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A beneficial by-product of ecological wastewater treatment: An evaluation of wastewater-grown duckweed as a protein supplement for sustainable agriculture

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ABSTRACT

Ecological wastewater treatment systems that incorporate aquatic plants (like duckweed) have the potential to recover nutrients and produce high-quality protein, simultaneously alleviating two global issues: hunger and lack of sanitation. Although protein production by duckweed in simple lagoon systems and laboratory trials has been reported, its physiology throughout more complex wastewater treatment systems containing a range of different environmental conditions has not been examined. In this study, a duckweed co-culture (*Lemna japonica/minor* and *Wolffia columbiana*) was grown on wastewater from four different stages of a pilot-scale ecological treatment system. Contrary to the literature, the protein content of duckweed did not consistently increase with increasing aqueous nitrogen concentrations, but rather appeared to also be dependent on chemical and microbial interactions. This study indicates that with proper management, duckweed grown in ecological wastewater systems can sustainably produce protein at rates exceeding those of common land-grown forage crops (10.1 ton ha^{-1} yr^{-1}).

1. Introduction

In the developing world, up to 90% of domestic wastewater is discharged without any treatment, releasing valuable nutrients into aquatic systems, leading to poor water quality, eutrophication, and dead zones (FAO UN, 2015). Often in similar locations, roughly 780 million people suffer from protein-energy undernourishment, due to scarcity of high-quality food (Swaminathan et al., 2012). As human population grows and developing countries become more affluent, the demand for animal-derived proteins is escalating: the Food and Agriculture Organization (FAO) estimates that dairy and meat consumption will increase by 82% and 102%, respectively, between 2000 and 2050 (Boland et al., 2013). In addition, protein-rich fodder to support livestock growth is often a limiting factor for meat production (Van Huis, 2013), and many regions with minimal arable land and water scarcity are being forced to import fodder, further decreasing the sustainability of local foods (USDA, 2013). There are also significant environmental concerns associated with the production of animal proteins, including land-use, greenhouse gas emissions, and degraded water quality, which all require careful consideration and proper management (McAllister et al., 2011).

Modern wastewater treatment technologies and intensive farming methods may at first appear to provide a solution to our growing sanitation and protein needs, but these are typically restricted to developed areas, require large amounts of energy, and often release excess nutrients and untreated contaminants into aquatic systems. Ecological wastewater treatment systems (ex., constructed wetlands; EcoMachines™) utilize diverse life forms, typically housed in a series of ponds or tanks, to clean wastewater to the same, or better, effluent quality as conventional wastewater treatment systems (ex., activated sludge plants). Compared to conventional technologies, ecological wastewater treatment systems have a smaller energy and chemical footprint, making them well suited for use in small or developing communities (US EPA, 2002). Moreover, certain aquatic plants grown in these systems (ex., duckweed) can sequester nutrients from wastewater, producing high-protein biomass that can be harvested and reused in sustainable agriculture.

The aquatic plants of the subfamily Lemnoideae (common name: duckweeds) require only sunlight to treat contaminated water, and simultaneously produce a concentrated source of protein and nutrients that can easily be harvested and reused for food production. Lemnoideae have been shown to grow rapidly on the surface of municipal, dairy, swine, industrial, and aquaculture wastewaters, reducing chemical oxygen demand (COD) and removing substantial amounts of

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nitrogen (N) and phosphorous (P) (Frederic et al., 2006; Adhikari et al., 2015; Cheng et al., 2002; Chaiprapat et al., 2003). Nitrogen uptake by duckweed is believed to be the most critical factor affecting its protein content, with a preference of ammonium (NH_4^+) over nitrate (NO_3^-) for synthesizing amino acids (Landesman et al., 2005).

