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The productivity effects of macroalgal biochar from *Ulva* (Linnaeus) bloom species on *Arabidopsis thaliana* (Linnaeus) seedlings

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ABSTRACT

Intensive agricultural practices and poor management of organic waste have adverse effects on aquatic ecosystems, where excessive macroalgal proliferation can occur to form ‘green tides’, with negative environmental, ecological and socio-economic impacts. One novel method for converting a problematic material into a valuable resource is to use excess algal biomass as a feedstock for biochar production. With a high elemental composition, such a resource might be suitable to redress soil deficiencies and to ameliorate soil fertility.

Green macroalgae from the *Ulva* genus, in bladed (predominantly *U. rigida*), tubular (predominantly *U. prolifera*) and mixed morphological (*U. rigida* and *U. prolifera*) phenotypes, were used to produce biochars. A pot trial within a controlled-environment chamber was carried out to determine the effects of amending high- and low-fertilizer compost with algal biochars (applied at 0, 0.5, 1, 2 and 5% w/w) on the growth rate of *Arabidopsis thaliana*. A commercial wood-based biochar was used under similar treatments as a control. Weekly imaging and final harvest weights provided additional growth data; composition data including ultimate and proximate analyses, pH, Brunauer–Emmett–Teller (BET) surface area and hydrolysis of the dried macroalgae and algal biochars were also conducted.

Significant enhanced growth in seedlings grown with biochar amendment were not observed in high- or low-fertilizer compost, and the addition of algal biochars at 5% w/w to high-fertilizer soil significantly reduced plant growth. Elemental analysis revealed that the algal biochars contained high quantities of alkaline elements including sodium. It was hypothesised that salinity was the primary factor affecting plant growth at higher biochar application rates, despite the algae being sourced from an estuarine environment. Biochar provenance and composition is highly significant: using the catch-all term ‘biochar’ ignores both the range of materials and composition that could be used to create it and its subsequent impact within the soil.

HIGHLIGHTS

- First plant trial using biochar predominantly from *Ulva* species.
- Negative impact seen with 5% algal biochar on plant growth.
- High sodium concentrations putatively identified as reduced plant growth cause.

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Introduction

Intensified agricultural production has accelerated the degradation of natural systems globally, including soils, the climate and aquatic habitats (Tilman *et al.*, 2002). Meanwhile, demand for food, feed and fibre continues to increase (Conijn *et al.*, 2018). Soil health is critical to crop production and environmental sustainability through its provision of ecosystem services (Blum, 2005), including support of plant production and regulation of climate (Lehmann *et al.*, 2020). Soil fertility describes the specific role of soils in supporting crop production (Bünemann *et al.*, 2018) and can be determined by measuring primary productivity (Bouma *et al.*, 2016).

In some cases, the incorporation of solid charred biomass, ‘biochar’, into soils has been shown to enhance soil fertility and carbon stocks over long

periods (Jeffery *et al.*, 2011; Cowie *et al.*, 2015). The feedstock and conditions of pyrolysis affect the properties of biochar and its effects on the physical, chemical and biological characteristics of soils, which determine fertility (Brown *et al.*, 2015; DeLuca *et al.*, 2015). Enhanced productivity has been attributed to the direct addition of plant nutrients, enhancement of soil properties such as water-holding capacity and cation exchange capacity (CEC), reduction of acidity and salinity through liming, and elevated microbial functioning via the provision of soil with an amenable structure and pH (Jeffery *et al.*, 2015).

However, yield reductions have also been observed, probably resulting from high sulphur content, high salinity, aluminium or manganese toxicity, or from uncharged activated carbon reducing nutrient availability, especially in fertile soils (Rees &

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Sidrak, 1956; Elseewi *et al.*, 1978; Deenik *et al.*, 2010). Labile carbon on the surface of biochar particles can adsorb nutrients upon contact, effectively reducing fertility by reducing the availability of nitrogen (N) and phosphorus (P) to plants (Sarkhot *et al.*, 2012; Mukherjee & Lal, 2014).

During pyrolysis, 70–90% of N present in any feedstock is volatilized; significant amounts remain in solid char, although most of this is in a form (heterocyclic) not readily released to the soil N pool (Rajkovich *et al.*, 2012). However, nutrient-rich biochars can both directly add ammonium (NH_4^+) salts to soils and raise the levels of N in soils by reducing losses via nitrous oxide (N_2O) emissions, ammonia (NH_3) volatilization and leaching (Taghizadeh-Toosi *et al.*, 2012). Biochar can also increase N-fixation rates and plant nutrition by enhancing the abundance and diversity of nutrient-cycling microorganisms and mycorrhizal fungi (Rondon *et al.*, 2007).

Potassium (K) and P are also added to the soil nutrient pool by biochars, mainly in amorphous PO_4^{3-} and K-containing salt forms (Camps-Arbestain *et al.*, 2015). P and K become available to plants after the weathering of minerals (Yamato *et al.*, 2006), but can also be rapidly sequestered in inorganic forms or taken up by soil microorganisms (Thies *et al.*, 2015). Most biochars have a neutral to basic pH so can ameliorate acidic soils, boosting productivity in these situations (Singh *et al.*, 2010; Jeffery *et al.*, 2017). Biochar's liming effect reduces the amount of iron (Fe) and aluminium (Al) in soil solution, releasing more P from its bonds with these cations (Cui *et al.*, 2011). However, a liming effect can be undesirable in alkaline soils, as Ca-P bonding increases, reducing P availability and the yields of acid-soil preferring crops (Jeffery *et al.*, 2015). Increases in CEC due to the biochar presence also allow better retention of nutrient ions such as P and K by the adsorption of these onto the surface of biochar particles (Slavich *et al.*, 2013). Biochar surface area is linked to its capacity to adsorb small molecules such as gases and solvents. Furthermore, biochar can increase the availability of micronutrients including calcium (Ca), magnesium (Mg), boron (B) and molybdenum (Mo) by altering pH levels (Rondon *et al.*, 2007; Major *et al.*, 2010) and it can reduce the presence of certain plant growth inhibitors by adsorbing them (Sheldrake *et al.*, 1978).

A meta-analysis by Jeffery *et al.* (2011) on 60 studies considering the effects of biochar application on crop yield showed that productivity effects were mixed in direction and magnitude, partially as a result of variability between the studies e.g. soil type, climate, crop type, biochar type (feedstock and pyrolysis conditions), trial type (field/pot), method of application (e.g. spread on fields or ploughed in) and application rate, which ranged from 1 t ha^{-1} to over 150 t ha^{-1} (approximately

equivalent to 0.05–7.7% w/w). On average, biochar increased crop productivity by 10%, with liming and enhancement of water-holding capacity identified as the major mechanisms for this increase.

Macroalgae are seemingly attractive as a biochar feedstock because they contain a high density of plant nutrients and because their excessive accumulation in eutrophic waterways incurs ecological and socio-economic costs (Smetacek & Zingone, 2013; Suutari *et al.*, 2015). Challenges associated with using seaweed include energy-intensive drying, seasonality of growth, and high temporal and spatial variability of elemental composition (Adams *et al.*, 2020). Algae with high elemental content of certain metals may also be unsuitable as biochar feedstock because these elements can accumulate in soils, generating levels toxic or detrimental to healthy crop growth. In comparison to biochars made from lignocellulosic feedstocks, algal biochars have a lower carbon content, surface area (SA) and cation exchange capacity but higher pH and nutrient content, including N and extractable inorganic nutrients such as P, K, Ca and Mg (Bird *et al.*, 2011; Yu *et al.*, 2017).

