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Dilution rates of cattle slurry affect ammonia uptake and protein production of duckweed grown in recirculating systems

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ABSTRACT

Duckweed is a potential collector of nitrogen from animal liquid manure and a source of protein suitable as feed for livestock and fish. Therefore, it provides opportunities for circular economy systems. Two duckweed species, *Spirodela polyrhiza* and *Landoltia punctata*, were grown in five recirculating systems each connected to a reservoir tank filled with water and graded organic cattle slurry concentrations. Fresh and dry biomass, protein production and amino acid profiles among the nitrogen removal were evaluated. *Spirodela polyrhiza* showed a significantly higher fresh biomass production but *L. punctata* dry matter content was generally higher resulting in similar dry biomass production for both species This study shows clearly that the crude protein content, ranging between 29.3 and 37.9% of dry matter, was positively correlated to slurry and total ammonia nitrogen (TAN) concentration of the substrate, independent of the duckweed species. Total crude protein yield was in the range of 1.37–1.95 g m^{-2} d^{-1}, following a quadratic function regarding slurry and TAN concentrations, with marginal differences between species. Biomass and crude protein yields were optimal for both duckweed species at a TAN concentration of 19 mg l^{-1}, which corresponded to a slurry dilution of 1:8. The results of this study provide important information for operation of recirculating duckweed production systems on slurry and operators should aim to keep TAN concentrations in that range for optimization of protein production in conjunction with TAN removal.

1. Introduction

Commercial feeding in our globally expanding terrestrial and aquaculture livestock operations is causing high environmental costs in many respects, including land use change, deforestation and overfishing for feed production, as well as increased greenhouse gas (GHG) emissions and environmental eutrophication caused by leached or emitted nitrogen (N) and phosphorus (P) compounds from animal excreta (Pelletier and Tyedmers, 2010; Schader et al., 2015; Cashion et al., 2017). Besides inducing environmental problems with unsustainable feed production, the expansion of intensive animal production and the resulting waste significantly contributes to global N and P emissions, which greatly contributes to exceeding the safe planetary boundaries (Steffen et al., 2015) as originally defined by Rockström et al. (2009).

For future sustainable animal production, establishing alternative protein sources for animal feed is required in order to reduce GHG emissions and eutrophication. Additionally, avoiding superfluous waste streams and efficient recycling of nutrients is demanded to reduce or mitigate excess emissions from livestock manure.

Duckweed (Family Araceae, Subfamily Lemnoideae) are small floating, aquatic flowering plants that have great potential as a future protein source in animal feeds (Sońta et al., 2019). Their crude protein (CP) levels are comparable to that of soybean, with reported concentrations between 30 and 40% (Xu et al., 2012; Zhao et al., 2014; Stadtlander et al., 2019) and maximum levels reaching 45.5% (Mbagwu and Adeniji, 1988). Additionally, the amino acid (AA) profile is considered to be of high quality and similar to terrestrial plants such as soybean and lupine (Appenroth et al., 2017; Stadtlander et al., 2019)

Abbreviations: AA, amino acids; CA, crude ash; CF, crude fiber; CL, crude lipids; CP, crude protein; DM, dry matter; FM, fresh matter; GHG, greenhouse gas; N, nitrogen; N_{tot}, total inorganic N; NO_3^-, nitrate; NO_2^-, nitrite; NH_3/NH_4^+, ammonia; P, phosphorous; TAN, total ammonia nitrogen; t, metric ton.

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and close to optimal for humans, broilers and pigs (Devlamynck et al., 2021). In more recent studies, valuable micro-nutrients such as α-tocopherol and different carotenoids (e.g. violaxanthin, zeaxanthin, lutein, β-carotene) have been identified in different duckweed species such as *Wolffia microscopica* (Appenroth et al., 2017), *Lemna minuta* (Sońta et al., 2020) or *Lemna gibba* (Stewart et al., 2020), providing additional benefits of duckweed as animal feed and even raising increased interest in duckweed for human nutrition.

Furthermore, duckweed species have the potential to produce very high biomass and simultaneously utilize high levels of N with a positive correlation between biomass production and ammonium (NH_4^+-N) concentration (Landolt and Kandeler, 1987). Under sub-optimal conditions, dry matter production can be as high as 23 t ha^{-1} a^{-1}, while under optimal conditions a dry matter production up to 79 t ha^{-1} a^{-1} has been reported (Leng et al., 1995).

In countries or regions with intensive animal production, manure and liquid slurry are often produced in excess of uptake capacity in surrounding agricultural land. Over-fertilization, however, leads to nutrient leaching into groundwater, especially with N in the form of nitrate (NO_3^-) (Mallin et al., 2015). Furthermore, a large share (around 84% in 2004) of the imported N into Europe is destined as feedstuffs such as soybean or other agricultural products (Leip et al., 2015).

Several duckweed species have proven their high efficiency in N and P removal when grown on nutrient rich substrates such as liquid manures or slurries and simultaneously produce high amounts of protein rich biomass in comparatively short time. For a sewage lagoon covered by *Spirodela* sp, Alaerts et al. (1996) reported a Kjeldahl-N and total-P reduction of 74–77%. For *Spirodela oligorrhiza* (most likely *S. polyrhiza*; Les et al., 2002), Xu and Shen (2011) reported a removal of 83.7% of total N and 89.4% of total P. *Landoltia punctata* was utilized to remove nutrients from swine wastewater and produce nutrient rich biomass by Mohedano et al. (2012) who reported 98.0% removal for Kjeldahl-N and 98.8% of total-P. Stadtlander et al. (2019) reported inorganic N reductions between 70.8 and 83.5% and inorganic P reductions between 68.4 and 79.2% for *L. punctata* and *S. polyrhiza* grown on either mechanically treated municipal sewage or diluted slurry, respectively. The recovery of N from either diluted digestate slurry or effluent from a biorefinery using *Lemna minuta* was between 75 and 81%, respectively. The recovery of P in the same experiment ranged between 45 and 55%, respectively (Sońta et al., 2020). . This provides an opportunity for a circular economy approach, if N and P are recycled into feed rather than contributing to ecosystem eutrophication.

