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Circular economy fertilization: Testing micro and macro algal species as soil improvers and nutrient sources for crop production in greenhouse and field conditions

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ABSTRACT

Nutrient losses from agricultural land to freshwater and marine environments contribute to eutrophication and often to the growth of algal blooms. However, the potential benefits of recycling this algal biomass back to agricultural land for soil quality and crop nutrition in a "circular-economy" has received little attention. We tested the effects of algal additions to arable soil in greenhouse-grown garden peas, and field plots of spring wheat, on plant growth and nutrition and physical and chemical properties of the soil. Representatives of five algal species, which contrasted in elemental composition, were applied at 0.2, 2 and 4 g kg^{-1} in the greenhouse and at 24 g m^2 in the field. These included the cyanobacteria *Arthrospira platensis* (*Spirulina*), the unicellular green algae *Chlorella* sp., the red seaweed *Palmaria palmata*, and the brown seaweeds *Laminaria digitata* and *Ascophyllum nodosum*. In the greenhouse at the highest application rates (4 g kg^{-1}), *Chlorella* sp., and *Spirulina* increased soil total nitrogen and available phosphorus, and *Spirulina* also increased soil nitrate concentrations. *P. palmata* and *L. digitata* significantly increased soil inorganic (NH_4^+ and NO_3^-) concentrations under all three application rates. *Chlorella* sp. significantly increased soil total P, N and C, available P, NH_4^+-N, and pea yield. Soil water-stable aggregates were unchanged by the algal additions in both the greenhouse and field study. In the field, 4 species (*Chlorella* sp. *Spirulina*, *P. palmata* and *L. digitata*) increased soil inorganic nitrogen concentrations, confirming their potential to recycle mineralizable nitrogen to agricultural soils, but no significant effects were found on wheat yields under the application rates tested.

1. Introduction

Soil quality plays a critical role in crop productivity and both soil and crop resilience to drought and heavy rainfall, but there is increasing concern that intensive arable farming has degraded soil water and nutrient holding-capacity as a result of organic matter loss (Department for Environment, Food and Rural Affairs, 2009; Graves et al., 2015). Soil quality constraints are implicated in the yield plateau seen in wheat and oilseed rape, the most important field-grown crops in the UK (Knight et al., 2012). Soil degradation is estimated to cost the UK between £0.9 billion and £1.2 billion annually, in onsite and offsite non-market 'external' costs (Graves et al., 2015). This value is mainly attributed to the loss of soil organic carbon (47%), compaction (39%) and erosion (12%) (Graves et al., 2015). These changes are reflected in soil physical and chemical attributes such as soil aggregate stability and nutrient status. Water-stable aggregates are key indicators of soil quality since they deliver good soil structure and function by: (i) physically protecting soil organic matter against rapid decomposition, (ii) increasing soil water-holding capacity, (iii) providing pore space for root growth and water infiltration, and (iv) enhance resistance to erosion, and ultimately reducing surface crusting and runoff, which leads to aquatic pollution (Paul et al., 2013).

Intensification of arable production with continuous annual cropping using high mineral nutrient inputs has depleted soil organic matter (Mulvaney et al., 2009), which is responsible for storing nutrients and maintaining soil structure, ultimately leading to nutrient losses to water bodies. This has been compounded by nutrient-rich topsoil being eroded from continuously cropped arable land at an average rate of 9.5 tonnes per hectare across the EU 28 countries (Eurostat, 2017). This has caused preferential loss of the finer particles, such as the nutrient-retaining organic matter and clays, exacerbating the risk of nutrient export from land to water bodies and eutrophication (Department for

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Environment, Food and Rural Affairs, 2014). Despite the implementation of the Water Framework Directive (WFD) and the active management of nitrate vulnerable zones, there has been a decrease in the overall number of water bodies in the UK being awarded high or good surface water status between 2011 and 2016 (Joint Nature Conservation Committee, 2017). In England alone, 28% of failures to meet the WFD standards are directly attributed to diffuse water pollution from agriculture and rural land use (Department for Environment, Food and Rural Affairs, 2014). The urgency of this situation has grown with increasing awareness of the fossil-fuel energy costs in the production and use of chemical fertilisers. Each year, 100 million tonnes of fertiliser is used globally, contributing to greenhouse gas emissions. A recent study conducted by the Grantham Centre for Sustainable Futures at The University of Sheffield showed that more than half the environmental impact of producing a loaf of bread is attributed to the use of ammonium nitrate fertiliser during the wheat cultivation process, which accounts for 43% of the sample loaf's greenhouse gas emissions (Goucher et al., 2017).

In order to reduce dependency on inorganic fertiliser use, organic fertilisers such as animal manure, biosolids from human wastes, anaerobic digestate, biochar and crop residues are used as alternatives (Farrell et al., 2014; Rady, 2011; Walsh et al., 2012). Of these, the manures, biosolids and digestates are potentially the most important nutrient sources, but these complex materials have caused pollution/ecological risks associated with veterinary antibiotics, use of growth promoting heavy metals such as (Cu and Zn) in pigfeed (Ciesinksi et al., 2018) and other contaminants such as arsenic (Heimann et al., 2015; Wuang et al., 2016; Zhang et al., 2015). Alternative sources of organic fertilisers that can provide plants with an optimal mix of macro and micronutrients as well as benefit the structural characteristics of soil would be hugely beneficial for the agricultural industry. The European Commission disclosed a legislative proposal in March 2016 on organic and waste-based fertilisers as part of their Circular Economy Action Plan (European Commission, 2016). The aim is to promote resource efficiency with regards to the fertiliser sector in order to create new business opportunities for farmers, as well as help them become more competitive in recycling organic nutrients compared to purchasing inorganic fertilisers (European Economic and Social Committee, 2016). It seeks to reduce waste, energy consumption and environmental damage (Messenger, 2016).

Algae are the main primary producers in most water bodies, and their growth is naturally stimulated by organic effluents and mineral nutrients (Sen et al., 2013). As incidences of diffuse pollution increase due to anthropogenic activity, the size and frequency of algal blooms is on the increase. Furthermore, climate change has been predicted to exacerbate the problem. One potential solution to limit the detrimental impacts of nutrient runoff from agriculture is to divert nutrients to water bodies where it is possible to exploit the natural ability of microalgae to grow much quicker than land plants (Wuang et al., 2016), and actively cultivate and harvest the biomass. The biomass can be used as a sustainable source of organic fertiliser, returning both nutrients and carbon to soil, potentially improving soil quality, crop growth and nutrition. Moreover, research in large-scale algal biomass production has increased in recent years, for diverse applications including biofuels, animal feed (Yaakob et al., 2014) and as nutrient scavengers in wastewater treatment processes (Zhu et al., 2013). This has also created opportunities for the development of by-products such as algal-based fertiliser that could contribute to a more sustainable circular-economy for nutrients in arable farming systems.

Chlorella sp. and *Spirulina* (*Arthrospira platensis* and *Arthrospira maxima*), which are commonly used microalgal species in the treatment of wastewater (Aslan & Kapdan, 2006), are reported to have high nutrient (N and P) removal capabilities from effluents, making them suitable candidates as soil conditioners. *Spirulina platensis* biomass has been shown to improve soil macronutrients (nitrogen, phosphorus and potassium) (Aly & Esawy, 2008), act as a biofortification agent, enhance plant protein content (Kalpana et al., 2014) and increase crop growth, i.e. 5 g *Spirulina* in 500 g^{-1} soil increased the height of Bayam red (red spinach) by 58.3% as well as fresh and dry weights by 110.1% and 155.8% respectively, when compared to the control group (Wuang et al., 2016). Dried algal biomass grown on anaerobic digestate from dairy manure increased plant available N and P in soils within 21 days and thereby improved cucumber and corn seedling growth (Mulbry et al., 2005). Additions of 2–3 g dried *Chlorella vulgaris* kg^{-1} soil significantly increased ($p < 0.0001$) fresh and dry weight of lettuce seedlings (Faheed & Abd-El Fattah, 2008). Extracts or composted marine algal seaweed species have been researched as amendments in crop production systems due to their biostimulatory potential on crop growth and their benefits as sources of organic matter and soil nutrients (Khan et al., 2009). Brown seaweeds (Phaeophyceae) have also been tested, with *Ascophyllum nodosum*, the most studied of the phaeophyceae, shown to improve growth and drought stress tolerance when used as a soil drench or foliar spray in container-grown citrus trees (Spann & Little, 2011). Other positive responses include early seed germination and establishment, improved crop performance and yield, as well as elevated resistance to biotic and abiotic stress (Khan et al., 2009). Brown seaweeds contain high amounts of polyuronides such as alginates and fucoidans, which are known for their gelling and chelating abilities and their ability to combine with metallic ions in the soil. They form high-molecular-weight complexes that absorb moisture and result in better soil aeration and moisture retention, and in turn boost soil microbial activity (Khan et al., 2009). The application of another brown seaweed, *Laminaria digitata*, has been shown to also improve soil physical properties including total pore volume and aggregate stability of a sandy soil (Haslam & Hopkins, 1996).