To date, there are few studies on the effects of amending soils with algal biochar on plant productivity (e.g. Bird *et al.*, 2012; Roberts *et al.*, 2015; Roberts & de Nys, 2016; Adams *et al.*, 2020; Ullah *et al.*, 2020). Biochar made from ensiled macroalgal brown kelps (*Laminaria* spp. and *Saccharina latissima*) had universally negative effects on the germination and health of lettuce plants (*Lactuca sativa*) and the growth of ryegrass *Lolium temulentum* L. seedlings (Adams *et al.*, 2020). The authors hypothesized that the biochar released compounds that collectively inhibited plant growth, although all elements analysed were below toxic levels. For green seaweeds, though, studies were more positive, for example in a study by Bird *et al.* (2012) who studied the effects of two green macroalgal biochars (produced mainly from *Cladophora* spp.) on the growth of sorghum (*Sorghum bicolor*). In this study algal biochars were applied at 0.35% w/w to two different soils, the first infertile and the second rich in organic carbon and nutrients. In low-fertility soil, observed growth was 15 and 32 times higher over the course of the trial with biochar compared with no-biochar controls, without and with additional inorganic fertilizer, respectively. In high-fertility soil, productivity improvements were much less pronounced but still significant. In both soil types, feedstock was found to have a stronger effect on yield than the addition of fertilizer, which was applied at a rate of 50 g m^{-2} . Bird *et al.* (2012) attributed the dramatic effect of biochar on growth rates in low-fertility soil to its direct nutrient contribution and the resulting reduction in root penetration resistance, provision of soil habitat and,

to a lesser degree, provision of labile carbon. These findings corroborate other studies which indicate that fertility benefits of biochar amendment are highly dependent on baseline soil health (Agegnehu *et al.*, 2017).

A study of the effects of pyrolysis conditions of green macroalgae *U. ohnoi* on the properties of its biochar found that lower pyrolysis temperatures generated biochar with greater capacity to ameliorate soil fertility/yields of radish *Raphanus sativus* (Roberts & de Nys, 2016). At higher temperatures, biochars were found to have higher salt and lower N content. A pyrolysis temperature of < 450°C produced char giving higher productivity than at higher temperatures, with 300°C optimal for carbon sequestration and soil improvement (Roberts & de Nys, 2016).

Others have investigated *Ulva* spp. char in its production or char quality characterization (for example Toth *et al.*, 2020; Wang *et al.*, 2020; Zhang *et al.*, 2021) but the majority of academic applications of *Ulva* spp.-derived biochar has been for the removal of specific dyes (Gokulan *et al.*, 2019; Priya *et al.*, 2020; Hanumanthu *et al.*, 2021; Kumar *et al.*, 2021) and other compounds, including from the soil (Keerthanam *et al.*, 2021).

The aim of this study was to explore further the potential of green macroalgae-derived, low-temperature biochar, both as a soil amendment and to determine the physico-chemical properties of the biochars generated. This was achieved by using green macroalgae from the *Ulva* genus green macroalgae, known to cause blooms in eutrophic waters, to generate low-temperature biochar. The chars were then used at a range of concentrations in compost mixes to assess how they affected the growth rate of the cress *A. thaliana* in a controlled-environment pot trial and analysed for composition data and properties including ultimate and proximate analysis, pH, Brunauer–Emmett–Teller (BET) surface area and hydrolysis.

Materials and Methods

Collection and identification of algae

Bulk collection (~2 kg per phenotype) of green macroalgae in bladed, tubular and mixed morphology (bladed and tubular mix at ~1:1) phenotypes were collected on 24 August 2020 from the intertidal zone of the upper Milford Haven estuary, Wales, UK at OS reference SN 01767 07627. Algae were washed thoroughly in a freshwater stream to remove mud and animals before being transported to Aberystwyth. The material was subsampled for identification with these samples stored at -20°C and the bulk remainder dried at 60°C in a Unitherm drying oven for 72 h until fully dry. This dry material was then stored in zip-lock bags under dark, ambient conditions prior to

pyrolysis. Macroalgae were identified as *U. rigida* (bladed morphology) and *U. prolifera* (tubular morphology) by microscopy (n = 9 samples; randomly selected from 45 samples for identification).

Production of biochars

Dry algae were pyrolysed in 100–200 g batches within a Eurotherm 2132 tube furnace (Carbolite Gero, Sheffield, UK) under nitrogen. Heating rate was 25°C min⁻¹ up to 350°C for the outer tube; the temperature was held for a further 20 min until internal temperature reached 350°C then held for 1 h. The tube was left to naturally cool after 1 h, the sample remaining under nitrogen throughout until the temperature was < 100°C. Samples were removed cold and stored in zip-lock bags in dark ambient conditions prior to processing. The resulting biochars were milled separately using a cutting mill (Retsch SM100, Haan, Germany), producing particles of < 1 mm diameter. Approximately half of each biochar was milled further using a handheld analytical mill (IKA A11 basic, Staufen, Germany) and passed through a 350 µm sieve. A commercial biochar (SoilFixer Biochar, SoilFixer Ltd, Royal Wootton Bassett, UK), produced from mixed European hardwoods (beech, oak, hornbeam) using a ring kiln at 350–550°C for 4–8 h, was similarly milled to produce material of < 1 mm and < 350 µm particle sizes.

Characterization of algae and biochars

Milled dry algae and biochars, each from bladed, tubular and mixed morphologies, underwent testing for elements and properties associated with soil fertility. Elemental composition and conductivity analyses were carried out by NRM Laboratories (Bracknell, Berkshire, UK) using standard test methods. Total nitrogen (N) was determined by total combustion in an oxygen-enriched atmosphere (Dumas technique) and combustion analyser (AOAC, 1990: JAS-373; JAS-476; JAS-656). Ammoniacal and nitrate-N were determined by methods involving reduction and spectroscopy (EPA, 1984: JAS-082); electrical conductivity (EC) was determined by EC meter in aqueous solution (JAS-058); mercury was determined by reduction to elemental vapour then detection by fluorescence following excitation (JAS-454); other elements were determined by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

Brunauer–Emmett–Teller (BET) surface area (SA) was determined by MCA (Meldreth, Cambridgeshire,

UK) for bladed, tubular and SoilFixer biochars milled to < 1 mm and < 350 μm powders. Processing conditions were as follows: sample mass 0.5 g; analysis adsorptive N_2 ; analysis bath temperature 77.3 K; equilibrium interval 10s. Additionally, samples of each biochar type underwent analytical hydropyrolysis (HyPy), a technique for determining the thermally stable black carbon and thermally labile fractions in biochar, using methodologies as described by Meredith *et al.* (2012). Bulk densities of the biochars (milled to < 1 mm) were also determined by weighing triplicate samples of 100 ml quantities of biochar.

Compost pH was determined for 0% and 5% char blends in triplicate, with each blend or compost only (0% char) weighing 10.0 g. Mimicking the plant trials, tap water was used; 13.6 ml were added to each compost blend in a 50 ml Greiner tube. Tubes were thoroughly vortexed horizontally, then shaken at 200 rpm at 23°C for 2 h. Samples were vortex-mixed as above and the pH of each sample determined.

Char pH was determined in triplicate. For each sample, 3.0 g char was added to a 50 ml Greiner tube; with 6.0 ml tap water to give equal water: solid ratios as in the plant trials. Samples were vortexed, incubated and analysed as above.

Pot preparation

Seedling compost mixtures were prepared as a blend of peat-based Bulrush compost (Magherafelt, County Londonderry, UK) with 10% w/w horticultural silver sand (RHS, London, UK). A compound fertilizer YaraMila Grower (Grimsby, Lincolnshire, UK) containing 15:9:20 (N:P:K) + 9.5% SO_3 + 1.8% MgO was added at 1.5 g l^{-1} or 0.25 g l^{-1} to give 'high' and 'low' fertilizer composts respectively.

The 64 pot treatments, each prepared in triplicate, consisted of the following: four different biochar sources (bladed algae, tubular algae, mixed morphology algae and SoilFixer control), at two particle sizes by milling (coarse, < 1 mm and fine, < 350 μm). Each of these eight biochar preparations were added to two compost mixes (high- and low-fertilizer based on YaraMila Grower addition) to produce 0.5%, 1%, 2% and 5% w/w biochar compost mixtures. Nine replicate control treatments of 0% biochar in both high- and low-fertilizer compost mixes were also produced. Each pot contained 40 g of compost-biochar blend in total.