The general composition of different duckweed species are quite similar to each other, consisting of macro-nutrients (ex., Ca, Na, K, Fe, Mg) and low fiber and lignin contents (Culley and Epps, 1973). However, the protein content of duckweed species can range from 15 to 45% by weight, depending on the quality of the water in which they are grown (Chantiratikul et al., 2010). When grown in nutrient-rich environments like wastewater, the resulting high protein content of Lemnoideae biomass can yield revenue as fodder for livestock and fish (Ansal et al., 2010; Mohedano et al., 2012; Fang, 2013; Zhao et al., 2014). Predicting the protein content of duckweed grown in different environments is necessary for effective implementation in various locations with different water quality characteristics. Previous lab and full-scale lagoon studies have examined the effect of nutrient concentrations on duckweed growth and protein content (Chaiprapat et al., 2003; Cheng and Stomp, 2009; Mohedano et al., 2012); however, an analysis of how duckweed is affected when grown on various stages of a more complex wastewater treatment system has yet to be investigated. The purpose of this study was to examine how N concentrations and N speciation affect the growth and protein content of duckweed grown in four different stages of an ecological wastewater treatment system.

2. Materials and methods

2.1. Experimental setup

Experiments were conducted in a pilot-scale ecological wastewater treatment system (Eco-Machine™) located in a 7500 m^2 greenhouse at The Pennsylvania State University (University Park, PA). This system operates year-round on primary influent municipal wastewater (following rag and grit removal), which is delivered several times per week from the local treatment plant to an underground outdoor holding tank (11.35 m^3, 4 d HRT). The wastewater is pumped from the holding tank (with a typical DO < 0.5 mg/L) into the greenhouse intermittently to achieve a flow rate of 2.65 m^3/day, and then flows by gravity through components of the system in the following order (Fig. 1A): closed Anaerobic tank (1.7 m^3; 15 h HRT); closed Anoxic tank (1.7 m^3; 15 h HRT); three open Aerobic tanks (each 3.79 m^3; 34 h HRT); a clarifier (1.4 m^3; 13 hr HRT); a horizontal subsurface flow wetland (44.6 m^3; 17 day HRT); and a final display pond (1.8 m^3; 16 hr HRT). The clarifier, horizontal subsurface wetland, and display pond were not part of this study. The Anaerobic and Anoxic tanks are identical and closed to the atmosphere. Wastewater from the Aerobic 3 tank, which is high in nitrate, is recycled to the Anoxic tank at 50% of the influent flow rate to achieve denitrification. Aerobic 1 and 2 are aerated periodically and have floating rafts vegetated with mature purple taro plants (*Colocasia esculenta*). Aerobic 3 is not aerated, and the water surface is covered with duckweed previously identified as a co-culture of *Lemna japonica/minor* and *Wolffia columbiana* (Calicioglu and Brennan, 2018).

Peristaltic pumps (Masterflex L/S 07528-10, Gelsenkirchen, Germany) were used to pump wastewater out of the sequential Anaerobic, Anoxic, Aerobic 1, and Aerobic 2 tanks at 27 mL/min (24 hr HRT) and into the head of four separate shallow growth trays (each 115 × 33 × 10 cm; Fig. 1B). The same pumps were used to pump wastewater out of the opposite end of each tray – the trays were hydraulically separated and not connected together. The trays were named for the tanks from which their water was supplied. The experiment was operated from January to May 2017, with daylight hours over this period ranging from 10 to 14 h.

2.2. Duckweed harvesting, growth rate, and protein analysis

To start the experiment, each tray was filled with water from its respective tank, and then one square foot of duckweed harvested from the Aerobic 3 tank was added. A two-week acclimation period (with continuous flow) was allowed before sampling was initiated. During the experiment, half of the duckweed (based on surface area) was harvested from each tray once per week using a net, rinsed with tap water, and dried at 45 °C in an Econotherm Lab Gravity Convection Oven (Precision Scientific, Winchester, IL) until a constant weight was achieved (2–3 days). The dried duckweed was stored at room temperature in an air tight desiccator for a maximum of six months, until it was ground into a powder and analyzed for crude protein content (Cumberland Valley Analytical Services, Hagerstown, MD).