Algae also represent a source of trace elements, which they acquire via biosorption and bioaccumulation (Michalak et al., 2017) and can therefore contribute to crop micronutrient uptake. Wheat, the second most important cereal crop globally, makes up about 28% of human dietary energy (Velu et al., 2016). It is the most important cereal crop in the UK where it is grown on 1.7 million hectares, yielding 15.2 million tonnes last year (Department for Environment, Food and Rural Affairs, 2017). The ability of algal-fertilisers to increase the often suboptimal concentration in wheat grains of zinc, iron and selenium (Broadley et al., 2006; Stroud et al., 2010) which are essential for human nutrition, needs to be investigated, as this could provide a cost effective, sustainable solution to micronutrient deficiencies (Velu et al., 2016).

There is increasing evidence that the deployment of algae biomass could act as a source of organic fertiliser. There are approximately 280,000 recognised algae species (Chojnacka & Kim, 2015), but the relative merits of different species and functional groups on soil quality and crop improvements, and their key attributes that control their effectiveness remain unclear. Algae vary greatly in their mineral and organic composition and consequently their impact on soil nutrients and aggregate stability are hypothesized to be strongly dependant on the initial concentration of nutrients in their biomass (Flavel & Murphy, 2006).

This study aims to investigate the use of chemically contrasting types (difference in elemental composition) of algal species biomass on soil aggregate stability, nutrients and ultimately growth and yields of crops. In addition, we explore the effects of different types of algae as soil amendments for improving micronutrient (e.g. zinc, iron and selenium) concentrations in wheat. To address these aims, bioassay greenhouse and field experiments were conducted with garden peas and wheat respectively. The five algal species chosen also represented different phylogenetic groups: the cyanobacterium *Spirulina*, the freshwater green alga *Chlorella* sp., a Chlorophyte, and three marine species namely *P. palmata* from the class Rhodophyta and *L. digitata* and *A. nodosum* both representing the class Phaeophyta.

Table 1
Physical and chemical characteristics of untreated soil used for greenhouse experiment.

pH	TN[a]	TP	TC	P_{AV}	K_{AV}	NH_4^+	NO_3^-	C:N	Stability[b] (1–2 mm)	WHC[c]
	g kg^{-1}	g kg^{-1}	g kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}	mg kg^{-1}		%	%
6.95 ± 0.02	1.73 ± 0.02	0.24 ± 0.08	21.9 ± 0.2	18.1 ± 1.1	16.3 ± 0.2	14.7 ± 0.2	14.9 ± 0.3	12.8 ± 0.1	1.71 ± 0.08	49.8 ± 0.3

[a] TN = total nitrogen, TP = total phosphorus, TC = total carbon, P_{AV} = available phosphorus, K_{AV} = available potassium, NH_4^+ = ammonium, NO_3^- = nitrate, n = 3.
[b] Water stable aggregates of the 1–2 mm size fraction.
[c] WHC = water holding capacity of soil.

2. Materials and methods

2.1. Experimental set-up

The experimental site and location of soil collected for the greenhouse experiment was Wise Warren at Spen Farm, Tadcaster, England (longitude 1°20′32.9″ W, latitude 53°51′40.7″ N). The field had been subjected to continuous cropping since 1985, mainly growing winter wheat, spring and winter barley, oilseed rape, sugar beet, winter beans, and potatoes. The soil is in the Aberford series (Calcaric Endoleptic Cambisol; (Cranfield University, 2017)). Results for the characterisation of initial topsoil conditions are shown in Table 1. At the beginning of the greenhouse experiment, the soil had a total phosphorus concentration of 0.238 g kg^{-1}, total nitrogen of 1.732 g kg^{-1}, carbon content of 21.94 g kg^{-1} and a pH of 6.95.

The dry biomass of five algal species: *Arthrospira platensis*, (*Spirulina*), *Chlorella* sp. *Palmaria palmata*, *Laminaria digitata* and *Ascophyllum nodosum* were individually added to separate soil samples. The algal biomass used was purchased commercially with the exception of *Ascophyllum nodosum* which was obtained from the strandline of a beach on the west coast of Ireland, rinsed (to remove sand), oven dried at 60 °C, ground and ball milled to pass a 600 µm sieve and mixed to ensure homogeneity. The contents of carbon, nitrogen and phosphorus in the algae biomass are shown in Table 2 and the micronutrients and heavy metal content are shown in Table 3. No supplemental nutrients/fertilisers were added, in order to compare the benefits of the different algal biomass types and varying application rates.

A pot experiment, using soil taken from the field at Wise Warren, was conducted in a GroDome greenhouse at the Arthur Willis Environment Centre (AWEC), The University of Sheffield, for 90 days (starting on 2nd April 2015 and ending on 1st July 2015) with pea maincrop (*Pisum sativa*). Prior to starting the experiment, the soil was air dried and homogenized by mixing in one large basin before being put into separate pots in equal amounts of 1 kg (± 0.05). The pots and plants were maintained under 12 h photoperiod, 200 µE m^{-2} s^{-1} light intensity, 21 °C:15 °C day:night temperatures. Dried algal biomass was added at low (0.2 g kg^{-1}) medium (2 g kg^{-1}) and high (4 g kg^{-1}) application rates, accompanied by controls with no algal additions. Application rates were chosen according to previous studies (Akhter et al., 2002; Nisha et al., 2007; Obana et al., 2007). Each pot was sown with four pea seeds, with four replicate pots of each treatment.

The field experiment was conducted the following year at Wise Warren Farm, from 27th April 2016 to 26th September 2016. The experiment was divided into 21 plots with three replications of each treatment. The square plots were 1 m^2 and each plot was divided equally into two. The algae were applied only once as topdressing, with application rates of 8 g and 16 g per half a metre square. This was equivalent to 29.81 kg N ha^{-1} for *Spirulina*, which had the highest N concentrations, down to 3.91 kg N ha^{-1} for *A. nodosum*, which had the lowest N concentrations. For phosphorus, algae application rates ranged from 0.65 kg P ha^{-1} under *Chlorella* sp., which had the highest P concentrations, down to 0.06 kg P ha^{-1} under *A. nodosum*. The plots were sown with spring wheat (*Triticum aestivum* L.) (Tybalt high yielding variety purchased from Limagrain) in April and harvested at maturation after 5 months in September. The measured response variables included soil total phosphorus, carbon and nitrogen, available phosphorus, potassium and nitrogen, water stable aggregates, crop yield and grain micronutrient content.

2.2. Analysis of soil physico-chemical properties

Quantitative analyses of specific soil physico-chemical properties were carried out for the greenhouse experiment before the start of the experiment and at the end after 90 days (~13 weeks). In the field experiment, in order to gain a better understanding of the nutrient dynamics of the algae biomass following addition onto soil, analysis was carried out on soil 2, 8 and 20 weeks following algae addition. Soil samples were air-dried, and sieved (2 mm) prior to analysis of soil nutrients and pH.

2.2.1. pH and soil nutrients

Soil pH was determined with 20 g of air-dried soil, mixed with 20 ml of distilled water to form a 1:1 ratio and measured using a pH electrode (Kalra, 1995). Total carbon (C) and nitrogen (N) were determined using a CN elemental analyser (Vario EL Cube, Langenselbold, Germany). Total soil phosphorus (P), including both organic and inorganic P (Carter & Gregorich, 2008), was determined for homogenized subsamples (20–50 mg, ± 0.001 mg). A catalyst of $LiSO_4$ and $CuSO_4$ (1:1) was added and the mixture digested in 1 ml of concentrated sulphuric acid at 365 °C for 6 h. Once cool, 9 ml of 18.2 MΩhm.cm (UHP) water

Table 2
Carbon content and macronutrients found in the different algal species.