Pots were labelled, watered and then pricked out with *A. thaliana* seedlings from inbred accession line Col-1 sown 3 weeks previously in a 2:1:1 mixture of John Innes seed compost (Westland, Huntingdon,

Cambridgeshire, UK), horticultural grit sand (RHS) and medium perlite (Sinclair, Ellesmere Port, Cheshire, UK), respectively.

Trial conditions and data collection

Immediately after pricking out, all pots were randomly positioned in trays and placed in a Fitotron controlled-environment cabinet (Sanyo Gallenkamp; now Weiss Technik UK) with an 8-h day with white LED lights (280 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ irradiance at pot height); the temperature fluctuated between 20°C and 22°C and humidity was unregulated. Pots were photographed from above at a fixed height using a Panasonic DMC-FZ48 camera after set-up (day 0) and subsequently on days 7, 14 and 21. Pots were covered for the first 2 days to mitigate seedling stress and the seedlings were watered approximately daily from below with additional watering from above as required. The growth trial was terminated on day 21. After being photographed, the plants were pinched off at ground level, cleaned, weighed, dried for 24 h at 50°C and re-weighed to gain wet and dry biomass weights.

Data analysis

Leaf surface area (SA) was determined from aerial photographs taken at weekly intervals. Images were processed in batches using Adobe Photoshop (San Jose, California, USA), Adobe Bridge and ImageJ software (Schneider *et al.*, 2012). Initial weights were estimated by multiplying initial SA by the mean dry weight per unit area (g mm^{-2}) of all plants at day 21. Two overall growth rates (GRs), based on final dry weight and SA (mg DW day^{-1} and $\text{mm}^2 \text{ day}^{-1}$) were calculated for each pot, averaged over the trial period. Weight-GR and SA-GR were found to correlate well ($R = 0.89$, $p < 0.001$).

Values associated with plants that had died were removed ($n = 4$), as these plants stopped growing early on in the trial period so had a disproportionate effect on treatment means. Growth rates were also square-root transformed to meet the assumptions of ANOVA. A four-way factorial ANOVA with a crossed design was carried out in R Studio (R Core Team, 2020), with biochar source and biochar size nested as fixed factors within biochar fraction to account for non-independence of factors (Zobel *et al.*, 1988; Bird *et al.*, 2012). Effect sizes associated with different factors were calculated based on 'generalized eta

squared' (Lakens, 2013). Significant main effects and interactions between factors were investigated further by post hoc testing between paired treatments with a Bonferroni adjustment. Foliar surface area after 7 days was similarly compared between selected treatments.

R was also used for the following analyses: Linear regression analysis to determine the correlation between specific biochar properties and plant growth rates. Pearson's product-moment correlation analysis was carried out on selected elements (Ca and Na) with elemental abundance values adjusted proportionately to biochar application rate.

Results

Characterization of algae and biochars

Composition analysis, detailed in Table 1, was conducted on the different algal phenotypic collections (bladed *U. rigida*, tubular *U. prolifera* and an ~50:50 mix of these phenotypes) in both their oven-dried forms and as biochar; and on commercially available hardwood-derived SoilFixer biochar. Results given were focused on elements known to affect plant productivity, nitrogenous molecules and selected heavy metals. For the chars, additional information was given regarding density, conductivity, C and C:N ratios, and char fractionation by hydrolysis to thermally stable and labile fractions.

All algal samples contained high amounts of N, P and K. The dried bladed algal sample contained 14% more total N per unit of mass ($33 \text{ g kg}^{-1} \text{ DW}$) than the tubular or mixed algal samples. Although bladed algae also contained the most ammoniacal N ($1.62 \text{ g kg}^{-1} \text{ DW}$), tubular algae contained the most nitrate N ($8.66 \text{ g kg}^{-1} \text{ DW}$). The mixed algal sample contained the least of both N types, which was unexpected as the mixed sample was predicted to exhibit an average of the bladed and tubular properties. The bladed sample also contained the most P ($3.05 \text{ g kg}^{-1} \text{ DW}$) and K ($30.5 \text{ g kg}^{-1} \text{ DW}$), although all samples had very similar amounts of K. Compared with the YaraMila fertilizer additive used to raise the compost fertility, the N is higher (17.2–25.7% versus 15% in nitrate and ammoniacal forms), P lower but comparative (2.09–3.08% versus 3.9% in P form) and K higher but comparative (18.9–30.5% versus 16.6% in K form). The N, P and K in the char may be in different forms to that in the fertilizer but the quantities present mean that these elements are not a source of deficiency in the chars.

Similar analyses conducted on the four biochars revealed substantial chemical differences between the algal biochars and the wood char, and some minor variation between the algal chars. As expected, the

algal biochars contained much more of the primary plant nutrients; on average, algal biochars had 67% more total N, 79% more P and 300% more K than the wood biochar. Like the dry algae, the bladed algal biochar had the most N, P and K out of the algal chars. On average, algal biochars contained 31% less total N, 8.5% less P and 15% less K than dry algae, indicating that the high concentrations of these elements, especially P, were largely conserved during pyrolysis.

Ca levels differed dramatically between algal samples. Tubular algae contained ~3 times as much Ca than bladed algae and 1.5 times as much as mixed algae. High levels and the variation between species are at least partly attributed to the presence of *Peringia ulvae* (laver spire shell or mudshell) which covered the algae (Fig. 1). The shell of such snails is mainly composed of calcium carbonate (Heller, 2015) and would artificially elevate Ca concentrations in the sample compared with seaweed alone but no large-scale processing would be capable of removing them all prior to charring the algae. Washing the biomass prior to drying removed some of the *Peringia*, but due to the three-dimensional overlap of the tubular algae fewer shells were removed from these than from the bladed algae. Na levels were relatively high in all algal samples compared with the SoilFixer control, particularly in the samples of tubular and mixed algae. Mg levels were similar between algal samples, with bladed algae having slightly more than the other samples. In contrast, the tubular sample had the most copper (Cu) and more zinc (Zn) than the bladed sample but less than the mixed sample. All algal samples contained much more Ca, Na, Mg and other plant micronutrients than would be expected in the wood control, which can partially be attributed to wood's higher carbon content.

Correspondingly, algal biochars contained much greater relative amounts of Ca, Na and Mg, similar amounts of Cu and lower amounts of Zn than the wood-derived SoilFixer biochar did. The charring process increased the Ca content of the chars $\times 3.2$ – 5.8 , again with this increase related to the retention of some *P. ulvae* present amongst the material. Biochar made from tubular algae, like the feedstock, had the highest levels of Ca at 198 g kg^{-1} . Other macroelements were present at approximately the same concentrations in the char as in the dried material. The amounts of the heavy metals lead (Pb) and cadmium (Cd) in the dry algae were within the expected range based on previous analyses (Bird *et al.*, 2012). Mercury (Hg) levels were less than 0.1 mg kg^{-1} in all samples. There were consistently low amounts of elements potentially toxic to plants, including Pb, Cd, Hg, nickel (Ni) and chromium (Cr) in algal and SoilFixer biochars.

Brunauer–Emmett–Teller (BET) surface area analysis revealed that the surface area of biochars

Table 1. Elemental composition and physical and chemical characteristics of dried *Ulva* algae and wood; and their subsequent biochars.

