

The fraction of applied N that was lost in the column tests as NH_4^+, NO_3^-, or NO_2^- was calculated by ($N_{treatment,leached} - N_{control,leached})/N_{treatment,added}$. Duckweed-amended soil leached the lowest fraction of total N added (2 ± 13%), followed by compost (4 ± 8%), and DAP (60 ± 14%). In the control, compost, and DAP treatments, >78% of the total N leached was in the form of NO_3^-. In the duckweed treatment, only 59% of the total N leached was as NO_3^-.

The fraction of applied P lost was calculated by ($P_{treatment,leached} - P_{control,leached})/P_{treatment,added}$. Cumulative PO_4^{3-}–P losses in leachate were 2.2 mg kg^{-1} in the DAP treatment, 1.2 mg kg^{-1} in the control, 0.9 mg kg^{-1} in the compost treatment, and 0.5 mg kg^{-1} in the duckweed treatment (Fig. 1C). Compost- and duckweed-amended soils leached the lowest fraction of total P added, at −3.2 ± 2.7 and −2.7 ± 1.0%, respectively. The DAP-amended soils leached 1.2 ± 0.4% of the added P. Microbial uptake may account for PO_4^{3-} and NO_3^- retention in duckweed and compost treatments; however, additional experimentation would be necessary to draw a definitive conclusion.

Continuous Simulated Rainfall Column Tests

Under the intermittent rainfall column tests, the duckweed and DAP treatments leached similar amounts of NH_4^+, but the DAP treatment leached substantially more NO_3^-. It was hypothesized that the C provided by the duckweed may have facilitated the consumption of O_2 and subsequently enabled denitrification, thus converting NO_3^- to N_2 and reducing leached NO_3^- compared with DAP treatments. In addition, the simulated intermittent rainfall likely created a cyclical and complex redox environment from repeated wetting and drying of the soil. For these reasons, a follow-up experiment was performed using simulated continuous rainfall with O_2–saturated water to develop a steady, aerobic environment and thereby minimize denitrification. For all treatments in the continuous rainfall tests, DO in the leachate ranged from 60 to 90% saturation, and ORP ranged from 175 to 280 mV throughout the experiment. No consistent differences between treatments were observed for DO. Although not statistically significant during all sample events, the leachate ORP from duckweed-amended soils was frequently lower than that of compost-amended soils.

Under continuous oxygenated rainfall conditions for 28 d (cumulative liquid/solid ratio = 25 mL g^{-1}), all treatments leached more cumulative NH_4^+ than under intermittent conditions, and the duckweed and DAP treatments leached substantially more: 31 ± 2.7 and 47.3 ± 6.5 mg kg^{-1} NH_4^+, respectively. Cumulative NO_3^- leached from the duckweed treatment remained the lowest overall (18.9 ± 0.2 mg kg^{-1} NO_3^-–N). At a cumulative loss of 25.7 ± 0.1 mg kg^{-1} NO_3^-–N, the DAP treatment leached less NO_3^- than the control or compost treatments, a dramatic decrease from the intermittent rainfall experiment in which the DAP treatment leached more than double all other treatments. The decrease in leached NO_3^- observed in the continuous flow column tests is attributed to the high rate of flow that flushed NH_4^+ out of the column before it could be oxidized.

Duckweed-amended soil lost less N than DAP-amended soils, primarily because less NO_3^- was lost from duckweed treatments. The mechanism for the lower NO_3^- in duckweed treatment leachate remains unclear, but this experiment suggests that denitrification was not the cause. Leachate DO and ORP measurements indicated that the bulk soil was not becoming anoxic, although anoxic micro-environments may have existed. It is also possible that duckweed supplied more labile C than the other treatments for microbial growth, which would subsequently cause NH_4^+ and NO_3^- to be incorporated into cell biomass.

Losses of PO_4^{3-} due to leaching under continuous rainfall conditions were minimal in all treatments. However, the duckweed and compost treatments leached less PO_4^{3-} than the control treatment, indicating that the organic matter, Fe, and Al in duckweed and compost may have immobilized PO_4^{3-}, thus reducing P flux to downgradient receiving waters.