Algae	C	N	P	Ca	Mg	C:N	N:P
	mg g^{-1}[a]						
Spirulina	543 ± 9^a	124 ± 2^a	2.08 ± 0.03^a	2.18 ± 0.06^a	2.12 ± 0.03^a	4.4	59.4
Chlorella sp.	511 ± 54^a	102 ± 13^a	2.67 ± 0.05^b	2.53 ± 0.02^a	2.07 ± 0.02^a	5.0	38.0
P. palmata	447 ± 7^{ab}	35.4 ± 0.2^b	0.87 ± 0.04^c	0.90 ± 0.06^b	1.7 ± 0.1^b	12.6	41.0
L. digitata	355 ± 6^b	18.8 ± 0.3^c	0.47 ± 0.02^d	7.0 ± 0.2^c	5.8 ± 0.1^c	18.9	39.6
A. nodosum	370 ± 1^b	16.3 ± 0.2^c	0.25 ± 0.02^e	19.8 ± 0.7^d	5.72 ± 0.03^c	22.8	65.4

Values with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, One way ANOVA).
[a] Mean ± standard error (n = 3). Data was log transformed where assumption of normality was not met.

Table 3
Micronutrients and heavy metals found in the different algal species.

Algae	Micronutrients and heavy metals									
	Zn	Fe	Se	B	Mn	Cu	Cd	Pb	As	Ni	Cr
	mg kg^{-1}[a]										
Spirulina	32 ± 3^{a}	1150 ± 17^{a}	0.15 ± 0.03^{a}	9.6 ± 0.2^{a}	31.2 ± 0.4^{a}	1.9 ± 0.1^{a}	0.05 ± 0.003^{a}	1.56 ± 0.05^{c}	2.6 ± 0.1^{a}	1.5 ± 0.7^{ab}	3.83 ± 0.07^{c}
Chlorella sp.	18 ± 3^{b}	886 ± 12^{b}	0.06 ± 0.004^{b}	1.9 ± 0.5^{b}	46.9 ± 0.9^{b}	1.94 ± 0.06^{a}	0.07 ± 0.001^{a}	0.04 ± 0.02^{a}	2.29 ± 0.05^{a}	1.1 ± 0.2^{ad}	1.33 ± 0.05^{a}
P. palmata	19.9 ± 0.7^{b}	146 ± 14^{ab}	0.83 ± 0.05^{c}	127 ± 7^{c}	8.7 ± 0.5^{c}	8.3 ± 0.5^{b}	0.95 ± 0.06^{b}	< 0.01^{a}	9.1 ± 0.7^{b}	4.4 ± 0.3^{c}	1.2 ± 0.1^{ab}
L. digitata	9 ± 1^{c}	43 ± 4^{c}	1.10 ± 0.06^{c}	87 ± 4^{c}	2.42 ± 0.06^{d}	1.10 ± 0.01^{c}	0.15 ± 0.0009^{a}	< 0.01^{a}	57.9 ± 0.2^{c}	0.11 ± 0.09^{bd}	0.97 ± 0.08^{b}
A. nodosum	69 ± 7^{d}	183 ± 7^{d}	0.91 ± 0.05^{c}	112.7 ± 0.6^{c}	75 ± 1^{e}	1.3 ± 0.2^{c}	0.70 ± 0.02^{c}	0.52 ± 0.002^{b}	35.7 ± 0.5^{d}	2.41 ± 0.09^{a}	1.32 ± 0.05^{a}

Values with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, One way ANOVA).
[a] Mean ± standard error (n = 3). Data was log transformed where assumption of normality was not met.

was added to samples and the samples analysed colorimetrically using the ammonium molybdate-antimony potassium tartrate-ascorbic acid method of Murphy and Riley (Murphy & Riley, 1962). Soil-available P was determined using the sodium bicarbonate ($NaHCO_3$) extraction method by extracting 2.5 g soil with 50 ml 0.5 M $NaHCO_3$ (Olsen et al., 1954), orthophosphate was then determined using the Murphy Riley method. Available nitrogen was analysed following extraction of 10 g soil with 40 ml 2 M KCl on a shaker for an hour. Samples were filtered using Whatman No.1 paper and then ammonium (NH_4^+-N) determined spectrophotometrically by means of a modified Berthelot reaction (Krom, 1980) and nitrate (NO_3^-N) using a rapid colorimetric determination by nitration of salicylic acid measured by absorbance at 405 nm (Cataldo et al., 1975; Matsumura & Witjaksono, 1999).

2.2.2. Physical properties

Aggregate stability was measured using the sequential wet sieving method adapted from Cambardella and Elliott (Cambardella & Elliott, 1993), to derive five size classes: > 2000 μm, (large macroaggregates) 1000–2000 μm (medium macroaggregates), 250–1000 μm (small macroaggregates), 53–250 μm (large microaggregates) and < 53 μm (small microaggregates and silt or smaller-sized particles. For each sample, 50 g ± 0.005 of soil was placed in a 2000 μm sieve and submerged in a bowl of distilled water filled up to 15 mm above the sieve mesh for 5 min, to allow for slaking. Subsequently, the sieve was moved up and down in 50 strokes over the period of approximately 2 min. Stones, roots and other organic material were removed and the aggregates placed into a pre-weighed tin cup. The remaining soil and water that passed through the 2000 μm sieve was then poured through a 1000 μm sieve and moved vertically for 40 strokes and transferred to a pre-weighed aluminium cup. The same steps were repeated for the 250 μm sieve (30 vertical strokes) and 53 μm (10 vertical strokes). The remaining water was allowed to settle overnight and poured into aluminium cups representing the < 53 μm fraction. Aluminium cups with soil samples were left in the oven at 105 °C for 24 h and the soil dry weight obtained.

2.3. Algae elemental ratio, crop biomass and grain micronutrient analysis

2.3.1. Algae elemental ratio

A Flash 2000 Elemental Analyser was used to obtain total C and N values and the total P of the algae and shoot (straw) biomass was measured as previously described for soil total P concentration.

2.3.2. Pea and wheat biomass

Harvested pea plants were weighed immediately to obtain fresh weight and then oven dried at 70 °C to obtain dry weights. Pea yield was analysed by counting the total number of pods per plant in each pot. Dry weight of wheat ears, straw and grain was obtained after drying in oven at 70 °C for 3 days. Wheat ears were threshed by hand and the grain subsequently passed through a riffle box to obtain a representative 10 g sample for nutrient analysis. Straw was powdered using a Retch s100 mill and subsamples analysed for total nutrients: C and N by CN elemental analyser, total P using the method as previously described for soil analysis.

2.3.3. Micronutrient analysis in wheat grain

Grain total micronutrients were analysed in wheat grains imbibed in UHP water and chopped into small pieces. 0.25 g was digested in aqua regia solution (3:1, HCl:HNO_3). The digested solution was filtered using a 0.2 μm syringe filter and diluted using UHP water to a fixed volume of 25 ml and the solution analysed using ICP-MS (Perkin-Elmer, Elan DRCII). All sample vessels were acid washed prior to analysis.

2.4. Statistical analysis of data

The means of the replicates for the 5 treatments ± standard error

are presented. Statistical analyses were conducted using the RStudio software version 3.1.0 and Graphpad Prism. To compare the effect of different algal species and application rates on measured soil characteristics, the Anderson-Darling test was used to check for distributional adequacy of the data and data was log transformed prior to analysis if it did not follow a normal distribution. The impact of the algae treatments on soil nutrients was tested using one-way ANOVA and Tukey post-hoc analysis to see how the treatments compared against each other and the control and a two-way ANOVA to see whether the treatments and their application rates had an impact on soil nutrients. Differences were considered significant at a probability level of ($p < 0.05$).