Analysis of initial feedstocks and reference wood													
Feedstock	P g kg ⁻¹	K g kg ⁻¹	Mg g kg ⁻¹	Ca g kg ⁻¹	Na g kg ⁻¹	Zn mg kg ⁻¹	Pb mg kg ⁻¹	Cd mg kg ⁻¹	Ni mg kg ⁻¹	Cr mg kg ⁻¹	Cu mg kg ⁻¹	Hg mg kg ⁻¹	
Bladed	3.05	30.5	29.0	19.1	18.2	41.8	10.5	0.10	9.15	10	12.1	< 0.1	
Tubular	2.44	29.6	26.3	62.0	23.1	49.1	12.6	0.12	12.3	16.3	16.6	< 0.1	
Mixed	2.55	29.4	24.6	41.6	24.3	57.2	14.4	0.14	14.2	18.5	16.6	< 0.1	
Wood	0.10	0.84	0.22	1.18	0.03	7.1	0.38	0.01	0.56	1.3	1.5	< 0.1	
Feedstock	Total N g kg ⁻¹	NO ₃ -N g kg ⁻¹	NH ₄ -N g kg ⁻¹										
Bladed	33.0	0.64	1.62										
Tubular	27.3	0.87	1.24										
Mixed	27.3	0.54	0.97										
Wood	2.80	-	-										
Analysis of generated and commercial biochars													
Feedstock	P g kg ⁻¹	K g kg ⁻¹	Mg g kg ⁻¹	Ca g kg ⁻¹	Na g kg ⁻¹	Zn mg kg ⁻¹	Pb mg kg ⁻¹	Cd mg kg ⁻¹	Ni mg kg ⁻¹	Cr mg kg ⁻¹	Cu mg kg ⁻¹	Hg mg kg ⁻¹	
Bladed	3.08	30.5	33.1	110	19.7	55.3	10.9	0.12	18.0	25.1	18.3	< 0.1	
Tubular	2.14	26.3	22.0	198	24.8	51.3	12.3	0.12	15.7	18.8	18.1	< 0.1	
Mixed	2.09	18.9	19.2	138	21.2	62.9	14.1	0.16	15.4	17.2	20.2	< 0.1	
SoilFixer	1.72	10.0	4.36	31.7	5.76	86.5	12.1	0.41	14.3	25.6	15.1	< 0.1	
Feed- stock	Bulk density g cm ⁻³	BET surface area <1 mm part. M ⁻² g ⁻¹	BET surface area <0.35 mm part. M ⁻² g ⁻¹	Cond. mS cm ⁻¹	Tot. N g kg ⁻¹	Mass loss %	C%	C:N	SPAC%				
Bladed	0.45	0.95	1.07	30.3	25.7	33.7	21.6	8.4	2.7				
Tubular	0.60	1.98	2.12	34.0	17.2	25.0	17.1	9.9	7.2				
Mixed	0.60	-	-	27.8	17.9	22.3	15.5	8.7	20.5				
SoilFixer	0.42	5.96	6.76	4.6	10.3	-	50.8	49.3	81.4				

NO₃-N = nitrate N; NH₄-N = ammoniacal N; - = not measured; bladed = *Ulva rigida*; tubular = *U. prolifera*; mixed = mixed *Ulva* spp. collected from Garron Pill (see text); Wood = uncharred wood composition is for reference only, see Chandrasekaran *et al.* (2012) except for deciduous wood total N from Martin *et al.* (2015) and P from Alakangas (2011). SoilFixer = commercial wood-derived char. Multipoint BET surface area measured by nitrogen absorption (volumetric technique/Micromeritics TriStar 3030 Plus; relative pressure (p/p^o) 0.057–0.200), part. = particle size; Cond. = electrical conductivity; mS = millisiemens; Mass loss = % loss of mass during pyrolysis; SPAC = stable polycyclic aromatic carbon, reported as a percentage of the total carbon.



Fig. 1. Mixed morphology *Ulva* spp. with *Peringia ulvae* present on and within the algal mass. *P. ulvae* may arise in groups (A) or singly (B–D); and can be on the outside (C) or harder to visualize due to being within the algal mass (B and D).

made from tubular algae was approximately double that of bladed algae. Biochars from the SoilFixer control had much higher (~ 6) surface areas than both algal feedstocks. Biochars of finer particle size had slightly higher surface areas than corresponding biochars ground to coarser powders; on average, biochars sieved to < 0.35 mm had 11% greater surface areas than biochars sieved to < 1 mm. The electrical conductivity of the chars showed the opposite alignment, with the similarly conductive algal chars $\times 6$ – $\times 7$ more than the wood-derived char control.

Analytical hydrolysis revealed that the amount and stability of carbon within each of the biochars differed strikingly. The algal biochars contained 57–69% w/w less carbon than the wood biochar and, of this, much less was stable polycyclic aromatic carbon (SPAC) (McBeath *et al.*, 2015). Although bladed algal biochar contained the highest amount of carbon out of the algal biochars, of total carbon only 2.7% was SPAC, compared with 81.4% in the SoilFixer biochar.

In solution, the algal biochar pH values were similar to one another but more alkaline, on average, than the wood biochar (Table 2). However, the algal biochar/compost blends had approximately equal pH to

the wood biochar/compost blends (pH 5.35–5.78), with this presumably due to buffering capacities of the composts, meaning that all seedlings, regardless of char concentration or type, were grown at a similar pH.

Plant growth trials

After transplanting (day 0) and at days 7, 14 and 21, high-resolution photographs taken from a fixed point were made of each seedling. Fig. 2 gives representative images showing seedlings through this period. The surface areas (SA) were determined from each image and combined to determine plant growth rates (GR) for those with algal char, controls (i.e. no char added) or wood char. Seedlings that died within the trial were excluded from this analysis ($n = 4$, three were grown in compost treatments amended with 5% (w/w) tubular algal biochar; the fourth was in a treatment with 1% (w/w) wood biochar). The two biochar particle sizes, < 1 mm and < 350 μm , showed no significant difference in SA or GR ($p < 0.001$) for any of the algal chars or the SoilFixer control; data for these particle sizes have therefore been combined for all analyses below to provide minimum $n = 6$ (excluding dead seedlings). Seedlings noticeably and significantly showed a difference between those grown in high- or low-fertilizer compost so were also presented under the different fertilizer regimes with these values plotted and shown in Fig. 3A. Fig. 3B shows the SA growth rate (SA-GR) for 5% (w/w) algal chars, again with no-char controls and 5% (w/w) wood char as comparators. Here plant growth was not significantly different from the controls under low compost fertilizer or low algal biomass additions; but retardation by high biochar loadings was apparent from week one, with plants growing in treatments with 5% bladed algal biochar and high fertilizer growing 23% less than the high-fertilizer controls. Likewise, treatments with 5% tubular algal biochar were associated with 28% less plant growth in week one than the controls.

Further comparisons can be seen in Fig. 4A showing SA growth rate for the combined algal biochar additions and Fig. 4B showing growth rates calculated

Table 2. pH analysis of compost-biochar and biochar blends.

Low compost fertilizer (0.25% YaraMila Grower)			High compost fertilizer (1.5% YaraMila Grower)			Char only	
Char loading (%w/w)	Char type	pH	Char loading (%w/w)	Char type	pH	Char type	pH
0% w/w	N/A (control)	5.08 ± 0.06	0% w/w	N/A (control)	4.57 ± 0.07	Bladed	8.51 ± 0.03
5% w/w	Bladed	5.55 ± 0.20	5% w/w	Bladed	5.26 ± 0.12	Tubular	8.42 ± 0.02
5% w/w	Tubular	5.65 ± 0.21	5% w/w	Tubular	5.31 ± 0.12	Mix	8.31 ± 0.03
5% w/w	Mix	5.66 ± 0.29	5% w/w	Mix	5.48 ± 0.08	SoilFixer	7.83 ± 0.03
5% w/w	SoilFixer	5.78 ± 0.16	5% w/w	SoilFixer	5.30 ± 0.09		

Bladed = *Ulva rigida*; Tubular = *U. prolifera*; Mixed = mixed *Ulva* spp.; all collected from Garron Pill, Milford Haven estuary, Pembrokeshire, UK; SoilFixer = commercial wood-derived char. $n = 3$ for all pH values with the mean value given; \pm standard deviation.