Since 91% of the NO_3^-–N in the DAP treatment was leached within the first 7 d of continuous rainfall, good management practices during DAP application may reduce N losses. Although the simulated rain event was larger than what would be expected during an average growing season in Centre County, Pennsylvania, and therefore DAP-N may not be lost in such large quantities in a field situation, the application of duckweed reduced NO_3^-–N losses even compared with the control. Assuming that increased microbial uptake of N occurs due to labile C in duckweed, then total soil N can be increased by applying duckweed, while minimizing nutrient pollution.

Field Runoff and Crop Yield

Runoff

Natural rainfall in the field ranged between 1.2 and 6.4 cm d^{-1}, compared with the 4.8 cm d^{-1} that was applied during the intermittent rainfall column experiment. Given the total volume

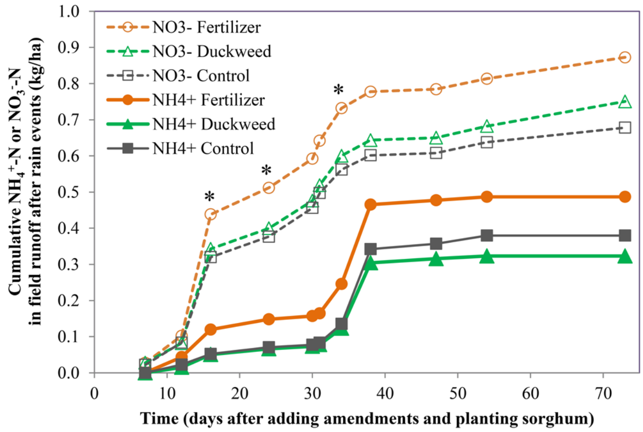

Fig. 3. Cumulative runoff losses of NH_4^+ and NO_3^- ($n = 5$) from a field treated with duckweed, a commercial blend 16–6–16 N–P–K fertilizer, or no treatment (control). Runoff events where the fertilizer treatment had significantly more NH_4^+–N than the other treatments are marked with an asterisk (*): Day 16 ($p = 0.03$), Day 24 ($p = 0.01$), and Day 34 ($p = 0.02$).

of rainfall applied in both experiments, nutrient losses from the field were expected to be smaller than the columns, which was indeed observed.

Cumulative NH_4^+–N in runoff from fertilizer plots was significantly higher ($p = 0.007$) than from duckweed and control plots throughout the experiment (Fig. 3). Variable NH_4^+–N concentrations were likely a result of variable rain intensities. The pH in runoff collection water was <7.5 throughout the experiment, suggesting that negligible quantities of NH_3 were lost through volatilization.

Although runoff NO_3^-–N concentrations were not significantly different between treatments ($p = 0.27$), cumulative NO_3^-–N in runoff was highest from the fertilizer plots (Fig. 3), followed by duckweed, and the control. Similar to the column leaching experiment, NO_3^-–N in the field runoff decreased over time throughout the experiment. The fertilizer plots lost 30% more combined NH_4^+–N and NO_3^-–N than the control or duckweed plots ($p = 0.09$).

Cumulative total P and PO_4^{3-}–P in the field runoff were statistically similar across all three treatments, which is in agreement with the laboratory column leaching results. At the last rainfall event, 73 d after planting, the cumulative PO_4^{3-}–P lost in the runoff was ~0.10 kg ha^{-1} ($p = 0.85$), and the total P lost was nearly 2.0 kg ha^{-1} ($p = 0.65$) for all treatments.

Forage Sorghum Yield

The dry mass yield of forage sorghum was highest in fertilizer plots (8.69 ± 0.90 Mg ha^{-1}), followed by duckweed (8.36 ± 1.26 Mg ha^{-1}) and control plots (7.93 ± 0.73 Mg ha^{-1}). Although less total N was applied to duckweed plots than to DAP plots (75 vs. 130 kg ha^{-1}, respectively), the data suggest that duckweed supports a crop yield increase within the expected response range. The yield response to N inputs (fertilizer and duckweed) was statistically similar ($p = 0.12$), which is likely due to significant background variability throughout the plots. The field had significant variation across blocks or repetitions ($p = 0.004$), with subplots on the downslope side of the field producing higher yields than the plots on the upslope side, suggesting that a previously unrecognized "fertility gradient" was present. The variability may be due in part to residual N (from grass tilled under) that supplied more N to the control treatment than would be present in a typical long-term agricultural field. Plot variability is also likely due to different drainage patterns throughout the field. Despite field variability, the data suggest that duckweed and fertilizer induced similar yield responses. The duckweed yield response was observed without a comparable increase in N and P runoff. Furthermore, it is likely that residual duckweed organic N will be mineralized in subsequent seasons, thus providing additional N beyond what was observed in this experiment, which will be examined in future work.

