Improvement of nitrogen uptake and dry matter content of Swiss chard by pre-incubation of duckweeds in soil

Tichaedza John Chikuvire[1] · Pardon Muchaonyerwa[1] · Rebecca Zengeni[1]

Abstract

Purpose Recovery of nutrients from water using duckweed and their reuse has significance in closing the loop on nutrient transfer from anthropogenic sources. This study investigated the effect of rate of application and pre-incubation period of duckweed on biomass and nutrient uptake of Swiss chard (Fordhook giant).
Methods Two glasshouse experiments were laid out in randomized complete block designs with three replicates. In the first experiment, Swiss chard was grown on two soils (ferralsol and regosol) amended with *Wolffia arrhiza* biomass at 0, 50, 100 and 200% of the recommended nitrogen rate. In the second experiment, the same vegetable was grown on the ferralsol amended with *W. arrhiza* and *Lemna minor* at recommended nitrogen rate, with pre-incubation periods of 0, 14 and 28 days.
Results Application of *W. arrhiza* biomass increased Swiss chard dry matter by 23–45% compared to the negative control. The positive control (urea at 100 kg N ha^{-1} rate) had highest Swiss chard biomass. Higher rates than 100 kg N ha^{-1} had no added benefit on dry matter accumulation and nitrogen uptake of Swiss chard. Pre-incubation of duckweed for 28 days improved nutrient uptake, resulting in higher dry matter than shorter periods. The Swiss chard dry matter after pre-incubation for 28 days was similar to that from urea application.
Conclusion Findings from this study suggest that duckweed is a resource with beneficial use for nutrient supply to vegetables, especially when appropriate rates are used with pre-incubation.

Keywords Duckweeds · *Lemna minor* · Nitrogen uptake · Pre-incubation · *Wolffia arrhiza*

Introduction

Nutrient transfer caused by anthropogenic activities from land to aquatic systems has been reported worldwide (May et al. 2006; Wang et al. 2007; Dubois et al. 2018). The nutrient transfer continuum model, comprising source–mobilization–delivery–impact phases, has been used to conceptualize this non-point nutrient transfer (Haygarth et al. 2005). However, Quilliam et al. (2015) criticized the model for failing to extend beyond impact (eutrophication), and advocated for, as part of the model, the inclusion of the phase on nutrient recovery for returning to land and reuse. This has significance partially in closing the loop on nutrient transfer from anthropogenic sources. Excessive growth of aquatic plants

on the nutrient rich water has potential to upset such ecosystems (Chislock et al. 2013). Meanwhile, evolving policy and regulatory imperatives that were designed to ensure long-term protection of ecosystems, health and wellbeing of society, have created new challenges and opportunities for efficient and cost-effective resource recovery from a wide range of waste streams (Heathwaite 2010; Shurin et al. 2013). While it is already a common practice to harvest aquatic plant biomass in heavily impacted freshwater bodies to facilitate drainage, flood conveyance, water quality, visual appeal, navigation and recreational amenities (Quilliam et al. 2015), there are limited studies on nutrient recovery from aquatic plants and their use as sources of plant essential nutrients.

The Midlands Region of KwaZulu-Natal province is one of the key agro-ecological regions of South Africa experiencing high nutrient loads in water bodies from anthropogenic activities (Isikhungusethu Environmental Services 2012; Hitayezu et al. 2016). In this region, a group of aquatic macrophytes called duckweeds is increasingly colonizing

✉ Tichaedza John Chikuvire
tjchikuvire@gmail.com

[1] School of Agricultural Earth and Environmental Sciences, University of KwaZulu-Natal, Private Bag X01, Scottsville 3209, South Africa

fresh and wastewater bodies. Duckweeds are among the floating aquatic macrophytes with huge capacity to absorb and even hyper-accumulate nutrients by doubling their biomass in 16–24 h under conducive environments (Leng 1999; Chaiprapat et al. 2005). They belong to the family of Lemnaceae with five genera and more than 37 species (Verma and Suthar 2015). Due to their desirable chemical and physiological traits, duckweeds have potential for phytoremediation of wastewater, energy production, feed supplement, bioplastics and phytotoxicity tests (Wang 1990; Radić et al. 2011; Kufel et al. 2012; Zeller et al. 2013; Gwaze and Mwale 2015). Although several studies have recommended duckweed biomass as an organic fertilizer based on its composition (Iqbal 1999; Leng 1999; Kostecka and Kaniuczak 2008), there is a paucity of literature on its use for soil fertility improvement. The use of duckweeds as a fertilizer could be an environmentally friendly option, although the suitability of duckweed species to supply nutrients to various crops on different soils has not been assessed.

Limited studies have been conducted to examine the potential of duckweed *Lemna minor* to support sorghum growth (Kraider 2015; Pulido 2016) and these studies concur that duckweeds may be viable sources of organic fertilizer, particularly supplying nitrogen (N) and phosphorus (P) to sorghum plants. Ahmad et al. (1990) applied *L. minor* as a complementary source of N and recorded increased plant height, straw and grain yields accompanied by increase in N, P and potassium (K) content of the rice plants. However, growth of duckweeds is affected by nutrient content and pH of the medium they thrive in, sunlight, temperature and species type (Landolt and Kandelar 1987). These factors, in turn, affect their elemental tissue composition. The differences in elemental composition of duckweeds could affect their effectiveness as sources of plant nutrients. Our preliminary studies have shown that the most common duckweed species found in the Midlands Region of KwaZulu-Natal province are *L. minor* and *W. arrhiza,* with the latter having a higher N content. Conversely, Swiss chard (*Beta vulgaris*) is a highly valued vegetable grown at a temperature range of 7–24 °C in this province (Directorate Agricultural Information Services 2008). The leafy vegetable has considerable amounts of vitamin C, potassium, calcium (Ca) and magnesium (Mg), (Brito et al. 2012). In South Africa, some studies reported enhanced cumulative yields of 50 t ha^{-1} of Swiss chard, in a prolonged growth season, using organic amendments such as compost and chicken manure (Agriculture Research Council 2013). To the best of our knowledge, no studies are available on growth performance of Swiss chard in soil amended with duckweed biomass. There is need for studies to provide answers to questions such as "Could the use of duckweed biomass, as an organic amendment, reduce the impact of non-point pollution from anthropogenic sources to adjacent water bodies, and benefit agro-ecosystems through plant nutrient supply? Does a period of pre-incubation of biomass of different duckweed species in soil improve their effectiveness as plant nutrient sources, given their low carbon (C) to N (C/N) ratio?" The objective of this study was to determine the effect of (1) duckweeds (*W. arrhiza*) as nitrogen source and (2) pre-incubation period of *W. arrhiza* and *L. minor* in soil, on Swiss chard biomass and nutrient uptake.

