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Optimizing the pre-treatment of marine biomass (*Laminaria pallida and Gracilariopsis funicularis*) for enhanced production of climate-smart agricultural biochar

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Abstract

Marine biomass has gained attention as a sustainable feedstock for biochar, but its high salt content limits its agricultural application. This study investigated the water-soaking pre-treatment of *Laminaria pallida* and *Gracilariopsis funicularis* to reduce salinity and improve biochar quality for agricultural applications. The experiment included three factors: water temperature (25 °C and 100 °C), soaking duration (0, 0.5, 2, and 6 h), and seaweed species, resulting in 16 treatment combinations. Significant effects ($P < 0.05$) of soaking conditions were observed on biochar pH, electrical conductivity (EC), macronutrient concentrations (C, N, P), cations (Na, K, Ca, Mg), and heavy metals. Cold-water soaking of *G. funicularis* for 6 h produced the most favorable biochar, with a reduced sodium concentration (from 23.9 g/kg to 12.2 g/kg), lower EC (29.3 mS/cm), moderate pH (8.6), and high nutrient content (N: 5.17%; P: 127 mg/kg). In contrast, *L. pallida* biochar retained higher sodium (46.5 g/kg to 62.6 g/kg) and strong alkalinity (pH 11.3), limiting its agronomic suitability. Hot water soaking reduced N and P but increased Zn concentrations (up to 5.5 mg/kg and 9.52 mg/kg in *L. pallida* and *G. funicularis*, respectfully), indicating trade-offs between nutrient retention and metal enrichment. Importantly, all heavy metals measured (Cd, Cr, Cu, Ni, and Zn) remained below permissible limits for organic soil amendments. Unlike previous studies, the current study systematically optimised a simple, low-cost pre-treatment that reduces salinity while preserving nutrient value in marine biomass-based biochar. These findings demonstrate that, compared to *L. pallida,* pre-treated *G. funicularis* biochar has strong potential as an eco-friendly soil amendment in arid regions, where salinity management and nutrient efficiency are critical. Future studies should integrate this approach with vermicomposting or microbial inoculation to further leach excess cations and stabilise biochar quality.

Keywords Seaweed biomass, Pre-treatment soaking, Salinity reduction, Nutrient retention, Soil amendment

1 Introduction

Climate change persists as one of the most urgent environmental challenges, primarily driven by the combustion of fossil fuels and the resultant accumulation of greenhouse gases, such as carbon dioxide (CO_2) and methane (CH_4), in the atmosphere [51]. The increase in greenhouse gas concentrations has exacerbated global warming and elevated the absorption of CO_2 by the oceans, leading to ongoing ocean acidification and disruption of marine ecosystems [33]. One of the emerging consequences of increased oceanic CO_2 concentrations is the excessive proliferation of marine macroalgae, as elevated CO_2 levels enhance their photosynthetic growth [11]. This problem is even more pronounced in countries such as Namibia, where the coast is influenced by the Benguela upwelling system, which is a cold nutrient-rich current that enhances marine productivity [29]. Consequently, large quantities of seaweed biomass accumulate along beaches, reducing coastal aesthetic value and negatively affecting local economies [49].

These challenges underscore the need for climate-smart technologies that can utilise the increased marine seaweed biomass through carbon sequestration and sustainable resource valorization [44]. Seaweeds, including kelp, *Gracilaria spp*., *Ulva spp*., and sargassum, have been traditionally utilized as organic fertilizers due to their abundant nutrient content [15]. However, their direct application to soil is often limited by their high salt content and possible heavy metal accumulation which can disrupt soil microbial activity, induce osmotic stress in pants, and endanger consumer health [52]. In this context, the pyrolytic conversion of seaweed waste into biochar, a carbon-rich, porous material produced by heating biomass under limited oxygen, offers a sustainable approach for both carbon sequestration and soil fertility improvement [6]. Biochar has been shown to enhance the physical and chemical properties of soil and hydroponic growth media [8]. Although most studies have focused on terrestrial biomass, the use of marine feedstocks for biochar production has recently gained momentum, with applications ranging from water purification and, soil amelioration to reducing agents in metallurgical industries [36, 43]. In arid regions such as Namibia, where terrestrial plant biomass is limited, seaweeds such as *Gracilariopsis funicularis*, *Laminaria pallida*, *and Macrocystis pyrifera* offer viable alternatives as agricultural biochar feedstocks owing to their abundance and nutrient content [15].

Literature shows that Namibia began harvesting seaweeds in the 1980 s, primarily for agar production, and conducted its first cultivation trial in the 1990 s on *Gracilaria spp.* [31]. In addition, the country is actively involved in kelp (*M. pyrifera*) seaweed cultivation in coastal industrial operations near Lüderitz owing to its commercial applications, such as biostimulants, and ecological benefits [4]. This presents a significant potential for sustainable seaweed production that could supply raw materials for biochar while supporting local coastal economies. However, the cultivation and utilisation of the naturally dominant species (*L. pallida* and *G. funicularis*) along the Namibian coast remain largely underexploited [15]. The potential of seaweed as a feedstock for agricultural biochar has been investigated, and research indicates that this amendment exhibits agronomic potential [22, 32].

Despite these benefits, marine biomass-derived biochar often exhibits challenges related to high salinity and the potential presence of heavy metals [12]. The physiochemical composition of biochar has been reported to vary with feedstock type, pyrolysis temperature, and residence duration [30]. For instance, [22] reported that at 400 °C

and a 1 h pyrolysis time, for *L. pallida* and *G. funicularis* biomass derived biochar yields stabilised, but the electrical conductivity (EC) exceeded 20 mS/cm. EC serves as a proxy for salinity; values exceeding 4 dS/m (equivalent to 4 mS/cm) are generally detrimental to sensitive crops, this highlights the need for salt management before field application [24].

Previous studies have reported several techniques for mitigating salinity in marine biochar feedstocks, including chemical washing or soaking using acidic or basic solutions, water rinsing or immersion, centrifugal dehydration, and vacuum desalination [37, 40, 43]. Emerging technologies, such as microbial enzymatic desalination, have also shown promise in removing salts from saline water and growing media, with potential applicability to media amendments [21, 28]. Although these methods are effective in removing soluble salts, some introduce chemicals or produce wastewater, while others are expensive, labor-intensive, or impractical for large-scale applications [21, 43]. Water-soaking pretreatment represents a more sustainable alternative, capable of effectively leaching salts without chemical additives or high costs and is potentially applicable on a large scale. Although this technique has demonstrated potential, its application to marine biochar feedstocks, particularly concerning the effects of water soaking at different temperatures on the qualities of the resultant biochar from seaweeds investigated in this study, remains largely unexplored.

Therefore, exploring pretreatment methods, such as cold and hot water soaking, to optimise the chemical properties of this seaweed-derived biochar represents both a novel and context-specific contribution to sustainable biomass valorization. It was hypothesised that seaweed species, water temperature, and soaking duration during water-based pretreatment would significantly influence the physicochemical characteristics of the resulting biochar, thereby improving its suitability for agricultural applications. Therefore, the objective of this study was to evaluate the effects of cold- and hot-water soaking pretreatment methods for *Laminaria pallida* and *Gracilariopsis funicularis* biomass at varied soaking durations on the selected chemical properties of biochar.

2 Materials and methods

2.1 Study site and source of materials

The study was conducted at the University of Namibia, Sam Nujoma campus in the Erongo region of Namibia (14°30'19"E, 22°55'27"S). The seaweeds *L. pallida* and *G. funicularis* were collected throughout June 2024 from the Henties Bay and Swakopmund Coast, Namibia, and their physicochemical characteristics are presented in Table 1. After collection, the seaweed biomass was carefully cleaned by removing shells, debris, and other foreign materials, including fragments of other seaweed species, to minimize contamination before being soaked in deionized water. The deionized water used in this study was collected from a water-deionizing machine at the University of Namibia, Sam Nujoma Campus. The chemical properties of the deionized water were as follows: pH, 7.2; EC, 42.7 mS/m; Na, 77 mg/L; K, 3.8 mg/L; nitrate, < 0.5 mg/L; nitrite, < 0.1 mg/L; Ca, 1.2 mg/L; and Mg, 2.5 mg/L. Sterile metal cylindrical cans (5 L) were used for seaweed biomass confinement at the time of biochar preparation in the muffle furnace.

Table 1 Physicochemical characteristics of raw seaweed biomass (dry weight basis) used for biochar production

Parameter	*Laminaria pallida*	*Gracilariopsis funicularis*
pH	5.73 ± 0.06	5.51 ± 0.02
EC (mS/cm)	37.37 ± 4.65	25.13 ± 0.67
Total N (%)	1.99 ± 0.03	3.44 ± 0.10
Total C (%)	25.53 ± 0.15	31.53 ± 0.59
Olsen P (g/kg)	0.95 ± 0.09	1.20 ± 0.05
NH_4-N (g/kg)	0.10 ± 0.05	0
NO_3/NO_2-N (g/kg)	0.19 ± 0.05	0.35 ± 0.03
Na (g/kg)	20.18 ± 1.40	7.86 ± 0.62
Ca (g/kg)	5.75 ± 0.27	1.80 ± 0.15
K (g/kg)	94.43 ± 4.23	67.51 ± 4.87
Mg (g/kg)	3.941 ± 0.260	1.87 ± 0.14
Cr (mg/kg)	4.38 ± 0.50	2.55 ± 0.20
Zn (mg/kg)	25.87 ± 4.52	30.52 ± 5.12
Ni (mg/kg)	3.24 ± 0.58	2.02 ± 0.86
Cd (mg/kg)	0.57 ± 0.01	4.52 ± 0.34
Pb (mg/kg)	3.45 ± 0.90	0.79 ± 0.05
As (mg/kg)	48.85 ± 2.15	5.01 ± 1.64

Values are presented as mean ± SEM ($n = 3$)

2.2 Treatment and experimental design

The experiment had three factors: two levels of deionized water temperature (room temperature 25 °C and hot water 100 °C), four soaking durations (0 min-without soaking, 30 min, 2 h, and 6 h), and two seaweed species (*L. pallida* and *G. funicularis*). The experiment used a $2 \times 4 \times 2$ factorial design, which was laid out in a completely randomized design (CRD) with three replicates. The experiment included 16 treatment combinations. The biomass of each seaweed species was individually treated and immersed in deionized water. Previous research has employed various soaking ratios, such as 1:10 w/v for *Eucheuma cottonii* [40] and 1:20 w/v for *Codium fragile* [37], demonstrating that the ratio is not constant but varies according to species and intended application. In this study, a ratio of 1:5 (w/v) was chosen to ensure complete submersion of the seaweed in water. Cold water treatment was performed by soaking the seaweeds in deionized water for different durations (0 min-without soaking, 30 min, 2 h, and 6 h). Hot water treatment was performed by soaking seaweed for 0 min, 30 min, 2 h, and 6 h in deionized water heated to the desired temperature on a hot plate and kept in an electric water bath to maintain a constant temperature. A total of 48 samples were obtained from the entire experiment and oven-dried at 70 °C for 48 h to remove excess water, ensuring uniform moisture content across all treatments before pyrolysis. To minimise experimental bias, treatments were randomly assigned using a random number generator. Each sample was handled independently to avoid cross-contamination during treatment, drying, and pyrolysis. Equipment, containers, and surfaces were thoroughly cleaned and rinsed with deionised water between treatments, and separate instruments were used when necessary to maintain sample purity.

