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Saltwater intrusion induces shifts in soil microbial diversity and carbon use efficiency in a coastal grassland ecosystem

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ABSTRACT

Salt accumulation and salinisation of coastal soils is a global issue. Further, climate change is likely to increase the amount of land affected by salinity due to the increasing frequency and severity of coastal flooding and brackish water ingress. The impact of this on the ability of soils to deliver ecosystem services, particularly carbon (C) storage, however, remains unclear. We hypothesized that coastal inundation would negatively affect C storage by lowering plant C inputs and by placing greater osmotic stress on the microbial community leading to a reduced C use efficiency (CUE). Here, we use a coastal grassland ecosystem, which is becoming increasingly subjected to sea and brackish water flooding, to explore the relationship between plant/microbial growth and CUE along a natural salinity gradient. To reflect steady state conditions, we traced the turnover and partitioning of a low (ambient) dose and high (growth stimulation) dose of ^{14}C-labelled glucose into microbial anabolic and catabolic pools, from which microbial CUE was calculated. This was supported by measurements of the diversity of the bacterial community across the salinity gradient using 16S metabarcoding. Our results showed that coastal flooding significantly reduced plant growth ($p < 0.005$), increased soil C content ($p < 0.05$) and induced an increase in microbial CUE under low glucose-C conditions ($p < 0.05$). Conversely, no significant difference in CUE or microbial growth was apparent when a high glucose-C dose was used. Soil bacterial community alpha (α) diversity increased with soil salinity while beta (*β*) diversity also shifted in response to the higher saline conditions. Our analysis suggests that the largest impact of coastal flooding on soil C cycling was the inability of the plant community to adapt, leading to higher plant residue inputs as well as the decline in soil structure. Conversely, the microbial community had adapted to the increased salinity, resulting in only small changes in the uptake and metabolic partitioning of C.

1. Introduction

It is estimated that 33% of irrigated land and up to 20% of total cultivated land globally is suffering from salinisation; the excess accumulation of salt in soils (Otlewska et al., 2020). This phenomenon is likely to be further exacerbated through climate change induced sea level rise and an increased frequency and severity of extreme weather events leading to vulnerability to coastal flooding in many regions of the world (Chen and Zong, 1999; Vitousek et al., 2017; Zemp et al., 2019). For example, in the UK, coastal flood risk is expected to increase over the next century and beyond, under all Intergovernmental Panel on Climate Change (IPCC) representative concentration pathway (RCP) climate change scenarios, mainly due to changes in time-mean sea level (predicted to be up to 0.5 m under RCP2.6–4.3 m under RCP8.5) (Met Office, 2018a). Consequently, coastal flooding is recognised as one of the top priority risks for many nations worldwide (Kirezci et al., 2020). Additionally, a large proportion of productive agricultural land globally is situated in low lying and reclaimed coastal regions (Gould et al., 2020). As such, soil salinisation in low lying coastal areas is likely to be an important global challenge in the context of food security and sustainable development for the foreseeable future (Karim and Mimura, 2008; Kirezci et al., 2020).

The progressive accumulation of salt in soil is known to directly impact soil quality, defined here as the capacity of the soil to function (Karlen et al., 1997). For example, the saline intolerance of unadapted plant roots and soil macrofauna is likely to lead to a reduction in

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bioturbation and aeration, eventually leading to a decline in the physical structure of soil (Otlewska et al., 2020). Additionally, salt-induced dispersion of soil particles can lead to a clogging of soil pores and a reduction in hydraulic conductivity (Qadir and Schubert, 2002) which alters the solubility and accessibility of soil organic matter (SOM) (Mavi et al., 2012; Wong et al., 2009, 2010). Consequently, a negative feedback mechanism may occur leading to a further downward trend in physical, chemical and biological soil quality, changing the nature of ecosystem service provision.

Salinity-induced changes (i.e., ion toxicity, osmotic and oxidative stress) to the size, structure, activity and functioning of the soil microbial community are known to have significant consequences on terrestrial carbon (C) cycling (Yan et al., 2015). In some circumstances, the controlled ingress of seawater onto former agricultural land (i.e., managed sea realignment) can lead to a major increase in soil organic C storage (Andrews et al., 2008), however, conversely it may also lead to a depletion of soil C stocks (Sjøgaard et al., 2017). Consequently, more research is needed to understand the complex feedbacks that may occur in the plant-soil C cycle upon coastal flooding.

Carbon use efficiency (CUE), the efficacy with which microorganisms metabolise available organic substrates into stable biosynthetic products, is critical to ecosystem C cycling and C storage (Geyer et al., 2016). The amount of additional biomass C produced per unit substrate C metabolised ultimately determines the rate of C accumulation (via SOM) or loss (via CO_2/CH_4 efflux) from a soil (Poeplau et al., 2019). Ultimately, CUE is a critical control on the capacity of soil and wider ecosystem to store C (Bradford and Crowther, 2013; Manzoni et al., 2012; Wang et al., 2021). Little work has been performed to evaluate the changes in microbial CUE in saline stressed soils. However, environmental drivers have been shown to potentially uncouple growth and respiration, changing CUE (Sinsabaugh et al., 2013).

Microbial community stability and resilience determines how a soil responds to and recovers from environmental stresses. Previous work on community stability has shown mixed effects, depending on the nature of the disturbance (e.g. heavy metal vs. salt loading) (Tobor-Kaplon et al., 2005, 2006). It has been suggested that in recently or currently stressed systems, organisms have less energy to cope with further disturbance, as in the first instance energy will be allocated to detoxification and repair, rather than be immediate growth (Griffiths and Philippot, 2013). However, there is evidence to show that communities have the potential to adapt or become tolerant to environmental conditions resulting in a more stable community, with a high proportion of energy available for growth (i.e. higher CUE) (Kallenbach et al., 2019). Previous studies have shown that a shift in the composition of the soil bacterial community and reduction in phylogenetic diversity occurs with increasing salinity in naturally saline environments (Canfora et al., 2014; Hollister et al., 2010; Rath et al., 2019a, 2019b; van Horn et al., 2014). However, the response of soils to periodic coastal inundation and salt stress remains poorly understood.