Methods and materials

Soil and duckweeds

The study was conducted at University of KwaZulu-Natal in Pietermaritzburg (29°37′33.9″S; 30°24′14″E), South Africa. The soil samples used for the glasshouse study were collected from Baynesfield Estate (29°45′S; 30°20′E) and Ukulinga (29°39′S; 30°24′E) research farm of the University of KwaZulu-Natal, in the Midlands region of KwaZulu-Natal province. Soil from Baynesfield Estates, classified as Rhodic Ferralsols (Dominy and Haynes 2002), was collected from land previously under maize and soyabean rotations for over 10 years. The soil from Ukulinga, classified as Dystric Regosols (McGranahan et al. 2016), was sampled from a piece of land that was fallow for the previous 5 years. Soil sampling was at the 0–20-cm depth, using picks and shovels. After air-drying, the soil was sieved (< 2 mm) before analyses. Prior to pot experiments, each soil was analyzed using composite samples replicated three times, following methods in the section on duckweeds, Swiss chard and soil analyses below, with results presented in Table 1.

Table 1 Means of selected physico-chemical properties of soils used for pot experiments

Parameter	Regosol	Ferralsol	Se
Clay (%)	26.7	43.3	3.73
pH (KCl)	4.8	4.6	0.10
Total C (%)	1.69	2.88	0.27
Total N (%)	0.16	0.25	0.02
Available P (mg kg^{-1})	10.6	13.7	0.85
K (cmol$_c$ kg^{-1})	0.18	0.48	0.07
Ca (cmol$_c$ kg^{-1})	7.47	4.85	0.60
Mg (cmol$_c$ kg^{-1})	3.82	1.75	0.47
Mn (mg kg^{-1})	53.7	43.2	4.71
Zn (mg kg^{-1})	5.07	6.53	0.34
Cu (mg kg^{-1})	6.16	5.89	0.25
EA (cmol$_c$ kg^{-1})	0.07	0.19	0.05

EA exchangeable acidity, *Regosol* Ukulinga soil, *Ferralsol* Baynesfield soil, *Se* standard error

At Baynesfield Estate, about 70-kg wet mass of duckweeds *W. arrhiza* and *L. minor* were randomly sampled from a pond that received an overflow of runoff water from upland fields and slurry lagoons. These upland fields were irrigated with pig slurry. The duckweeds were transported to the laboratory as a dense suspension, and extraneous materials such as insects, grass and small sticks were removed by passing the suspension through a set of sieves (2000–1000 μm), before rinsing with distilled water. Separation of *L. minor* from *W. arrhiza* was by the 1000-μm screen that only allowed the latter to pass through. The biomass was oven dried at 60 °C to constant weight. Three composite samples of each of the duckweed species were analyzed following methods in the section on duckweeds, Swiss chard and soil analyses below, and results are presented in Table 2.

Pot experiment 1

A 2×5 factorial experiment, laid out in a randomised block design with three replicates, was set in a glasshouse. The components of the two factors used in this experiment were two soil types (ferralsol and regosol) and five rates of *W. arrhiza* biomass, as soil amendment. Blocking of moisture and temperature gradients was based on distance from the walls of the glasshouse humidifier. The soils were weighed (3.0 kg) into pots, with inner diameter of 20 cm and height of 17 cm. Biomass of *W. arrhiza* was added at 0, 50, 100 and 200% of the N recommended rate for Swiss chard. The Soil and Analytical Services Laboratory of the KwaZulu-Natal Department of Agriculture provided the N recommendation (100 kg N ha^{-1}). Urea fertilizer was included as a positive control at 100 kg N ha^{-1}, split-applied with 50% application at transplanting and 3 weeks later. The rate per pot was converted into mass basis by matching the total N in duckweed

or urea to the required treatment level using the recommended N rate per hectare and soil bulk density of 1.50 and 1.62 g cm^{-3} (ferralsol and regosol, respectively), for the 0–20-cm depth. The P and K for Swiss chard were supplemented as the difference between the recommended rates based on soil test values from the ammonium bicarbonate extraction method (The Non-Affiliated Soil Analysis Work Committee 1990) and duckweed tissue P and K content, using sodium dihydrogen phosphate and potassium chloride.

Swiss chard (Fordhook giant) seedlings were obtained from Sunshine seedlings. Two seedlings (56 days old) per pot were transplanted. Thinning to a single plant was after 2 weeks. The minimum and maximum temperatures in the glasshouse were 18 and 23 °C, respectively. The pots were watered periodically to prevent drought stress of the plants. Care was taken to avoid over watering, facilitated by general guidance from field capacity values of 25 and 30% for ferralsol and regosol, respectively, as determined in a separate study. Weeds were handpicked and incorporated into the soil. At 8 weeks after transplanting, shoots were harvested and dried at 60 °C to constant weight and ground. The soil was air dried and roots were separated using a set of sieves.

Pot experiment 2

The second pot experiment was set up in a randomised complete block design (for one-way analysis of variance) with eight treatments and replicated three times in a glasshouse. The treatments included combinations of two duckweed species (*W. arrhiza* and *L. minor*) at three pre-incubation periods and two controls. The ferralsol was used in this experiment and was weighed (2 kg) into pots with inner diameter of 20 cm and height of 17 cm. Duckweed treatments were added at the recommended rate of 100 kg N ha^{-1}. The amended soils were pre-incubated for 0, 14 and 28 days before planting Swiss chard seedlings, while maintaining moisture at field capacity. The periods were selected on the basis of a preliminary incubation study that indicated increase in nitrate levels after 28 days (Chikuvire et al. 2018). The pre-incubation was timed in such a way that all planting was done at the same time. The controls were not pre-incubated. Urea fertilizer was included as a positive control at 100 kg N ha^{-1}, split-applied with 50% application at transplanting and 3 weeks later. No N was added to the negative control. The P and K for Swiss chard were supplemented as in pot experiment 1. Transplanting, watering, weed control, harvesting and soil and plant analyses were as in pot experiment 1.

Duckweeds, Swiss chard and soil analyses

Duckweed, soil and Swiss chard samples were analyzed for C and N using the LECO Trumac CNS Auto-analyser

Table 2 Means ($\pm$ se) of elementary composition of duckweed species

Element	*Wolffia arrhiza*	*Lemna minor*
C/N	8.38 ± 0.01^{a}	8.81 ± 0.15^{a}
N (%)	5.01 ± 0.003^{a}	4.58 ± 0.009^{b}
Ca (%)	0.50 ± 0.003^{b}	1.12 ± 0.007^{a}
Mg (%)	0.49 ± 0.003^{a}	0.50 ± 0.006^{a}
K (%)	2.81 ± 0.02^{a}	1.92 ± 0.02^{b}
P (%)	0.62 ± 0.003^{b}	0.67 ± 0.006^{a}
Al (mg kg^{-1})	1511 ± 18^{b}	7394 ± 38^{a}
Fe (mg kg^{-1})	3517 ± 50^{b}	6201 ± 43^{a}
Mn (mg kg^{-1})	277 ± 2^{a}	237 ± 1^{b}
Zn (mg kg^{-1})	61 ± 5^{b}	142 ± 1^{a}
Cu (mg kg^{-1})	19 ± 5^{a}	10 ± 2^{b}

Means followed by the same letter in a row are not significantly different at $p < 0.05$

Version 1.1x (LECO Corporation, 2012). Selected physico-chemical properties of soil, residual pot soil analyses and tissue P, K, Ca, Mg, iron (Fe), manganese (Mn), zinc (Zn) and cupper (Cu) were determined, in triplicate. Ammonium bicarbonate extraction solution was used for soil analyses (The Non-Affiliated Soil Analysis Work Committee 1990) and nitric acid for plant tissue digestion. The P and metal concentrations were measured by an inductively coupled plasma-optical emission spectrometer (Varian 720-ES ICP-OES). Swiss chard uptake results were obtained from the product of tissue nutrient concentrations and dry matter (g pot^{-1}).