2.3 Biochar production

The dried seaweed biomass from various treatments was subsequently converted into biochar through pyrolysis in a sterile, airtight stainless-steel container equipped with a small vent hole to release volatile gases and prevent pressure build-up, while allowing

minimal oxygen entry. The container was heated in a muffle furnace (with a digital temperature controller) at 400 °C for 1 h, as recommended by [22]. No inert carrier gas, such as N_2, was introduced. These conditions facilitated a low-oxygen environment characteristic of pyrolysis rather than complete combustion. After 1 h, the container was removed from the furnace using heatproof gloves and allowed to cool to room temperature before being opened to prevent ignition. Subsequently, the biochar samples obtained were further crushed using a mortar and pestle and sieved with a 2 mm sieve before analysis.

2.4 Sample analysis

2.4.1 pH and electrical conductivity (EC)

The biochar sample (5 g) was thoroughly mixed with deionized water at a ratio of 1:10 (w/v) and shaken on a reciprocating shaker at 120 rpm for 30 min. pH and EC were measured using a multimeter with a glass electrode (HQ40d, Aqua Services & Engineering, Germany). Prior to measurement, the pH probe was calibrated using standard buffer solutions of pH 4.0 and 7.0, while the EC probe was calibrated with a sodium chloride (NaCl) standard solution of 1000 ± 10 µS/cm. The pH and EC were measured by placing multimeter probes in the solution [3].

2.4.2 Total carbon and nitrogen

The total carbon and nitrogen contents were quantified using the dry combustion method with a Vario EL Cube Elemental Analyzer (Elementar, Germany). The analyser was calibrated using a certified reference material, specifically sulfamethazine, which contains 20.13% total nitrogen and 51.78% total carbon. The limits of detection (LOD) for the analyser were determined to be 0.01% for nitrogen and 0.25% for carbon. To conduct the analysis, a 0.1 g biochar sample was precisely weighed into an aluminum foil boat, along with approximately 5 mg of Tungsten Oxide (WO_3) powder additive, using an analytical balance. The aluminum foil boat containing the weighed biochar sample was then placed inside an operational Vario EL Cube Elemental Analyzer, which automatically determined the total carbon and nitrogen content in the sample.

2.4.3 Extractable P

Extractable phosphorus was extracted using the Olsen method, and phosphorus was analyzed using the ascorbic acid method [25]. All analytical-grade reagents were supplied by Merck (Pty) Ltd, South Africa. Extractable P was extracted from 5 g of biochar sample using 50 mL of 0.5 M $NaHCO_3$ (pH 8.5) by shaking for 30 min at 180 rpm [44]. Whatman No. 2 filter paper was used to filter the extract, and the ascorbic acid method, as described by [25], was used to analyze the P in the extracts The ascorbic acid method involved the development of a blue color and the measurement of absorbance at a wavelength of 880 nm using a UV-Vis spectrometer (Gynesis 10 S UV-Vis, Thermo Fisher Scientific, USA)[25].

2.4.4 Extractable ammonium, nitrate and nitrite

Extractable ammonium, nitrate and nitrite were extracted using 0.5 M potassium sulfate and analyzed calorimetrically [35]. All analytical-grade reagents were supplied by Merck (Pty) Ltd, South Africa. To extract ammonium nitrate and nitrite, a 5 g biochar sample was mixed with 50 mL of 0.5 M potassium sulfate and shaken for 1 h using a mechanical

reciprocal shaker at 180 rpm. The extract was filtered through the same grade of filter paper (Whatman No. 2)Colorimetric determination of NH_4^+-N and NO_2/NO_3-N in the extracts was performed following the procedures described by [35]. Briefly, for NH_4^+-N, the filtered extract was reacted sequentially with N1 and N2 reagents, and the resulting blue color intensity was measured at 655 nm using a UV-Vis spectrometer (Genesys 10 S, Thermo Fisher Scientific, USA), calibrated with standards ranging from 0 to 25 µg NH_4^+-N mL^{-1}.

For NO_2/NO_3-N determination, the extract was reacted with salicylic acid and NaOH solutions according to [35],and absorbance was read at 419 nm using the same spectrometer calibrated with standards ranging from 0 to 10 µg NO_3-N mL^{-1}.

2.4.5 Extractable cations

Extractable cations (Ca, Mg, Na, and K) were extracted using the ammonium acetate method [3]. A 5 g biochar sample was extracted with 1 M ammonium acetate, adjusted to pH 7.0 using 1 M NaOH. The mixture was shaken on a reciprocating shaker at 180 rpm for 30 min, and the extracts were filtered using the same grade of filter paper prior to analysis with an Atomic Absorption Spectrometer (PinAAcle™ 900 F, PerkinElmer Inc., USA). For quality assurance, certified standard solutions were used for calibration, and analytical blanks were included to detect possible contamination. Quality control (QC) standards were analysed after every ten samples to verify analytical precision and accuracy.

2.4.6 Total concentrations of heavy metals

To measure the total concentration of heavy metals (Cd, Pb, Cr, As, Zn, Cu and Ni), biochar samples were digested using the wet digestion method as described by [3]. A 0.5 g sample of biochar was digested with 10 mL of nitric acid (60%), heated at 150 °C for 30 min, before adding 4 mL of perchloric acid (65%), and heated again for 30 min at the same temperature. After the digestion process, the mixture was allowed to cool before being diluted in a 100 mL volumetric flask. The diluted solution was filtered using the same filter paper type before analysis with a calibrated Atomic Absorption Spectrophotometer (AAS) (PinAAcleTM900F, PerkinElmer Inc., USA). er Inc., USA). Quality assurance and quality control followed same protocol used for cations analysis.

2.5 Data analysis

The data were analyzed using a three-way analysis of variance (ANOVA) using Jamovi statistical software version 2.6.13 (The Jamovi project, 2024). jamovi. (Version 2.6) [Computer Software]. Retrieved from https://www.jamovi.org. Normality of the data was assessed using the Shapiro–Wilk test in Jamovi, and all variables met the assumption of normality ($P > 0.05$). Differences among treatment means were considered statistically significant at $P \leq 0.05$. For parameters that showed significant differences, post-hoc comparisons were performed using the LSmeans Differences Student's t-test in JMP statistical software (JMP Student Edition, version 18.2.2) at the same significance level ($\alpha = 0.05$). Microsoft Excel and GraphPad Prism version 10.2.2 for Windows (GraphPad Software, www.graphpad.com) were used to plot graphs.

3 Results

3.1 Biochar pH and EC

There was a significant influence ($P < 0.05$) of all the three factors on the pH of seaweed biochar (Table 1). Furthermore, a significant interaction ($P < 0.05$) was observed between soaking duration and seaweed species, as well as between water temperature and seaweed species, on the pH of biochar (Table 1). However, there was no significant interaction ($P > 0.05$) between water temperature and soaking duration, and also between water temperature, soaking duration, and seaweed species on pH of the biochar (Table 1). For both seaweed species, the pH increased with soaking time in both the cold and hot water treatments (Fig. 1A and B). The pH of biochar derived from *L. pallida* was higher than that from *G. funicularis* in both the cold and hot water treatments (Fig. 1A and B). Specifically, the pH of the biochar from *L. pallida* in cold water ranged from 10.98 to 11.30, with the lowest pH observed at 0 min and the highest at 6 h of soaking (Fig. 1A). Under cold water treatment, the lowest pH in *G. funicularis* was observed at 0 min with 8.6, while the highest was recorded at 6 h with 9.33 (Fig. 1B). In hot water, the pH of *L. pallida* ranged from 11.03 to 11.35 (Fig. 1A), while for *G. funicularis* it ranged from 8.94 to 9.47 (Fig. 1B). Hot water treatment slightly increased the biochar pH for the same soaking duration, although the overall trend remained consistent.

There was a significant influence ($P < 0.05$) of all three factors on the electrical conductivity (EC) of seaweed biochar (Table 1). The three-way interaction between the factors had a significant effect ($P < 0.05$) on the EC of seaweed biochar (Table 1). In both seaweed species, the EC decreased with increasing soaking duration in cold water (Fig. 1A and B).