2.3. Water sampling and analyses

Temperature, conductivity, dissolved oxygen (DO), pH, and

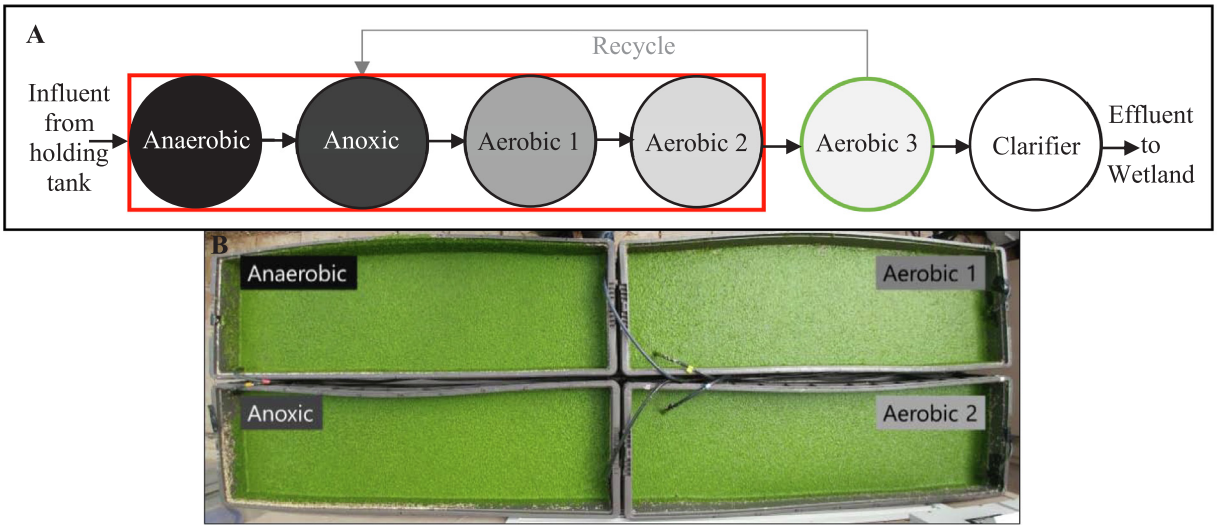

Fig. 1. (A) Plan-view schematic of the Eco-Machine™ (arrows indicate the direction of flow). Red square indicates the four tanks from which water was diverted for this experiment. Green outline on Aerobic 3 tank indicates source of duckweed used to inoculate each growth tray. (B) Plan-view photograph of the four, hydraulically separated duckweed growth trays. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

oxidation–reduction potential (ORP) within each tray were determined weekly on site using a YSI 556 multi-probe system (YSI Inc., Yellow Springs, OH). At the same sampling events, water samples were taken from the influent and effluent tubing of each duckweed tray, collected into 50 mL centrifuge tubes, placed on ice in a cooler, and transported to the lab. NH_4^+ was measured using an Orion Star Series portable meter and electrode (Thermo Scientific, Waltham, MA). Chemical oxygen demand (COD) was measured according to Standard Methods (5220 D; Clesceri et al., 1998). Total nitrogen (TN) was determined using a Shimadzu TOC-VCSH/CSN analyzer (Shimadzu, Columbia, MD). After filtering (0.45 µm), NO_3^-, phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) were quantified using a Dionex ICS-1100 ion chromatograph equipped with an AS-18 column and 30 mM potassium hydroxide eluent (Dionex, Sunnyvale, CA). All analyses were performed within two hours of collection.