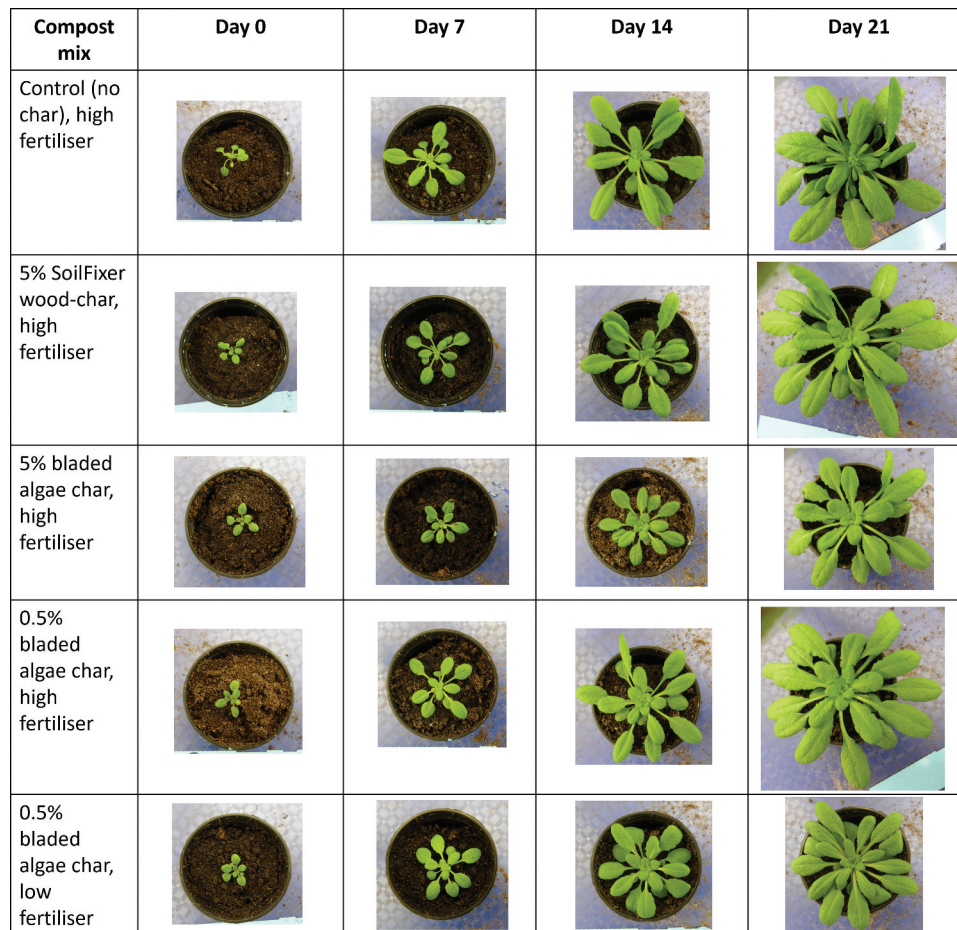


Fig. 2. Representative seedling images grown in varied composts over time. Images shown are the same replicate at days 0, 7, 14 and 21; camera height is fixed throughout. All composts with char contained the larger (< 1 mm) fraction.

using the dry weight yields taken at harvest. Both show that a significant growth reduction occurs in the 5% (w/w) algal char addition, but not when lower additions of biochar (0.5–2% w/w) were added. By calculating growth rates based on the final dry weights (Fig. 4B) a highly significant difference was seen between the 5% (w/w) algal char and the controls in high fertilizer compost ($p < 0.001$) and a significant difference for the 5% (w/w) algal biochar in low fertilizer compost ($p < 0.01$). Amending composts with 5% w/w algal biochar reduced SA-GR by 36% ($p < 0.001$) and weight-GR by 52% ($p < 0.001$) compared with control treatments, on average. None of the wood biochar treatments significantly affected plant growth rates compared with control treatments.

Data were also broken down within algal char type (Fig. 5). When plants were growing in high-fertilizer composts containing 5% (w/w) bladed algal biochar they grew 23% less, on average, than plants in a high-fertilizer compost without char present. Likewise, seedlings grown with a 5% (w/w) tubular algal biochar, high-fertilizer compost were associated with

28% less plant growth. Generally, it was found that algal biochar significantly affected growth rates compared with control treatments in high-fertilizer composts but not in low-fertilizer composts. An exception to this is that seedlings grown in 5% (w/w) mixed algal biochar, low-fertilizer compost were associated with 40% lower SA-GR ($p < 0.05$) and 48% lower dry weight-GR ($p < 0.01$) compared with control treatments. In high-fertilizer compost, the application of 5% w/w algal biochar reduced SA-GR by 43% ($p < 0.001$) and weight-GR by 60% ($p < 0.001$), on average. This observation was mainly attributable to the low growth rates associated with bladed and tubular algal biochars. Tubular algal biochar had the most severe growth-limiting effect of all algal feedstocks: at 5% (w/w) addition in high-fertilizer compost, it reduced SA-GR by 63% ($p < 0.001$) and dry weight-GR by 78% ($p < 0.001$), on average, compared with the control seedlings. Similarly, seedlings grown in 5% (w/w) bladed algal biochar, high-fertilizer compost reduced SA-GR by 58% ($p < 0.001$) and dry weight-GR by 71% ($p < 0.001$).