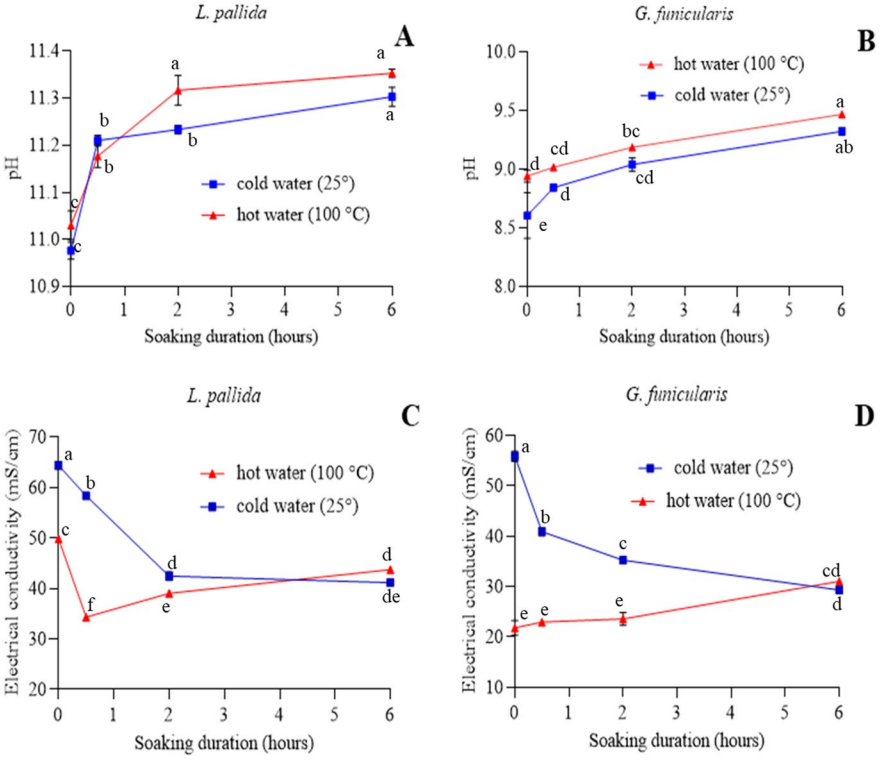

Fig. 1 Effect of soaking seaweed (*L. pallida* and *G. funicularis*) biomass at different water temperatures and soaking durations on the pH (**A** and **B**) and electrical conductivity (**C** and **D**) of the biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

Conversely, in the hot water treatment, EC increased with soaking time (Fig. 1C and D). For both seaweed species, EC was higher in cold water than in hot water for equivalent soaking times (Fig. 1C and D). In the hot water treatment, biochar derived from *L. pallida* exhibited an EC range of 34.3 mS/cm to 47.8 mS/cm, with the lowest EC observed after 30 min of soaking (Fig. 1C). In the cold water treatment, EC ranged from 41.1 mS/cm to 64.3 mS/cm, with the lowest EC recorded after 6 h of soaking (Fig. 1C). The highest EC in *L. pallida* was reported at 0 min in both cold and hot water treatments. For *G. funicularis* biochar in the hot water treatment, EC ranged from 21.8 mS/cm to 31 mS/cm, with the lowest EC at 0 min and the highest at 6 h (Fig. 1D). Under cold water treatment, the lowest EC in *G. funicularis* was observed at 6 h with a value of 29.3 mS/cm, while the highest EC was observed at 0 min of soaking with 55.9 mS/cm (Fig. 1D).

3.2 Macronutrient content (C, N, P)

There was a significant influence ($P < 0.05$) of all three factors on the total percentage of carbon in seaweed biochar (Table 2). The three-way interaction between the factors had a significant effect ($P < 0.05$) on the total C content of the biochar (Table 2). In both seaweed species, cold water soaking showed that the percentage of carbon increased with soaking duration, whereas hot water soaking showed that the total C decreased with increasing soaking duration (Fig. 2A and B). Notably, compared with hot water soaking, cold water soaking resulted in a lower total C content in both seaweed species for the same soaking duration, except for extended durations (6 h) (Fig. 2A and B). Under cold water treatment, the total C of *L. pallida* ranged from 27.94% to 35.72%; the longest soaking time produced the highest percentage (Fig. 2A). In the hot water treatment of *L. pallida*, the highest percentage C was observed at 30 min with the value of 37.75%, whereas the lowest total C was observed after 6 h of soaking with the value of 33.66% (Fig. 2A). In *G. funicularis* subjected to cold water soaking, total C ranged from 36.10%

Table 2 Analysis of variance (ANOVA) of various chemical properties of biochar prepared from *L. pallida* and *G. funicularis* seaweeds

Parameter	Source of variation						
	Water temperature (°C) (T)	Soaking duration (h) (D)	Seaweed species (S)	D × T	D × S	T × S	D × T × S
pH	< 0.001	< 0.001	< 0.001	ns	0.001	0.008	ns
EC (mS/cm)	< 0.001	< 0.001	< 0.001	< 0.001	0.004	< 0.001	< 0.001
Total C (%)	< 0.001	< 0.001	< 0.001	< 0.001	0.003	< 0.001	< 0.001
Total N (%)	< 0.001	0.031	< 0.001	ns	< 0.001	< 0.001	< 0.001
C: N ratio	< 0.001	< 0.001	< 0.001	ns	ns	< 0.001	ns
Extractable P (mg/kg)	0.008	0.02	ns	ns	ns	ns	ns
NH_4^+-N (mg/kg)	ns	ns	< 0.001	ns	ns	< 0.001	< 0.001
NO_2/NO_3-N (mg/kg)	0.038	ns	ns	ns	ns	ns	ns
Na (mg/kg)	< 0.001	0.002	< 0.001	0.004	ns	ns	ns
Ca (mg/kg)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.021
Mg (mg/kg)	0.01	< 0.001	< 0.001	0.001	< 0.001	< 0.001	< 0.001
K (mg/kg)	< 0.001	< 0.001	< 0.001	< 0.001	ns	< 0.001	< 0.001
Cr (mg/kg)	ns	< 0.001	ns	ns	0.008	< 0.001	ns
Cd (mg/kg)	ns	ns	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Cu (mg/kg)	< 0.001	ns	ns	ns	0.043	ns	0.043
Zn (mg/kg)	< 0.001	< 0.001	< 0.001	0.008	< 0.001	ns	ns
Ni (mg/kg)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.007

ns = not significant ($P > 0.05$)

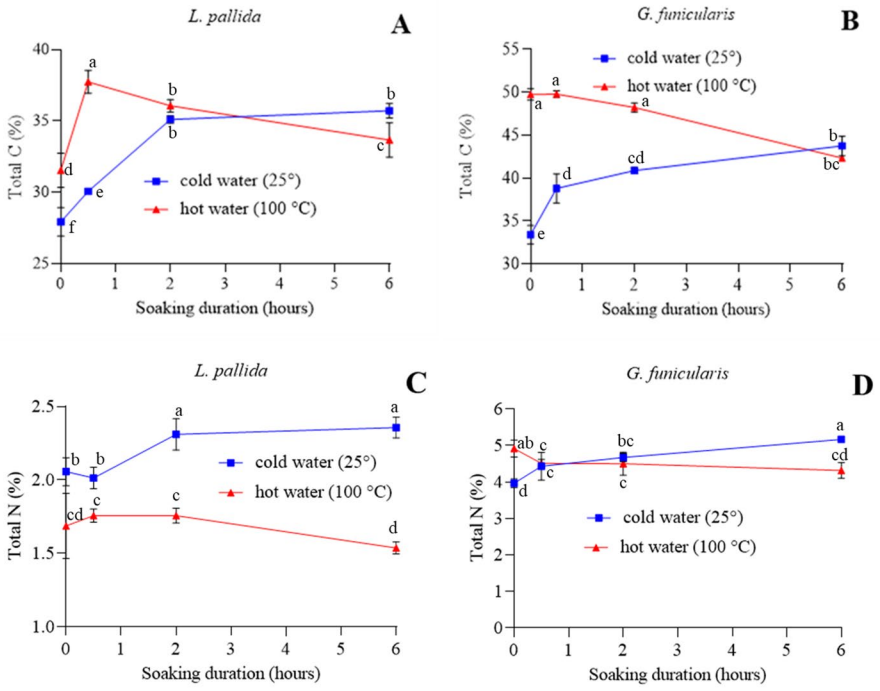

Fig. 2 Effect of soaking seaweed (*L. pallida* and *G. funicularis*) biomass at different water temperatures and soaking durations on the total C (**A** and **B**) and total N (**C** and **D**) of biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

at 0 min to 43.74% after 6 h of soaking, whereas under hot water treatment, total C in biochar ranged from 42.32% at 6 h to 49.77% at 30 min of soaking (Fig. 2B).

There was a significant influence ($P < 0.05$) of all the three factors on the total percentage nitrogen of seaweed biochar (Table 2). There was a significant interaction ($P < 0.05$) between seaweed species and other factors on total N, though there was no significant interaction ($P > 0.05$) between soaking duration and water temperature on total N of the biochar (Table 2). In both seaweed species, the percentage of nitrogen increased with the duration of soaking in the cold water treatment. In contrast, in the hot water treatment, total N decreased as the soaking duration increased (Fig. 2C and D). Generally, cold water treatment produced biochar with a higher percentage of nitrogen than hot water treatment for the same soaking duration in both seaweeds (Fig. 2C and D). In the cold water treatment, the total N of *L. pallida* exhibited an upward trend, starting with 2.06% at 0 min and rising to 2.36% after 6 h of soaking (Fig. 2C). The total N of biochar derived from *L. pallida* biomass in the hot water treatments ranged from 1.54% at 6 h to 1.76% at 30 min (Fig. 2C). For *G. funicularis*, the cold water treatment resulted in the highest total N of 5.17% at 6 h and the lowest total N of 3.97% at 0 min, whereas in the hot water treatment, total N exhibited a declining trend, starting at 4.92% at 0 min and reaching its lowest value of 4.32% at 6 h (Fig. 2D).