2.4. Metals analysis

Duckweed dried at 45 °C for 48 h was ground using a mortar and pestle and digested in 70% OmniTrace nitric acid in a MARS 6 microwave digestion system for 25 min (CEM Corporation, Matthews, NC). Digested samples were diluted to 3% nitric acid and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) at the Penn State Laboratory for Isotopes and Metals in the Environment (LIME). Metals targeted in the analysis were: Aluminum (Al); Arsenic (As); Cadmium (Cd); Chromium (Cr); Copper (Cu); Iron (Fe); Lead (Pb); Nickel (Ni); Silver (Ag); and Zinc (Zn).

2.5. Statistical analysis

SAS 9.4 was used to conduct a repeated measure analysis of covariance (ANCOVA) with an autoregressive lag 1 covariance structure to determine if a significant difference existed between the duckweed protein content, growth rate, and nutrient removal observed in each tray. Linear regressions were also conducted for duckweed protein content versus ammonia, total nitrogen, and dissolved oxygen in each tray to determine if a significant trend was observed.

3. Results and discussion

3.1. Wastewater and duckweed biomass characteristics

In general, the wastewater characteristics entering the Anaerobic tray (Table 1) were similar to that of low-strength domestic wastewater, with the exception of COD. The lower than average COD values entering the Anaerobic tray can be attributed to removal in the holding tank of the Eco-Machine™: an average of 36.7% COD removal was observed from the wastewater delivery truck to the holding tank, which is comparable to COD removal in septic tanks (US EPA, 2002). Temperature, conductivity, and pH stayed relatively constant through each tray, while DO and ORP were higher in the Aerobic trays due to aeration of their respective tanks, consistent with trends observed in conventional wastewater treatment.

Nitrogen removal in the duckweed trays was similar to that measured in other studies (Zhao et al., 2014; Mohedano et al., 2012). As expected, TN and NH_4^+ concentrations decreased from the Anaerobic to the Aerobic trays, but interestingly, NO_3^- production did not correspond directly with NH_4^+ removal (Table 1). Previous studies have shown simultaneous nitrification/denitrification occurring in duckweed growth ponds, and have speculated the occurrence of denitrification in the benthic layer (Zimmo et al., 2003). Although the duckweed trays used in this study did not have a sediment layer, wastewater solids did accumulate on the bottom of each tray over the course of the experiment, coincident with decreasing NO_3^- concentrations in the effluent. These observations suggest that as solids accumulated, an anoxic zone was formed at the bottom of the trays that provided a niche environment for denitrifying bacteria.

TN removal was similar for all trays (20–25%). In ecological wastewater treatment systems, a combination of N transformation mechanisms are responsible for N removal, including: mineralization of organic nitrogen (ON) to NH_4^+; sedimentation of ON; regeneration of NH_4^+ from sediment; decay of plant biomass to ON; volatilization of NH_3; nitrification of NH_4^+ to NO_3^-; denitrification of NO_3^- to nitrogen gas (N_2); plant uptake of NH_4^+ and NO_3^-; and microbial uptake of NH_4^+ and NO_3^- (Kadlec and Wallace, 2008). It is likely that a combination of these processes was contributing to N removal in the duckweed trays, making it difficult to attribute N removal to any one mechanism; however, the % TN removal by duckweed uptake in each tray was estimated to be: 13.8% for Anaerobic; 16.6% for Anoxic; 16.9% for Aerobic 1; and 13.2% for Aerobic 2, based on the calculations described in Eq. (1)–(3):

$$TN\ removed\ by\ duckweed\left(\frac{g\ N}{wk}\right) = \left(\frac{g\ dry\ duckweed}{wk}\right) * \left(\frac{g\ protein}{g\ dry\ duckweed}\right) * \left(\frac{g\ N}{6.25\ g\ protein}\right) \quad (1)$$

$$TN\ removed\ in\ trays\left(\frac{g\ N}{wk}\right) = \left(\frac{L\ wastewater}{d}\right) * \left[\left(\frac{mg\ TN_{in}}{L}\right) - \left(\frac{mgTN_{out}}{L}\right)\right] * \left(\frac{1\ g}{1000\ mg}\right) * \left(\frac{7\ days}{1\ wk}\right) \quad (2)$$

$$\%\ TN\ removed\ by\ duckweed = \frac{TN\ removed\ by\ duckweed}{TN\ removed\ by\ trays} * 100\% = \frac{Eq.\ (1)}{Eq.\ (2)} * 100\% \quad (3)$$