The application of duckweed and fertilizer resulted in increased uptake of N and P by plants (Table 3). The fraction of N in forage sorghum biomass was significantly higher ($p = 0.002$) in the fertilizer plots (1.20% N) than in duckweed (1.05% N) and control (1.03% N) plots. The fraction of P in forage sorghum biomass was similar ($p = 0.10$) in all treatments (fertilizer = 0.254% P, duckweed = 0.256% P, and control = 0.254% P). Fertilizer efficiency, calculated as $[(\text{Yield}_{\text{treatment}} \times N_{\text{fraction,treatment}}) - (\text{Yield}_{\text{control}} \times N_{\text{fraction,control}})]/N_{\text{applied,treatment}}$, was 8 ± 22% for duckweed and 17 ± 9% for fertilizer. No significant difference was observed in the mass of individual sorghum grains between treatments ($p = 0.63$).

A mass balance of N and P in the field plot indicates that the measured values for runoff and crop uptake are reasonable (Table 4). The high value of baseline TN in the soil is likely to have supported the crop yield observed in the control treatment.

Table 3. Forage sorghum yield and uptake of N and P as affected by application of duckweed and fertilizer.

Treatment (applied N)	Yield†	N uptake	P uptake
	kg ha^{-1}		
Control (0 kg ha^{-1})	7930a‡	82a	20a
Duckweed (75 kg ha^{-1})	8360a	88a	21a
Fertilizer (130 kg ha^{-1})	8690a	104b	22a

ANOVA

Source of variation	df	Significance	df	Significance	df	Significance
Treatment (trt)	2	ns§ (0.11)	2	** (0.006)	2	ns (0.10)
Block (rep)	4	** (0.004)	4	ns (0.06)	4	* (0.013)

* Significant at the 0.05 probability level.

** Significant at the 0.01 probability level.

† Yield = (harvested forage sorghum mass, Mg) × (dry matter fraction)/(plot area, ha).

‡ Within columns, means followed by the same letter are not significantly different according to LSD (0.05).

§ ns, nonsignificant.

Table 4. Mass balance of N and P in field plots planted with forage sorghum and treated with either duckweed, a commercial blend 16–6–16 N–P–K fertilizer, or no amendment (control). Mass concentration was generated by an assumed soil depth of 20 cm (n = 5).

Treatment	Soil total N	Added N	Runoff total N	N uptake	Soil total P	Added P	Runoff total P	P uptake
	mg kg^{-1}							
Control	2700	0	3.1	36	30	0	1.0	9.0
Duckweed	2700	25	3.2	39	30	8.8	0.9	9.5
Fertilizer	2700	58	4.2	46	30	9.5	0.9	9.8

Nevertheless, the addition of duckweed and fertilizer caused measurable increases in N runoff and plant uptake of N.

The transfer of metals from treated wastewater into duckweed, and subsequently into sorghum, was extremely low (below detection for most metals, Table 1), but Hg, Mo, and Se should be monitored in the future to ensure that wastewater-grown duckweed meets safety standards for agricultural applications.

Conclusions

This work indicates that duckweed applied to agricultural soils may effectively supply plant-available N and P at a rate comparable with mineral fertilizers, yet contribute substantially less N and P pollution.

Duckweed amendments in the microcosm experiment provided the same or more mineral N than compost amendments for an equivalent mass of organic N. In column experiments, the duckweed treatment leached the lowest quantity of NO_3^-. If denitrification was negligible, as the follow-up experiment suggests, then the most likely mechanism for NO_3^- retention is microbial uptake, supported by labile carbon in duckweed, which is a desirable method to retain N in agricultural soils. Verifying the mechanism by which duckweed-treated soil leached less NO_3^- and PO_4^{3-} would help quantify the benefit of duckweed.

In field tests, the total sorghum yield and cumulative N and P runoff for all three treatments were statistically similar, which was likely due to the significant variability throughout the plots. However, the trends indicate that duckweed does provide a yield benefit without a comparable increase in N and P runoff. Future work should focus on repeating the experiment in a uniform field, as well as over multiple growing seasons.

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