Water temperature and soaking duration had a significant influence ($P < 0.05$) on extractable phosphorus concentration in seaweed biochar, though there was no significant difference ($P > 0.05$) between the two seaweed species (Table 2). There was no significant interaction ($P > 0.05$) between all the three factors on extractable P concentration in the seaweed biochar. Biochar from *L. pallida* showed extractable P which decreased with soaking time, regardless of the water temperature (Fig. 3A). The extractable P

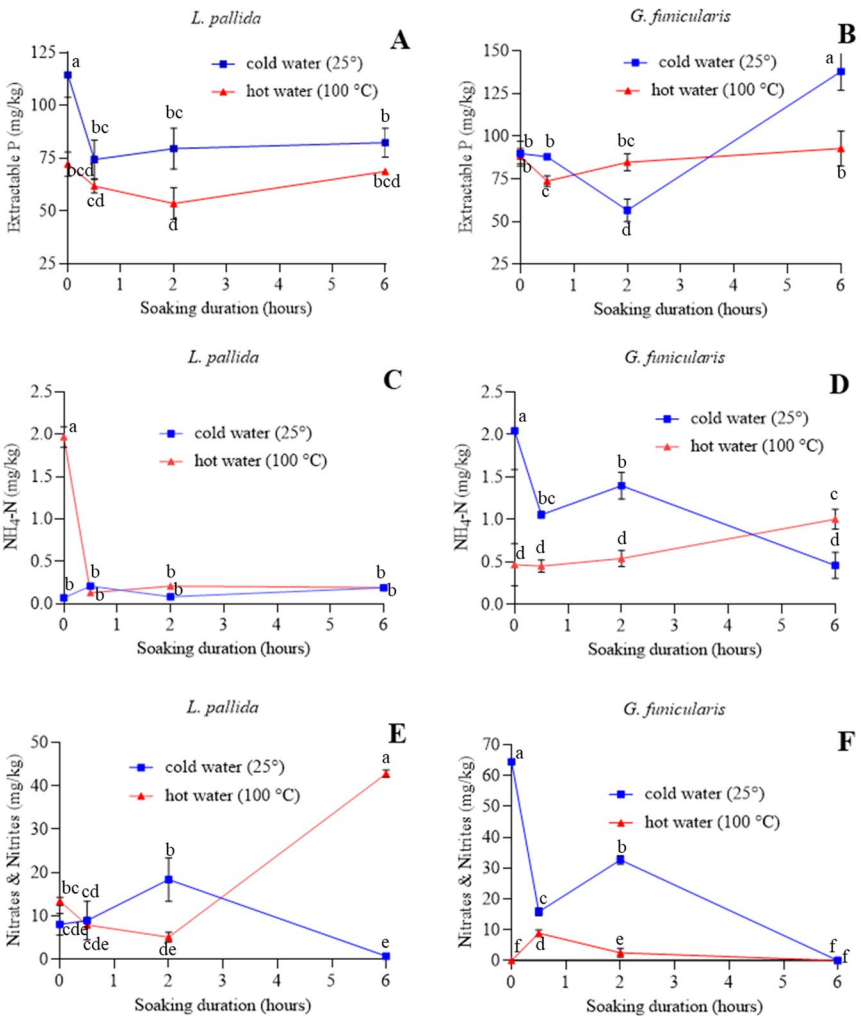

Fig. 3 Effect of soaking seaweed (*L. pallida & G. funicularis*) biomass under different water temperatures and soaking durations on the extractable P (**A** and **B**), NH_4-N (**C** and **D**), NO_3/NO_2-N (**E** and **F**) of biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

concentration was higher in the cold water treatment than in the hot water treatment for the same duration (Fig. 3A). In cold water, *L. pallida* biochar had the highest extractable P content at 0 min with the value of 114.50 mg/kg and the lowest was observed at 30 min with a value of 74.37 mg/kg (Fig. 3A). In hot water, 0 min observed the highest extractable P in *L. pallida*, with the value of 72.20 mg/kg, while the lowest value of 53.52 mg/kg was recorded after 2 h of soaking (Fig. 3A). In *G. funicularis*, extractable P showed an increasing trend over time, regardless of the water temperature (Fig. 3B). *G. funicularis* biochar had the highest extractable P of 127.01 mg/kg at 6 h in cold water, and the lowest value of 56.60 mg/kg at 2 h (Fig. 3B). In hot water, extractable P in *G. funicularis* ranged from 77.29 mg/kg at 30 min to 92.86 mg/kg at 6 h (Fig. 3B).

Seaweed species had a significant influence ($P < 0.05$) on the ammonium (NH_4^+-N) concentration in biochar, though water temperature and soaking duration had no significant ($P > 0.05$) influence on NH_4^+-N concentration in biochar (Table 2). Furthermore, there were significant interactions ($P < 0.05$) between water temperature and seaweed

species as well as between water temperature, soaking duration, and seaweed species on NH_4^+-N concentration in the biochar (Table 2). Under cold water soaking, biochar from *L. pallida* demonstrated an increasing trend over time, starting at 0.068 mg/kg at 0 min to 0.19 mg/kg after 6 h (Fig. 3C). Conversely, *G. funicularis* exhibited a contrasting pattern under the same water temperature conditions, displaying a decline over time, beginning at 2.05 mg/kg at 0 min and decreasing to 0.46 mg/kg at 6 h (Fig. 3D). In hot water, *L. pallida* derived biochar showed a declining trend over time, starting at 1.97 mg/kg at 0 min, ending at 0.61 mg/kg after 6 h, while *G. funicularis* showed an increasing trend under this treatment, rising from 0.47 mg/kg at 0 min to 1.00 mg/kg after 6 h of soaking (Fig. 3C and D).

Water temperature had a significant influence ($P < 0.05$) on the nitrate and nitrite (NO_2/NO_3-N) concentrations of seaweed biochar, though seaweed species and soaking duration had no significant ($P > 0.05$) influence on NO_2/NO_3-N concentration in the biochar (Table 2). In both seaweed species, under cold water conditions, the NO_2/NO_3-N concentration showed a declining trend over time (Fig. 3E and F). In the hot treatment, the NO_2/NO_3-N concentration decreased with increasing time; however, in *L. pallida*, it increased with prolonged duration (Fig. 3E and F). In cold water, *L. pallida*, NO_2/NO_3-N concentration ranges from 0.71 mg/kg (6 h) to 18.38 mg/kg at 2 h as shown in Fig. 3E. In hot water, *L. pallida*, the highest NO_2/NO_3-N concentration was observed after 6 h (42.82 mg/kg) and the lowest of 5.10 mg/kg was observed at 2 h (Fig. 3E). In the biochar made from *G. funicularis* under cold water soaking, the NO_2/NO_3-N concentration starts at 64.44 mg/kg (no-soaking), decreasing to 0 mg/kg at 6 h (Fig. 3F). In the hot water treatment, the NO_2/NO_3-N concentration in *G. funicularis*-derived biochar ranged from 0 mg/kg (0 and 6 h) to 8.92 mg/kg (30 min) (Fig. 3F).

3.3 Exchangeable cations (Na, K, Ca, Mg)

There was a significant influence ($P < 0.05$) of all three factors on sodium concentrations in seaweed biochar (Table 2). There was a significant three-way interaction ($P < 0.05$) between factors on the Na concentration in seaweed biochar (Table 2). In *L. pallida*, cold water treatment showed a declining trend, while the hot water treatment showed an increasing trend (Fig. 4A). In *G. funicularis*, the sodium concentration showed a declining trend regardless of the water temperature (Fig. 4B). In both species, cold water treatment resulted in a higher Na content than hot water treatment for the same soaking duration (Fig. 4A and B). In *L. pallida*, the sodium concentration in biochar decreased from 62.55 g/kg at 0 min to 46.52 g/kg after 6 h of cold water treatment (Fig. 4A). Conversely, in hot water treatment, the Na concentration in *L. pallida*-derived biochar increased from 30.22 g/kg at 0.5 h to 39.87 g/kg at 6 h (Fig. 4A). In *G. funicularis*, the sodium concentration under cold water soaking decreased from 23.85 g/kg without soaking to 12.16 g/kg after 6 h (Fig. 4B). Under hot water treatment, the Na concentration in *G. funicularis*-derived biochar ranged from 2.77 g/kg at 2 h to 12.01 g/kg at 0 min (Fig. 4B).

There was a significant influence ($P < 0.05$) of all three factors on calcium concentrations in seaweed biochar (Table 2). There was a significant three-way interaction ($P < 0.05$) between factors on the Ca concentration in seaweed biochar (Table 2). In *L. pallida*, the Ca concentration showed a decreasing trend over time, regardless of the water temperature (Fig. 4C). Biochar derived from *G. funicularis* showed an increasing

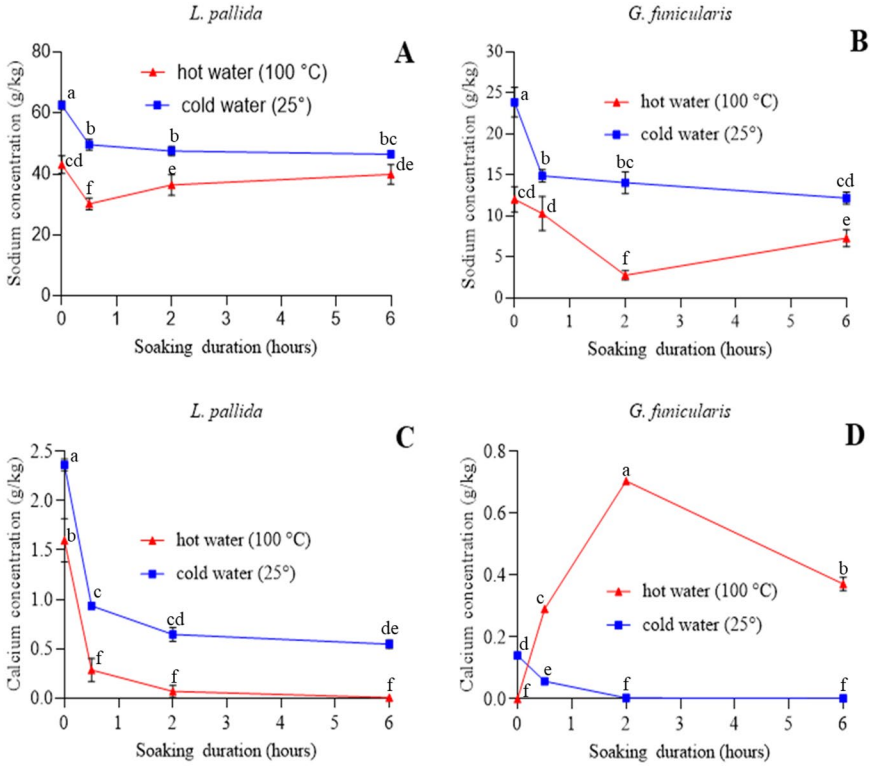

Fig. 4 Effect of soaking seaweed (*L. pallida* and *G. funicularis*) biomass at different water temperatures and soaking durations on the Na (**A** and **B**) and Ca (**C** and **D**) concentrations of biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