Here, we present the results of a greenhouse experiment utilizing a setup comprising five identical recirculating duckweed systems with reservoir tanks providing substrate to ten duckweed growing containers. The aim of this experiment was to identify the optimum total ammonia nitrogen (TAN) concentration for fresh and dry biomass and protein production in duckweed cultures in reservoir-based recirculating systems. To achieve this, *S. polyrhiza* and *L. punctata* were grown in five different cow-slurry to water concentrations (1:20, 1:10, 1:8, 1:6 and 1:4, representing TAN concentrations of 5.57, 14.2, 19.4, 26.3 and 48.6 mg l^{-1}, respectively); and we investigated the effects on biomass (fresh and dry matter, FM and DM, respectively) and CP production, AA profiles and the N and P retention potentials.

2. Material and methods

2.1. Greenhouse experiment

The experiment was conducted in a greenhouse at the Research Institute of Organic Agriculture in Frick, Switzerland. For that, five identical recirculating systems were prepared. In each recirculating system, one tank served as reservoir (inner dimensions: 76.5 cm × 55.5 cm x 40.0 cm, filled to 26 cm or 110 l) for the respective liquid slurry substrate. A submersible pump inside each reservoir tank was used to continuously pump the substrate (flow rates between 3 and 4.5 l h^{-1} per container) into the ten equal duckweed growing containers (inner dimensions: 36.5 cm × 27 cm x 16.5 cm, filled to 4 cm or 4 l) of each recirculating system. The surface area of the duckweed containers equalled 985 cm^2. The inlets into the duckweed containers tended to clog, leading to reduced inflow, but were checked daily and cleaned regularly. Via a central drain pipe, the substrates from the growing containers were refluxed into the respective reservoir tank. Each recirculating system had a total volume of 150 l (110 l + 10 × 4 l). Five of the ten containers per recirculating system were initially stocked with 20 g fresh matter of *S. polyrhiza* and the other five containers were stocked with 20 g fresh matter of *L. punctata*. Both duckweed species were provided by the Landolt Duckweed Collection (Zurich, Switzerland; *S. polyrhiza*: collection number 9346; *L. punctata*: collection number 9426). One of five different slurry water dilutions (substrates) was allocated to an individual recirculating system, resulting in treatments 1:20, 1:10, 1:8, 1:6 and 1:4, corresponding to an average of 5.57 mg l^{-1}, 14.2 mg l^{-1}, 19.4 mg l^{-1}, 26.3 mg l^{-1} and 48.6 mg l^{-1} TAN, respectively, in the fresh substrate. After seven days, the used substrates from the reservoir tanks were removed using a submersible sewage pump, the reservoir tanks cleaned and filled with fresh substrate (slurry and water) of the respective dilution. Cattle slurry was taken from a nearby organic dairy farm and stored in a 1000 l IBC container adjacent to the greenhouse during the experimental period. The experiment lasted four weeks from early April to early May 2018. Air temperature was monitored at 15 min-intervals inside the greenhouse and the global radiation was recorded at hourly intervals by a weather station on the premises of the institute. The time course of both parameters is presented in Fig. 1. Duckweed was stirred manually twice a day during the week and once per day during weekends to prevent microalgae from growing over the fronds.

2.2. Sampling schemes and analytical methods

For determination of biomass gain, 50% of the duckweed surface area was harvested by hand once per week during the experiment and 100% of the area was harvested at the end of the experiment using a scoop net. FM was determined by weighing after spinning twice for 30 s each in a salad spinner to remove excess water. The duckweed harvested from each container was then frozen container-wise at −20 °C until further analysis, resulting in five replicates per treatment and species. Proximate composition analysis was conducted for duckweed samples collected before the experiment started, and after the first and fourth experimental week. For analysis, duckweed samples were thawed and DM was determined in a sub-sample of around 2 g FM by drying at 105 °C for 6 h. The remaining sample was dried at 40 °C for two days. After the two days, the dried sample was finely ground using an electrical household coffee grinder. Total N was determined in pulverized dried duckweed samples by the Dumas method in a C/N analyzer (vario Max CUBE, Elementar Analysensysteme GmbH, Langenselbold, Germany) and a conversion factor of N × 6.25 = CP was used. For total inorganic N (N_{tot}; TAN + NO_2^-–N + NO_3^-–N) and TAN determination of substrate, substrate samples were taken from the reservoir tank at the beginning and end of the first, third and fourth weeks, frozen at −20 °C and stored until analysis. For N_{tot} and TAN analysis, substrate samples were thawed and 2 ml subsamples centrifuged for 3 min at 20.000 rpm (13.000×*g*) and the supernatants analyzed for TAN, NO_2^-–N and NO_3^-–N (combined) in a Smartchem 450 (AMS Alliance, Frepillon, France). Phosphorous was determined as PO_4–P in centrifuged subsamples with the Spectroquant test kit (Merck KGaA, Darmstadt, Germany) using a Genesys 150 spectrophotometer (Thermo Fisher Scientific, Waltham, USA). The pH of the samples was determined with a WTW pH 7110 (Xylem Analytics Germany Sales GmbH & Co. KG, Weilheim, Germany).