Data analysis

All data were subjected to analysis of variance (ANOVA) using GenStat 14th edition. Least significant differences (LSD) (at $p < 0.05$) were used to separate the treatment means of the first experiment. Multiple comparisons of means using Tukey's honest significance test were carried out for the second experiment, where the LSD was not appropriate, since the treatments exceeded six (Gomez and Gomez 1984).

Results and discussion

Shoot dry matter and elemental uptake of Swiss chard

There were no significant interaction effects of soil type and rate of duckweed application on dry matter yield and uptake for most nutrients. Effects of rates of application of duckweed biomass and soil type, as main factors, were significant on Swiss chard dry matter and uptake of N, Ca, Mg, Mn, Zn and Cu (Fig. 1, Tables 3, 4). The increase

Fig. 1 Effect of rate of duckweed application on dry matter and N uptake (mg pot^{-1}) of Swiss chard. Vertical error bars represent standard errors
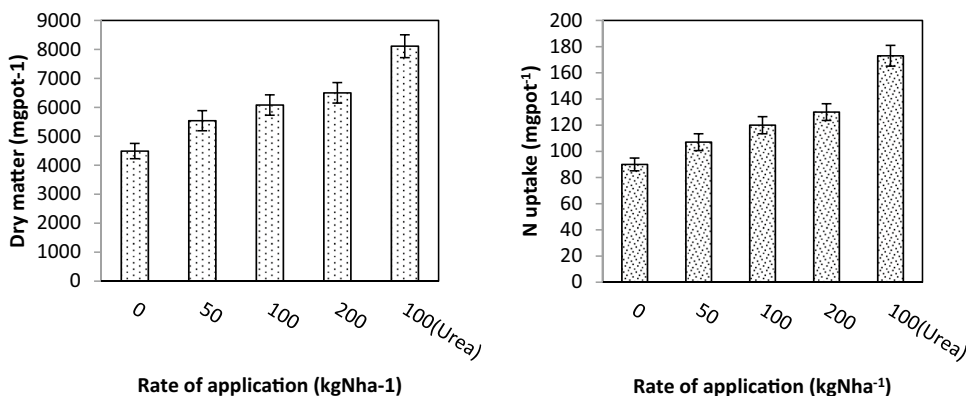
Table 3 Effect of rates of duckweed application on means ($\pm$ se) of nutrient uptake of Swiss chard

Rate (kg N ha^{-1})	P (mg pot^{-1})	Ca	Mg	Mn	Zn	Cu
0	31 ± 4.1[a]	66 ± 9.5[c]	54 ± 6.4[d]	3.85 ± 0.4[b]	0.94 ± 0.1[d]	0.09 ± 0.01[b]
50	36 ± 5.6[a]	77 ± 10.2[bc]	65 ± 7.6[cd]	4.40 ± 0.3[b]	1.15 ± 0.1[c]	0.11 ± 0.01[ab]
100	35 ± 5.7[a]	88 ± 12.1[ab]	76 ± 9.1[bc]	5.18 ± 0.4[a]	1.27 ± 0.1[cb]	0.12 ± 0.01[a]
200	36 ± 5.5[a]	93 ± 13.5[ab]	80 ± 9.2[b]	5.20 ± 0.5[a]	1.34 ± 0.1[b]	0.12 ± 0.01[a]
100 (Urea)	32 ± 4.6[a]	97 ± 14.4[a]	97 ± 10.1[a]	5.63 ± 0.3[a]	1.54 ± 0.2[a]	0.13 ± 0.01[a]

Means followed by the same letter in a column are not significantly different at $p < 0.05$

Table 4 Effect of soil type on means ($\pm$ se) of dry matter yield and uptake of N, Ca, Mg, Mn, Zn and Cu (mg pot^{-1}) by Swiss chard

Soil	DM	N uptake	P	Ca	Mg	Mn	Zn	Cu
Ferralsol	7935 ± 347[a]	160 ± 7.3[a]	20 ± 1.0[b]	114 ± 6.1[a]	94 ± 5.3[a]	5.56 ± 0.22[a]	1.59 ± 0.06[a]	0.13 ± 0.006[a]
Regosol	4357 ± 297[b]	89 ± 5.8[b]	48 ± 1.8[a]	55 ± 3.2[b]	54 ± 3.5[b]	4.14 ± 0.25[b]	0.90 ± 0.05[b]	0.09 ± 0.004[b]

Means followed by the same letter in a column are not significantly different at $p < 0.05$

DM dry matter

in Swiss chard biomass with rates of application of duckweed biomass could be explained by relative increase in nutrient uptake, compared to the control. Application of duckweed biomass in all treatments significantly increased the Swiss chard dry matter by 23–45% compared to the negative control (Fig. 1).

The positive control produced the highest Swiss chard dry matter. Application of duckweed at an equivalent rate of 200 kg N ha^{-1} produced significantly higher dry matter than at 50 kg N ha^{-1} and negative control. The recommended N rate (100 kg N ha^{-1}) had similar dry matter as the 50 and 200 kg N ha^{-1} rates. The difference between the dry matter of Swiss chard from the positive control and at duckweed rates of 100 and 200 kg N ha^{-1} was due to the type of the N source. Hammad et al. (2007) reported that nitrogen levels and sources influenced dry mass of spinach, while other studies generally maintained that the source of N did not influence the yield of leafy vegetables (Wang and Li 2004; Engelbrecht et al. 2010). Increasing duckweed rate of application to an equivalent of 100 kg N ha^{-1} improved the dry matter of Swiss chard compared to the negative control but it remained lower than that of the positive control. This suggested that some N was yet to mineralize. Application of urea as a straight fertilizer provided readily available N for uptake (Brito et al. 2012), resulting in highest dry matter of the positive control.