trend in hot water and a declining trend in cold water (Fig. 4D). Generally, Ca content in *L. pallida-derived* biochar was higher in the cold treatment than in the hot water treatment; however, in *G. funicularis*, the results showed an opposing trend (Fig. 4C and D). The initial calcium concentration in *L. pallida* subjected to cold water soaking was 2.36 g/kg at no soaking, which decreased progressively to 0.55 g/kg after 6 h (Fig. 4C). Similarly, in the hot water treatment, the calcium concentration in *L. pallida*-derived biochar started at 1.60 g/kg at no soaking and declined to 0 g/kg after 6 h (Fig. 4C). For *G. funicularis*, the calcium concentration in the biochar under hot water conditions ranged from 0 g/kg at no soaking to 0.71 g/kg at 2 h (Fig. 4D). Conversely, cold water treatment exhibited a decreasing trend, with calcium concentration reducing from 0.14 g/kg at 0 min to 0 g/kg after 6 h of soaking (Fig. 4D).

There was a significant influence ($P < 0.05$) of the three factors on magnesium concentrations in seaweed biochar (Table 2). Moreover, there was a significant three-way interaction ($P < 0.05$) between factors on Mg concentration in seaweed biochar (Table 2). In *L. pallida*, magnesium levels were higher with hot water than with cold water treatment, peaking at moderate soaking durations before decreasing (Fig. 5A). After cold water treatment, the magnesium content in *L. pallida* rose from 4.48 g/kg at no-soaking to 4.99 g/kg after 2 h and then dropped to 4.68 g/kg after 6 h (Fig. 5A). Hot water treatment in *L. pallida* led to a maximum of 5.52 g/kg at 0.5 h, decreasing to 5.03 g/kg after 6 h of soaking (Fig. 5A). In *G. funicularis*, the magnesium content decreased over time with both treatments, although there was a slight recovery with hot water (Fig. 5B). When subjected to cold water, magnesium levels in *G. funicularis* dropped from 0.56 g/kg at

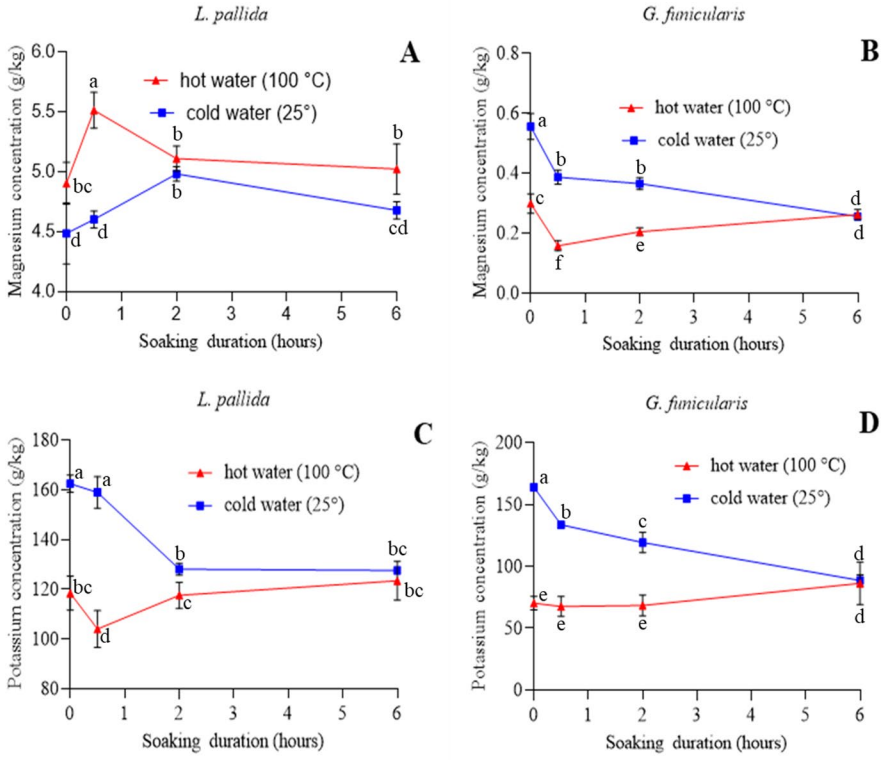

Fig. 5 Effect of soaking seaweed (*L. pallida* and *G. funicularis*) biomass at different water temperatures and soaking durations on the Mg (**A** and **B**) and K (**C** and **D**) concentrations of biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

no-soaking to 0.26 g/kg after 6 h (Fig. 5B). In contrast, with hot water, the concentration decreased from 0.30 g/kg at 0 min to 0.16 g/kg at 0.5 h, then increased to 0.26 g/kg at 6 h (Fig. 5B).

There was a significant influence ($P < 0.05$) of all three factors on potassium concentrations in seaweed biochar (Table 2). There was also a significant interaction ($P < 0.05$) between water temperature and soaking duration, water temperature and seaweed species, water temperature, soaking duration, and seaweed species on the potassium concentration in biochar (Table 2). However, there was no significant interaction ($P > 0.05$) between soaking duration and seaweed species on K concentration in biochar (Table 2). In both species, cold water soaking resulted in higher potassium concentrations than hot water soaking for the same soaking duration (Fig. 5C and D). Both cold and hot water caused an initial drop in the K concentration, followed by a slight increase at extended soaking durations (Fig. 5C and D). In cold water soaking, *L. pallida* indicated that K concentration decreased from 162.47 g/kg at 0 min to 127.51 g/kg after 6 h (Fig. 5C). In contrast, in the hot water treatment, the K concentration ranged from 10.40 to 12.40 g/kg at 0 min and 6 h, respectively (Fig. 5C). In *G. funicularis*, cold water treatment showed a decreasing trend in K concentration, whereas in hot water treatment, the concentrations showed an increasing trend over time (Fig. 5D). The potassium concentration in *G. funicularis*-derived biochar under cold water soaking decreased from 163.9 g/kg at 0 min to 88.64 g/kg after 6 h of soaking (Fig. 5D). Meanwhile, the hot water soaking K concentration ranged from 70.19 g/kg at 0 h to 86.32 g/kg at 6 h, with only minor variations.

3.4 Heavy metals (Cr, Cd, Cu, Zn, Ni)

There was a significant influence ($P < 0.05$) of soaking duration on the Chromium concentration in biochar, however, seaweed species and water temperature did not result in any significant influence ($P > 0.05$) on Cr concentration of the biochar (Table 2). There was a significant interaction ($P < 0.05$) between the soaking duration and seaweed species, and also between water temperature and seaweed species on Cr content of the biochar (Table 2). However, soaking duration and water temperature, soaking duration, water temperature and seaweed species did not result in any significant interaction ($P > 0.05$) on Cr concentration in the biochar (Table 2). In both *L. pallida* and *G. funicularis*, Cr increased with time, regardless of the water temperature (Fig. 6A and B). In *L. pallida*, cold water treatment maintained a lower concentration than hot water treatment for the same soaking duration, in contrast to the trend observed in *G. funicularis* (Fig. 6A and B). In *L. pallida*, Cr concentration under hot water treatment starts at 0.22 mg/kg at 0 min, fluctuates, and then gradually increases to 0.39 mg/kg at 6 h

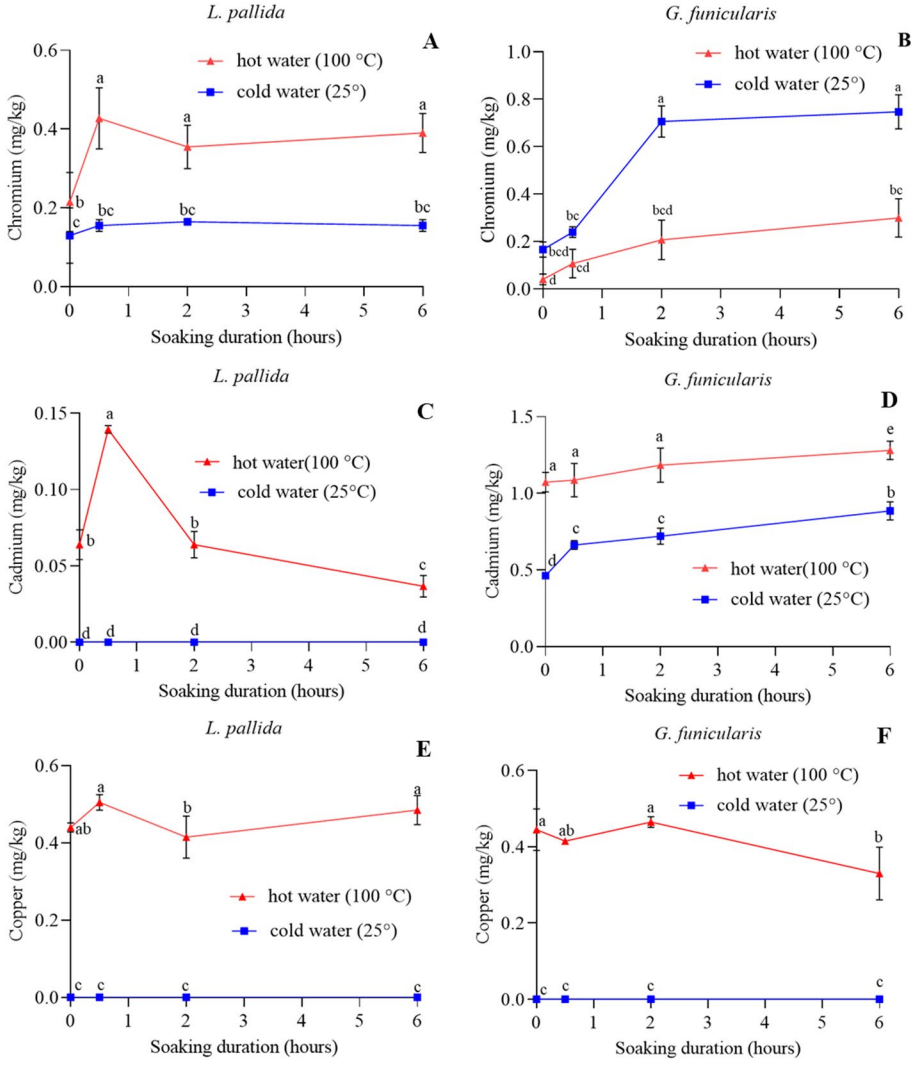

Fig. 6 Effect of soaking seaweed (*L. pallida* and *G. funicularis*) biomass under different water temperatures and soaking durations on the Cr (**A** and **B**), Cd (**C** and **D**), and Cu (**E** and **F**) concentrations in biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