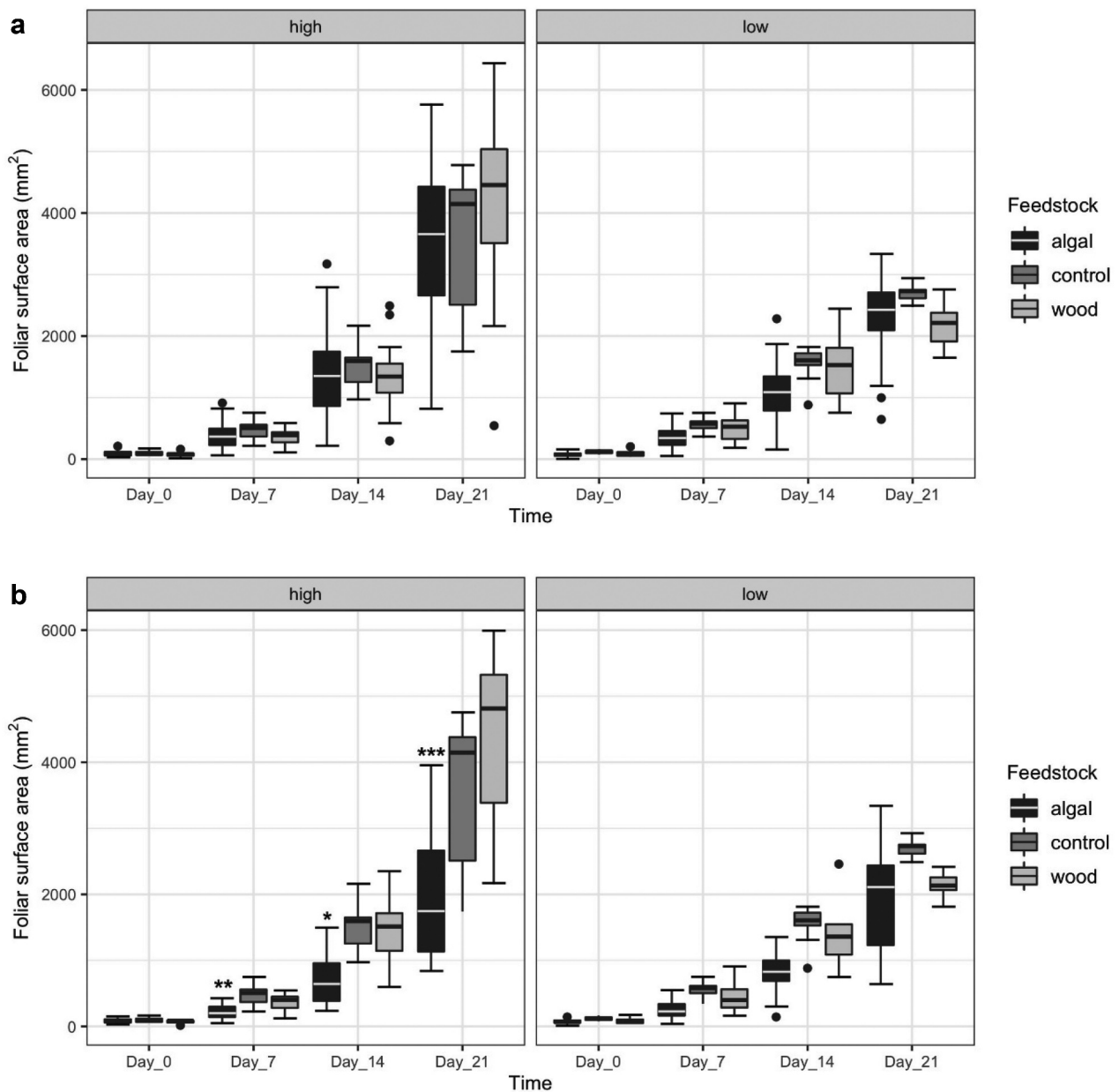


Fig. 3. Leaf surface area (SA) of plants at weekly intervals during the growth trial. A: mean growth rate for all biochar application rates tested; B: only treatments with 5% w/w biochar application (plus controls). Left, high fertilizer; right, low fertilizer. Boxplots show median, interquartile range (IQR), furthest value from median within $1.5 \times$ IQR (whiskers) and outlier values (dots). Shading shows biochar feedstock; algal includes bladed, tubular and mixed. Significant differences between treatments and controls, based on Bonferroni-adjusted pairwise comparisons of transformed data, are illustrated with asterisks (* = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.001$).

Seedling surface area (SA) and dry weight data were statistically analysed with the treatments that differed significantly from control groups presented in Table 3. The analysis identified a significant three-way interaction effect on SA- and dry weight-based growth rates between compost fertilizer level, biochar application rate and char feedstock (SA-GR: $F_{(3, 187)} = 4.03$, $p < 0.01$; dry weight-GR: $F_{(3, 187)} = 4.18$, $p < 0.01$). Additionally, there were significant two-way interactions between compost fertilizer level and biochar application rate (SA-GR: $F_{(1, 187)} = 5.04$, $p < 0.05$; weight-GR: $F_{(1, 187)} = 8.98$, $p < 0.01$), compost fertilizer level and char feedstock (SA-GR:

$F_{(4, 187)} = 4.23$, $p < 0.01$) and char feedstock and biochar application rate (SA-GR: $F_{(3, 187)} = 4.72$, $p < 0.01$; weight-GR: $F_{(3, 187)} = 5.12$, $p < 0.01$). Significant main effects were observed for the compost fertilizer level, biochar application rate and (weakly) feedstock used, but biochar coarseness (i.e. the two particle sizes) had no significant effect on plant growth.

The fertilizer addition in the compost had the strongest effect on plant growth rates of all controlled factors; it was associated with 33% of the total variance in growth rates (SA-GR: $F_{(1, 187)} = 114$, $p < 0.001$; weight-GR: $F_{(1, 187)} = 71.9$, $p < 0.001$). On average, plants grown in

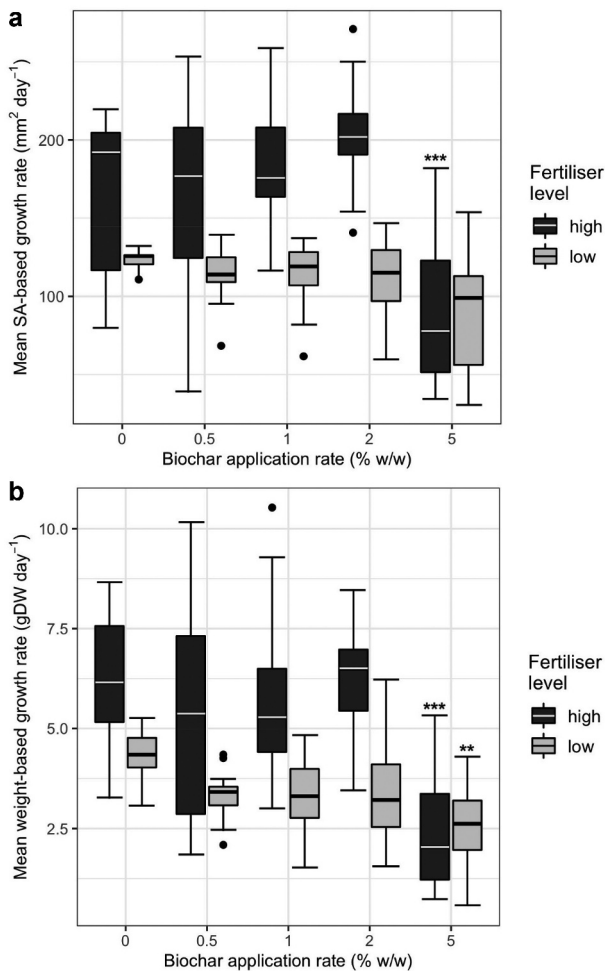


Fig. 4. The effects of fertilizer and biochar application rate (for all algal feedstocks) on leaf surface area-growth rate (SA-GR, A) and dry weight-growth rate (B). Boxplots show median, interquartile range (IQR), furthest value from median within $1.5 \times$ IQR (whiskers) and outlier values (dots). Significant differences between treatments and controls, based on Bonferroni-adjusted pairwise comparisons of transformed data, are illustrated with asterisks (* = $p < 0.05$, ** = $p < 0.01$; *** = $p < 0.001$). DW = dry weight.

pots with high-fertilizer compost had 59% greater surface area ($p < 0.001$) and were 55% heavier ($p < 0.001$) than those grown in low-fertilizer compost.

Overall, feedstock was associated with just 4% of the total variance in growth rates and did not significantly affect SA-GR (weight-GR: $F_{(4, 187)} = 2.63$, $p < 0.05$). Biochar application rate had the second largest influence on plant growth rates; it was associated with 11% of the total variance in growth rates (SA-GR: $F_{(1, 187)} = 22.7$, $p < 0.001$; weight-GR: $F_{(1, 187)} = 24.3$, $p < 0.001$).

Correlation analysis

Pearson's product-moment correlation analyses were carried out for several different chemical elements by plotting the relative amount of an element in the biochar against the mean growth rate for all treatments. Initially, the amounts of chemical elements were not scaled relative

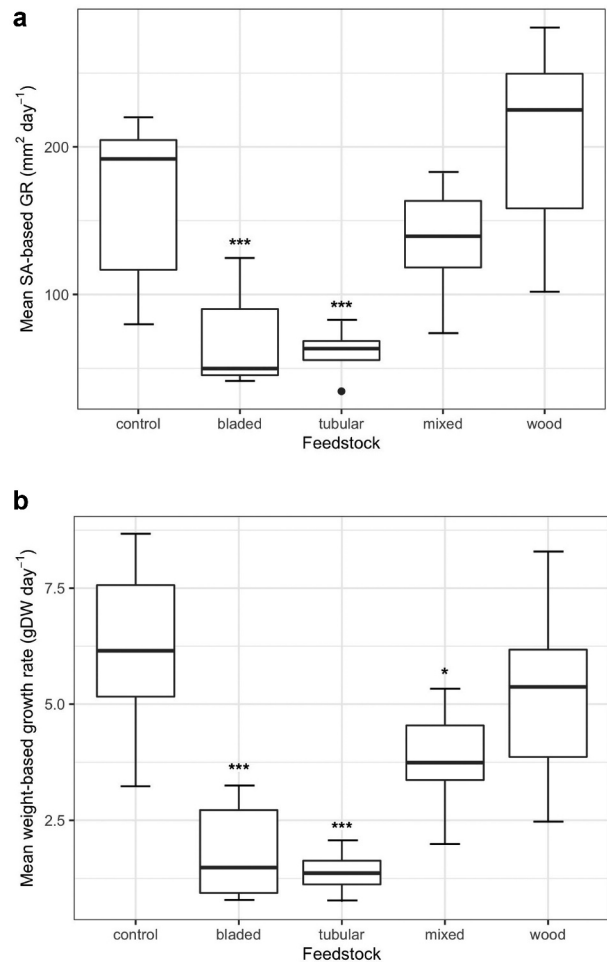


Fig. 5. The effects of biochar feedstock on leaf surface area-growth rate (SA-GR, A) and dry weight-growth rate (B) when biochars were applied at 5% w/w. Boxplots show median, interquartile range (IQR), furthest value from median within $1.5 \times$ IQR (whiskers) and outlier values (dots). Significant differences to control groups, based on Bonferroni-adjusted pairwise comparisons of transformed data, are illustrated with asterisks (* = $p < 0.05$; *** = $p < 0.001$). DW = dry weight.