The 100 and 200 kg N ha^{-1} rate had similar N uptake by Swiss chard that was lower than that of the positive control. The negative control had the lowest N uptake rate. Nitrogen uptake of Swiss chard generally increased from the negative control, emphasizing the essential role of N in plant growth as highlighted by Engelbrecht et al. (2010). The similarity of uptake of nutrients at the 100 and 200 kg N ha^{-1} implied that N uptake generally influenced the growth of the vegetable and affected uptake of other nutrients (Table 3) in a similar pattern, such that there was no comparative improvement of dry matter yield at 200 kg N ha^{-1}. This observation is supported by studies that reported lack of improvement of Swiss chard dry matter yield at high N rates between 150 and 200 kg N ha^{-1} (Kołota and Czerniak 2010) and also absence of substantial yield enhancement after increments of N dose from 100 to 200 kg N ha^{-1} (Kołota et al. 2017). The increased availability of N from the decomposition of duckweed at high rates and the positive control enhanced plant growth that facilitated higher uptake of other essential nutrients. At low application rates, uptake of K, Ca, Mg, Mn and Zn declined, resulting in limited biomass accumulation. While the positive control had higher Ca uptake than the 50 kg N ha^{-1} rate, the negative control had lower levels than the 200 kg N ha^{-1} rate and the positive control. The positive control had the highest uptake of Mg. Uptake of Mg at 200 kg N ha^{-1} was higher than at 50 kg N ha^{-1} and the negative control.

Uptake of Mn at 50 kg N ha^{-1} and negative control was lower than the rest of the treatments. Copper uptake of the negative control was lower than that of the positive control and 100 and 200 kg N ha^{-1}. Rates of duckweed application did not affect P uptake of Swiss chard.

The ferralsol had significantly higher Swiss chard dry matter and uptake of N, Ca, Mg, Mn, Zn and Cu than the regosol (Table 4). Uptake of P by Swiss chard was higher for the regosol than the ferralsol. The high nutrient uptake on ferralsol was consistent with its relatively higher fertility status than the regosol (Tables 1, 4). Uptake trends of Fe by Swiss chard were not influenced by N rates for regosol possibly due to low soluble Fe at the prevailing soil pH (Ranade-Malvi 2011; Brady and Weil 2008). This suggestion is supported by relatively higher uptake of P in the regosol than ferralsol, where availability could have been limited by fixation. The relatively low soil pH, high clay content and availability of soluble Fe and Al could have resulted in more P fixation (Lucas and Davis 1961) of ferralsol than the regosol. Relative to the control, addition of high rates of N from duckweed and urea contributed to soil acidification due to possible nitrification. Sanchez-Monedero et al. (2001) observed a decline in pH during organic waste composting due to nitrification and Turmel et al. (2015) confirmed that the overall effect of N mineralization is acidifying.

Significant interactions of rates of application of duckweed biomass and soil type existed for Swiss chard uptake of K and Fe (Fig. 2). Uptake of these two elements was higher in the ferralsol than the regosol. For regosol, uptake of K by Swiss chard at the 200 kg N ha^{-1} rate and positive control was higher than that of the negative control and 50 kg N ha^{-1}. The regosol amended with duckweed at 100 kg N ha^{-1} had similar K uptake to that at 50 and 200 kg N ha^{-1}. For ferralsol, the highest Swiss chard uptake of K was from the positive control, while the lowest was from the negative. Uptake of K from ferralsol at 50 kg N ha^{-1} was similar to that at 100 and 200 kg N ha^{-1}. Uptake of Fe was not significantly different at all rates on regosol. However, for ferralsol, it was higher for 50, 200 kg N ha^{-1} and positive control than the negative control; while that at 100 kg N ha^{-1} was similar to other rates.

## Residual soil chemical properties after growth of Swiss chard

Interaction effects of soil type and rates of application of duckweed biomass were significant on residual soil N, P, K, Ca and Cu but not on C, Zn and exchangeable acidity. This implied that the levels of most residual nutrients depended on the soil type and rates of application of duckweed biomass. The low uptake of these elements in treatments with little dry matter resulted in elevated levels of these elements in the residual soils. The residual N content of ferralsol was

Fig. 2 Effect of soil and rate of application of duckweed and urea on K and Fe uptake of Swiss chard. Vertical error bars represent standard errors
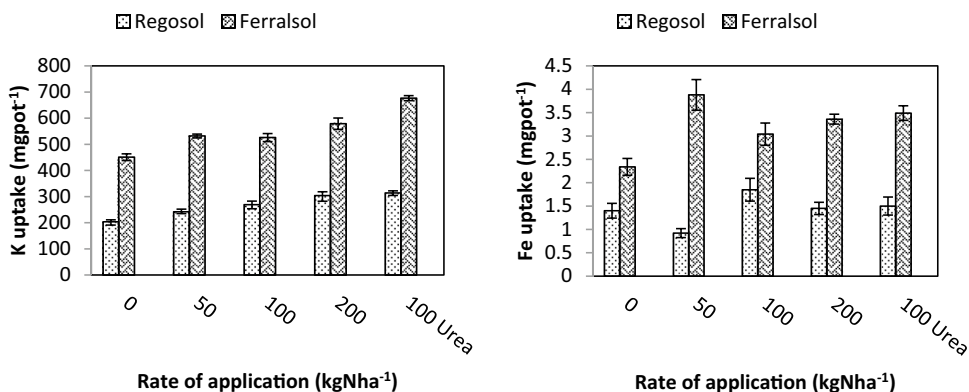
higher than that of the regosol at all rates except the negative control, which had a similar level (Fig. 3a). The regosol's residual N was similar at all rates except for the negative control that had higher levels. The increase in supplied N, as a result of duckweed addition, generally made N more available for plant growth with limited effects on residual levels. Malepfane and Muchaonyerwa (2018) reported a decrease in residual nutrients related to vigorous growth of plants, resulting in uptake of these nutrients from the soil. The ferralsol had the highest residual N in the negative control treatment, while the 50 kg N ha^{-1} rate had the lowest, with the remaining rates having similar levels. The residual