This study aimed to assess the impact of salinisation on the soil microbial community and its resultant CUE across a gradient of salinity in a coastal soil becoming increasing susceptible to periodic saltwater flooding from sea level rise and storm surges. We hypothesized that; i) CUE would decrease under higher salt conditions, due to increased levels of environmental stress, and ii) the soil microbial community under higher salt-stress would exhibit change and increased tolerance compared to the unstressed community.

2. Materials and methods

2.1. Study site

The study site was located at the Henfaes Agricultural Research Station, Abergwyngregyn, North Wales (53°14′ 30′′N, 4°01′22′′W). It comprises a sheep-grazed grassland agricultural field located next to the Menai Strait, which forms part of the Irish Sea (Fig. S1) and is adjacent to the Afon Rhaeadr-fawr river. The site has experienced an increased frequency of tidal storm surges and associated brackish riverine flooding over the last decade, leading to the progressive ingress of salt water onto productive agricultural land (Ganguli and Merz, 2019; Hendry et al., 2019; Met Office, 2018b). This area has also been identified by Welsh Government as an area of very high risk from coastal flooding (NRW, 2014, 2020) due to its low-lying nature (<2 m a.s.l) and lack of coastal protection. The site is under permanent grassland, infrequently grazed with livestock, vegetation is dominated by *Lolium perenne* L.. The soil is classified as a sandy loam textured Eutric Cambisol (Typic Hapludalf) developed on a mixture of glacial till and windblown sand. The site has a temperate-oceanic climate regime with mean annual rainfall of 1060 mm and mean annual temperature of 10 °C (10 y average).

Three separate areas with clear salt accumulation at the soil surface were chosen for this study. Within each of these individual field areas, three individual linear transects were demarcated at least 3 m from each other (i.e. nine transects in total). Each transect had a visually clear gradient in salt accumulation and vegetation cover (Fig. 1, Fig. S2). Samples of soil were collected from the Ah horizon (0–10 cm depth) at four locations along this gradient, representing different levels of visual vegetation damage and surface salt accumulation (Fig. 1). The percentage vegetation cover along the transect was assessed using gridded 60 × 60 cm quadrats with 400 individual measurement squares. The relative amount of vegetated and bare soil squares was measured for each quadrat alongside a photographic record of the transects.

2.2. Soil characterisation

Bulk density cores (0–5 cm, 100 cm^3) were oven dried (105 °C, 24 h) before being sieved, to 2 mm, to remove stones, and weighed. After collection, fresh soil was homogenised and sieved to pass 8 mm to remove stones, mesofauna and roots. This sieve size was chosen to minimise changes in microbial activity (Jones and Willett, 2006). Additionally, a subsample of fresh sieved soil was further sieved to 2 mm, to further homogenise and remove stones and vegetation, before being stored at −80 °C to await bacterial sequencing. Soil pH and electrical conductivity (EC) were measured on 1:5 (w/v) soil-to-ultrapure water (UPW; >18 MΩ cm^{-1} resistance) suspensions by submerging standard electrodes. Soil salinity across the patch gradients is visualized in Fig. 2. Subsequently, 1:5 (w/v) soil-to-0.5 M acetic acid (AcOH) and 1:5 (w/v) soil-to-UPW extracts were performed to measure nutrient availability (MISR/SAC, 1985). Extractable nitrate (NO3–N) and ammonium (NH4–N) concentrations within the UPW extracts were determined by the colorimetric methods of Miranda et al. (2001) and Mulvaney (1996), respectively. Bioavailable phosphate (PO_4–P) concentrations within the AcOH extracts were determined using the molybdate blue colorimetric method of Murphy and Riley (1962). Exchangeable soil cations (Na, K and Ca) were measured on the AcOH extracts using a Sherwood Model 410 Flame Photometer (Sherwood Scientific Ltd, Cambridge, UK). Dissolved organic C (DOC) and total dissolved N (TDN) were determined on UPW extracts using a Multi N/C 2100S Analyzer (AnalytikJena, Jena, Germany). Soil moisture content was determined gravimetrically on the sieved soils by oven drying (105 °C, 24 h). The C and N content of the soil was determined on oven-dried soil using a TruSpec CN analyzer (Leco Corp, St Joseph, MI). All chemical and physical analysis described was performed within 48 h of soil collection, during which soil samples were stored at 4 °C.

2.3. Soil microbial activity and carbon use efficiency

To determine microbial activity and CUE, we measured the mineralization of ^{14}C-labelled glucose in each soil sample. Briefly, 5 g of each soil was placed in individual sterile 50 cm^3 polypropylene tubes. Subsequently, 0.5 ml of uniformly ^{14}C-labelled glucose solution (10 kBq ml^{-1}) with either a low (100 μM) or high concentration (100 mM) was added to the soil surface. After addition of the ^{14}C-labelled glucose, a 1

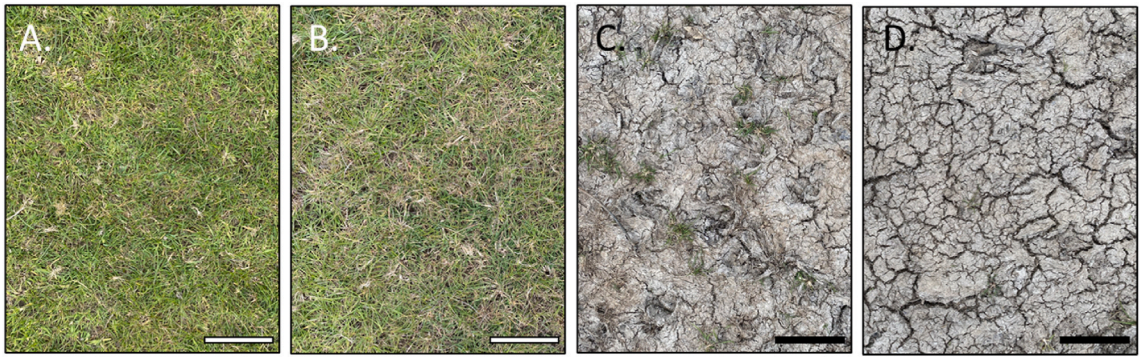

Fig. 1. Visual representation of the salinity gradient sampled. A) Control, B) Control Edge, C) Salt Edge and D) Salt patch, scale bars represent 10 cm.