The average TN removal from the influent of the Anaerobic tank to the effluent of the Aerobic 2 tank in the pilot-scale Eco-Machine™ system was 45%, similar to the removal typically observed in free water surface wetlands (US EPA, 2000), suggesting that duckweed can play a significant role in N recovery from these systems.

Phosphate concentrations throughout the duckweed trays showed very little change (Table 1). Duckweed is known to concentrate P up to 1.5% of its dry weight (Leng et al., 1994). However, duckweed is capable of drawing P from its biomass, and has been known to grow on waters devoid of P once it has been accumulated (Leng, 1999). Since the duckweed in this experiment was originally grown on wastewater within the Eco-Machine™, it is likely that the biomass had already accumulated a sufficient amount of P to continue to reproduce, thus not needing to utilize the P present in the wastewater of the growth trays. Also, it has been shown that P in the biomass of duckweed is highly soluble and is rapidly released into the medium upon death of the plant (Stambolie and Leng, 1994). It is possible that due to the harvesting frequency used within this experiment (7 days), some of the duckweed may have been dying and releasing P back into the wastewater. Further studies examining P removal with varying harvesting frequencies of duckweed grown on domestic wastewater should be conducted.

Sulfate concentrations showed little change from the influent to the effluent of each growth tray, with exception of the Anaerobic tray, which had a significant increase ($p = 0.002$; Table 1). It is likely that the increase in SO_4^{2-} observed within the Anaerobic tray can be attributed to the changing environmental conditions between the Anaerobic Eco-Machine™ tank and the Anaerobic duckweed growth tray. The shallow depth of the tray likely enabled some dissolution of oxygen from the atmosphere into the water, leading to higher DO and ORP values than in the Anaerobic tank. However, the DO and ORP values found in the bulk liquid of the Anaerobic duckweed growth tray are below the values required to oxidize reduced sulfur (S) to SO_4^{2-} (Bouroushian, 2010). This suggests that duckweed could be providing a niche aerobic environment for sulfur-oxidizing bacteria in its

Table 1
Average water quality characteristics from the influent, effluent, and within each duckweed growth tray, and duckweed biomass characteristics throughout the experiment (n = 18; ± one standard deviation). Trays are named for the Eco-Machine™ tanks from which their flow is derived.