soil P was similar for both soils at all rates except at the positive control where the regosol had higher levels than the ferralsol (Fig. 3b). The residual soil P of the negative control for the regosol was higher than that of the positive control, 50 and 200 kg N ha^{-1} rates. Soil at the 100 kg N ha^{-1} rate had higher residual N than at 200 kg N ha^{-1}. For ferralsol, the lowest residual P was from the positive control followed by the 200 kg N ha^{-1}, while the negative control had the highest. The residual soil K was higher for ferralsol than regosol at all rates except for the positive control, where the two soils had similar levels (Fig. 3c). The negative control of the regosol had residual K that was higher than that of the
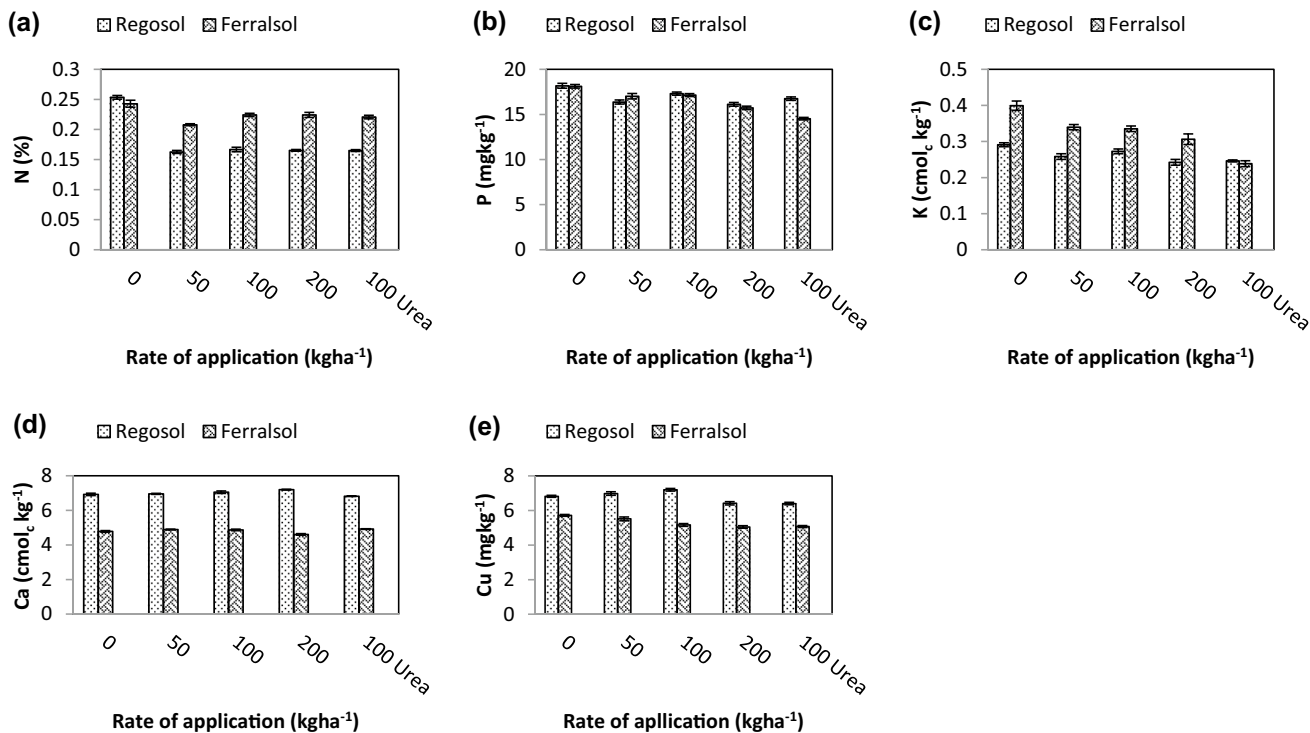
Fig. 3 Residual N (**a**), extractable P (**b**) exchangeable K (**c**) and Ca (**d**), and extractable Cu (**e**) in two soils treated with increasing rates of duckweed. Vertical error bars represent standard errors

positive control and the 200 kg N ha^{-1} rate. Treatments of the ferralsol that incorporated duckweed had similar residual K, while it was lowest for the positive control and highest for the negative control. Residual soil Ca at all rates was higher for the regosol than ferralsol (Fig. 3d). At the 200 kg N ha^{-1} rate, the residual Ca for the regosol was similar to that at 100 kg N ha^{-1} and higher than that of both controls and 50 kg N ha^{-1}. At this rate (200 kg N ha^{-1}), residual Ca for the ferralsol was lower than that of the positive control, 50 and 100 kg N ha^{-1} rates. The regosol had higher residual Cu than the ferralsol at all rates (Fig. 3e) but was similar at 50 and 100 kg N ha^{-1}. Residual Cu for ferralsol at 200 kg N ha^{-1} and positive control was significantly lower than that of the negative control.

Rates of duckweed application had no significant effect on residual soil C content and Zn (Table 5). This might be due to contribution by the Swiss chard's below ground biomass, despite separation of roots, and low levels of Zn taken up by the plants relative to quantities in soil.

Residual Mg from the negative control and 50 kg N ha^{-1} was similar and significantly higher than that of the 100 kg N ha^{-1} and positive control. At 200 kg N ha^{-1}, it was similar to that at 100 N ha^{-1} and positive control. At 100 kg N ha^{-1}, residual Mn was significantly higher than at 200 kg N ha^{-1} and the positive control. The residual exchangeable acidity of the positive control was higher than the negative control due to nitrification. The residual soil pH of the negative control was higher than at 100 and 200 kg N ha^{-1} rates, while the positive control had the lowest. Treatments of duckweed biomass had similar residual soil pH values.

Soil type had significant residual effects on C, Mg, Mn, exchangeable acidity and pH after growth of Swiss chard (Table 6). The regosol had significantly higher Mg, Mn and pH than ferralsol, while the later soil had significantly higher C and exchangeable acidity. The residual Zn levels were similar for both soils. These trends were consistent with those of the initial levels in the soils.

Shoot dry matter and nutrient uptake of Swiss chard after pre-incubation of duckweed

Pre-incubation of duckweed biomass had a significant effect on dry matter of Swiss chard (Table 7). *W. arrhiza* and *L. minor* biomass pre-incubated for 28 days produced similar Swiss chard dry matter to that of the positive control. Pre-incubation of *W. arrhiza* for 28 days had higher Swiss chard dry matter than other treatments, besides the positive control and *L. minor* pre-incubated for the same duration.

Pre-incubation of *L. minor* for 28 days produced Swiss chard dry matter that was higher than that of the negative control and both duckweed species that were not pre-incubated. The highest Swiss chard N uptake was after pre-incubating *W. arrhiza* for 28 days followed by the positive control and *L. minor* pre-incubated for the same duration. Pre-incubation of duckweed for 28 days might have been essential in facilitating uptake of N and other nutrients, resulting in dry matter of Swiss chard that was similar to the positive control. The incubation period was appropriate for mineralization of N in duckweed, as most nutrients were initially unavailable and had to be slowly released through microbial degradation (Brito et al. 2012). This observation was confirmed by low dry matter content of Swiss chard that was similar to that of the negative control, after incorporation of biomass from both duckweed species at planting. The finding indicates that a relatively long

Table 5 Effect of rates of duckweed application on means ($\pm$ se) of residual soil C, Mg, Mn, Zn, exchangeable acidity and pH

Rate (kg N ha^{-1})	C (%)	Mg (cmol$_c$ kg^{-1})	Mn (mg kg^{-1})	Zn	EA (cmol$_c$ kg^{-1})	pH
0	2.25 ± 0.17^a	2.56 ± 0.32^a	46.61 ± 5.89^{ab}	6.10 ± 0.37^a	0.20 ± 0.04^b	4.45 ± 0.09^a
50	2.26 ± 0.17^a	2.55 ± 0.33^a	49.53 ± 7.00^{ab}	6.22 ± 0.32^a	0.22 ± 0.05^{ab}	4.43 ± 0.09^{ab}
100	2.32 ± 0.17^a	2.45 ± 0.32^b	50.64 ± 7.65^a	6.30 ± 0.46^a	0.21 ± 0.05^{ab}	4.41 ± 0.09^b
200	2.22 ± 0.16^a	2.53 ± 0.32^{ab}	46.55 ± 6.23^b	5.67 ± 0.07^a	0.23 ± 0.05^{ab}	4.41 ± 0.10^b
100 (Urea)	2.22 ± 0.17^a	2.43 ± 0.32^b	46.16 ± 5.83^b	6.59 ± 0.68^a	0.24 ± 0.05^a	4.34 ± 0.09^c