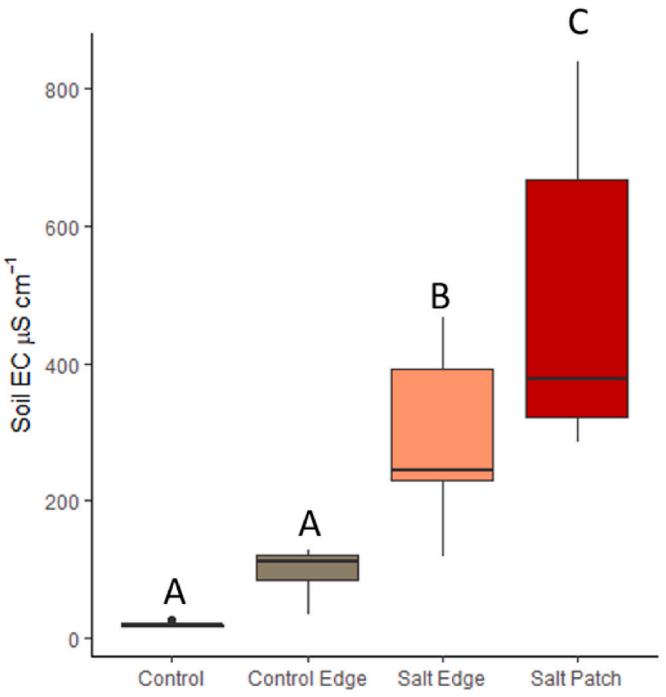

Fig. 2. Variation in electrical conductivity (EC) between location treatments along a natural salinity gradient caused by coastal flooding ($n = 9$). Horizontal lines show the median, boxes the 25th to 75th percentiles, whiskers the 5th to 95th percentile range. Letters denote significant differences between location treatments.

M NaOH trap (1 ml) was suspended above the soil to catch any respired $^{14}CO_2$. The tubes were then hermetically sealed and incubated at room temperature (20 ± 1 °C). The NaOH traps were replaced periodically (after 1, 3, 6, 9, 24, 34, 48, 58, 72 h and subsequently every 24 h) for one week after glucose application. The efficiency of the NaOH traps was >98% (as determined by collecting $^{14}CO_2$ generated from adding excess 0.1 M HCl to 0.001 M $NaH^{14}CO_3$). The amount of ^{14}C in the NaOH traps was measured using Optiphase HiSafe 3 liquid scintillation cocktail (PerkinElmer Inc., Waltham, MA, USA) and a Wallac 1404 scintillation counter (Wallac EG&G, Milton Keynes, UK) with automated quench correction. At the end of the incubation period, the soils were extracted with ice-cold 1 M NaCl (200 rev min^{-1}, 15 min), centrifuged (24,000 g, 5 min, 4 °C) and the ^{14}C in the supernatant measured by liquid scintillation counting as described above (Rousk and Jones, 2010). Glucose was used as the C substrate due to its almost ubiquitous use by soil microorganisms and it represents the largest C input to soil in a polymeric form (Gunina and Kuzyakov, 2015). The low glucose concentration was chosen to reflect ambient soil solution concentrations measured at the same location (Boddy et al., 2007) and which is not predicted to induce significant microbial growth. The high glucose concentration reflects that in root cell sap (Jones and Darrah, 1996) and is predicted to induce microbial growth.

2.4. 16S amplicon sequencing

Fresh soil was placed into a MoBio PowerMag Soil DNA Isolation Bead Plate (MoBio Laboratories Inc., Carlsbad, CA). DNA was extracted following MoBio's instructions on a KingFisher Flex robot (Thermo Fisher Scientific Corp, Waltham, MA). Bacterial 16S rRNA genes were PCR-amplified with dual-barcoded primers targeting the V4 region (515F 5′-GTGCCAGCMGCCGCGGTAA-3′, and 806R 5′-GGACTACHVGGGTWTCTAAT-3′), as per the protocol of Kozich et al. (2013). Amplicons were sequenced with an Illumina MiSeq using the 300-bp paired-end kit (v.3) (Illumina Inc., San Diego, CA). Sequences were denoised, taxonomically classified using Silva (v. 138) as the reference database, and clustered into 97%-similarity operational taxonomic units (OTUs) with the mothur software package (v. 1.44.1) (Schloss et al., 2009).

The potential for contamination was addressed by co-sequencing DNA amplified from samples and from template-free controls (negative control) and extraction kit reagents processed the same way as the samples. A positive control consisting of cloned SUP05 DNA, was also included. OTUs were considered putative contaminants (and were removed) if their mean abundance in controls reached or exceeded 25% of their mean abundance in the samples. OTUs were filtered if they had fewer than 3 counts and occurred in fewer than 10% of the samples. Sequencing read files analysed in this study can be accessed from the National Center for Biotechnology Information (project PRJNA818181).

2.5. Statistics and data analysis

To describe glucose mineralization, a double first order kinetic decay equation model was fitted to the loss of ^{14}C from the soil (S_{min}; i.e. the inverse of $^{14}CO_2$ accumulation) where

$$S_{min} = (P_1 \times \exp_1^{-k \times t}) + (P_2 \times \exp_2^{-k \times t}) \quad (1)$$

And where P_1 describes the amount of ^{14}C allocated to the first mineralization pool and k_1 is the rate constant for P_1. Similarly, P_2 is the proportion partitioned into the second slower C mineralization pool described by rate constant k_2. The equation was fitted to the experimental data using a least squares iterative model in SigmaPlot v12.3 (Systat Software Inc., San Jose, CA). Dependency values for each model parameter were used to indicate whether the parameter values were strongly dependent on one another. To critically evaluate which decay model best described the experimental data, the following criteria were employed: An r^2 value of 0.90 was deemed acceptable for assessing the fit of the model to the experimental data. To check for model overfitting, a dependency value cut-off of 0.98 was selected. The half-life

($t_{½}$) for the mineralization pool P_1 was calculated as follows:

$$t_{½} = \ln(2)/k_1 \quad (2)$$

Further details of the modelling approach and its assumptions are provided in Glanville et al. (2016). Microbial C use efficiency for glucose (CUE_{mic}) was calculated according to Jones et al. (2018) where

$$CUE_{mic} = P_2 / (P_1 + P_2) \quad (3)$$

where P_1 is the amount of ^{14}C allocated to catabolic processes and P_2 is the amount of ^{14}C allocated to anabolic processes. Differences in substrate half-life and CUE_{mic} between location treatments was tested using an ANOVA model followed by a posthoc Tukey HSD test.