(Fig. 6A). Similarly, cold water starts at 0.13 mg/kg at 0 min and then increases progressively to 0.16 mg/kg at 6 h (Fig. 6A). For *G. funicularis*, under cold water treatment, the observed Cr concentration was 0.17 mg/kg, 0.24 mg/kg, 0.71 mg/kg, and 0.76 mg/kg at 0, 0.5, 2, and 6 h, respectively (Fig. 6B). Under hot water treatment, the concentration starts at 0.04 mg/kg at no-soaking and rises progressively to 0.3 mg/kg at 6 h (Fig. 6B).

There was a significant influence ($P < 0.05$) of seaweed species on cadmium concentration in the biochar, though water temperature and soaking duration did not result in any significant influence on Cd concentration of the biochar ($P > 0.05$) (Table 2). There was a significant three-way interaction ($P < 0.05$) between the factors on Cd content of biochar (Table 2). In *L. pallida*, Cd concentration under hot water treatment decreased over time, whereas in *G. funicularis*, it increased over time (Fig. 6C and D). Under cold water treatment, no Cd was detected in *L. pallida*, whereas in *G. funicularis*, Cd increased with time while maintaining a lower concentration than hot water treatment for the same duration of soaking (Fig. 6C and D). In *L. Pallida*, no Cd was detected in the biochar for any of the soaking durations tested (Fig. 6C). Meanwhile, cadmium concentrations in hot water treatment showed a decreasing pattern from 0.064 mg/kg at 0 h to 0.037 mg/kg at 6 h (Fig. 6C). Cadmium concentration for *G. funicularis* ranged from 0.46 mg/kg at 0 h to 0.89 mg/kg at 6 h for cold water treatment, and from 1.07 mg/kg at 0 h to 1.28 mg/kg at 6 h for hot water treatment (Fig. 6C and D).

Water temperature had a significant influence ($P < 0.05$) on the copper content of biochar, though soaking duration and seaweed species had no significant influence ($P > 0.05$) on the Cu content of biochar (Table 2). There was a significant interaction ($P < 0.05$) between the soaking duration and seaweed species, soaking duration, seaweed species, and water temperature on the Cu concentration in biochar (Table 2). However, there was no significant interaction ($P > 0.05$) between soaking duration and water temperature, and also between water temperature and seaweed species on the Cu content of biochar (Table 2). Copper was not detected in any of the seaweed species under cold-water soaking treatment (Fig. 6E and F). Under hot water, the Cu content in *L. pallida* increased over time, while in *G. funicularis* it decreased over time; however, with fluctuations at intermediate soaking durations (Fig. 6E and F). Cu concentration in *L. pallida*, hot water treatment, Cu concentration increased from 0.44 mg/kg to 0.49 after 6 h (Fig. 6E). For *G. funicularis*, hot water treatment, the highest observed Cu concentration was 0.47 mg/kg after 2 h, and the lowest was 0.33 mg/kg after 6 h of soaking (Fig. 6F).

There was a significant influence ($P < 0.05$) of all three factors on the Zn concentration of seaweed biochar (Table 2). There was a significant interaction ($P < 0.05$) between soaking duration and water temperature, as well as between soaking duration and seaweed species, on the Zn concentration of seaweed biochar (Table 2). However, there was no significant interaction ($P > 0.05$) between water temperature and seaweed species, and also between water temperature, soaking duration and seaweed species on the Zn concentration of biochar (Table 2). In both species, the concentration of Zn increased with prolonged soaking duration, irrespective of water temperature (Fig. 7A and B). However, the cold water treatment resulted in a lower concentration compared to the hot water treatment for equivalent soaking durations. In *L. pallida*, under cold-water treatment, the lowest Zn concentration was observed at no-soaking with the value of 1.44 mg/kg, and the highest was observed at 6 h with the value of 3.09 mg/kg (Fig. 7A). Similarly, under the hot water treatment, no-soaking still had the lowest Zn concentration with

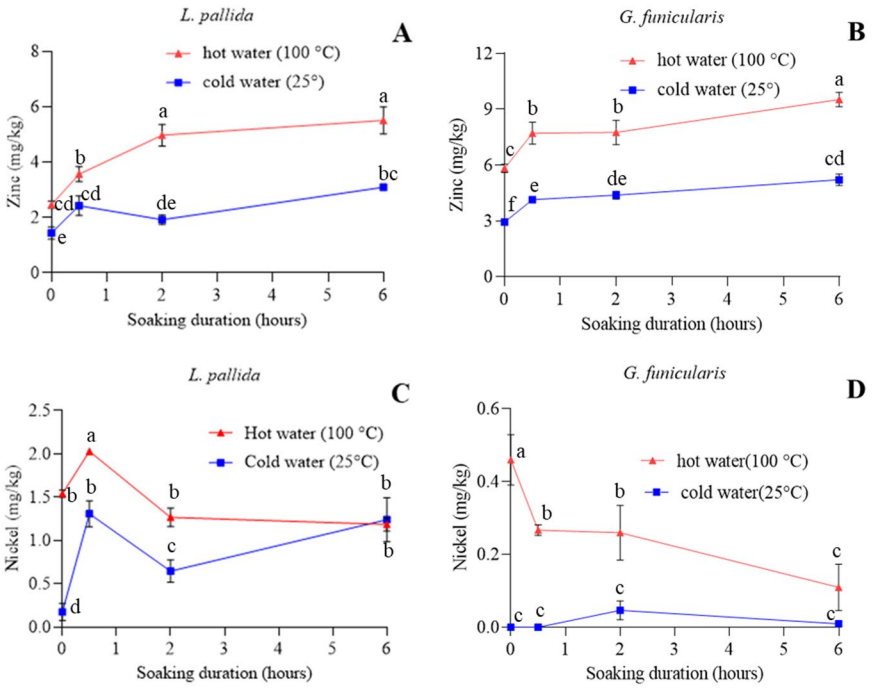

Fig. 7 Effect of soaking seaweed (*L. pallida* and *G. funicularis*) biomass under different water temperatures and soaking durations on the Zn (**A** and **B**) and Ni (**C** and **D**) concentrations in biochar. Error bars indicate standard deviation, while data points within each graph having different lowercase letters are significantly different ($P < 0.05$)

the value of 2.46 mg/kg, and 6 h had the highest Zn concentration with the value of 5.5 mg/kg. For *G. funicularis*, under cold water soaking, the Zn concentrations increased from 2.94 mg/kg at 0 min to 5.21 mg/kg after 6 h of soaking, whereas, under hot water, it increased from 5.83 mg/kg to 9.52 mg/kg for the corresponding soaking durations (Fig. 7B).

There was a significant influence ($P < 0.05$) of all three factors on the nickel content of seaweed biochar (Table 2). There was a significant three-way interaction ($P < 0.05$) between the factors affecting Ni concentration in the biochar (Table 2). For *L pallida*, in hot water, the Ni content initially increased and then decreased over time, whereas in cold water, it showed an increasing trend (Fig. 7C). Generally, cold water resulted in biochar with a lower Ni concentration than hot water for both seaweed species (Fig. 7C and D). For *L. pallida*, under the hot water treatment, the Ni concentration started at 1.54 mg/kg without soaking, fluctuated, and then decreased to 1.18 mg/kg after 6 h of soaking (Fig. 7C). On the other hand, in cold water treatment, the concentration started at 0.18 mg/kg, fluctuated, and then increased to 1.24 mg/kg after 6 h of soaking. For *G. funicularis*, hot water showed a decreasing trend over time, starting at 0.46 mg/kg at no-soaking, reaching 0.11 mg/kg after 6 h of soaking (Fig. 7D). In the cold-water treatment, no Ni was detected in *G.funicularis*-derived biochar; however, after extended durations, it varied slightly (Fig. 7D).

3.5 Optimal treatment summary

Based on the evaluated parameters, the most agronomically suitable biochar from *Laminaria pallida* was produced after hot-water soaking (100 °C) for 6 h. This treatment yielded a moderately high pH (11.35) and an acceptable electrical conductivity of

43.73 mS/cm, indicating reduced soluble salt content compared with unsoaked biomass. The biochar exhibited high extractable nitrogen (43.02 mg/kg), extractable potassium (123.39 g/kg), and phosphorus (68.70 mg/kg), providing a balanced nutrient profile. Exchangeable sodium (39.87 g/kg) was lower than in several treatments, minimising salinity risk. Heavy metals remained below the International Biochar Initiative, IBI, [18] permissible limits, (Cd < 38 mg/kg, As < 100 mg/kg, Ni < 420 mg/kg, Cr < 1200 mg/kg, Pb < 300 mg/kg, Zn < 7400 mg/kg, and Cu < 600 mg/kg) confirming their safety for soil application.