3. Results

3.1. Algae biomass elemental characterisation

The elemental composition of the algal species varied considerably and for some elements these differences were greatest between freshwater and marine algae (Table 2). For example, C concentrations were significantly higher in *Chlorella* sp. and *Spirulina* compared to *L. digitata* ($p < 0.01$) and *A. nodosum* ($p < 0.01$). Total N concentrations also varied significantly ($p < 0.001$) among the algae species, but were similar between *Chlorella* sp. and *Spirulina* and between *L. digitata* and *A. nodosum* ($p > 0.05$). All algae species differed in their total P concentrations ($p < 0.001$), while *A. nodosum* had the lowest C, N and P concentrations. Mg concentrations varied among all 5 species, with *L. digitata* and *A. nodosum* having higher concentrations in their biomass (5.8 and 5.7 mg g^{-1} respectively) compared to *P. palmata*, which had the lowest concentrations (1.7 mg g^{-1}).

For the micronutrients analysed (Table 3), all the algae species differed significantly in their Zn, Ca and Fe concentrations ($p < 0.0001$), except for *P. palmata*, *Spirulina*, *Chlorella* sp. and *A. nodosum*, which all had similar Zn, Ca and Fe concentrations, respectively. Se concentrations were significantly higher in macroalgae species compared to microalgae ($p < 0.0001$), with *L. digitata* having the highest concentration at 1.1 mg kg^{-1}.

Heavy metals analysed were cadmium (Cd), lead (Pb), arsenic (As), nickel (Ni) and chromium (Cr). Apart from in *P. palmata* and *A. nodosum*, the algal species contained negligible concentrations of Cd. The Pb concentrations in *P. palmata* and *L. digitata* were both below limits of detection, but low concentrations (0.04 mg kg^{-1}) were detected in *Chlorella* sp. *Spirulina* was found to have significantly higher concentrations of Cr (3.83 mg kg^{-1}) than the other algal species, and *L. digitata* had high concentrations of As (57.9 mg kg^{-1}), just above the lower guideline value of 50 mg kg^{-1} for agricultural land (Toth et al., 2015).

3.2. Part I greenhouse experiment

3.2.1. Effect of algal biomass on soil total nitrogen, carbon and phosphorus concentrations

Total P under both *Chlorella* sp. and *P. palmata* treatments were significantly lower ($p < 0.01$) in comparison to the control (Fig. 1). There was a significant interaction between treatment and application rate ($p < 0.01$), which was only evident under low application rates (0.2 g kg^{-1}) of *P. palmata* treatment, which had significantly lower concentrations of total P ($p < 0.05$) in comparison to low and high (4 g kg^{-1}) application rates of *A. nodosum*. Highest amounts of total N in the greenhouse experiment were observed under *Chlorella* sp. and *Spirulina* (both 1.9 g kg^{-1}) treatments, which both increased by approximately 12% from initial soil N concentration of 1.7 g kg^{-1}. Both these treatments were found to have significantly higher ($p < 0.001$) concentrations of total N than the control treatments. High application rates (4 g kg^{-1}) of *Spirulina* and *Chlorella* sp. significantly ($p < 0.05$) increased soil total N concentrations in comparison to the control

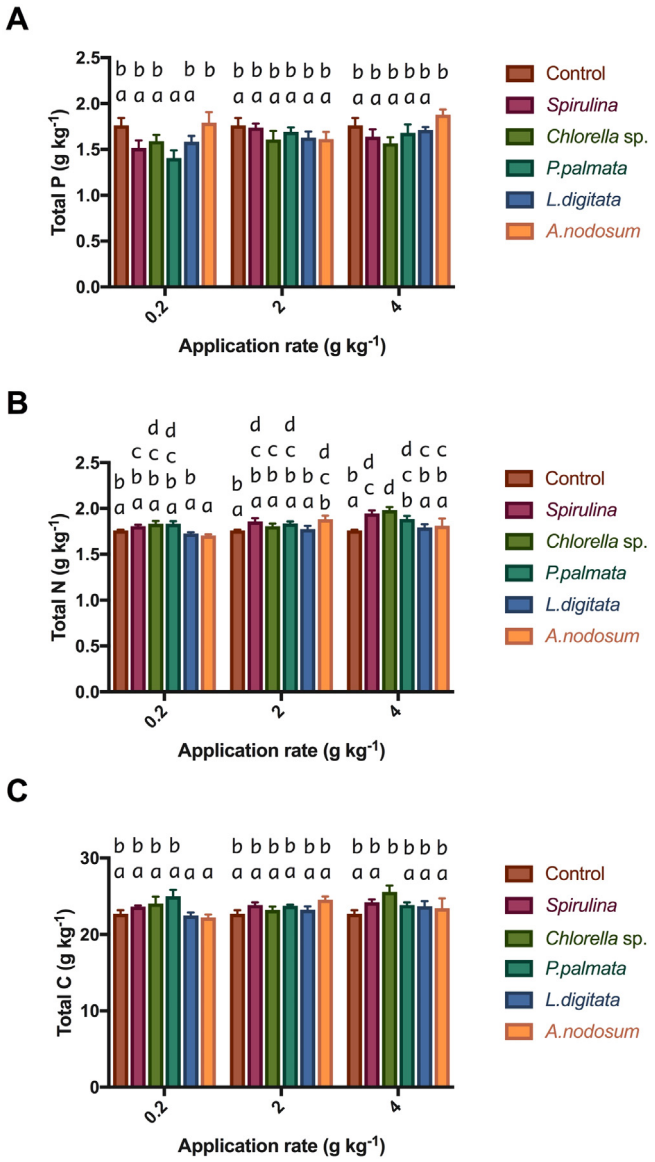

Fig. 1. (a) Soil total phosphorus ([F (17,54) = 2.607, $p = 0.004$] two-way ANOVA), (b) total N ([F (17,54) = 4.956, $p < 0.0001$] two-way ANOVA), (c) total C concentrations ([F (17,54) = 2.255, $p = 0.01$] two-way ANOVA) at harvest (13 weeks) as affected by application of different algae species at 3 application rates (0.2, 2 and 4 g kg^{-1}). Boxplots represent mean concentrations, with the bars on the columns representing standard error of the mean, n = 4. Means which do not share the same letter e.g. a, b etc., are significantly different ($p < 0.05$, two way ANOVA).

treatments. Total soil C concentrations significantly increased under both *Chlorella* sp. and *P. palmata* treatments in comparison to the control ($p < 0.05$), with concentrations under highest application rates of *Chlorella* sp. treatment increasing by 17% from the initial soil C concentrations. Additionally, under high application rates of *Chlorella* sp. soil C concentrations were significantly higher ($p < 0.05$) than they were under low application rates of *A. nodosum* and *L. digitata*.

3.2.2. Effect of algal biomass on available nitrogen and phosphorus concentrations

After 13 weeks (Fig. 2), *Chlorella* sp. *Spirulina*, and *P. palmata* treatments significantly increased ($p < 0.05$) soil available P in comparison to the control. Both *L. digitata* and *A. nodosum* had the lowest increases in soil available P concentrations. A significant interaction ($p < 0.0001$) was also observed between application rate and algae