From the 16S data, alpha diversity was calculated using the Shannon index on raw OTU abundance tables after filtering out contaminants, as described in section 2.4. The significance of diversity differences between location treatments was tested using an ANOVA model followed by a posthoc Tukey HSD test.

To obtain the overall variance in microbial composition, the similarities in microbial diversity across samples and location treatments were visualized by nonmetric multidimensional scaling (NMDS) ordinations based on Bray-Curtis dissimilarity. Ellipses for each location treatment were calculated using the 'veganCovEllipse' function. Significant environmental variables ($p < 0.05$) based on permutated data were selected and fitted onto the NMDS ordination space using the 'envfit' function in the '*vegan*' R package (Oksanen et al., 2020), significances of correlations were tested with 999 permutations. The NMDS results were quantitively evaluated with permutational multivariate analysis of variance (PERMANOVA) using the 'adonis 'function in '*vegan*', followed by posthoc pairwise comparisons to evaluate microbial diversity differences between location treatments with the function "pairwise.perm.manova" from '*RVAideMemoire*' (Hervé, 2021). Although a PERMDISP test conducted with the 'betadisper' function in '*vegan*' identified non-homogenous dispersion between location treatments ($F = 3.15$, $p = 0.038$) PERMANOVA was performed as it is robust against non-homogeneous dispersions with balanced designs (Anderson, 2017).

Normality and homoscedasticity of the data were first checked using Anderson Darling and Levene's tests, respectively. Above-ground biomass was subjected to natural log transformation to ensure normality was met. Mixed-effect models were performed for each measured variable with the '*lme4*' package (Bates et al., 2018). The models included the fixed factor EC (as a proxy for salt stress) and patch number as a random factor to account for spatial variation. Predicted fitted values from the mixed-effect model were calculated with *predictInterval* with the '*merTools*' package (Knowles et al., 2020). The statistical significance cut-off for all analysis was set at $p < 0.05$.

3. Results

3.1. Impact of salinity on glucose mineralization rate and microbial CUE

Overall, a double exponential kinetic model fitted well to the $^{14}CO_2$ mineralization data for both the low ($r^2 = 0.985$; mean dependency 0.73 ± 0.01) and high glucose treatments ($r^2 > 0.980$; mean dependency 0.90 ± 0.01). Exploring the ^{14}C-labelled glucose mineralization rates across the saline gradient showed variation with both patch location (i.e., salinity) and glucose (low vs. high) dose (Fig. 3). Under low glucose addition (100 μM), the rate of mineralization was more rapid in the control soil samples than the samples with the highest salinity ($F_{(3,8)} = 20.2$, $p < 0.001$; Fig. 3). This was evidenced by the shorter half-life for the fast catabolic C pool (P_1) in the control treatment ($t_{½} = 0.70 \pm 0.03$ h) relative to those with highest salt concentrations ($t_{½} = 1.00 \pm 0.05$ h) ($p = 0.012$). No major effect of salt was seen on the rate of processing of ^{14}C through the slow metabolic pool (P_2) attributable to the turnover of the microbial biomass ($F_{(3,8)} = 4.1$, $p = 0.051$). Under the low ^{14}C-glucose-C availability, salt stress increased soil microbial CUE from 0.749 ± 0.003 in the control treatment to 0.809 ± 0.008 in the highest salinity treatment ($p < 0.05$) (Table S1).

Under high glucose addition (100 mM), a greater proportion of glucose was mineralised to $^{14}CO_2$ relative to the low glucose treatment (paired *t*-test $p < 0.001$). In addition, the half-life for the fast catabolic C pool (P_1) was much slower under the high glucose treatment ($t_{½} = 14.1 \pm 1.2$ h) relative to the low glucose treatment, however, soil salt content had no effect on the turnover rate of this pool ($F_{(3,8)} = 3.0$, $p = 0.095$). Salinity had less impact on the final cumulative amount of glucose-C respired, although the control samples exhibited the highest cumulative respiration rates (Fig. 3). In contrast to the low-glucose treatment, soil salinity status had no effect on CUE in the high glucose treatment ($p = 0.73$) or on the rate of turnover of the microbial biomass, Pool P_2 ($F_{(3,8)} = 0.95$, p = 0.462).

3.2. Impact of salinity on 16S bacterial community structure

In total, 10442 bacterial operational taxonomic units (OTUs) were identified across all 16S rRNA gene reads. There was some variation in the proportional abundance of OTUs between location treatments,

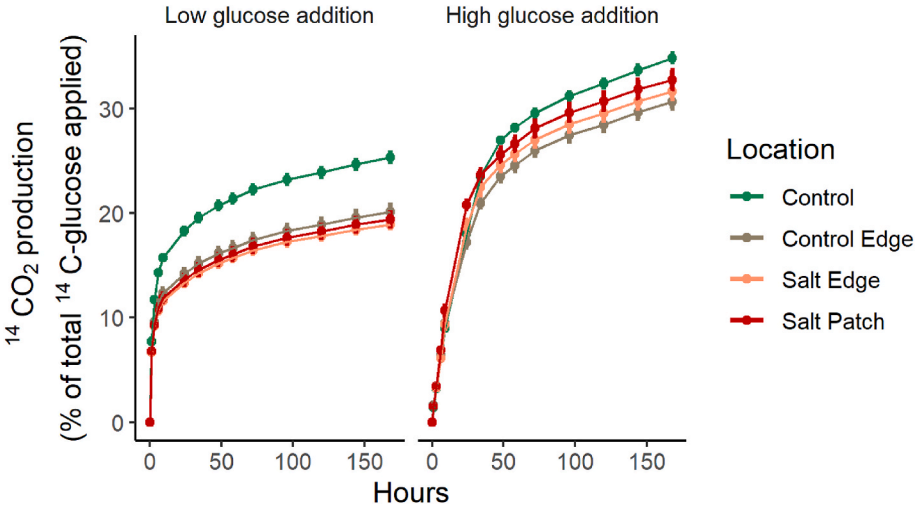

Fig. 3. A comparison of the ^{14}C-labelled glucose mineralization rates of soils across a saline gradient dosed with either 100 μM (CUE Low) or 100 mM (CUE High). Points represent cumulative ^{14}C respired over time ($n = 9$), error bar bars represent SEM. This data was used to calculate CUE presented in Table S1 using the double first order kinetic decay equation model described in section 2.5.