to biochar application rate, to avoid false correlations being observed due to the presence of higher amounts of other elements. Unfortunately, this reduced the power of correlation analysis to the extent that no significant correlations were observed. Correlation analysis was carried out again for selected elements (Ca and Na); these were present in dramatically different quantities between the different biochars and negatively correlated with SA-GR ($R = -0.30$, $p < 0.001$; $R = -0.30$, $p < 0.001$, respectively) and dry weight-GR ($R = -0.34$, $p < 0.001$; $R = -0.35$, $p < 0.001$, respectively).

Discussion

Characterization and composition of biochars

Key fertilizer component N most commonly limits primary productivity in terrestrial ecosystems, although

Table 3. Significant pairwise comparisons between treatments and controls, showing the effects of fertilizer and biochar application on plant growth rates.

Controls											
Fert.	BAR % w/w	Mean SAGR mm ² day ⁻¹	SD mm ² day ⁻¹	Mean wtGR mg day ⁻¹	SD mg day ⁻¹						
low	0	122.9	± 7.50	4.36	± 0.71						
high	0	162.8	± 53.3	6.21	± 1.85						
Effect of fertilizer application											
BAR % w/w	Feedstock	Fert. A	SAGR A mm ² day ⁻¹	wtGR A mg day ⁻¹	Fert. B	SAGR B mm ² day ⁻¹	wtGR B mg day ⁻¹	p SAGR (adj.)	Sig. SAGR	p wtGR (adj.)	Sig. wtGR
0	n/a	high	162.8	6.21	low	122.9	4.36	0.10	ns	0.03	*
all	blad.	high	154.0	4.65	low	113.8	3.36	0.02	*	0.04	*
all	mix.	high	170.1	5.26	low	99.1	4.36	5.6 × 10 ⁻⁷	***	1.6 × 10 ⁻⁵	***
all	tub.	high	159.7	5.02	low	109.2	3.02	2.1 × 10 ⁻³	**	6.4 × 10 ⁻⁴	***
all	SoilFixer	high	195.9	5.15	low	98.6	3.38	1.2 × 10 ⁻⁹	***	8.7 × 10 ⁻⁴	***
Effect of biochar application											
Fert.	Feedstock	BAR A % w/w	SAGR A mm ² day ⁻¹	wtGR A mg day ⁻¹	BAR B % w/w	SAGR B mm ² day ⁻¹	wtGR B mg day ⁻¹	p SAGR (adj.)	Sig. SAGR	p wtGR (adj.)	Sig. wtGR
high	blad.	0	162.8	6.21	5.0	68.9	1.82	2.1 × 10 ⁻⁵	***	3.2 × 10 ⁻⁸	***
high	tub.	0	162.8	6.21	5.0	60.8	1.39	6.0 × 10 ⁻⁵	***	5.9 × 10 ⁻⁸	***
high	mix.	0	162.8	6.21	5.0	136.2	3.81	0.19	ns	0.047	*
high	alg.	0	162.8	6.21	5.0	92.1	2.46	1.4 × 10 ⁻⁴	***	1.0 × 10 ⁻⁸	***
low	tub.	0	122.9	4.36	5.0	86.3	2.11	0.56	ns	0.01	*
low	mix.	0	122.9	4.36	5.0	73.5	2.29	0.03	*	0.01	*
low	alg.	0	122.9	4.36	5.0	89.6	2.57	0.15	ns	6.2 × 10 ⁻³	**

Fert. = fertilizer application rate; BAR = biochar application rate; SAGR = surface area-based growth rate; wtGR = weight-based growth rate; SD = standard deviation; p (adj.) = Bonferroni-adjusted probability value (for each growth rate); Sig. = significance code (n, not significant, p ≥ 0.05; * = p < 0.05; ** = p < 0.01; *** = p < 0.001); blad. = bladed algae; mix. = mixed bladed and tubular algae; tub. = tubular algae; alg. = average of algal feedstocks. SoilFixer = commercial wood-derived char. Only comparisons with at least one significant difference are shown; significance values are based on Bonferroni-adjusted pairwise comparisons of transformed data.

P-limitation is sometimes observed (LeBauer & Treseder, 2008). Usually, non-weathered biochar produced from nutrient-rich feedstocks, such as algae, delivers a source of highly available nutrient salts that provide a direct, short-term nutritional boost to plants (Chan & Xu, 2009). As a relatively low pyrolysis temperature of 350°C was used to produce the algal biochars in this study, and chemical analysis of the biochars confirmed that levels of N were approximately proportional to those in the feedstock, it is highly likely that nutrient salts, particularly ammonium (NH_4^+), accumulated as residue on the surface of the algal biochar (Neary *et al.*, 2005).

The algal biochars had lower SPAC contents (2.7%) than wood biochar because of the lower carbonization temperature (350°C) and residence time (1 h) used to produce the algal biochars (McBeath *et al.*, 2015). In contrast, the SoilFixer biochar had a very high SPAC content of 81.4% of the total carbon, due to both the higher production temperature (up to 550°C) and residence time (up to 8 h). The composition of the algal biochars is also consistent with the findings of McBeath *et al.* (2015), who reported that, like manures, macroalgal feedstocks have relatively low carbon and high ash contents.

BET surface areas of algal biochars were also lower than that of the wood biochar, probably reducing the capacity of the algal biochars to adsorb nutrient ions such as P and K. This also meant a lower quantity of pores within the biochar, reducing functional group presentation and interactions with microbes present. These could be both forming a platform for microbial immobilization (Luo *et al.*, 2015); and as mediators for direct interspecies electron transfer between microbes, enhancing utilization of compounds within the compost (Wu *et al.*, 2020). Despite the finer-milled particles having an average of 11% greater surface area than larger char fractions, these had no significant impact on the surface areas or weights of the *A. thaliana* seedlings. This indicates that these microbial interactions were of low significance in comparison to the elements contained within and on the char.

Most elements measured, including those that are toxic in trace amounts, were present at similarly low concentrations in the wood control and algal biochars, with Cd, Cr, Cu, Hg, Ni, Pb and Zn in all biochars well below UK limits for biosolids applied to agricultural land (BAS, 2017). However, calcium and sodium were present in dramatically different concentrations between the algal and wood biochars; the algal biochars consisted of 11–20% (w/w) Ca and 1.9–2.5% (w/w) Na, compared with just 3.2% (w/w) Ca and 0.5% (w/w) Na in the wood biochar. The combined highest concentrations of these elements were observed in the tubular algal biochar with the bladed algal biochar the lowest. These values were

particularly affected by the Ca concentration with the tubular biochar > ×3 the concentration of the bladed biochar. It is reasonable to assume that the majority of this increase is in the greater propensity to trap *P. ulvae* between strands of tubular algae over the flattened surface of the bladed algae.

The C:N ratio of the wood biochar was (49.3), a lot higher than the C:N ratios of the algal biochars (8.4–9.9). These differences in C:N ratio may be partially responsible for the differences in growth rates observed following the addition of 5% (w/w) biochar, as microorganisms are known to immobilize N in most organic substrates with C:N ratios less than 20 (Leeper & Uren, 1993). However, the algal char ratios are comparable to those published in the literature (e.g. 5.8–8.4; who previously saw positive impacts from their algal chars on plant growth).