Parameter	Duckweed Growth Trays							
	Anaerobic		Anoxic		Aerobic 1		Aerobic 2	
	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
COD ($mg\,L^{-1}$)	114 ± 21	72.7 ± 20	70 ± 18	61.7 ± 22	39 ± 9.4	33.7 ± 12	38 ± 14	35.4 ± 15
TN ($mg\,L^{-1}$)	32.9 ± 8.1	25.3 ± 7.9	25.7 ± 7.8	20.4 ± 7.2	25.6 ± 7.2	20.6 ± 7.5	22.9 ± 7.1	17.7 ± 7.8
NH_4^+-N ($mg\,L^{-1}$)	24.2 ± 9.1	17.6 ± 7.0	18.3 ± 7.0	12.9 ± 6.7	17.8 ± 6.8	11.0 ± 6.2	9.1 ± 5.4	5.1 ± 4.3
NO_3^--N ($mg\,L^{-1}$)	0.0 ± 0.0	1.0 ± 1.5	0.0 ± 0.1	2.3 ± 2.0	1.6 ± 1.3	4.7 ± 4.4	8.8 ± 3.9	8.8 ± 4.4
PO_4^{3-}-P ($mg\,L^{-1}$)	3.2 ± 1.2	3.0 ± 1.2	2.9 ± 1.1	3.1 ± 0.9	2.9 ± 1.0	3.3 ± 0.9	3.3 ± 1.0	3.2 ± 0.7
SO_4^{2-} ($mg\,L^{-1}$)	18.3 ± 6.9	25.5 ± 5.7	31.7 ± 10.5	29.6 ± 4.8	31.7 ± 4.3	30.3 ± 3.5	30.6 ± 3.7	30.2 ± 3.6
Temp. (°C)	19.6 ± 3.4		19.5 ± 3.1		20.0 ± 3.3		20.0 ± 2.6	
Cond. (mS/cm)	2.3 ± 0.9		2.3 ± 0.7		2.2 ± 0.5		2.1 ± 0.5	
pH	7.3 ± 0.2		7.4 ± 0.2		7.4 ± 0.2		7.5 ± 0.2	
DO ($mg\,L^{-1}$)	0.5 ± 0.1		1.0 ± 0.6		1.7 ± 0.9		2.9 ± 1.4	
ORP (mV)	−197 ± 79		−15 ± 113		110 ± 70		151 ± 37	
Duckweed growth rate ($g\,m^{-2}\,d^{-1}$)	8.0 ± 4.0		7.0 ± 3.6		8.2 ± 3.9		6.9 ± 3.5	
Duckweed protein content (% DM)	37.0 ± 1.9		37.4 ± 2.3		37.0 ± 1.6		36.0 ± 2.2	
Protein yield ($g\,m^{-2}\,d^{-1}$)	2.9 ± 1.4		2.6 ± 1.2		3.0 ± 1.5		2.5 ± 1.3	
Fe ($mg\,kg^{-1}$)	408 ± 100		397 ± 82		298 ± 66		199 ± 30	
Al ($mg\,kg^{-1}$)	84.4 ± 24		73.3 ± 32		52.5 ± 33		65.7 ± 64	
Zn ($mg\,kg^{-1}$)	44.1 ± 8.8		42.2 ± 8.2		49.8 ± 9.8		62.8 ± 23	
Cu ($mg\,kg^{-1}$)	19.5 ± 8.7		15.5 ± 5.3		12.2 ± 3.4		14.3 ± 3.5	
Pb ($mg\,kg^{-1}$)	2.7 ± 1.5		2.6 ± 1.5		2.4 ± 1.3		3.1 ± 1.6	
Ni ($mg\,kg^{-1}$)	2.5 ± 1.5		1.8 ± 0.4		0.99 ± 0.2		0.89 ± 0.3	
Cr ($mg\,kg^{-1}$)	0.37 ± 0.1		0.36 ± 0.1		0.25 ± 0.1		0.27 ± 0.2	
As ($mg\,kg^{-1}$)	0.23 ± 0.06		0.25 ± 0.06		0.18 ± 0.05		0.15 ± 0.07	
Cd ($mg\,kg^{-1}$)	0.03 ± 0.03		0.02 ± 0.01		0.02 ± 0.01		0.03 ± 0.01	

Ag within the duckweed biomass was below the detection limit for all samples.

rhizosphere via photosynthesis, enabling the oxidation of S to SO_4^{2-} and thereby higher concentrations of SO_4^{2-} in the effluent of the Anaerobic tray than the influent. Little research has been done on the S requirements of duckweed; however, high levels of S-amino acids have been observed when growth rate is high and NH_4^+ in non-limiting (Leng et al., 1994), suggesting that low S concentrations have the potential to limit growth and protein content. However, since S is abundant in wastewaters, it is an unlikely candidate for deficiencies in practical applications.