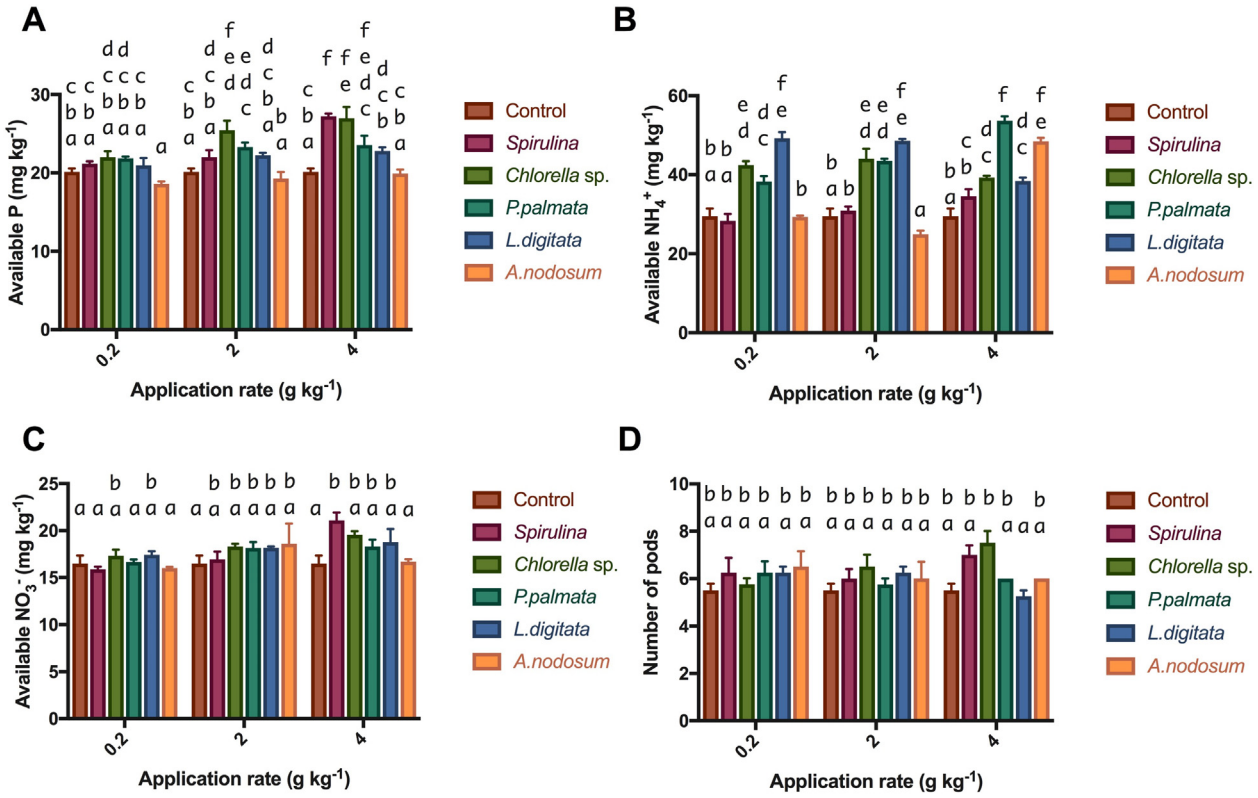

Fig. 2. (a) Soil available phosphorus ([F (17,54) = 11.66, p < 0.0001] two-way ANOVA), (b) available NH_4^+-N ([F (17,54) = 40.05, $p =$ < 0.0001] two-way ANOVA), (c) available NO_3^-N concentrations ([F (17,54) = 2.763, $p = 0.002$] two-way ANOVA) and (d) yield at harvest (13 weeks) ([F (5,66) = 2.478, $p = 0.04$] One-way ANOVA) as affected by application of different algae species at 3 application rates (0.2, 2 and 4 g kg^{-1}). Boxplots represent mean concentrations, with the bars on the columns representing standard error of the mean, n = 4. Means which do not share the same letter e.g. a, b etc., are significantly different ($p < 0.05$, two way ANOVA).

treatment where under high (4 g kg^{-1}) application rates, *Chlorella* sp. was observed to have increased soil available P by ~50% from 18.1 mg kg^{-1} at the start of the experiment to 27 mg kg^{-1} at harvest (13 weeks). Medium application rates of *Spirulina* were also found to significantly improve soil available P concentrations in comparison to the control ($p < 0.0001$).

At harvest, the concentration of NH_4^+-N in the soil significantly increased ($p < 0.0001$) under *Chlorella* sp. *P. palmata* and *L. digitata* treatments, which were found to have higher concentrations than the control. An increase from initial soil concentrations of 180%, 200% and 200% was observed respectively under *Chlorella* sp. *P. palmata* and *L. digitata*. There was no difference in NH_4^+-N concentrations between *Spirulina* treatment and *A. nodosum* in comparison to the control. None of the algae treatments had any significant impact on soil NO_3^--N except for *Spirulina*, where high application rates increased NO_3^--N concentrations by 42%. This increase was significantly higher ($p < 0.05$) than the NO_3^--N concentrations measured under the lowest application rates of *A. nodosum*, *P. palmata* and the control.

3.2.3. Effect of algae biomass on soil aggregate stability

There was no significant difference between any of the treatment means and the control. Data showed a lot of variability under all the different algal treatments and application rates.

3.2.4. Effect of algae biomass on pea yield

Chlorella sp. treatment had the highest average pea yield with 6.6 pods, which was significantly higher ($p < 0.05$) than yields under the control treatments. The control had the lowest overall average yield of 5.5 pods. Pea yield under a high application rate of *Chlorella* sp. were significantly higher ($p < 0.05$) than yields under high application rates of *L. digitata* treatment.

3.3. Part II field experiment

In the field experiment with spring wheat (*Triticum aestivum* L.) the focus was to see whether the effects on soil available nutrients in the soil were replicable and more specifically how this would affect crop yield and nutritional value. Furthermore, to gain a better understanding of the degradation of the algae biomass in soil, temporal measurements were taken after: 2, 8 and 20 weeks (at harvest), when the wheat crop had reached maturation.

3.3.1. Temporal effects of algal biomass on soil available phosphorus concentrations

Soil available P concentrations 2 weeks after the addition of the algae biomass were highest under the control and *Chlorella* sp. treatments in comparison to *A. nodosum* ($p < 0.05$). However, application rate did not have any significant impact on available P concentrations between any of the algal treatments (Table 4).

After 8 weeks, soil available P concentrations increased from their concentrations at 2 weeks. However, there was no significant difference between any of the treatments means and no effect of application rates on soil available P concentrations ($p > 0.05$). After 20 weeks, P concentrations were found to have decreased and were highest under control treatments and significantly higher ($p < 0.05$) in comparison to *A. nodosum* and *L. digitata* treatments under which the lowest concentrations of available P were recorded. There was also no significant interaction between application rate and treatment.

3.3.2. Temporal effects of algal biomass on soil available NH_4^+-N concentrations

Soil NH_4^+-N concentrations under *Chlorella* sp. increased by 8% two weeks after the addition of algae treatments (Table 5) and were

Table 4
Soil available phosphorus dynamics in arable soil during 20 weeks of wheat crop growth, as affected by application of different algal species.

Treatment	Time[a]		
	2	8	20
	mg kg^{-1}[b]		
Control (1)	54 ± 8^{a}	67 ± 7^{a}	44 ± 3^{a}
Control (2)	53 ± 7^{a}	67 ± 6^{a}	40 ± 4^{a}
Spirulina (8 g)	50 ± 3^{a}	65 ± 6^{a}	35 ± 4^{a}
Spirulina (16 g)	48 ± 2^{a}	60 ± 4^{a}	33 ± 2^{a}
Chlorella sp. (8 g)	53 ± 5^{a}	66 ± 4^{a}	33 ± 2^{a}
Chlorella sp. (16 g)	55 ± 3^{a}	65 ± 8^{a}	38 ± 3^{a}
P. palmata (8 g)	50 ± 2^{a}	62 ± 3^{a}	33 ± 3^{a}
P. palmata (16 g)	48.3 ± 0.7^{a}	58 ± 2^{a}	33 ± 3^{a}
L. digitata (8 g)	48 ± 4^{a}	58 ± 4^{a}	32 ± 4^{a}
L. digitata (16 g)	46 ± 4^{a}	56 ± 6^{a}	31 ± 5^{a}
A. nodosum (8 g)	45 ± 2^{a}	54.90 ± 0.002^{a}	32 ± 1^{a}
A. nodosum (16 g)	47 ± 3^{a}	56 ± 1^{a}	31 ± 3^{a}

[a] Weeks after addition of algal biomass. Initial available P concentrations = 63 ± 2 mg kg^{-1}.
[b] Mean ± standard error (n = 3). Values with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, two way ANOVA).

Table 5
Soil available nitrogen (NH_4^+) dynamics in arable soil during 20 weeks of wheat crop growth, as affected by application of different algal species.