For *Gracilariopsis funicularis*, the optimal biochar was derived from cold-water soaking for 6 h. This treatment produced biochar with a moderately alkaline pH (9.3) and the lowest EC (29.3 mS/cm) among all cold-water treatments, indicating effective desalination of the biomass. It also achieved the highest total carbon (43.74%), total nitrogen (5.17%), and extractable phosphorus (127.01 mg/kg), reflecting enhanced nutrient retention and agronomic potential. Exchangeable Na (12.6 g/kg), Ca (0.55 g/kg), and Mg (0.26 g/kg) were comparatively low, further reducing salinity risk. Additionally, a notably high Zn concentration (5.21 mg/kg) was recorded, enhancing the potential to improve soil micronutrient availability. All heavy metal concentrations were below the IBI [18] limits.

Overall, among the two species, *G. funicularis* biochar from cold-water soaking (6 h) emerged as superior owing to its lower EC and Na content, more moderate pH, and balanced macro- and micronutrient composition, which collectively enhance its agronomic suitability while minimising salinity hazards. In contrast, *L. pallida* biochar, despite its higher nutrient content (notably N, K, and P), exhibited excessively high pH and EC which may restrict its immediate application in neutral or slightly alkaline soils without further pre-treatment techniques. Therefore, *G. funicularis* represents a more promising feedstock for producing agronomically and environmentally favourable biochar under the tested pre-treatment conditions.

4 Discussion

4.1 Effects of seaweed soaking pre-treatment on the pH, EC, total C, and total N of resultant biochar

Nutrient availability, microbial activity, and crop performance depend on the soil pH [20]. This study illustrated that the pH increased with soaking duration, likely due to the progressive leaching of soluble acidic compounds (e.g. organic acids, sulfates, and halides) from the seaweed biomass, resulting in a more alkaline biochar [9, 50]. Soaking in hot water resulted in higher pH values than in cold water, likely due to enhanced diffusion and solubilisation of mineral-bound salts and cations, which promoted the formation of basic oxides during pyrolysis [9, 50]. Biochar derived from the two seaweed species exhibited distinct pH responses to the treatments. *G. funicularis* produced moderately alkaline biochar (pH 8.6–9.47), consistent with the values reported by [6] for beach-cast seaweed biochar. In contrast, *L. pallida* showed higher alkalinity (pH 10.98–11.35), aligning with the findings of [36], who reported similar increases in pH following pyrolysis of brown algal biomass. This indicates that the pH response of biochar to soaking is influenced by the intrinsic chemical composition of each species, the thermal effects associated with soaking, and the duration of exposure. In addition, the differences in biochar pH between species likely reflect variations in the initial pH of the raw

biomass, with *L. pallida* showing higher pH than *G. funicularis* (Table 1). [22] reported that *G. funicularis*-derived biochar exhibited a neutral pH, which contradicts the current study's findings, which showed the lowest pH of 8.6 for the same species and pyrolysis temperature. The higher pH observed in our study may be attributed to the water soaking pre-treatment, as well as the higher alkali minerals content, as observed in Figs. 4 and 5. Pre-treatment potentially reduces acidifying agents such as chlorine and sulfur, allowing more alkaline elements such as Ca, K, Mg, and Na to predominate and increase the pH of the biochar [17, 41]. However, to some extent, this study demonstrated results similar to those of [22] because both studies observed that *L. pallida*-derived biochar has a higher pH than *G. funicularis*-derived biochar, and this may be linked to species-specific properties of seaweed. Brown macroalgae, such as *L. pallida*, typically contain higher levels of ash-forming minerals (e.g. Na, K, Ca, and Mg) and structural polysaccharides, such as alginate, which decompose during pyrolysis to release basic oxides and carbonates, thereby elevating the biochar pH [43]. In contrast, red macroalgae, such as *G. funicularis*, are richer in sulfated polysaccharides (e.g. agar and carrageenan) and therefore tend to yield biochar with relatively lower alkalinity [9].

The findings show that biochar from *G. funicularis* and *L. pallida* with moderately alkaline pH can be effectively used in agriculture as a liming material for acidic soils or can be used to adjust the pH in hydroponics, where acidification due to nitrogenous fertilizer applications is an issue [19, 47]. Alternatively, integrating biochar with moderate alkalinity, such as that derived from *G-funicularis*, cold water, 6 h treatment, into vermicomposting technologies may buffer its pH to a range of 5.5–7.5, which is optimal for most vegetables [27], while simultaneously formulating ideal amendment. The effectiveness of incorporating this biochar into vermicomposting systems to produce a well-balanced soil amendment should be validated in future experimental studies. Electrical conductivity quantifies the availability of soluble salts in soils and is a strong indicator of fertility and salinity in amendments [7]. EC values were species specific, with higher levels in *L. pallida* biochar than in *G. funicularis*, consistent with the raw biomass EC of 37.4 mS/cm and 25.1 mS/cm, respectively (Table 1). This study showed that cold water soaking treatments decreased the EC of seaweed biochar, particularly with extended soaking durations, while showing opposite trend under hot water treatment. This emphasizes that the trend in EC during soaking is significantly affected by a three-way interaction among soaking time, species, and temperature ($P < 0.05$). For instance, in cold-water treatment, EC declined over time, likely due to leaching of soluble cations and other ionic compounds from the seaweed biomass, facilitated by cell wall swelling [2, 17]. In contrast, in the hot-water treatment, EC increased with time, possibly due to enhanced solubilization of mineral salts and thermal breakdown of cell wall structures releasing additional cations into the biochar matrix [17]. The high EC observed in biochar derived from untreated biomass (0 min) corresponds to the high EC (73.5 mS/cm-107.4 mS/cm) observed in untreated biochar as reported by [2]. Meanwhile, the reduced EC (29.3 mS/cm-41.1 mS/cm) in seaweed biochar at prolonged soaking durations in cold water treatment contradicts [2] findings which reported higher EC values (73.5 mS/cm-107.4 mS/cm) in marine algae-derived biochar. [5] also supported the findings of the current study, as they indicated that marine seaweed biochar had an elevated EC (42 mS/cm-53 mS/cm), possibly because of its higher mineral composition. [22] reported that biochar from *G. funicularis* had an EC of 24.3 mS/cm, while *L. Pallida*

showed 36.6 mS/cm. These align with current findings for 0-minute treatments, showing average EC value 25.3 mS/cm for *G. funicularis* and 37.7 mS/cm for *L. pallida*.The high EC observed in the reduced cold water soaking may be attributed to the chemical composition of seaweed, such as the high cation and ash content in biochar, as insufficient soaking time limits ion release from the biomass [2]. All EC values recorded in this study exceeded the maximum permissible limit of 4 dS/m for most vegetables [10]. This finding suggests that the direct application of seaweed biochar may pose agronomic risks, such as osmotic stress, impaired germination, and reduced root water uptake in salt-sensitive crops [7]. Nevertheless, such biochar could be beneficial for amending acidic soils or cultivating salt-tolerant species (e.g. wheat, barley, and date palm) and halopytes, where moderate salinity can enhance ionic balance and nutrient exchange [10]. Integrating water-soaking with post-treatments such as vermicomposting or blending with low-salinity organic matter could further buffer salinity and expand the safe applicability of seaweed biochar across a wider range of crops [38]. The higher carbon content in biochar allows it to enhance soil aeration, water retention, and drainage, whereas nitrogen is a vital element for chlorophyll, amino acids, and DNA/RNA synthesis in plants [53]. However, the suitability of biochar amendment depends on its C/N ratio [14]. Generally, the C/N ratio in seaweed-derived biochar increased with prolonged soaking for both species. This is likely due to the leaching of nitrogen-rich soluble compounds, such as proteins and amino acids, while carbon, mostly present in structural forms, remains largely unaffected, as reported for beach-cast seaweed biochars [6]. Species-specific composition and treatment conditions further modulate these changes. Most treatments in this study showed C/N ratios < 20, which characterizes a suitable biomass for soil amendment [41]. These findings align with those of [41], who reported that biochar from red seaweed has a C/N ratio < 20; however, these findings contradict the C/N ratios obtained for commercially cultivated brown seaweed (C/N ratio up to 74) used by [41]. Moreover, the current study noted that *G. funicularis* derived biochar yielded a slightly lower C/N ratio than *L. pallida*. These findings are supported by [22], who reported about a 15.6 C/N ratio and an 8.4 C/N ratio in *L. pallida* and *G. funicularis*, respectively. Abu-Zied et al. [1] have documented that brown macroalgae, such as *L. pallida*, typically possess higher carbon content and C/N ratios compared to red species, such as *G. funicularis*. However, the current study reveals that *G. funicularis* exhibits marginally higher total carbon and nitrogen than *L. Pallida* (Table 1). Despite this discrepancy, the C/N ratio of *G. funicularis* remains lower than C/N ratio for *L. pallida*, likely attributable to its relatively elevated nitrogen content, the findings are partially in line with the pattern identified by Mondal et al. [30]. The results for Laminaria, which exhibit a C/N ratio > 20 under hot water, are not consistent with the findings of Cárdenas-Aguiar et al. [6], who reported that all seaweed biochar C/N ratios were strictly < 20.The differences between the current study and others were attributed to the use of different seaweed species, environmental growth conditions, pyrolysis methods, and pre-treatment employed in the current study. The findings of this study provide insights that seaweed-derived biochar can be an effective amendment for locking organic carbon in the soil, particularly that from *L. pallida*, due to its high carbon content. Moreover, a C/N ratio of 8.41–11.11, similar to that of *G. funicularis*, indicates a nitrogen-rich biochar that promotes immediate nutrient availability, making it ideal for boosting short-term plant growth without risking nitrogen immobilization [45]. These C/N characteristics also indicate differing

effects on microbial activity and soil fertility. Biochars with lower C/N ratios, such as that of *G. funicularis*, tend to stimulate microbial growth and enhance nutrient turnover, whereas those with higher C/N ratios, such as *L. pallida*, may contribute more to carbon stabilisation and long-term soil organic matter buildup [14].