Means followed by the same letter in a column are not significantly different at $p < 0.05$

EA exchangeable acidity

Table 6 Effect of soil type on means ($\pm$ se) of residual Soil C, Mg, Mn, Zn, exchangeable acidity and pH

Soil	C (%)	Mg (cmol$_c$ kg^{-1})	Mn (mg kg^{-1})	Zn	EA (cmol$_c$ kg^{-1})	pH
Ferralsol	2.74 ± 0.02^a	1.54 ± 0.02^b	28.9 ± 0.40^b	6.33 ± 0.20^a	0.37 ± 0.01^a	4.14 ± 0.01^b
Regosol	1.77 ± 0.01^b	3.47 ± 0.03^a	66.9 ± 1.51^a	6.03 ± 0.32^a	0.07 ± 0.004^b	4.69 ± 0.01^a

Means followed by the same letter in a column are not significantly different at $p < 0.05$

EA exchangeable acidity

Table 7 Effect of period of duckweed incorporation on dry matter yield and elemental uptake (mg pot^{-1}) by Swiss chard grown in the ferralsol

Tr	Pre-inc (days)	DM	N	P	K	Ca	Mg	Mn	Zn	Cu	Fe
− Control	0	4940[cd]	76[cd]	18[a]	345[ab]	59[b]	56[c]	3.95[ab]	0.96[b]	0.11[ab]	3.72[a]
+ Control	0	6753[abc]	124[b]	15[a]	443[ab]	89[ab]	98[abc]	5.29[ab]	1.31[ab]	0.15[a]	5.25[a]
LM	0	4164[d]	67[d]	17[a]	310[b]	55[b]	54[c]	3.45[ab]	0.90[b]	0.08[b]	3.23[a]
LM	14	5444[bcd]	93[c]	16[a]	398[ab]	65[b]	63[c]	3.35[b]	0.96[b]	0.08[b]	2.77[a]
LM	28	7385[ab]	137[b]	15[a]	442[ab]	109[ab]	120[ab]	4.82[ab]	1.75[a]	0.14[ab]	4.43[a]
WF	0	4384[d]	78[cd]	15[a]	330[b]	54[b]	54[c]	3.23[b]	0.78[b]	0.08[b]	3.32[a]
WF	14	5994[bcd]	95[c]	13[a]	385[ab]	75[b]	79[bc]	4.02[ab]	0.90[b]	0.11[ab]	2.34[a]
WF	28	8218[a]	177[a]	14[a]	483[a]	145[a]	144[a]	5.81[a]	1.88[a]	0.16[a]	2.04[a]
Se		353	8	0.7	15	7	7	0.2	0.09	0.07	0.4

Means followed by the same letter in a column are not significantly different at $p < 0.05$ using Tukey's honest significance test

Tr treatment, *Pre-inc* pre-incubation period, *LM Lemna minor*, *WF Wolffia arrhiza*, − *Control* no addition of amendment, + *Control* urea applied at recommended rate at transplanting and 3 weeks after transplanting, *Se* pooled standard error of the mean

pre-incubation period is required to derive maximum benefits from duckweed as a nutrient source. Pre-incubation for 14 days proved to be less effective at influencing Swiss chard dry matter probably due to low initial amounts of mineralized nutrients and lack of synchrony between N crop demand and N mineralization (Sainju et al. 2006).

There were no significant differences in uptake of P and Fe for all treatments. The non-responsive uptake of these nutrients by Swiss chard over the period of pre-incubation indicated limited ability of the plant to utilize P and relative abundance of Fe in soil. High Fe and Al levels in the ferralsol, at relatively low pH, coupled with additions from duckweed decomposition and the acidifying nature of the nitrification process resulted in P fixation on Al and Fe oxyhydroxide surfaces and precipitation of Al and Fe phosphates (Chikuvire et al. 2018). This might have affected P uptake values that were even lower for the 28-day incubation period. This observation agrees with Ahmadil et al. (2010) who reported an increase in Ammonium-N application resulting in a decrease in P uptake by spinach.

The uptake of K after pre-incubation of *W. arrhiza* for 28 days was higher than that for both duckweed species without pre-incubation. Although all treatments were corrected for K, Swiss chard had higher K uptake from soil pre-incubated with *W. arrhiza* for 28 days than that not pre-incubated, as a result of synergistic effects with higher N released through mineralisation (Ranade-Malvi 2011). This trend is not exhibited with *L. minor* treatments possibly due to high Ca levels, released from its biomass, that antagonized uptake of K. The Ca and Mg uptake after pre-incubating *W. arrhiza* for 28 days was higher than the rest of the treatments besides the positive control and the *L. minor* pre-incubated for the same duration. Magnesium uptake from soil pre-incubated for 28 days with *L. minor* was higher than the negative control, *L. minor* pre-incubated for 14 days and both duckweed species without pre-incubation. The uptake of Mn from *W. arrhiza* pre-incubated for 28 days was higher than that for *W. arrhiza* incorporated at transplanting and *L. minor* pre-incubated for 14 days. Pre-incubation of both duckweed species for 28 days resulted in higher Swiss chard uptake of Zn than all treatments except the positive control. The uptake of Cu for the positive control and *W. arrhiza* pre-incubated for 28 days was higher than for *L. minor* pre-incubated for 14 days and for both species without pre-incubation. Generally, high uptake of nutrients by Swiss chard after pre-incubation of *W. arrhiza* for 28 days could partly be explained by the amendment's elemental composition. *W. arrhiza* had a narrower C/N ratio and higher N content than *L. minor*. This could have facilitated more rapid decomposing of *W. arrhiza* and release of nitrogen (Kumar and Goh 2000; Tejada et al. 2008) that was readily taken up by the plants, thereby influencing uptake of most nutrients.

Residual soil chemical properties after growth of Swiss chard

Pre-incubation of duckweed had no significant residual effect on soil C, N, K, Ca, Mn, Cu and exchangeable acidity (Table 8). The high dry matter content of Swiss chard at longer pre-incubation periods, in response to greater mineral N, resulted in greater uptake of other plant essential nutrients including K, Ca, Mg, Mn and Zn from the soil, especially where *W. arrhiza* biomass was incorporated. This might have resulted in lack of effect of pre-incubation period on most residual soil nutrients.