Treatment	Time[a]		
	2	8	20
	mg kg^{-1}[b]		
Control 1	16.6 ± 0.7^{ab}	11.9 ± 0.6^{ab}	12.0 ± 0.3^{a}
Control 2	16.8 ± 0.6^{ab}	13 ± 2^{ab}	11.8 ± 0.3^{a}
Spirulina (8 g)	17.4 + 0.8^{ab}	12.3 ± 0.5^{ab}	12.10 ± 0.007^{a}
Spirulina (16 g)	19.1 ± 0.1^{a}	12.7 ± 0.6^{ab}	12.1 ± 0.3^{a}
Chlorella sp. (8 g)	19.1 ± 0.6^{a}	18 ± 2^{ab}	12.4 ± 0.4^{a}
Chlorella sp. (16 g)	19.0 ± 0.8^{a}	18 ± 3^{ab}	12.2 ± 0.3^{a}
P. palmata (8 g)	17.1 ± 0.4^{ab}	19 ± 2^{a}	11.6 ± 0.3^{a}
P. palmata (16 g)	17.6 ± 0.7^{ab}	18 ± 2^{ab}	12.2 ± 0.5^{a}
L. digitata (8 g)	16.8 ± 0.4^{ab}	20 ± 2^{a}	12.5 ± 0.3^{a}
L. digitata (16 g)	16.6 ± 0.8^{ab}	10.1 ± 0.6^{b}	12.5 ± 0.4^{a}
A. nodosum (8 g)	15.5 ± 0.4^{b}	10 ± 1^{b}	11.8 ± 0.3^{a}
A. nodosum(16 g)	15.5 ± 0.8^{b}	10.7 ± 0.8^{b}	12.20 ± 0.08^{a}

[a] Weeks after addition of algal biomass. Initial NH_4^+-N concentrations = 17.6 ± 0.2 mg kg^{-1}.
[b] Mean ± standard error (n = 3). Values with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, two way ANOVA).

significantly higher ($p < 0.01$) than the NH_4^+-N concentrations under the control, *L. digitata* and *A. nodosum* treatments. Both low (8 g) and high (16 g) application rates of *Chlorella* sp. and high application rates of *Spirulina* significantly increased ($p < 0.0001$) NH_4^+-N concentrations in comparison to low and high application rates of *A. nodosum*. 8 weeks after the addition of algae, *P. palmata* increased NH_4^+-N by 5%, significantly higher ($p < 0.05$) than concentrations under the control, *Spirulina* and *A. nodosum* treatments. Low application rates of *P. palmata* had higher ($p < 0.001$) NH_4^+-N concentrations in comparison to low application rates of *L. digitata* and both low and high application rates of *A. nodosum*. By harvest at 20 weeks, soil NH_4^+-N concentrations decreased under all treatments, to values lower than at the beginning of the experiment. There was no significant difference between any of the treatment means and no significant interaction between treatments and application rates on NH_4^+-N concentrations.

Table 6
Soil available nitrogen (NO_3^-) dynamics in arable soil during 20 weeks of wheat crop growth, as affected by application of different algal species.

Treatment	Time[a]		
	2	8	20
	mg kg^{-1},[b]		
Control 1	18.4 ± 0.2^{b}	7.62 ± 0.01^{a}	20.3 ± 0.5^{a}
Control 2	18.3 ± 0.3^{b}	8.0 ± 0.2^{a}	19.4 ± 0.3^{ab}
Spirulina (8 g)	19.9 ± 0.2^{b}	8.1 ± 0.3^{a}	18.0 ± 0.6^{b}
Spirulina (16 g)	23 ± 1^{a}	7.9 ± 0.3^{a}	18.2 ± 0.2^{ab}
Chlorella sp. (8 g)	20.2 ± 0.7^{ab}	8.0 ± 0.3^{a}	19.3 ± 0.8^{ab}
Chlorella sp. (16 g)	20.8 ± 0.1^{ab}	7.9 ± 0.1^{a}	18.6 ± 0.5^{ab}
P. palmata (8 g)	18.2 ± 0.4^{b}	8.3 ± 0.2^{a}	20.3 ± 0.2^{a}
P. palmata (16 g)	18.4 ± 0.3^{b}	8.5 ± 0.1^{a}	19.9 ± 0.6^{ab}
L. digitata (8 g)	18.3 ± 0.1^{b}	8.22 ± 0.05^{a}	20.0 ± 0.3^{ab}
L. digitata (16 g)	18.1 ± 0.4^{b}	8.4 ± 0.2^{a}	19.4 ± 0.2^{ab}
A. nodosum (8 g)	18.6 ± 0.4^{b}	8.0 ± 0.2^{a}	20.12 ± 0.07^{ab}
A. nodosum (16 g)	19.0 ± 0.2^{b}	7.90 ± 0.05^{a}	19.8 ± 0.4^{ab}

[a] Weeks after addition of algal biomass. Initial NO_3^--N concentrations = 18.1 ± 0.5 mg kg^{-1}.
[b] Mean ± standard error (n = 3). Values with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, two way ANOVA).

3.3.3. Temporal effects of algal biomass on soil available NO_3^--N concentrations

After 2 weeks, soil NO_3^--N concentrations increased significantly under *Spirulina* and *Chlorella* sp. treatments ($p < 0.05$) in comparison to the control (Table 6). Under high application rates of *Spirulina*, NO_3^--N was higher ($p < 0.001$) in comparison to NO_3^--N concentrations under both high and low application rates of control, *L. digitata*, *P. palmata* and *A. nodosum*. After 8 weeks, NO_3^--N concentrations were highest under *P. palmata* treatments and were significantly higher ($p < 0.05$) in comparison to the control. There was no interaction between the treatments and their application rates on soil NO_3^--N concentrations ($p > 0.05$). Twenty weeks after the addition of algae treatments, soil NO_3^--N concentrations increased again under all treatments. The lowest increase was observed under *Spirulina*, where NO_3^--N concentrations were significantly lower ($p < 0.01$) than control, *A. nodosum*, *L. digitata* and *P. palmata*.

3.3.4. Temporal effects of algal biomass on soil aggregate stability

Two weeks after the addition of algae the highest increase in water stable macro-aggregates (250–2000 μm) was observed under *L. digitata* treatment (Table 7) and was found to be significantly higher ($p < 0.05$) than under *A. nodosum* treatment. However, the % dry weight of water stable macro-aggregates under *L. digitata* were found to be no different from the control and all the other algae treatments. After 8 weeks, water stable macro-aggregates appeared to increase following the addition of *A. nodosum*, and *P. palmata*, however, there were no significant differences between any of the treatment means. After 20 weeks, under *L. digitata* treatment, water stable macro-aggregates increased significantly ($p < 0.05$) in comparison to *A. nodosum* treatment. However there was no difference between *L. digitata* and other algae treatments or the control. There was no apparent relationship between treatment and application rate on water stable macro-aggregates after 2, 8 or 20 weeks.

3.3.5. Effect of algal biomass on wheat parameters and micronutrients

The effects of the algae on wheat parameters are presented in Table 8. Total shoot biomass was calculated from the combined dry weight of the harvested straw and wheat ears. Total shoot biomass was highest under *Chlorella* sp. treatment (444 g m^{-2}). There were no significant differences in total shoot biomass between *Chlorella* sp. and the control, *Spirulina*, *P. palmata* and *L. digitata* treatments. Wheat ear count

Table 7
Water stable aggregates (250 μm– > 2000 μm) dynamics in arable soil during 20 weeks of wheat crop growth, as affected by application of different algal species.

Treatment	Time[a]		
	2	8	20
	% dry weight[b]		
Control 1	52 ± 1[a]	58 ± 2[a]	47 ± 1[a]
Control 2	55 ± 2[a]	59 ± 2[a]	46 ± 2[a]
Spirulina (8 g)	57 ± 1[a]	59 ± 1[a]	48 ± 1[a]
Spirulina (16 g)	55 ± 4[a]	59 ± 4[a]	46 ± 2[a]
Chlorella sp. (8 g)	56 ± 1[a]	61 ± 2[a]	50 ± 4[a]
Chlorella sp. (16 g)	53 ± 3[a]	58 ± 1[a]	49 ± 3[a]
P. palmata (8 g)	55 ± 2[a]	62 ± 2[a]	55.1 ± 0.6[a]
P. palmata (16 g)	52 ± 2[a]	61 ± 1[a]	48 ± 3[a]
L. digitata (8 g)	62 ± 1[a]	59 ± 0.9[a]	54 ± 1[a]
L. digitata (16 g)	57.3 ± 0.8[a]	63 ± 1[a]	51 ± 3[a]
A. nodosum (8 g)	52 ± 3[a]	54 ± 2[a]	46 ± 3[a]
A. nodosum(16 g)	41 ± 10[a]	58 ± 2[a]	45 ± 2[a]

[a] Weeks after addition of algal biomass. Initial % dry weight of macro aggregates = 57 ± 1.
[b] % dry weight of soil macro aggregate (250–2000 μm) fraction. Mean ± standard error (n = 3). Values with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, two way ANOVA).