Duckweed FM, DM and CP production were calculated for the different treatments and species per area and time. For production calculations, the mean FM, DM and CP contents between the first and final samplings were used. Amino acid determination was conducted

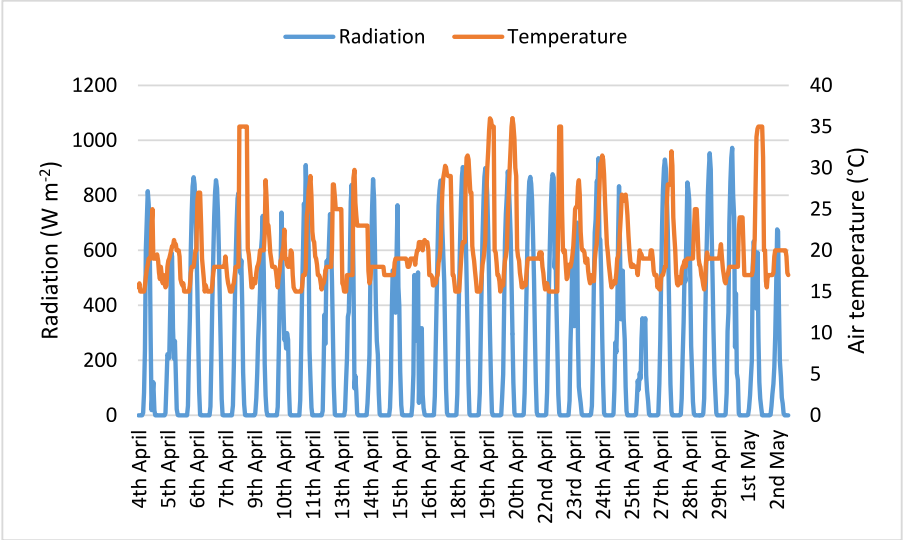

Fig. 1. Sun radiation and air temperature inside the greenhouse during the experimental period.

according to Bidlingmeyer et al. (1984) in pooled samples (separated by species and treatment) harvested in the last experimental week. The samples were hydrolysed with hydrochloric acid before derivatization using a mixture of ethanol, triethylamine, water and phenylisothyocyanate (7:1:1:1) for 20 min at ambient temperature. Amino acids were determined using liquid chromatography and two solvents, an aqueous buffer (0.14 M sodium actetate containing 0.15 ml l^{-1} trimethylamine, pH 6.35) and 60% acetonitrile in water.

To compare the N reduction from the respective substrates with N retention in duckweed biomass, the N content of duckweed biomass of both species harvested in the last week as measured by Dumas was compared with the respective N reduction in the substrates during the last experimental week in the respective treatment and is presented in Fig. 3. To calculate the relative N uptake by both duckweed species as percentage of total available N the following formula was used:

$$\frac{N_{DW}}{N_{tot}} \times 100$$

Where:

N_{DW} = total N in dry duckweed biomass produced during the 4-week trial per treatment – total N in dry duckweed of the inoculum.

N_{tot} = total N (sum of TAN, NO_2^- and NO_3^-) provided via fresh substrates – N_{tot} left in old substrates.

2.3. Statistical analysis

For statistical analysis, SPSS vers. 24.0 (IBM Corporation, Armonk, USA) was used. Differences between species were tested for each slurry dilution by Student's t-test. Significance of linear and curvilinear correlations between TAN concentrations (treatment) and FM, DM (%), CP (%), DM production (g m^{-2} d^{-1}) and CP production (g m^{-2} d^{-1}) content were tested for each species by simple linear regression models (linear regression) and quadratic linear regression models. Homoscedasticity and normal distribution were tested visually by scatter plots and histograms and by Levene and Kolmogorrov-Smirnoff tests, respectively. Significance level for all tests was $\alpha = 0.05$.

3. Results

During April, the daytime was around 11–12 h and the average daily global radiation ranged from 191 W m^{-2} to 579 W m^{-2}. The air temperatures inside the greenhouse ranged from 15 °C to 36 °C with an overall average of 20 °C (Fig. 1).

The average N_{tot}, TAN and P concentrations and the pH of the substrates at the beginning and end of a 7-day cycle are shown in Fig. 2. After seven days of recirculation through the duckweed growing containers, the TAN concentrations in the substrates sampled from the different reservoir tanks varied on average from 0.11 ± 0.02 mg l^{-1} (1:20) to 14.1 ± 3.99 mg l^{-1} (1:4). The reduction of TAN ranged from 98.5% (1:10) to 71% (1:4) with higher declines observed in lower slurry concentrations and lower declines in higher slurry concentrations. The