The residual soil P after *L. minor* pre-incubated for 14 days was higher than that of the two duckweed species pre-incubated for 28 days. Residual soil Mg for the negative control and *L. minor* incorporated at transplanting (not pre-incubated) were significantly higher than that of both duckweed species pre-incubated for 28 days. The two duckweed species incorporated on the day of transplanting had higher residual soil pH than the positive control and both

Table 8 Effect of period of duckweed incorporation on residual soil chemical properties after Swiss chard grown on the ferralsol

Biomass	Pre-inc (days)	C (%)	N	P (mg kg^{-1})	K	Ca	Mg	EA	pH	Mn	Zn	Cu
					(cmol$_c$ kg^{-1})					(mg kg^{-1})		
− Control	0	3.10[a]	0.22[a]	20.7[ab]	0.37[a]	4.99[a]	1.77[a]	0.43[a]	4.23[ab]	34.2[a]	5.81[ab]	5.29[a]
+ Control	0	3.31[a]	0.27[a]	20.9[ab]	0.33[a]	4.99[a]	1.57[abc]	0.51[a]	4.16[bc]	34.5[a]	5.70[b]	5.50[a]
LM	0	3.22[a]	0.25[a]	20.2[ab]	0.39[a]	5.18[a]	1.81[a]	0.39[a]	4.29[a]	36.5[a]	6.39[ab]	6.03[a]
LM	14	3.19[a]	0.26[a]	22.6[a]	0.36[a]	4.94[a]	1.62[ab]	0.37[a]	4.22[ab]	30.2[a]	5.55[b]	5.26[a]
LM	28	3.21[a]	0.25[a]	18.4[b]	0.26[a]	5.08[a]	1.45[bc]	0.56[a]	4.14[bc]	31.2[a]	5.70[b]	5.61[a]
WF	0	3.09[a]	0.24[a]	19.6[ab]	0.38[a]	4.93[a]	1.67[ab]	0.37[a]	4.27[a]	36.6[a]	6.69[a]	5.50[a]
WF	14	3.06[a]	0.23[a]	21.5[ab]	0.33[a]	5.00[a]	1.62[ab]	0.46[a]	4.19[abc]	32.8[a]	5.66[b]	5.40[a]
WF	28	3.20[a]	0.25[a]	19.1[b]	0.22[a]	4.81[a]	1.32[c]	0.53[a]	4.10[c]	32.9[a]	5.67[b]	5.00[a]
Se		0.31	0.004	0.37	0.02	0.05	0.04	0.02	0.02	0.64	0.10	0.09

Means followed by the same letter in a column are not significantly different at $p < 0.05$ using Tukey's honest significance test

Pre-inc pre-incubation period, *LM Lemna minor*, *WF Wolffia arrhiza*, *+ Control* urea applied at recommended rate at transplanting and 3 weeks after transplanting, *EA* exchangeable acidity, *Se* pooled standard error of the mean

duckweed species pre-incubated for 28 days. The ferralsol was highly weathered, well drained and fertile (Table 1) and facilitated nitrification, as confirmed by residual soil pH that mostly declined when duckweed was pre-incubated for 28 days. The residual soil Zn for *W. arrhiza* and *L. minor* incorporated at the day of transplanting was similar and among the highest levels due to depressed plant uptake.

Conclusion

Swiss chard's nutrient uptake and dry matter, together with residual concentrations of soil nutrients, depended on initial soil properties, elemental composition and rate of duckweed application and pre-incubation period. The relatively high N content in duckweed species *W. arrhiza* makes it a suitable organic N source to improve Swiss chard yield. Higher application rates of duckweed than 100 kg N ha^{-1} had no added advantages on dry matter accumulation and N uptake by Swiss chard. Pre-incubation of duckweed biomass for at least 28 days improved nutrient availability and uptake, resulting in dry matter of Swiss chard that was as good as that from the positive control (urea application). Although duckweed had a low C/N ratio, findings suggest that synchronization of crop nutrient demand with nutrient release from duckweed is critical for use of this resource. In addition, further assessment of field experiments on different soil types should provide important insight.

References

Agriculture Research Council (2013) Production guidelines for summer vegetables. ARC-Vegetable and Ornamental Plant Institute, Pretoria

Ahmad Z, Hossain NS, Hussain SG (1990) Effect of duckweed (*Lemna minor*) as complement to fertilizer nitrogen on the growth and yield of rice. Int J Trop Agric 8:72–79

Ahmadil H, Akbarpour V, Dashti F, Shojaian A (2010) Effect of different levels of nitrogen fertilizer on yield, nitrate accumulation and several quantitative attributes of five Iranian spinach accessions. Am Eurasian J Agric Environ Sci 8:468–473

Brady NC, Weil R (2008) The nature and properties of soils, 14th edn. Pearson Prentice Hall, Upper Saddle River

Brito LM, Pinto R, Mourão I, Coutinho J (2012) Organic lettuce, rye/vetch, and Swiss chard growth and nutrient uptake response to lime and horse manure compost. Org Agric 2:163. https://doi.org/10.1007/s13165-012-0032-9

Chaiprapat S, Cheng JJ, Classen JJ, Liehr SK (2005) Role of internal nutrient storage in duckweed growth for swine wastewater treatment. Trans Am Soc Agric Eng 48:2247–2258. https://doi.org/10.13031/2013.20088

Chikuvire TJ, Muchaonyerwa P, Zengeni R (2018) Decomposition of *Wolffia arrhiza* residues rapidly increases mineral nitrogen and decreases extractable phosphorus in acidic soils. Environ Monit Assess 190:510. https://doi.org/10.1007/s10661-018-6895-9

Chislock MF, Doster E, Zitomer RA, Wilson AE (2013) Eutrophication: causes consequences, and controls in aquatic ecosystems. Nat Educ Knowl 4:10. http://www.nature.com/scitable/knowledge/library/eutrophication-causes-consequences-and-controls-in-aquatic-102364466. Accessed 13 Mar 2017

Directorate Agricultural Information Services (2008) Vegetable production in a nutshell. Department of Agriculture, Pretoria

Dominy CS, Haynes RJ (2002) Influence of agricultural land management on organic matter content, microbial activity and aggregate stability in the profiles of two Oxisols. Biol Fertil Soils 36:298–305. https://doi.org/10.1007/s00374-002-0542-9

Dubois N, Saulnier-Talbot É, Mills K et al (2018) First human impacts and responses of aquatic systems: a review of paleolimnological

records from around the world. Anthr Rev 5(1):28–68. https://doi.org/10.1177/2053019617740365

Engelbrecht GM, Ceronio GM, Motseki PC (2010) Effect of nitrogen levels and sources on production of Swiss Chard (*Beta vulgaris* var. *Cicla*). S Afr J Plant Soil 27:229–234. https://doi.org/10.1080/02571862.2010.10639991

Gomez KA, Gomez AA (1984) Statistical procedures for agricultural research, 2nd edn. Wiley, New York

Gwaze RM, Mwale M (2015) The prospect of duckweed in pig nutrition: a review. J Agric Sci 7:189. https://doi.org/10.5539/jas.v7n11p189

Hammad SA, Abou-Seeda MA, El-Ghamry AM, Selim EM (2007) Nitrate accumulation in spinach plants by using N-fertilizer types in alluvial soil. J Appl Sci Res 3:511–518

Haygarth PM, Condron LM, Heathwaite AL, Turner BL, Harris GP (2005) The phosphorus transfer continuum: linking source to impact with an interdisciplinary and multi-scale approach. Sci Total Environ 344:5–14. https://doi.org/10.1016/j.scitotenv.2005.02.001