Table 8
Effect of algae biomass on various parameters of spring wheat.

Treatment	Total shoot biomass		Ear number per m^2		Yield	
	Mean[a]	SE[b]	Mean	SE	Mean	SE
	(g^{-1})				($t\,ha^{-1}$)	
Control	359[ab]	14	156[a]	9	4.6[a]	0.2
Spirulina	377[ab]	20	145[a]	14	4.2[a]	0.3
Chlorella sp.	444[a]	13	172[a]	9	5.0[a]	0.3
P. palmata	403[ab]	25	158[a]	17	4.6[a]	0.5
L. digitata	385[ab]	33	150[a]	17	4.4[a]	0.6
A. nodosum	341[b]	25	143[a]	10	4.2[a]	0.4

[a] Mean values (n = 3), with different superscript e.g. a, b etc., in the same column are significantly different ($p < 0.05$, one way ANOVA).
[b] Standard error.

Table 9
Inputs and output amounts of total N and P in field experiment.

Treatments	Total nitrogen	Total phosphorus
	Input[b]	
	$mg\,N\,m^{-2}$[a]	$mg\,P\,m^{-2}$
Spirulina	2980 ± 4.3[c]	50.1 ± 0.8[a]
Chlorella	2446 ± 302[c]	64 ± 1[b]
P. palmata	850 ± 6[b]	21 ± 1[c]
S. latissima	450 ± 7[a]	11.4 ± 0.5[a]
A. nodosum	391 ± 5[a]	6.0 ± 0.5[a]
	Output[c]	
Control	24,380 ± 1411[a]	536 ± 28[a]
Spirulina	27,312 ± 1786[a]	520 ± 26[a]
Chlorella	31,241 ± 1094[a]	561 ± 58[a]
P. palmata	26,472 ± 1912[a]	533 ± 49[a]
S. latissima	26,033 ± 2480[a]	504 ± 63[a]
A. nodosum	23,895 ± 1505[a]	487 ± 35[a]

[a] Mean ± standard error (n = 3).
[b] Total N and P in amount of algae added.
[c] Total N and P in aboveground (shoot and grain) dry biomass after harvest.

Table 10
Micronutrient concentrations in wheat grain.

Treatment	Micronutrients and heavy metals											
	Ca	Mg	Zn	Fe	Se	B	Mn	Cu	Cd	Pb	As	Ni	Cr
	$mg\,kg^{-1}$[a]												
Control	336 ± 12[a]	< 0.01[a]	27 ± 2[a]	35 ± 1[a]	< 0.05[b]	6.4 ± 0.5[b]	29.3 ± 0.9[a]	3.7 ± 0.3[a]	0.02 ± 0.001[a]	0.08 ± 0.02[a]	8.6 ± 0.2[ab]	2.1 ± 0.6[ab]	1.87 ± 0.03[a]
Spirulina	289 ± 28[ab]	< 0.01[a]	29 ± 2[a]	32 ± 2[a]	0.13 ± 0.05[a]	12.8 ± 0.6[ab]	31.0 ± 0.9[a]	3.7 ± 0.4[a]	0.03 ± 0.003[a]	0.27 ± 0.06[a]	8.0 ± 0.4[a]	2.6 ± 0.7[ab]	2.19 ± 0.07[b]
Chlorella sp.	251 ± 8[b]	< 0.01[a]	26 ± 1[a]	33 ± 1[a]	< 0.05[b]	16 ± 2[a]	31.3 ± 0.9[a]	3.0 ± 0.1[a]	0.02 ± 0.003[a]	0.17 ± 0.05[a]	8.4 ± 0.1[ab]	3 ± 1[b]	2.22 ± 0.05[b]
P. palmata	285 ± 9[ab]	< 0.01[a]	25 ± 1[a]	35 ± 2[a]	< 0.05[b]	15 ± 2[a]	30 ± 1[a]	3.1 ± 0.2[a]	0.02 ± 0.003[a]	0.11 ± 0.06[a]	9.17 ± 0.09[b]	0.5 ± 0.1[a]	2.22 ± 0.06[b]
L. digitata	322 ± 15[ab]	< 0.01[a]	25 ± 1[a]	35.0 ± 0.5[a]	< 0.05[b]	9 ± 2[ab]	31.1 ± 0.6[a]	3.27 ± 0.08[a]	0.04 ± 0.02[a]	0.6 ± 0.6[a]	9.2 ± 0.1[b]	1.7 ± 0.7[ab]	2.17 ± 0.04[b]
A. nodosum	310 ± 21[ab]	< 0.01[a]	27 ± 1[a]	36 ± 1[a]	< 0.05[b]	7 ± 2[b]	29.4 ± 0.6[a]	3.3 ± 0.1[a]	0.02 ± 0.002[a]	0.2 ± 0.1[a]	8.6 ± 0.2[ab]	0.6 ± 0.2[a]	2.1 ± 0.1[ab]

[a] Mean ± standard error (n = 3). Values with the same letters in a column are not significantly different ($p < 0.05$, one way ANOVA).

was also highest under *Chlorella* sp. (172, n = 3), though there was no significant difference between any of the other treatments. The highest grain yield was obtained under *Chlorella* sp. with an average yield of 5 t ha^{-1} and the lowest was observed under *A. nodosum* (4.2 t ha^{-1}). The control treatment had an average grain yield of 4.6 t ha^{-1}. There was no significant difference in yield between any of the treatments. Total N and P measured in the aboveground biomass, which included the wheat shoot and grain (Table 9), showed no significant differences for both macronutrient amounts between any of the algal treatments and control. Despite this, *Chlorella* sp. had the highest amounts of N and P in its aboveground biomass in comparison to all the other treatments and the control.

Specific micronutrients were measured in the wheat grain at harvest, 20 weeks after algal biomass additions (Table 10). There were no significant differences in wheat grain micronutrient concentrations, namely, Zn, Fe, Mn, and Mg between any of the algae treatments. Ca concentrations in wheat grain were similar under all treatments except for control, which was significantly higher ($p < 0.05$) than *Chlorella* sp. B concentrations were highest under *Chlorella* sp. and *P. palmata* in comparison to the control and *A. nodosum* treatments ($p < 0.001$). Se concentrations in wheat grain were found to be significantly higher ($p < 0.0001$) under *Spirulina* treatments in comparison to all the other treatments. Se concentrations were 0 mg kg^{-1} under the algae treatments and control, but was 0.13 mg kg^{-1} under *Spirulina*.

4. Discussion

The present study compared the effect of five chemically different algae species on restoring soil physicochemical properties and improving crop yield. Prior to the start of the experiment, the concentrations of total C, N, P, and micronutrients in the algae biomass were quantified, based on the hypothesis that their initial nutrient composition would have an impact on nutrient concentrations in the soil.

Net mineralisation and immobilisation of nutrients are dependent on whether the C:N ratio of the substrate (biomass) is above or below the critical value of *c.* 20, where ratios > 20 indicate net immobilisation and ratios < 20 favour net mineralisation (White, 2006). The C:N ratios of the algal biomass (Table 2) show that *Spirulina*, *Chlorella* sp., *P. palmata* and *L. digitata* all have C:N ratios < 20. *A. nodosum* was the only algae to have C:N ratio above 20. C:N ratios of both freshwater and marine algae are reflective of their individual growth conditions and indicate whether they have been grown in nutrient replete or deficient conditions (Geider & La Roche, 2002). The C:N:P ratio of marine algae is tightly linked to the inorganic pool of C, N and P in the ocean interior (i.e. the Redfield ratio) and this ratio may differ within and among taxa in response to variation in the abiotic environment (Yvon-Durocher et al., 2015). The composition of microalgae typically found in freshwater lakes is also highly variable: the ratio of C:N:P varies with the ratio supplied in the water as well as the pH of the water (Krebs, 2008).