however, generally Alphaproteobacteria (Gram-negative) and Bacilli (Gram-positive) were the most abundant classes, although their abundances reduced under more saline locations compared to the control (Fig. 4, Fig. S3). Shannon diversities also differed between location treatments as tested by ANOVA ($F_{(3,32)} = 7.41$, $p < 0.01$). Subsequent posthoc tests revealed that diversity was significantly higher in the Salt Edge ($p < 0.01$) and Salt Patch ($p < 0.05$) compared to the Control treatment.

Based on the relative abundance of taxa ordered by class, beta-diversity analysis by NMDS and variance comparison of Bray-Curtis distances also determined differences in soil microbial diversity between location treatments (Fig. 5, PERMANOVA; $F = 11.07$, $p = 0.001$). Pairwise comparisons of the microbial diversity revealed significant differences between all locations along the salinity gradient (Table S2). The salt stress gradient also correlated strongly with the ordination, as indicated by the fitted vectors of environmental variables (Fig. 5). Here, soil EC, a direct measure of salt stress, had the highest squared correlation coefficients (r^2) compared to other environmental variables (Table S3).

3.3. Plant biomass and soil physicochemistry

Exploring relationships through mixed effects models (Fig. 6) shows that salt stress significantly reduces aboveground biomass ($p < 0.005$), where the mean aboveground biomass difference between samples collected from the control locations and the salt patch locations was 96% (Table S4). Overall, we found that soil nutrient availability increased along the salt stress gradient as evidenced by the positive soil carbon, nitrogen, ammonium, nitrate, and phosphate relationship with salt stress (Fig. 6 and Table S5). As an artifact of both total soil C and N % increasing with increased salt stress and N having a slightly stronger relationship than C (t value for N = 3.99 ad t value for C = 3.12), we found a strong positive relationship between soil C:N ratios and salt stress ($t = 32.86$, $p = 0.03$). Soils also became more acidic and compact with increased salt stress, as demonstrated by the negative soil pH ($t = -4.98$, $p < 0.05$) and bulk density relationship ($t = -4.98$, $p < 0.001$) with salt stress, respectively.

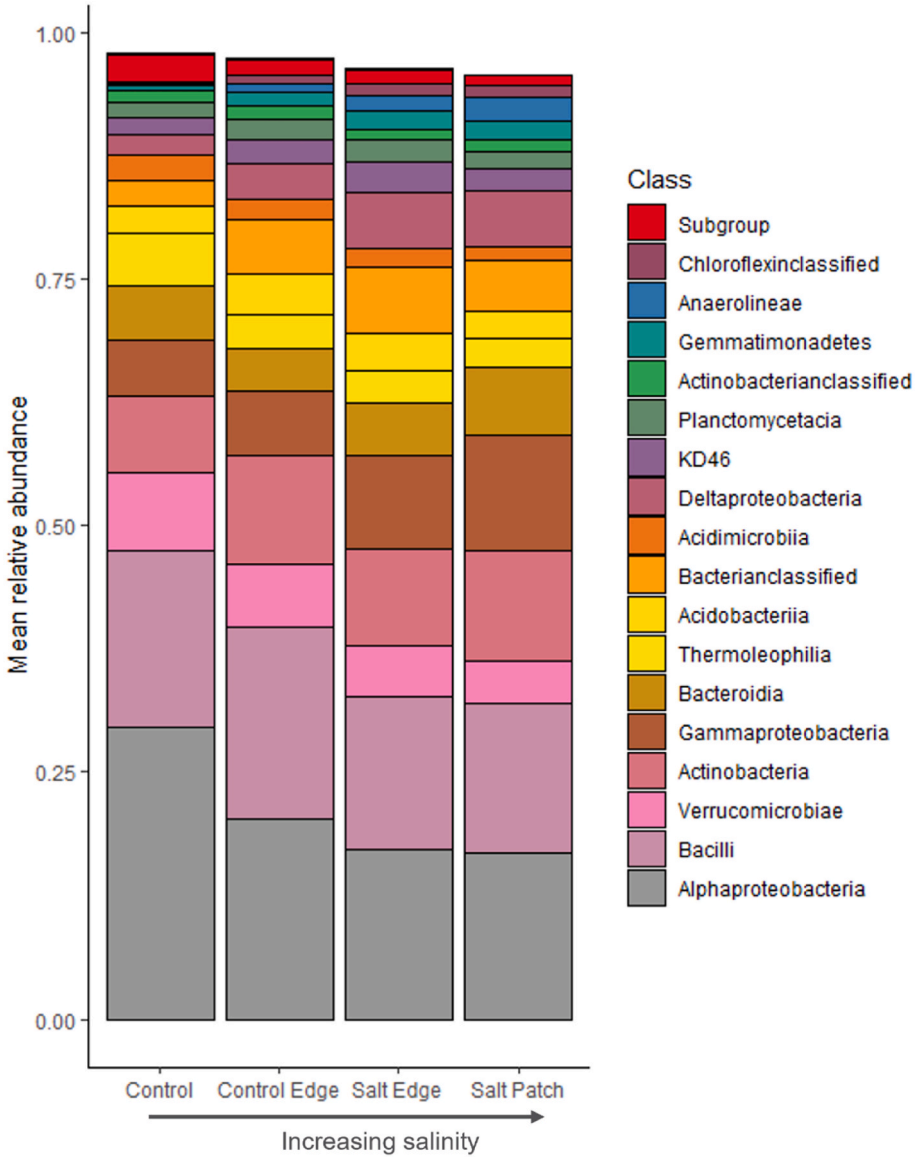

Fig. 4. Mean relative abundance of the dominant soil biota by class (>1%) within each salt gradient treatment.