4.2 Effects of seaweed soaking pre-treatment on the macro elements (P, N, and cations) of resultant biochar

Macro elements are required by plants in large amounts, and their shortage can negatively affect crop growth and development; however, in excess, some cations, such as Na, can harm soil and crop health [39, 48]. In this study, the water-soaking treatment increased the extractable P content in seaweed biochar while reducing NH_4-N, NO_3/NO_2-N, and cations, particularly at prolonged durations. Although extractable P increased with soaking duration, the extractable P values (53.52 mg/kg- 127.01 mg/kg) observed were generally lower than those reported by [22] (140 mg/kg –1200 mg/kg), despite the same seaweed species being used. This is likely because the water-soaking treatment possibly leached soluble phosphorus from the biomass before pyrolysis. The increasing trend in extractable P is likely due to the water soaking pre-treatment, which probably removed salts and allowed P mobilization in the biomass, which, in turn, increased extractable P in the biochar [17]. Similar to the extractable P case, our study reported lower values of NH_4-N and NO_3/NO_2-N (0 mg/kg- 64.44 mg/kg) compared to [22], who reported NH_4-N and NO_3/NO_2-N within the range of 390 mg/kg-1230 mg/kg at the same pyrolysis temperature for the same seaweed species. This was attributed to the high solubility of NH_4-N and NO_3/NO_2-N in water and ion exchange between seaweed biomass and water due to the concentration gradient [50]. The relatively higher N and extractable P retention under cold-water soaking likely reflects reduced leaching losses, as elevated temperatures enhance their solubility and volatilization [50].

Regarding cations, our study findings align with those by [46], which reported that biochar from seaweed contains K and Na as a large proportion of cations, with *L. pallida* showing higher concentrations than *G. funicularis*. The cation values in this study were slightly lower than those reported by [2], potentially because of the mineral content variability in the seaweed used. It is important to note that the control (no-soaking) had higher cation values than biochar subjected to water-soaking treatments. This was also attributed to the pre-treatment used in this study, as water soaking possibly dissolved cations and initiated cation exchange between biomass and water owing to the concentration gradient [50]. Despite the pre-treatment, relatively elevated sodium concentrations were still detected in both biochars, with the highest concentration observed in *L. pallida*. Similar persistence of Na, Ca, and Mg has been reported by [43] and is attributed to the presence of alginate salts in brown algal cell walls, where these cations are structurally bound to alginic acid and therefore not easily removed by water treatments. Such Ca concentrations in our study showed lower values than the findings of [2], who reported that macro-algae biochar contained Ca ranging from 15.1 g/kg-79.4 g/kg. This may be linked to different seaweed species used in their study, as well as the solubility of Ca ions in water [16]. The observed significant interactions ($P < 0.05$) among species, soaking time, and temperature suggest that the behaviour of macro-elements in seaweed-derived biochar is collectively influenced by the intrinsic chemical composition of each species, the duration of soaking, and the thermal conditions. This implies that

the impact of soaking time on nutrient retention varies between species and between cold and hot treatments, highlighting the complex compositional and physicochemical interactions within the biomass.

These findings indicate that seaweed-derived biochar can serve as a good amendment for enriching.

soils with extractable P and cations. Biochar amendment for *G. funicularis* when subjected to cold treatment for 6 h showed potential agronomic use, illustrating a better combination of macronutrients, that is, higher extractable P, moderate salinity in terms of Na content (12.16 g/kg), and elevated K content. Although the 2 h hot-water treatment recorded the lowest Na concentration, the 6 h cold-water treatment was preferred for its overall balanced physicochemical properties, mainly favorable pH, EC, TN, TC, extractable P. However, the reported lower values for Ca, NH_4-N, and NO_3/NO_2-N of up to 0 mg/kg, particularly for extended soaking durations, necessitate the integration of Ca- and N-rich amendments, such as vermicompost, to optimize crop nutritional requirements. The residual sodium present in seaweed biochar, can be mitigated through vermicomposting before its application to the soil [5, 22].

4.3 Effects of seaweed soaking pre-treatment on the heavy metal content of resultant biochar

Heavy metal accumulation, including elements such as Cr, Cd, Pb, Ni, Cu, As, and Zn, is associated with reduced plant growth, interference with soil microbial processes, and severe human health issues, such as cancer and kidney failure [23]. Nonetheless, certain heavy metals such as Cu and Zn play essential roles in biological processes, especially when present in trace amounts [34]. According to the IBI, (2015), heavy metals in amendments are considered more detrimental when they exceed the maximum permissible limit; otherwise, below those levels, they are safe for soil applications. In our study, all the selected heavy metals had concentrations below the maximum permissible limits for organic amendments: Cd < 38 mg/kg, As < 100 mg/kg, Ni < 420 mg/kg, Cr < 1200 mg/kg, Pb < 300 mg/kg, Zn < 7400 mg/kg, and Cu < 600 mg/kg in all treatments (IBI, 2015). Although all metal concentrations were below safety thresholds, their accumulation in the biochar was significantly influenced by the interactions of seaweed species, soaking duration, and water temperature, with notable two- and three-way interactions observed for Cu, Zn, Ni, Cd, and Cr ($P < 0.05$). These interactions suggest that the concentration of each metal is contingent not solely on an individual factor but also on the combination of species and treatment conditions. This finding implies that the selection of seaweed species and soaking conditions can mitigate heavy metal risks, thereby ensuring that marine biomass derived biochar remains safe for agricultural applications. In addition, due to these interactions, distinct patterns emerged across treatments and species, likely reflecting the variations in intrinsic metal content, solubility, and leaching dynamics among different species.

These findings are in line with the results of [22], who reported that heavy metals in seaweed biochar were below the maximum permissible limit; however, for most elements in our study, heavy metal concentrations were slightly lower than those reported by [22]. These discrepancies in heavy metal levels may be attributed to the water pretreatment applied in our study; the soaking process likely facilitated ion exchange and leaching of loosely bound metal ions from the seaweed matrix, thereby reducing the

heavy metal content in the resulting biochar [50]. In addition, the lower heavy metal concentrations observed in this study can be attributed to the inherently low levels present in the raw seaweed biomass, which were already below the maximum permissible limits prior to pre-treatment (Table 1). Our findings are further supported by [30], who reported that macro-algae-derived biochar is safe for use as a soil ameliorator owing to its negligible amounts of heavy metals. The observed differences in retention and leaching of metals such as Cu, Zn, Cd, and Cr are influenced by their chemical speciation, solubility in water, and binding affinity within the biochar matrix [12, 13]. Biochar can immobilise metals through adsorption onto surface functional groups or incorporation into stable mineral complexes, while soaking selectively removes labile metal fractions [12, 13]. For instance, Zn and Ni were partially retained under prolonged soaking, whereas Cu was more readily leached under cold-water treatment, reflecting differences in metal bioavailability within the biochar as reported by [12]. These mechanisms provide insights into how soaking treatments and species-specific biomass composition influence the safety and agronomic value of seaweed-derived biochar.

Therefore, the findings of this study imply that seaweed biochar can be safely used in the soil environment without harming living organisms, owing to its low heavy metal content, particularly when subjected to optimized water-soaking treatments. This suggests that seaweed biochar has the potential to enrich soils with Zn, which is crucial for the functioning of various enzymes that facilitate metabolic functions in crops, particularly that from *G. funicularis* that was subjected to cold water treatment for 6 h [34]. Cold water soaking treatments completely leached out Cu, despite the crucial role of Cu in plants [26]. This emphasizes that hot water treatment may be significant due to its ability to conserve Cu in the amendment, especially in *L. pallida* at 6 h of soaking, while considering soil safety precautions. Additionally, applying cold water-treated biomass-derived amendments in combination with other eco-friendly Cu-supplier amendments may render it a perfect amendment.

5 Conclusions and recommendations

This study demonstrated that incorporating a simple water-soaking step before pyrolysis can reduce salinity and enhance nutrient availability in seaweed biochar, making it more suitable for agricultural use. Biochar from *G. funicularis* soaked in cold water for 6 h showed the most favorable characteristics, including high nutrient content, reduced salinity (EC 29.3 mS/cm), and a moderately alkaline pH (9.3). Although sodium levels and EC remained high, further optimization through techniques such as vermicomposting is recommended in future studies. *L. pallida* biochar exhibited nutrient potential; however, its higher salinity and alkalinity may limit application to salt-sensitive crops. It could, however, serve as soil amendment for growing halophytes or other salt-tolerant crops. On the other hand, the proposed water-soaking pre-treatment presents potential limitations, particularly regarding the management and reuse of the effluent water streams generated during the washing process. Effective recycling or treatment of this wastewater is essential to prevent nutrient loss and minimise environmental impact; therefore, it needs to be explored in future studies. Future work should focus on improving desalination strategies, including microbial pretreatments, to enhance the agronomic value of marine biochar, and evaluating field-level performance of marine biomass-derived biochar in sustainable crop production systems. Future research

should investigate the cultivation of these seaweeds species to support sustainable large-scale biochar production and evaluate the ecological impacts of seaweed harvesting on marine ecosystems.

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Author contributions
E.N.M.R wrote the main manuscript text, including all figures and tables; B.H. helped in experimental setup, data collection, and sample analysis. H.A.M- wrote the main manuscript text; reviewed the manuscript; sourced for funding and supervision and S.H reviewed the manuscript and funding acquisition.

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Data availability
Data from this study will be made available on request.

Declarations

Declarations of generative AI and AI-assisted technologies in the writing process
During the preparation of this work, the author(s) used Open Paper Pal in order to improve the English readability of the manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Ethics approval and consent to participate
The experiments conducted in this study were performed in accordance with ethical standards of the University of Namibia, under ethical clearance reference number OGC0033. The experiments conducted in this study involving plant materials were performed in accordance with the ethical standards of the University of Namibia, as well as international guidelines and legislation. Adherence was done to the Convention on Biological Diversity (CBD), the Nagoya Protocol on Access and Benefit-sharing, and the International Plant Protection Convention (IPPC). We confirm that all experiments were conducted with due regard for minimizing harm and maximizing the ethical treatment of plant materials.
The procurement, handling, and disposal of plant materials were carried out in accordance with the highest ethical standards.

Consent for publication
The authors declare that this is the original research study and that it has neither been submitted nor been concurrently submitted in any other journal for publication.

Competing interests
The authors declare no competing interests.

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