Metals concentrations within the duckweed biomass from the four growth trays are provided in Table 1. All metals were below the regulatory limits for fodder set by the European Parliament and US FDA. Of the analyzed metals, Fe had the highest biomass concentration, ranging from 160 to 600 mg/kg duckweed biomass, which is higher than many other crops used for livestock feed, including barley, corn, soy, and wheat (Skinner and Peterson, 1928). The low availability of Fe in soils is a key limiting factor for crop production in arid and semiarid regions, leading to Fe-deficient crops (Khan et al., 2011); thus, wastewater-grown duckweed could provide a valuable source of Fe-rich fodder in these areas. These results indicate that wastewater grown duckweed meets the requirements for metals in fodder, and may be beneficial in areas with Fe deficiencies.

3.2. Duckweed protein trends

Contrary to previous work (Landesman et al., 2005; Leng et al., 1994), which showed that duckweed protein content increased with increasing aqueous N concentrations up to 60 mg N/L, duckweed crude protein content in this study did not consistently increase with increasing N concentrations in the aqueous phase (Fig. 2A & B). Instead, differences in duckweed crude protein content and growth rates between trays were statistically insignificant. The average crude protein content and growth rate of duckweed in this study was 36.9% dry matter (DM) and 27.5 $ton/ha^{-1}\,yr^{-1}$, respectively (n = 32; 8 from each tray). A statistically significant positive correlation between NH_4^+ and crude protein was observed in the Aerobic 1 and Aerobic 2 trays (Fig. 2A; $p \leq 0.05$), while a negative correlation was observed in the Anaerobic tray (Fig. 2A; $p \leq 0.05$). Interestingly, a strong positive correlation between the crude protein content of duckweed and DO in the Anaerobic tray was observed (Fig. 2C; $p \leq 0.01$). Since microbial communities in wastewater change with DO, it is likely that plant-microbe interactions had a substantial effect on duckweed protein content.

The rhizosphere of aquatic plants experiences different chemical conditions from the surrounding environment due to a range of processes induced by the plant roots and their associated microbial activity. During photosynthesis, water is converted to oxygen at the roots, providing a niche environment for ammonia-oxidizing bacteria (AOB), increasing the potential for N transformations, and potentially affecting nutrient uptake. Thick biofilms were visible on the roots of the duckweed harvested from the Anaerobic and Anoxic trays, whereas the duckweed roots from the Aerobic 1 and Aerobic 2 trays did not have a visible biofilm (Fig. S1). It is speculated that the dense biofilms present on the duckweed roots in the Anaerobic and Anoxic trays may have contained a large population of AOB, which may have out-competed duckweed for the NH_4^+ in the wastewater, resulting in lower than anticipated protein yields. To confirm this hypothesis, the expression of NH_4^+ oxidizing genes from the duckweed rhizospheric microbial communities will be analyzed in future work.

3.3. Duckweed protein value

Based on the average growth rate and protein content of duckweed measured over this 5-month study, an annual protein yield of 10.1 ton protein $ha^{-1}\,yr^{-1}$ was estimated as shown in Eq. (4):