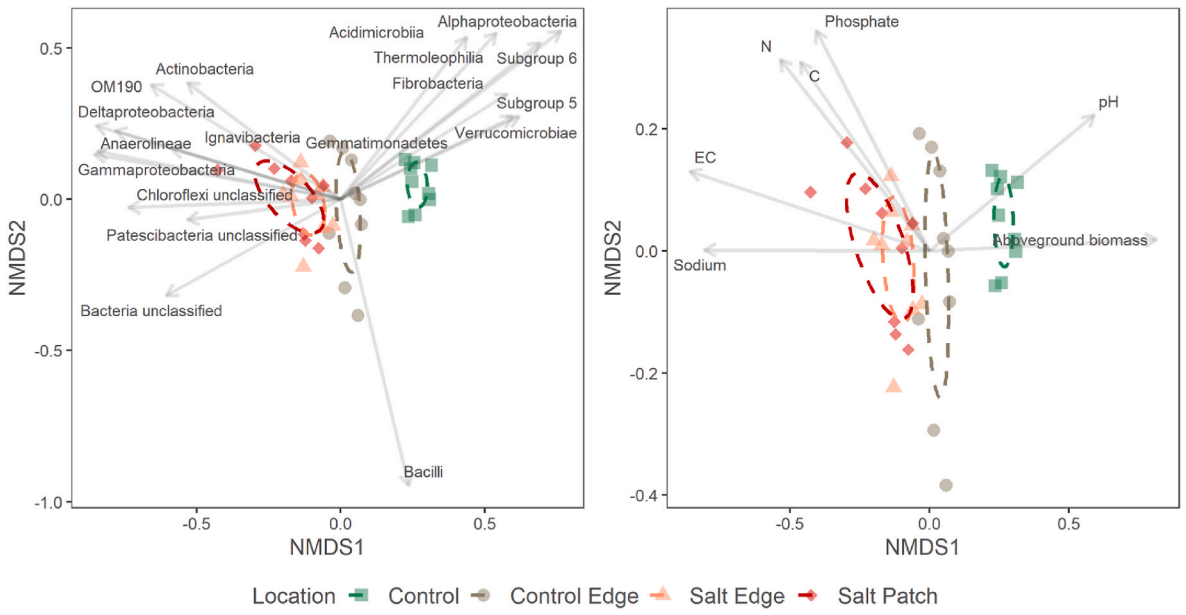

Fig. 5. Non-metric dimensional scaling ordination (stress = 0.07) of community diversity across salt gradient treatments denoted by colours. Results of PERMANOVA ($F = 11.07$, $p = 0.001$) and dispersion of variances of groups ($F = 3.15$, $p = 0.031$) were significant. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

4.1. CUE along a soil salinity gradient

Bacterial growth and respiration have both been shown to decrease with higher salinity, particularly in soils under agricultural management (Rath et al., 2019b; Rath and Rousk, 2015). Here, we used a low concentration (100 μM) of ^{14}C-labelled glucose to measure the ability of the intrinsic community to metabolise labile substrate, i.e., maintenance. As well as a high concentration (100 mM) of ^{14}C-labelled glucose to assess the ability of the community to grow under unlimited substrate, i.e., growth. There was no significant relationship between CUE under the high ^{14}C-labelled glucose application and soil EC (Table S1), suggesting that fast growing members of the microbial community were not inhibited by excess salt in the soil (i.e., adaptation of the copiotrophic community, *r*-strategists) (Fierer et al., 2007). This also suggests that the community did not divert a large amount of C towards osmoprotectant production or the operation of Na^+ efflux pumps. However, there was a significant positive correlation between CUE under low ^{14}C-labelled glucose application and soil EC (Fig. 3). This clearly suggests that metabolic activity was shifted under higher salt stress. We speculate that while the high glucose dose only targets the *r*-strategists, the low glucose dose provides a more representative view across the whole community (i.e., includes slow growing *K*-strategists) (Fierer et al., 2007). This CUE pattern under high and low glucose doses was contrary to our expectations as we hypothesized that the fast-growing community would be more affected by salt stress and would have a reduced glucose uptake rate (P_1, k_1) and CUE under salt stress (Luo et al., 2020), which was not the case. We also hypothesized that C turnover through the microbial biomass (i.e. P_2, k_2) would be slower in the salt-affected soils due to a reduction in mesofaunal abundance and microbial grazing. Comparison of the CUE values in the low and high glucose doses may therefore indicate that the *K*-strategists are less adapted to salt stress.

The structure of the soil microbiome and its intrinsic CUE underpins the ability of a soil to store C, as well as its functional ability to cycle and retain nutrients. In this study we clearly showed that storm surge-induced coastal inundation resulted in a major shift in bacterial community structure and functioning in terms of C turnover. Metabolic C partitioning in the microbial biomass is fundamentally controlled by a range of environmental factors; determining the level of community stress, and soil nutrient stoichiometry (Manzoni et al., 2012). Increasing the microbial CUE of agricultural soils is seen as beneficial, potentially increasing C storage while reducing C system losses (Kallenbach et al., 2019). However, increasing CUE is also likely to enhance the retention of N and P through stoichiometric balance with C. Previously, it has been shown that soil communities under high abiotic stress have a lower CUE, as more C is respired as organisms attempt to maintain normal cell function while producing stress mitigation compounds e.g., osmolytes (Empadinhas and da Costa, 2008; Manzoni et al., 2012), or to repair stress-induced damage (Jones et al., 2019; Xu et al., 2018). In this soil we have previously shown that osmotic stress induces the transitory production of osmoprotectants by the microbial biomass (Brown et al., 2021; Miura et al., 2020), which would be consistent with a low CUE and a decreased potential for long term C storage and sequestration (Sinsabaugh et al., 2013). This is also supported by the CUE data presented here for the low glucose additions.