Impact of biochars on growth trial

In general, no positive effects of algal biochar amendment were found at any addition concentration 0.5, 1, 2, 5% (w/w) compared with the control plants 0% char addition and commercial wood char at 0.5, 1, 2, 5% (w/w) concentrations. In fact, the application of 5% (w/w) algal biochars to compost significantly reduced plant growth rates, especially when added to high-fertilizer compost. This is in direct contradiction to earlier studies using green algae-derived biochars such as in Bird *et al.* (2012) where 3.5% (w/w) low-temperature chars of seawater-derived green macroalgae (predominantly *Cladophora coelothrix*; 89%) outperformed freshwater-derived green algae and a lignocellulose control char in 'low fertility' soils, improving growth rate by ~90%. In comparison, for this trial fertilizer addition improved surface area by 59% and final weight by 55%.

On average, control plants grown in high-fertilizer compost grew 32–42% more rapidly compared with control plants grown in low-fertilizer compost, indicating that plant growth was nutrient limited during the trial period, at least in the low-fertilizer composts. However, the introduction of plant nutrients (N, P and K) in algal biochars did not similarly enhance plant growth. A 5% (w/w) bladed algal biochar contributed ~51.4 mg N to the compost mix, a similar amount to that contributed by the inorganic fertilizer to create the high-fertilizer compost (~51.8 mg N). However, it seems that nutrients delivered by the algal biochar were not readily available for plant uptake or that another factor counteracted any benefits of nutrient addition. Stunted seedling growth in compost treatments with 5% (w/w) algal biochars indicates that the biochars also introduced elements or additional compounds detrimental to plant productivity.

Our trial used a different base for the composts and a cress (*A. thaliana*) rather than a food crop in the trial. However, these mixes were based on standard combinations used in trials within the National Plant Phenomics Centre (www.plant-phenomics.ac.uk/) which have been shown to represent high and low fertilizer conditions well. As further confirmation, the main growth improvement in our trial was seen through the addition of a fertilizer, indicating that the 'low fertilizer' compost was indeed that. The biochar at temperatures were prepared well within the recommended maximum temperature of 450°C (Roberts & de Nys, 2016). In Roberts & de Nys's (2016) study, they also considered a washing step on *U. ohnoi*. Rinsing the *U. ohnoi* reduced char yield and electrical conductivity but did not significantly affect radish seedling germination, hypocotyl length or total production. However, their *U. ohnoi* was grown within an on-shore prawn farm and the Na concentration was not reported; it is likely that the salinity was lower than that within a lower-estuarine environment where our *Ulva* spp. were collected. The differences between the previously reported and our current results must therefore be at least partially attributable to the increased Na in the biochar and the impact of this on the compost and seedlings.

Salinity and liming effects

Soil salinity is well known to have several detrimental effects on plants (Spokas *et al.*, 2012). *Arabidopsis thaliana* is a known glycophyte, meaning that it will only thrive in soils with low NaCl content (Møller & Tester, 2007). Bird *et al.* (2012) showed that salts contained within biochar readily leach into the soil solution, where they can have deleterious effects on plant physiology, especially in more fertile soils. At high concentration, salt ions interfere with water absorption through root cells, affect nutrient uptake and inhibit cellular metabolism (Knight *et al.*, 1997). The algal biochars used in this growth trial contained, on average, around 2% Na by weight with the tubular algae biochar containing the highest concentrations of Ca and Na; and also the most severe growth-limiting effect including mortality of three plants when mixed at 5% (w/w) to the compost mixture. In treatments with 5% (w/w) algal biochar, any benefit conferred by elevated nutrient levels (from inorganic fertilizer or biochar) was negated. The salinity tolerance threshold of *A. thaliana* is predicted to have been exceeded when the biochar application rate became > 2% (w/w).

The Na⁺ ion transport processes that underlie salt tolerance have been well studied in *A. thaliana* and are thought to also occur in major food crops including rice (*Oryza sativa*; Ren *et al.*, 2005) and wheat (*Triticum aestivum*; Byrt *et al.*, 2007). Therefore, the effects of

saline biochars on *A. thaliana*, such as osmotic stress, and ultimately reduced growth, are relevant to the use of biochars for amending agricultural soils. Algal biochar salinity could be reduced by increased washing of the feedstock with fresh water to remove salts.

Ca occurs in natural soils at widely differing concentrations (Burstrom, 1968) and is recognized as an essential intracellular regulator of growth and development in plants, with the capacity to impart signalling specificity during responses to environmental changes (Hepler, 2005). To this end, high Ca-nitrate fertilizers are produced by Yara at ~26% CaO presence (Yara, 2022) so the chars containing 11–19.8% Ca would not negatively impact plant growth. Ca signalling is involved in salt-stress signalling in plants; the availability of Ca²⁺ ions affects the capacity of plants to respond to high salinity (Davies *et al.*, 1981). Knight *et al.* (1997) demonstrated that salt stress causes a rapid increase in cytosolic Ca²⁺ levels in *A. thaliana*, which is at least partially due to an influx of Ca²⁺ from the soil.

Kaya *et al.* (2002) found that high Ca²⁺ availability ameliorated the adverse effects of salinity on strawberry yields, probably by competing with Na⁺ ions for membrane binding sites. Ca can constitute more than 10% of the dry weight of some tissues (particularly in cell walls) of some plants without causing deleterious effects to growth (Marschner, 2012), although cytosolic concentrations rarely exceed the 0.1–0.2 μM level (Hirschi, 2004). Therefore, high Ca levels might have partially mitigated salinity issues imposed by biochar application but not to a sufficient degree. A final point is that NO₃⁻ and PO₄³⁻ anions in soil solution bind to Ca²⁺ and Na⁺ cations, reducing the availability of these nutrients for plant uptake (Barrow, 1984) so excess concentrations of both Ca and Na may have had an additional nutritional reduction impact.

The algal biochars were more alkaline than the wood biochar, probably due to the retention of Ca-rich *P. ulvae* shells, but their pH was at the low end of the expected range for algal biochars in general (Tag *et al.*, 2016). High alkalinity inhibits the growth of some plants, including *A. thaliana*, mainly by affecting the rhizosphere and hindering primary root elongation (Xu *et al.*, 2012). However, algal char/compost blends remained acidic after the addition of alkaline chars at 5% w/w, indicating that over-liming was unlikely to have slowed plant growth in this experiment.

Correlation data

Pearson-product moment correlation analyses between elemental concentration in the biochars and corresponding plant growth rates did not produce any significant ($p > 0.05$) correlations when application rate was ignored. Therefore, it was not

possible to separate the effects of multiple different factors (chemical elements and other properties) in this way. However, following the identification of high Ca and Na levels in the algal biochars, further analysis on the amounts of these elements, scaled relative to biochar application rates, indicated a negative correlation with plant growth rates.

It is postulated that high Na levels in the algal biochars were the primary factor reducing plant growth in this study, by increasing compost salinity and interfering with plant physiology. This has far-reaching repercussions as the main potential source of green algae is from estuarine and off-shore locations, as algal blooms; and the algae would be in saline waters in all such locations. If possible, harvesting in the upper inter-tidal estuary reaches and rinsing with fresh water could partially or wholly mitigate against the high salt concentrations. The control of such salt concentrations must therefore be a key future consideration for green algal bloom biochar production.

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Author contributions

F. Kenneth: designed, coordinated and conducted the experiments reported; collected the sample material and prepared it for pyrolysis; conducted the data analysis and wrote the first draft of this manuscript. J. Adams: designed, coordinated and conducted the experiments reported; gained the funding award for this project and arranged external sample analysis; reviewed and edited the manuscript. C. Joniver: collected the sample material and prepared it for pyrolysis; conducted species identification on the samples; reviewed and edited the manuscript. W. Meredith: prepared, analysed and interpreted the hydrolysis data; reviewed and edited the manuscript.

Disclosure statement

No potential conflict of interest was reported by the authors.

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