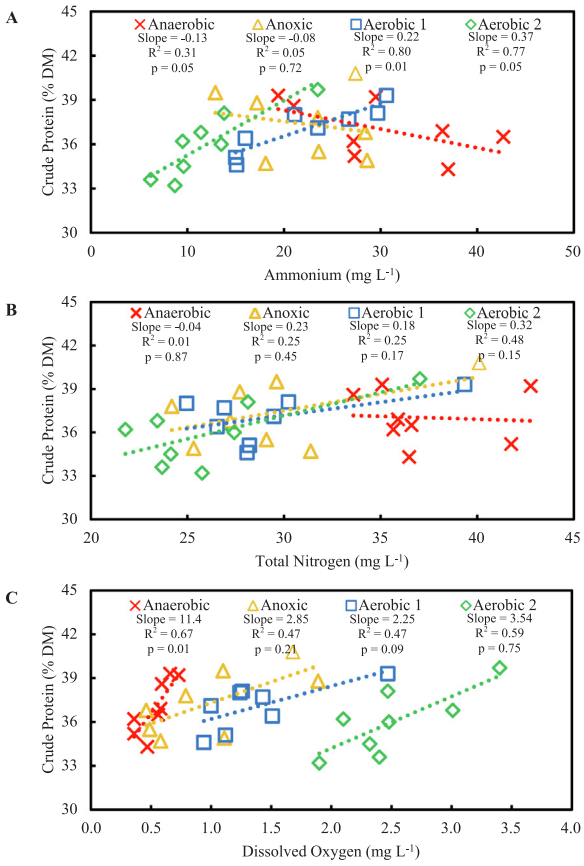

Fig. 2. Duckweed crude protein content versus aqueous ammonium (A), total nitrogen (B), and dissolved oxygen (C) concentrations in each duckweed growth tray (n = 8 per tray).

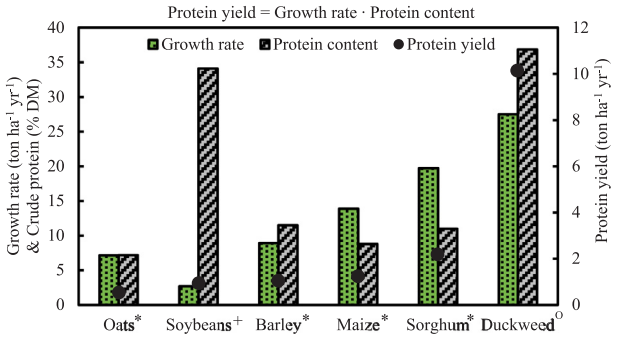

Fig. 3. Comparison of growth rate, crude protein content, and protein yield of duckweed and common land-grown forage crops (*Kennely et al., 1995; +Masuda & Goldsmith, 2009; °this study).

$$protein\ yield\left(\frac{ton}{ha * yr}\right) = \left(\frac{g\ dry\ duckweed}{m^2 * wk}\right) * \left(\frac{g\ protein}{g\ dry\ duckweed}\right) * \left(\frac{52\ wk}{yr}\right) * \left(\frac{10,000\ m^2}{ha}\right) * \left(\frac{1\ ton}{10^6\ g}\right) \quad (4)$$

This protein yield was used to estimate the potential monetary value of duckweed at $4800 USD $ha^{-1}\ yr^{-1}$ using the current market value of soybean meal ($465/ton) as a surrogate for duckweed, since it has similar protein quality and content (World Bank, 2018). This duckweed protein yield dwarfs that of common land-grown forage crops (Fig. 3), while not occupying arable land. The yield of duckweed protein achieved in this experiment can be used as an estimation for full-scale, year-round operation in controlled indoor environments or subtropical or tropical regions (since the Eco-Machine™ is operated at a minimum air temperature of 20 °C); however, lower protein yields should be anticipated in temperate regions in which indoor production, or year-round outdoor operation, is not feasible. Thus, incorporating wastewater grown duckweed into animal fodder can simultaneously: 1) improve nutrient removal from wastewater streams; 2) serve as a protein source for supporting livestock growth; and 3) reduce land dependence for forage crops.

4. Conclusion

Recovering nutrients from wastewater to produce protein-rich plant biomass may improve water and food security while simultaneously decreasing nutrient pollution and lowering demands on prime agricultural land. Understanding wastewater characteristics that promote high protein duckweed is imperative for optimizing protein production. This study indicates that, contrary to previous work, N concentrations in actual wastewater cannot be solely used to predict protein content in duckweed biomass, but rather a suite of chemical and microbial interactions must also be understood. The potential uptake of trace organic contaminants into duckweed should also be considered when selecting growth locations, which will be evaluated in future work. Growing duckweed for protein on the end stages of a wastewater treatment system may be a viable approach for reducing the risk of contamination from pathogens and chemicals, while not sacrificing protein yield.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoena.2019.100004.

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