In this study, while soil total C increased with salinity, there was no strong statistical relationship between total organic C and salinity ($p = 0.8$; Fig. 6 and Table S5). Elevated soil salinity has been shown to have mixed effects on soil C, either leading to an increase (Chambers et al., 2013; Servais et al., 2019) or decrease in C mineralization (Ardón et al., 2018; Herbert et al., 2018). The relative change appears to depend on several factors including experimental location and prevailing environmental conditions. As previously discussed by de la Reguera and Tully (2021), soil moisture fluctuations will be a major control on C flux, determining the anaerobicity of soils and speed of decomposition rates. Similarly, the rate at which salt is removed from the soil profile by rainfall and plant uptake is also likely to be an important determinant (Isayenkov and Maathuis, 2019; Li et al., 2018; Munns and Gilliham, 2015). CUE in this experiment was performed on field-moist soil, to be as representative as possible of field conditions on collection. However, it is highly likely that the CUE of the soil across the saline gradient explored here is dynamic and will vary with soil moisture i.e., the degree of inundation and subsequent rainfall (Stark et al., 2019). It is therefore recommended that future research explores the relationship between frequency and degree of saline and brackish water inundation and soil CUE.

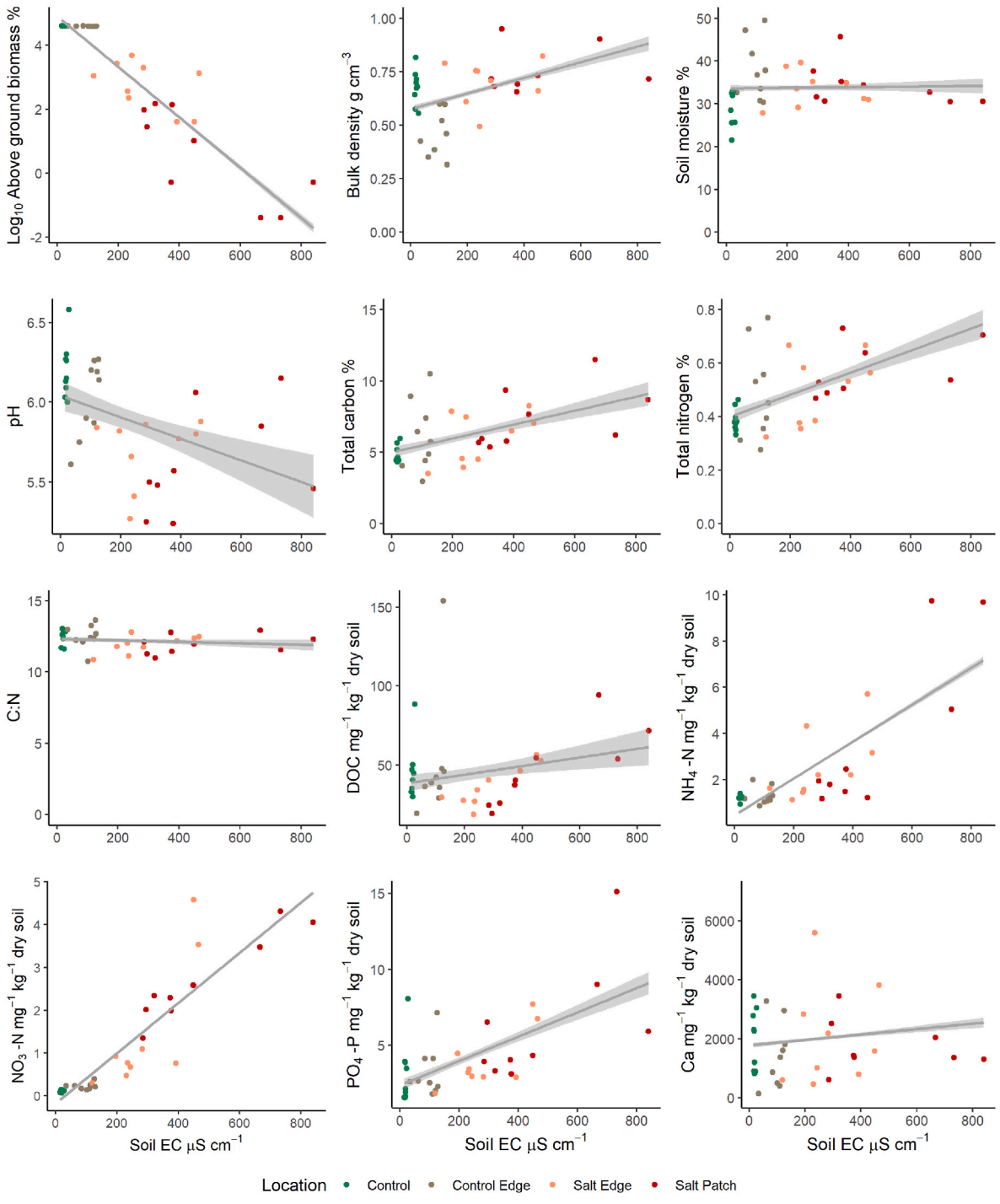

Fig. 6. Trends in above ground biomass, soil bulk density, pH, carbon, nitrogen, C: N ratio, total organic carbon (TOC), ammonium, nitrate and phosphate across a saline gradient. Points represent individual sampling points where colour denotes sampling location to ensure a saline gradient was captured. The trend lines represent the predictive fitted ratio change values based on the mixed effects models, where coloured shaded areas represent 95% upper and lower confidence intervals of the mean. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

4.2. Changes in the 16S bacterial community across the saline gradient

Salt significantly affected the structure (Figs. 4 and 5, Table S2, and Table S3) and likely also the function of the soil bacterial community. We ascribe this change to salt toxicity (and associated changes in soil pH and EC; Fig. 5), as well as physical changes to soil structure and a reduction or change in plant primary productivity changing the dynamic of rhizosphere-associated bacteria (Rath and Rousk, 2015). Indeed, several studies have previously concluded that salinity negatively affects the diversity and community composition of microorganisms in soils and sediments, consistently across coastal ecosystems (Behera et al., 2017; Kim et al., 2019; Zhao et al., 2020).