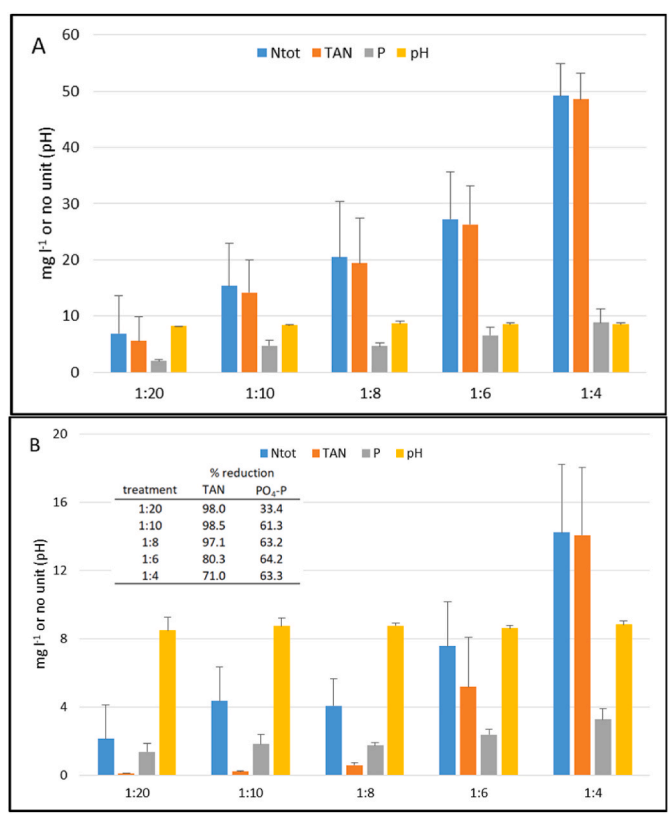

Fig. 2. N_{tot} (TAN + NO_3^-–N + NO_2^-–N), TAN (NH_4^+–N + NH_3–N), PO_4–P and pH in the fresh (A) and used (B) substrates and an inlayed table with the observed reductions in TAN and P concentrations during a 7-day cycle; N = 3, values = mean + SD.

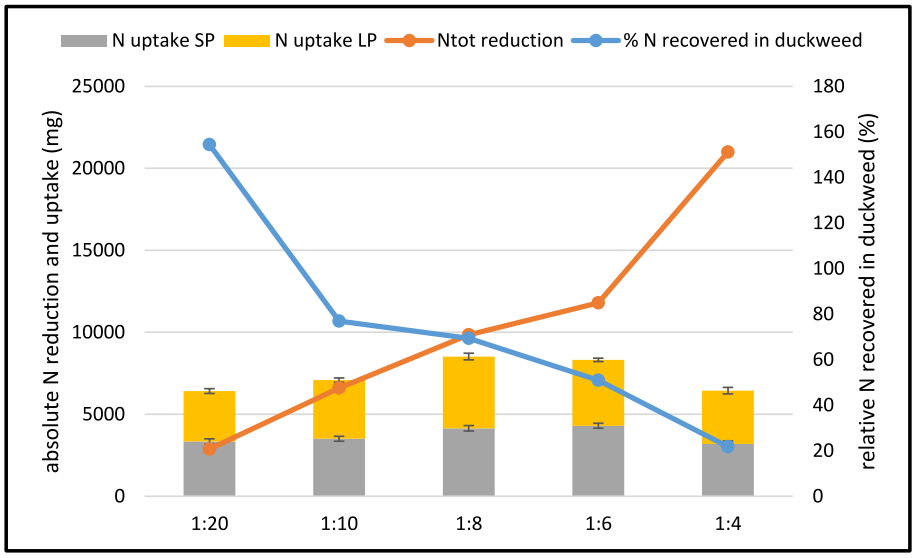

Fig. 3. Absolute N uptake (mg) by *S. polyrhiza* (SP) and *L. punctata* (LP), N reduction in the substrates and relative N recovery (%) by combined duckweed biomass for the different treatments. N = 5, values = mean ± SD; no SD provided for N_{tot} reduction and % N recovered.

contribution of NO_2^-–N and NO_3^-–N to N_{tot} was very low throughout all treatments and measurements. The P concentration declined during a 7-day cycle and reached on average between 1.73 mg l^{-1} (1:8) to 3.25 mg l^{-1} (1:4). The decline in P-concentration was with 33.4% very low in the lowest slurry concentration (1:20) while it was in all other treatments between 61.3% (1:10) and 64.2% (1:6).

With increasing slurry concentration, also the absolute N reduction increased. The absolute N retention in the duckweed biomass did not increase significantly and therefore an increasing gap occurred between the N reduction in the substrates and N uptake into duckweed (Fig. 3).

The FM production was significantly higher in *Spirodela polyrhiza* compared to *Landoltia punctata* while DM content was significantly higher in *L. punctata* (Fig. 4). Total dry matter yields were, however, similar between species. In both species, the lowest FM and DM production was observed in the highest slurry concentrations (treatment 1:4), while the highest FM and DM production occurred in treatment 1:8, for both duckweed species. With slurry concentrations lower than 1:8, FM and DM yields declined again. For both species, highly significant curvilinear correlations were found for total FM production (g), DM content (g 100 g^{-1} FM) and DM production (g m^{-2} d^{-1}) (Fig. 4).

For both species, there was a clear and highly significant positive linear correlation between the TAN concentration in the slurry and the CP content (g 100 g^{-1} DM) in the dried duckweed (Fig. 5) and CP content did not differ between species. Total crude protein yield (g m^{-2} d^{-1}) also showed a curvilinear function, which was generally similar for both species. However, CP yields in treatments 1:20 and 1:6 were significantly higher for *S. polyrhiza* compared to *L. punctata* (Fig. 5).

The AA profiles of both duckweed species show a very similar distributional pattern. No treatment related tendencies could be observed (Fig. 6). For *S. polyrhiza*, most amino acids tended to be highest in treatment 1:10, followed by treatment 1:4 and lowest in treatment 1:6. In *L. punctata* treatment 1:6 showed mostly the highest AA concentration with no clear tendency for a treatment with lowest AA concentration. For both duckweed species, glutamic and aspartic acid were the dominant AA, followed by alanine, leucine and valine. The AA with the lowest concentrations were methionine, tyrosine and histidine.