In the greenhouse study, *Chlorella* sp. and *Spirulina* were shown to increase total soil N. Evidence of algae increasing soil N is not uncommon: certain cyanobacteria e.g. *Nostoc* and *Anabaena* have been recognised as significant contributors to soil N through their atmospheric N-fixing abilities (Akhter et al., 2002). This property has been predominantly observed in cyanobacteria species and in experiments carried out using *Nostoc muscorum*, total N has been reported to increase by 111–120% (Rogers & Burns, 1994), under inoculum rates ranging from equivalents of 2 kg ha^{-1} to 5 kg ha^{-1}. Akhter et al. (Akhter et al., 2002) also reported an increase in total N in rice soil inoculated with 2 g of a mixture of five cyanobacterial species, and an increase of 50% total N after inoculation with live *Nostoc* cells was reported by Maqubela et al. (Maqubela et al., 2009). Our results show that the green alga *Chlorella* sp. was just as effective, highlighting its possible use in increasing soil total N concentrations, particularly in UK agricultural soils.

Chlorella sp. and *P. palmata* also significantly increased soil total C. Algae are known to help in the accumulation of C in the soil, for example, *Nostoc* strains added to soil at a rate of 0.02 g cm^{-2}, were shown to increase soil organic C after 90 days in an experiment conducted by Obana et al. (Obana et al., 2007). In another previous experiment conducted by Rogers and Burns (Rogers & Burns, 1994), smaller doses of live *Nostoc muscorum* (4.04×10^5 equivalent to 5 kg ha^{-1} cell dry weight) recorded an increase of 50–63% of total C in a poorly structured silt loam soil. This is a much larger increase in comparison to the increase observed in the present experiment under high application rates of *Chlorella* sp. treatment, which increased by 17% under highest application rates (4 g kg^{-1}). Nevertheless, the benefits of adding *Chlorella* sp. to improve soil C concentrations are evident. This was also expected as *Chlorella* sp. had the second highest concentration of C stored in its biomass.

Total P in soil decreased significantly under both *Chlorella* sp. and *P. palmata* treatments in the greenhouse. There was no significant difference between any of the algae treatments and their impact on soil total P concentrations in comparison to the control, suggesting that the soil was already rich in phosphorus (0.24 g kg^{-1} ± 0.08) and the algae treatments had little impact on altering the natural concentrations in the soil. Due to factors such as adsorption, precipitation or conversion to the organic form (Moonrungsee et al., 2015), only a very small portion of total P is available to plants in the form of orthophosphate or easily mineralized organic P. The addition of algal biomass was expected to increase mineralisation of organic P by soil microbes thereby releasing orthophosphate anions (HPO_4^{2} and $H_2PO_4^{-}$) into the soil solution (Richardson et al., 2009). Medium and high application rates of *Chlorella* sp. and *Spirulina* significantly increased soil available P concentrations in the greenhouse compared to the control. Under field conditions however, soil available P concentrations under the algae treatments did not change significantly at 2, 8 or 20 weeks after algal addition. The significance of their impact in the field could have been lost as a result of the larger variability in soil conditions. Results from a flask study conducted by Mulbry et al. (Mulbry et al., 2005) showed increasing available P in soils with increasing algal additions, with responses being affected by existing soil P concentrations. In the present study, available P concentrations had declined by the end of the field experiment, most likely due to depletion by the crop growth. The control soils had higher P concentrations compared to the algal treatments, which was possibly due to microbial immobilisation as a result of the carbon supplied by the algal necromass.

Soil NH_4^+-N concentrations in the greenhouse study increased under *Chlorella* sp., *P. palmata* and *L. digitata*. Similar results were observed in the field, where high NH_4^+-N concentrations were also recorded under both *Chlorella* sp. and *P. palmata*, suggesting mineralisation of algal necromass N. *Spirulina*, along with *Chlorella* sp. and *P. palmata* also increased soil NO_3^--N concentrations in the greenhouse and field experiment. Soil NO_3^--N decreased from 2 to 8 weeks most likely due to plant uptake. Concentrations increased again by week 20 possibly as a result of both nitrification, and due to the crop N demand decreasing as it reached maturity and stopped growing, leaving higher concentrations in the soil as residual nitrogen. With the addition of nitrogen-rich organic matter, soil NO_3^--N concentrations would be expected to increase. Most studies have focused on the impact of algal amendments on soil total nitrogen concentrations, while only few have looked at available N, particularly NO_3^--N, which is the preferred form of N taken up by crops like wheat. One of these studies was conducted by Possinger and Amador (Possinger & Amador, 2016), where the addition of a seaweed mixture including *A. nodosum* and *L. digitata*, among others, elicited a decrease in soil NO_3^- concentrations over time. The study concluded that the addition of seaweed did not improve NO_3^- concentrations. This contrasts our findings where algae, particularly *Spirulina*, caused an increase in soil NO_3^--N concentrations both in the field and greenhouse study.

Algal biomass had little effect on soil aggregate stability in the

greenhouse experiment after 13 weeks. In the field soil, % dry weight of soil macro aggregates (250– > 2000 μm) increased under *L. digitata* after 2 weeks, however this was not significantly different to the control. Maqubela et al. (Maqubela et al., 2009) reported an increase in soil macroaggregates when live *Nostoc* was added to non-cropped soils compared to cropped soils, implying that the addition of dried biomass, as undertaken in this study, has minimal impact on soil aggregate stability. Other studies support this, where improvements in soil aggregate stability were observed when adding live cultures, producing a subsequent enmeshing effect of the growing inoculated cyanobacterium filaments and the gluing effect of excreted polysaccharides (Maqubela et al., 2009).

In terms of micronutrients, Se was only found in wheat grain grown under *Spirulina* treatment. Se is a micronutrient normally deficient in wheat crops. UK grown wheat consumption has increased, but Se concentrations remain low, or exist in forms not chemically available to the crop in the UK (Hart et al., 2011). Increased Se concentrations in wheat will be beneficial for human nutrition and health since it is often deficient in UK diets (Hart et al., 2011). Other studies have shown the capacity of *Spirulina* to take up micronutrients including Se (Wuang et al., 2016), thus highlighting its potential as a source of Se for wheat crop.

It is clear that the addition of algal biomass, particularly at higher application rates of 4 g kg^{-1} have significant effects on soil total C and N as well as available P, NH_4^+-N and NO_3^--N. The algal amendments did show consistently significant improvements on soil NH_4^+-N in the greenhouse and field. However, in terms of available P, where improvements were seen only in the greenhouse and not in the field, a higher application rate may have altered this. Conversely, the purpose of soil conditioning is not only to improve the characteristics of the soil, but should also be translatable to crop growth and yield. *Chlorella* sp. was the only algal species to significantly increase pea yield in comparison to the control in the greenhouse, although in the field, the effects on wheat were not as significant. *A. nodosum* had no significant impact on any of the soil characteristics. This was most likely because it was applied as a dried amendment and had low N and P concentrations in its biomass. Materials with high C:N (low N) ratios tend to decompose more slowly as the N is less readily available to plants due to it being immobilized in microbial biomass. Previous studies on the impacts of *A. nodosum* on plants and soil typically use an extract rather than the dried biomass, possibly allowing for the nutrients to be released and taken up more rapidly by the plants.

5. Conclusion

There is a growing interest in the use of algal-based biofertilisers to increase crop productivity. Capturing nutrient run off using algae could also counter eutrophication of natural water bodies (Michalak et al., 2016), a more sustainable method for “closed-loop” nutrient cycling. Algae have previously been shown to improve soil characteristics such as C content and aggregate stability, and cyanobacteria (e.g. *Nostoc muscorum*) have been shown to improve soil N in desert environments as well as rice paddy fields through their N-fixing abilities. In the present study, it was shown that the algae had a significant impact on agricultural soils, through the addition of soil nutrients. However they did not show any significant improvement on soil aggregate stability under the conditions tested and it is suggested that the addition of live algal biomass needs to be investigated for effects on soil aggregation. *Chlorella* sp. and *Spirulina* had immediate impact on inorganic N, with *Chlorella* sp. increasing NH_4^+-N concentrations and *Spirulina* increasing NO_3^--N; *P. palmata* was also shown to influence soil available N concentrations at a later stage during crop growth. The outcome of both experiments highlight the importance of chemical composition of algae in supplying plant available nutrients, providing insights into selecting appropriate species for arable soil nutrient management strategies. Overall, the results show the benefits and potential of using algae as a sustainable organic fertiliser with the aim of increasing soil total N content and in particular improving N mineralisation rates in the soil.

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