In this study, where saline inundation is relatively infrequent, but its legacy is long lasting (i.e. salt deposition and salinisation; Fig. 1, Fig. S2), the importance of soil ecological resistance (ability to withstand disturbance) and resilience (ability to recover from disturbance) must be considered (Griffiths and Philippot, 2013). We know from field observations that our field site has experienced unprecedented and repeated

storm-surge saline intrusions in recent years. Although the dynamics of salt accumulation have not been measured, we know that surface salt accumulation has been a progressive process providing time for the microbial community to adapt. In contrast, the plant community has not been able to adapt leading to the loss of vegetation. We assume that creating a new quasi-stable state essentially selecting for bacterial with higher salt tolerances, seen here in the significant difference in beta-diversity particularly between the Control and Patch sampling. Further research is required to understand the effects of salt exposure on the temporal dynamics of the soil microbial community and the effect on C cycling as it is highly likely that the frequency and severity of these events will increase in the future due to climate change.

Plants are soil-ecosystem engineers, with their root exudates creating nutrient hotspots within the soil, and consequently influencing the soil microbial community (Berendsen et al., 2012; Pathan et al., 2020). The grassland soil studied here can essentially all be classed as rhizosphere due to the high intrinsic root density (~25 cm root cm^{-3} soil; 0–10 cm). In comparison to bulk soil, rhizosphere-associated bacterial communities are denser, have larger cells (Lopes et al., 2016) and increased microbial activity (Reinhold-Hurek et al., 2015). Plants use their rhizosphere to select (generally beneficial) microbial communities (Dawson et al., 2017; Yin et al., 2021), with specific plant species root exudate cocktails having been shown to encourage bacteria with matching substrate uptake preferences (Zhalnina et al., 2018). This pre-selection has often been associated with a decrease in species richness and evenness (Peiffer et al., 2013; Shi et al., 2015). Here we saw a significant reduction in plant aboveground biomass with increasing salinity (Figs. 1 and 6) as the species within the grassland were not halotolerant and had senesced within the saline patches. With the death of plants and therefore loss of the rhizospheric pre-selection of the bacterial community, changes in bacterial alpha- and beta-diversity occurred (Fig. 5). It is likely that, with time, halotolerant plant species would begin to colonise the salt patches, leading to further changes in the soil physicochemistry and the associated biological community.

4.3. Soil chemistry as affected by soil salinity

Saline inundation is likely to have a large effect on soil chemistry; as a result of the reduction in plant biomass, a change in the soil porewater chemistry (Herbert et al., 2018), physical structure (Oster and Shainberg, 2001), and changes in exoenzyme activity (Singh, 2016). As shown here (Fig. 6, Table S5), as plant biomass decreases as a result of saline stress, induced by ion toxicity and osmotic stress and liming nutrient uptake (Bidalia et al., 2019; Shrivastava and Kumar, 2015), demand for soil available nutrients is impaired (particularly P, NH_4^+ and NO_3^-), leading to significant accumulation in soil porewater. This is likely to reduce the nitrification within salt affected soil due to the increased availability of available nitrogenous compounds (Yao et al., 2022), however further work is required to understand the effect of salt on N transformations in soil. Equally, with a reduction in root biomass and perturbation and the likely flocculation of clay particles, bulk density increases (Fig. 6) (Imadi et al., 2016). Equally, Na and other saltwater cations can desorb NH_4^+ from soil exchange sites (Jun et al., 2013; Weston et al., 2010) while shifting microbial nitrate metabolism from denitrification to NO_3^- reduction to NH_4^+ (Giblin et al., 2010), further leading to NH_4^+ accumulation. Increases in SO_4^{2-} in the porewater are likely to be reduced by sulphate-reducing bacteria, leading to HS and H_2S formation. Orthophosphate may also be released from Fe–P minerals though the subsequent complexation of H_2S with Fe^{2+} (van Diggelen et al., 2014). This study took place >2 months after inundation, allowing NH_4^+ and P to accumulate relative to the control (Fig. 6) (Herbert et al., 2018; Sánchez-Rodríguez et al., 2018). Additionally, microbial extracellular enzyme activity is likely to be affected by salinity with inhibitory effects having been shown on a range on enzymes (e.g. dehydrogenase, β-glucosidase, urease, protease, alkaline phosphatase, acidic phosphatase and arylsulphatase) (Rietz and Haynes, 2003; Tripathi et al., 2007; Zheng et al., 2017), potentially leading to slower nutrient cycling rates and lowering microbial growth and biomass (Singh, 2016), although these were not measured in this study. While soil pH is likely to increase initially after inundation, as seawater is alkaline (pH 8.1), we ascribe the decrease in pH with salinity across the gradient to anion accumulation (e.g. NO_3^-, PO_4^{3-} and SO_4^{2-}) and cation exchange between Na^+ and H^+ (Fig. 6) (van Tan and Thanh, 2021).

5. Conclusions

Climate change is highly likely to increase the frequency and severity of coastal flooding in low lying areas in the near future. Understanding the effects of salt exposure on the soil microbial community and the associated effect on soil C cycling is therefore of high impetus, affecting the provision of a large number of ecosystem services, including nutrient cycling and soil fertility. Here, we showed that a gradient of salinity caused by infrequent brackish water flooding to a coastal grassland significantly altered the soil bacterial community. However, CUE of the soil biological community was relatively unchanged (i.e. functionally resilient) under 'growth' conditions and higher under 'maintenance' conditions suggesting adaption of the microbial community to the higher salt conditions, thus, allowing it to rapidly respond to relatively small labile C inputs. We suggest that soil microbial community strategy (*r* vs. *K*) was potentially driving differences in CUE under different C availability. This indicates that ecosystem service provision related to C storage and cycling is likely be dependent on the availability and quality of organic substrate, as well as the level of salt stress. Perhaps the most significant driver of changes across the gradient was change in loss of plant biomass with increasing salinity, which is likely to have influenced soil chemistry as well as the structure of the soil microbial community. Therefore, we conclude that most of the observed effects of salinity on soil microbial functioning are indirect, being mediated by the lack of plant C inputs, nutrient uptake and maintenance of soil structure.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2022.108700.

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