4. Discussion

Animal slurry can vary in terms of nutrient content, microbial community including pathogens, heavy metal and drug residue concentrations between livestock species and individual animals. The main factors influencing slurry quality are animal species, type of feed and feeding intensity, type and duration of storage before emptying slurry tanks, and water inflow from sources such as barn or house roofs (Wilkinson, 1979; Kupper et al., 2020).

Emissions from agriculture, including gas emissions (e.g. ammonia, nitrous oxide, methane and carbon dioxide) and leaching of phosphates and nitrates, contribute significantly to external costs of primary food production (Pretty et al., 2000). Using duckweed to capture higher proportions of N and P and recycle them into biomass could help reduce those emissions and thus potentially lower the external costs of agriculture, especially in connection to animal production.

Duckweed species are well known for their highly efficient N-uptake and P-uptake, with reported efficiencies of up to 98% for N and 98.8% for P, respectively (Xu and Shen, 2011; Mohedano et al., 2012; Zhao et al., 2014; Iatrou et al., 2015; Stadtlander et al., 2019). In this study, a slurry concentration up to 1:8 resulted in near total reduction of TAN concentrations in the slurry after one week (Fig. 2 B) and either incorporation into duckweed biomass as CP or non-protein N, emitted as gaseous N emissions (such as NH_3, NOx or N_2O) or bound in either bacterial biomass or particulate matter in the sediment. Besides incorporation into CP, duckweed can also store N in the form of NO_3^- in the roots and fronds, although the relative percentage did not exceed 0.8% of total N in roots and 0.1% of total N in fronds (Lehman et al., 1981). However, in the two highest cow slurry concentrations (1:6 and 1:4) of the current study, the concentration of the remaining TAN in 7-day old substrates increased with increasing TAN concentration of fresh substrate, indicating that the duckweed biomass was not able to fully utilize the TAN after one week (Fig. 2).

Ammonium and ammonia (TAN), depending on pH, are forming the principal N fraction in cattle slurry (Ndegwa et al., 2008), as could also be observed in our study where TAN was by far the largest fraction of inorganic N in fresh substrates (Fig. 2). Duckweed species are known to prefer NH_4^+-N as N source over NO_3^--N, although in the absence of NH_4^+-N, they can utilize NO_3^--N (Cedergreen and Madsen, 2002) which is the likely explanation for the excess nitrogen fixed in duckweed in the 1:20 treatment. NH_4^+ and NH_3 occur in an equilibrium, depending on pH and temperature, with a shift towards NH_3 at basic pH. Throughout our experiment, the pH of all substrates was above 8 and ranged between 8.21 and 8.83. The estimated relative contribution of NH_3 to TAN, based on an assumed average substrate temperature of 20 °C and the measured average pH of the respective treatment, would be between 5.7% at pH 8.21 and 20.1% at pH 8.83. Formation of volatile NH_3 and loss through

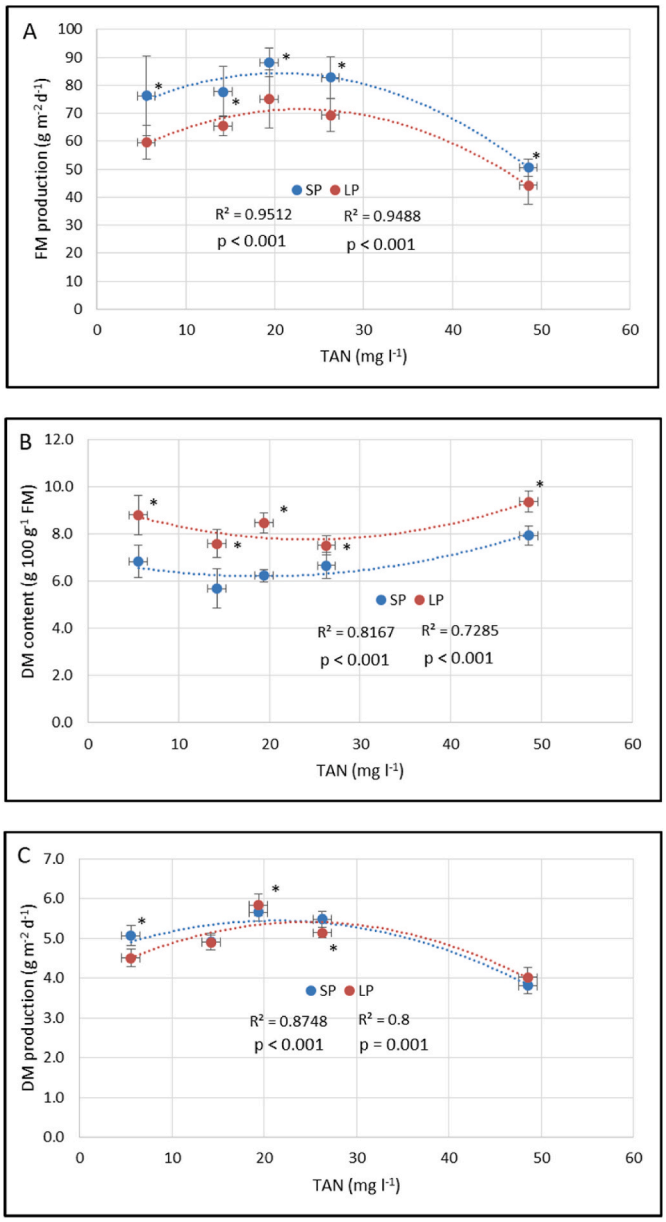

Fig. 4. (A) Correlations between TAN concentration and FM production (g m^{-2} d^{-1}), (B) DM content (g 100 g^{-1} FM) and (C) DM production (g m^{-2} d^{-1}) for *S. polyrhiza* (SP) and *L. punctata* (LP). * = significant difference between species ($p < 0.05$, Student's t-test); R^2 = coefficient of determination and p-value = significance of regression model; N = 5.