Heathwaite AL (2010) Multiple stressors on water availability at global to catchment scales: understanding human impact on nutrient cycles to protect water quality and water availability in the long term. Freshw Biol 55:241–257. https://doi.org/10.1111/j.1365-2427.2009.02368.x

Hitayezu P, Zegeye EW, Ortmann GF (2016) Farm level crop diversification in the Midlands region of KwaZulu-Natal South Africa: patterns, microeconomic drivers, and policy implications. Agroecol Sustain Food Syst 40:553–582. https://doi.org/10.1080/21683565.2016.1156595

Iqbal S (1999) Duckweed aquaculture, potentials, possibilities and limitations for combined wastewater treatment and animal feed production in developing countries. SANDEC report no. 6/99

Isikhungusethu Environmental Services (2012) Status Quo report for the uMgungundlovu district municipality strategic environmental assessment and strategic environmental management. Isikhungusethu Environmental Services (Pty) Ltd, Pietermaritzburg

Kołota E, Czerniak K (2010) The effects of nitrogen fertilization on yield and nutritional value of Swiss chard. Acta Sci Pol Hortorum Cultus 9:31–37

Kołota E, Adamczewska-Sowińska K, Balbierz A (2017) Response of Swiss chard (*Beta vulgaris* L. var. *cicla* L.) to nitrogen fertilization. Acta Sci Pol Hortorum Cultus 16:47–56

Kostecka J, Kaniuczak J (2008) Vermicomposting of duckweed (*Lemna minor* L.) biomass by *Eisenia fetida* (Sav.) earthworm. J Elementol 13:571–579

Kraider AN (2015) Behaviour of duckweed as an agricultural amendment: N mineralization, leaching and sorghum uptake. Dissertation, The Pennsylvania State University

Kufel L, Strzałek M, Wysokińska U, Biardzka E, Oknińska S, Ryś K (2012) Growth rate of duckweeds (Lemnaceae) in relation to the internal and ambient nutrient concentrations—testing the Droop and Monod models. Pol J Ecol 60:241–249

Kumar K, Goh KM (2000) Crop residues and management practices: effects on soil quality, soil nitrogen dynamics, crop yield, and nitrogen recovery. Adv Agron 68:197–319. https://doi.org/10.1016/S0065-2113(08)60846-9

Landolt E, Kandelar R (1987) The family of Lamnaccae—a monographic study: phytochemistry, physiology, application and bibliography, vol 4. Stiftung Ruebel, Zurich, p 638

LECO Corporation (2012) TruMac CNS/NS determinators. LECO Corporation, St. Joseph, MI

Leng RA (1999) Duckweed: a tiny plant with enormous potential for agriculture and environment. FAO, Rome

Lucas RE, Davis JF (1961) Relationships between pH values of organic soils and availability of 12 plant nutrients. Soil Sci 92:177–182

Malepfane NM, Muchaonyerwa P (2018) Pre-incubation in soil increases the nitrogen fertiliser value of hair waste. Environ Monit Assess 190:1–9. https://doi.org/10.1007/s10661-017-6429-x

May AM, El-Fadel M, Scrimshaw MD, Lester JN (2006) Factors influencing development strategies for the Abou Ali River in Lebanon I: spatial variation and land use. Sci Total Environ 362:15–30. https://doi.org/10.1016/j.scitotenv.2005.09.056

McGranahan DA, Ramaano R, Tedder MJ, Kirkman KP (2016) Variation in grassland fuel curing in South Africa. Fire Ecol 12:40–52. https://doi.org/10.4996/fireecology.1203040

Pulido CF (2016) Duckweed as a sustainable soil amendment to support crop growth, enhance soil quality, and reduce agricultural runoff. Dissertation, The Pennsylvania State University

Quilliam RS, Niekerk Van, Chadwick DR, Cross P, Hanley N, Jones DL, Vinten AJA, Willby N, Oliver DM (2015) Can macrophyte harvesting from eutrophic water close the loop on nutrient loss from agricultural land? J Environ Manag 152:210–217. https://doi.org/10.1016/j.jenvman.2015.01.046

Radić S, Stipaničev D, Cvjetko P, Rajčić M, Širac S, Pevalek-Kozlina B, Pavlica M (2011) Duckweed *Lemna minor* as a tool for testing toxicity and genotoxicity of surface waters. Ecotoxicol Environ Saf 74:182–187. https://doi.org/10.1016/j.ecoenv.2010.06.011

Ranade-Malvi U (2011) Interaction of micronutrients with major nutrients with special reference to potassium. Karnataka J Agric Sci 24:106–109

Sainju UM, Whitehead WF, Singh BP, Wang S (2006) Tillage cover crops and nitrogen fertilization effects on soil nitrogen and cotton and sorghum yields. Eur J Agron 25:372–382. https://doi.org/10.1016/j.eja.2006.07.005

Sanchez-Monedero MA, Roig A, Paredes C, Bernal MP (2001) Nitrogen transformation during organic waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. Bioresour Technol 78:301–308. https://doi.org/10.1016/S0960-8524(01)00031-1

Shurin JB, Abbott RL, Deal MS, Kwan GT, Litchman E, McBride RC, Mandal S, Smith VS (2013) Industrial-strength ecology: trade-offs and opportunities in algal biofuel production. Ecol Lett 16:1393–1404. https://doi.org/10.1111/ele.12176

Tejada M, Gonzalez JL, García-Martinz AM, Parrado J (2008) Effects of different green manures on soil biological properties and maize yield. Bioresour Technol 99:1758–1767. https://doi.org/10.1016/j.biortech.2007.03.052

The Non-Affiliated Soil Analysis Work Committee (1990) Handbook of standard soil testing methods for advisory purposes. Soil Science Society of South Africa, Pretoria

Turmel M-S, Speratti A, Baudron F, Verhulst N, Govaerts B (2015) Crop residue management and soil health: a systems analysis. Agric Syst 134:6–16. https://doi.org/10.1016/j.agsy.2014.05.009

Verma R, Suthar S (2015) Utility of duckweed as source of bioenergy: a review. Bioenergy Res 8:1589–1597. https://doi.org/10.1007/s12155-015-9639-5

Wang W (1990) Literature review on duckweed toxicity testing. Environ Res 52:7–22. https://doi.org/10.1016/S0013-9351(05)80147-1

Wang Z, Li S (2004) Effects of nitrogen and phosphorus fertilization on plant growth and nitrate accumulation in vegetables. J Plant Nutr 27:539–556. https://doi.org/10.1081/PLN-120028877

Wang X, Lu Y, Han J, He G, Wang T (2007) Identification of anthropogenic influences on water quality of rivers in Taihu watershed. J Environ Sci 19:475–481. https://doi.org/10.1016/S1001-0742(07)60080-1

Zeller MA, Hunt R, Sharma S (2013) Sustainable bioderived polymeric materials and thermoplastic blends made from floating aquatic macrophytes such as duckweed. J Appl Polym Sci 127:375. https://doi.org/10.1002/app.37555