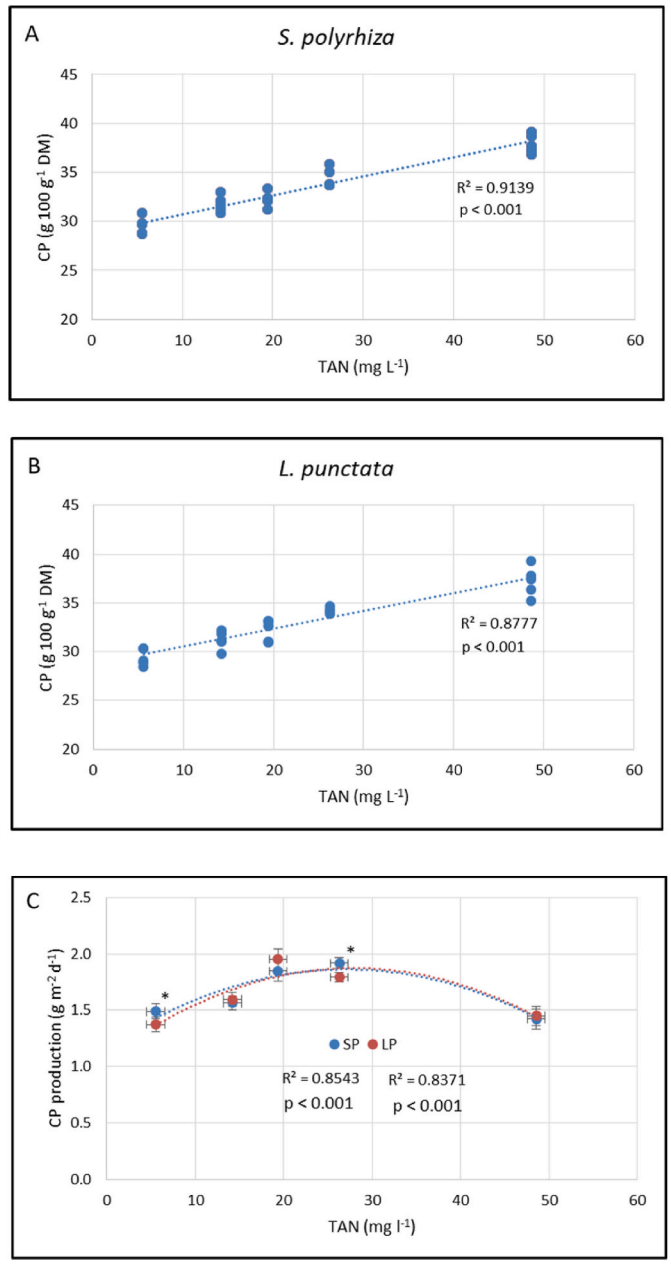

Fig. 5. Correlations between CP concentration (g 100 g^{-1} DM) for (A) *S. polyrhiza* (SP) and (B) *L. punctata* (LP) and (C) TAN concentration and resulting CP production (g m^{-2} d^{-1}). * = significant difference between species ($p < 0.05$, Student's t-test); R^2 = coefficient of determination and p-value = significance of regression model; N = 5.

agitation of substrates due to pumping and refluxing is a likely explanation for some of the increasing gap between N reduction in the substrates and the N recovered in duckweed biomass. .

That was shown for volatile N emissions, both in the form of NH_3 and N_2O, which increased significantly in slurry storage tanks when the slurry was aerated, and were as high as 49.4% for NH_3 and 91% for N_2O, respectively (Amon et al., 2006). Kupper et al. (2020) reported that the baseline NH_3 emissions from cattle and pig slurry are 0.12 g m^{-2} h^{-1} for cattle slurry and 0.15 g m^{-2} h^{-1} for pig slurry when stored in lagoons. When slurry is stored in tanks, the NH_3 emissions for cattle slurry were reduced to 0.08 g m^{-2} h^{-1} but increased to 0.21 g m^{-2} h^{-1} for pig slurry (Kupper et al., 2020). To our knowledge, the loss of volatile N-compounds has not yet been measured or estimated in slurry or sewage-based duckweed production systems. Assuming the unaccounted TAN from the high slurry concentration treatments of the present study have been primarily lost as gaseous emissions, the choice of appropriate slurry dilution rates is of high importance for a sustainable recycling system.

Besides fixation of N, duckweed species can efficiently fix P with uptake rates as high as 98.8% (Mohedano et al., 2012). The reduction of P between fresh and used substrates in this study made a sudden jump from treatment 1:20 (33.4% P reduction) to treatment 1:10 (61.3% P reduction) and increased only slightly in higher slurry concentrations. . Mohedano et al. (2012) reported, that the main route for P removal in duckweed ponds is via duckweed biomass uptake but that sedimentation processes could also contribute to P removal from the liquid phase. Another possibility for reductions of N and P concentrations could be microbial activity. In wastewater systems, microbial activity contributes to nitrogen removal by nitrification, denitrification and subsequent

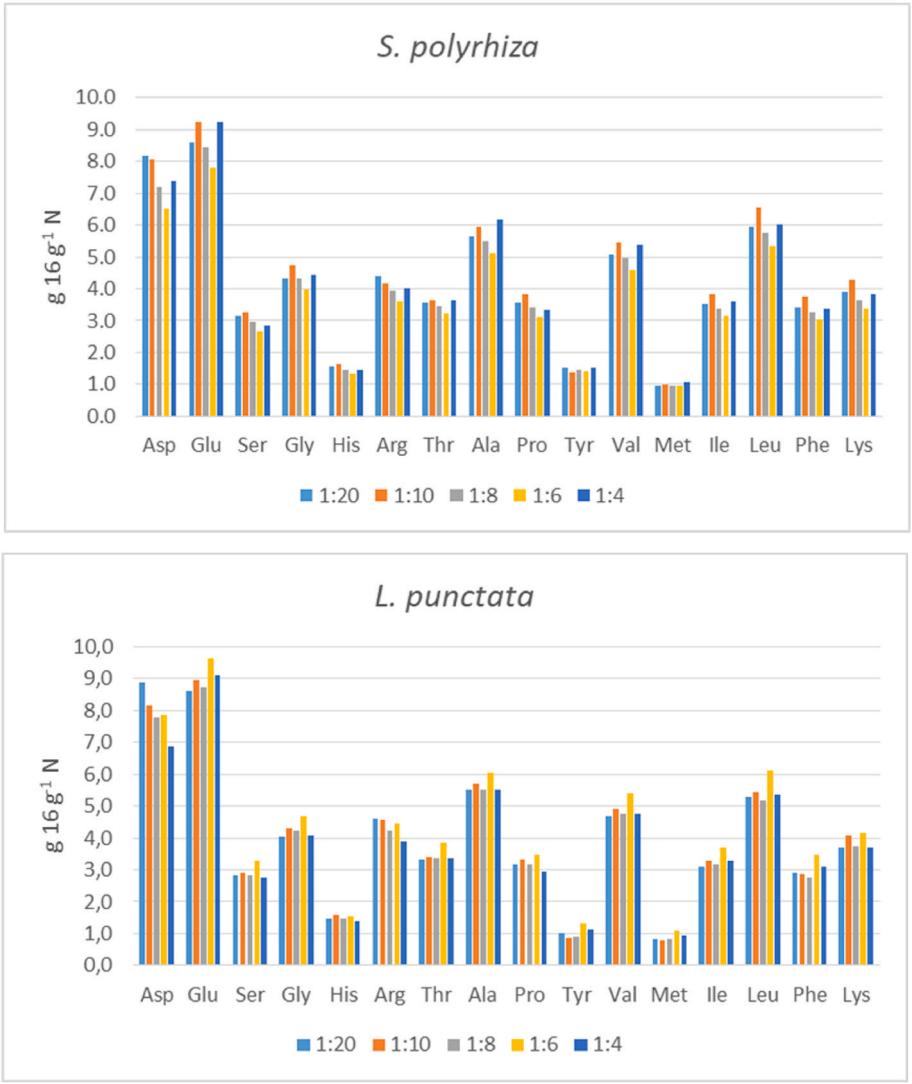

Fig. 6. Amino acid composition (g 16 g^{-1} N) of *S. polyrhiza* and *L. punctata* grown on different slurry dilutions.

build-up of microbial biomass (Shen et al., 2019) which will also incorporate P. The relative contribution of duckweed towards N removal or uptake, however, is considered to be around 75% and thus has a far larger influence compared to microbial processes (Körner and Vermaat, 1998; Zhao et al., 2015). Contrary to N, where gaseous emissions either as $NH_{3,\ NOx}$ or as N_2O certainly contribute at a varying degree, especially depending on pH, to an apparent reduction of N concentrations in the slurry, P does not occur in chemical states prone to gaseous emissions. Therefore all P not recovered by duckweed must either be bound in the sediment or utilized by bacterial biomass.

A range of DM content of 4–14% for various duckweed species has been described, with the majority ranging between 4 and 9% (Landolt and Kandeler, 1987). A difference of 4%-points (e.g. from 4% DM to 8% DM) would result in two times more absolute dry biomass production. Therefore, DM content is of major interest for a targeted duckweed production. A higher DM content has been reported to be achieved with warmer temperature and slower growth while fast growth rates usually tended to decrease DM (Landolt and Kandeler, 1987). The results presented in this study support this statement, as the u-shaped curvilinear relationship observed between TAN concentration and DM content (Fig. 2) and the inversely u-shaped curvilinear relationship between TAN concentration and total FM production (Fig. 2) demonstrate comparable trends. However, the optimum productivity of both species utilized in this study was observed to be at a TAN level of 19 mg l^{-1}, which was two orders of magnitude lower than the generally tolerable NH_4^+-N level of 1350 mg l^{-1} mentioned by Devlamynck et al. (2021) for duckweed. Furthermore, these two factors (DM production and DM content) need to be balanced for an optimized biomass production. In this experiment the DM content of *S. polyrhiza* was observed to be around 6–7%, while the DM content of *L. punctata* tended to be higher (7.5–8.5%), which both correspond to reported ranges (Landolt and Kandeler, 1987; Cheng et al., 2002).

The maximum CP production of 1.92 g m^{-2} d^{-1} for *S. polyrhiza* in the 1:6 treatment would extrapolate to 5.18 t ha^{-1} a^{-1} of CP production assuming a 9-month growing period. For *L. punctata* the CP production of 1.95 g m^{-2} d^{-1} in the 1:8 treatment would extrapolate to 5.27 t ha^{-1} a^{-1} in a 9-month growing period. This is considerably lower than the 7.23 t ha^{-1} a^{-1} reported by Xu et al. (2012) for *S. polyrhiza* and a 9-month time period despite a lower reported average CP content of 26.5%. Another study reported a protein production ranging between 2.9 and 3.5 t ha^{-1} a^{-1}, which is lower compared to the results reported here or by Xu et al. (2021), especially since they considered a 12-month growing period (Devlamynck et al., 2021).

The observed differences in CP content and CP yield between both duckweed species were relatively small and reported CP content was generally around or above 30% CP in the DM (Fig. 4). In contrast, large differences were observed in an earlier study between the same two species with *S. polyrhiza* showing 30.6% CP and *L. punctata* only showing

13.8% CP (Stadtlander et al., 2019). However, the authors applied a static system with weekly substrate exchanges and a much lower volume to surface area ratio compared to the reservoir-based recirculation system reported in this study.

To consider duckweed as productive animal feed, it must contain a high CP concentration and protein quality (i.e. high essential amino acid concentrations and good digestibility). In addition, duckweed should ideally be a competitive alternative to terrestrial crops such as soybeans (average CP of 35.2% on as fed basis), lupine (average CP of 30.4% on as fed basis), peas (average CP of 25.3% on as fed basis when shelled and extruded) and canola meal (average CP of 38% on as fed basis when solvent extracted) (NRC, 2011). Crude protein content in different duckweed species tends to be in the range of 25–35%, while values as high as 45% have been reported (Landolt and Kandeler, 1987; Mbagwu and Adeniji, 1988). In this study, the CP content of both duckweed species was between 29.3 and 37.9%, and could therefore compete favorably with most other terrestrial plant protein sources.

No marked influence of increasing slurry and thus TAN concentrations was observed for the AA profile of the tested duckweed species (Fig. 5). However, the AA content in *S. polyrhiza* appeared to be highest in treatments 1:10 and 1:4, although this could not be analyzed statistically. Both species showed mostly similar AA concentrations. When comparing the amino acid profiles of *S. polyrhiza* observed in this study with that of *S. polyrhiza* reported by Stadtlander et al. (2019), lower concentrations of several amino acids such as serine, valine, methionine, leucine, tyrosine, phenylalanine, lysine, arginine and proline in the CP were revealed.

Duckweed species show a great potential for several applications including N and P recycling from animal manures into feed- or foodstuffs for animals and humans due to their potentially very high growth rates and well balanced nutrient content. Besides for nutritional purposes, duckweed species have also been successfully used for bio-remediation (Bharti and Banerjee, 2012), as green manure (Yao et al., 2017) or for production of bioethanol (Xu et al., 2011; Verma and Suthar, 2015). Some of the intended utilizations contradict each other to a certain degree. A high protein content and good amino acid profile is necessary for a utilization as animal feed (Sońta et al., 2019) while a high starch content is desirable for a utilization as energy plant (Verma and Suthar, 2015; Appenroth et al., 2021). Regardless of the intended use of produced duckweed, certain challenges and open questions remain, especially when duckweed is to be produced on animal slurries. Formation and emission of gaseous NH_3 could lead to high N emissions from large scale duckweed-slurry production systems and need to be investigated in detail.

Duckweed has many potential applications, here, we explored explicitly the potential of *S. polyrhiza* and *L. punctata* for protein production and the optimal TAN concentration for highest DM and CP gains. A system as described in this study would create certain challenges such as an uncertain amount of volatile N emissions. Slurry treatments such as acidification could reduce the amount of volatile N losses (Kupper et al., 2020) but detailed studies exploring all gaseous N emissions have to be conducted. The risk of other potentially hazardous or unwanted gaseous emissions such as GHG (e.g. CO_2 or CH_4) has been shown to be minimal or that slurry-duckweed systems even fix several-fold more CO_2 than they do emit (Mohedano et al., 2019).

5. Conclusion

The present study provides new and robust data showing the positive correlation between TAN concentration in slurry and CP content of *S. polyrhiza* and *L. punctata*. Furthermore, it provides an optimum TAN concentration (19 mg l^{-1}), which is similar for both duckweed species, for balancing differing FM and DM concentrations to maximize CP production. The knowledge about the optimum TAN concentration is important for operators of duckweed-slurry systems. Reduced biomass production in the lowest slurry concentration could be a sign for N limitation while reduced biomass production in the highest slurry concentration could result from increased NH_3 concentrations and potential toxicity effects on duckweed, especially given the high pH (>8.2) of slurries.

For *S. polyrhiza* and *L. punctata*, the optimum TAN concentration for balancing was at 19 mg l^{-1}, which corresponded to a slurry dilution rate of 1:8 in this study. However, the optimal slurry dilution rate will largely depend on the quality and origin of the slurry. Duckweed systems operated within the reported range of TAN concentration could produce more than 5 t of dry crude protein per hectare and year, assuming a 9-month growing period. For future studies, determination of volatile N emissions from slurry-duckweed systems should be considered in order to enable comparisons between duckweed production and traditional food or feed producing agricultural system regarding the N balance and N use efficiencies.

CRediT authorship contribution statement

T. Stadtlander: project coordinator, main study design, implementation, data, Formal analysis, and, writing of manuscript. **J. Bandy:** implementation, sample collection, handling and analytics, critical manuscript corrections. **D. Rosskothen:** implementation, sample collection, handling and analytics. **C. Pietsch:** project preparation, study design, manuscript corrections. **F. Tschudi:** project preparation, study design, manuscript corrections. **M. Sigrist:** project preparation, study design. **A. Seitz:** project preparation, study design. **F. Leiber:** study design, critical evaluation of results and manuscript.

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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