Rhizosphere 34 (2025) 101115

Contents lists available at ScienceDirect

Rhizosphere

journal homepage: www.elsevier.com/locate/rhisph

# Upregulating of organic acids in contrasting plant species results in moderation of high pH stress

Felipe E. Sepúlveda Olea [a,*], Ian T. Burke [b], Robert D. Hancock [c], Simon D.A. Pont [d], Douglas I. Stewart [a]

[a] School of Civil Engineering, University of Leeds, Leeds, LS2 9JT, UK
[b] School of Earth and Environment, University of Leeds, Leeds, LS2 9JT, UK
[c] Cell and Molecular Sciences, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK
[d] Environmental and Biochemical Sciences Department, The James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK

ARTICLE INFO

*Keywords:*
Phytostabilization
Root exudation
Rhizosphere
Waste management
Contaminated land

ABSTRACT

Development of vegetative cover on the surface of polluted waste deposits provides a physical protective layer and an input of organic compounds via root exudation. The monocot yorkshire fog (*Holcus lanatus*) and eudicot red clover (*Trifolium pratense*) are plant species that have been observed to grow successfully in alkaline waste, managed with phytostabilization. In this work the effects of pH-stress conditions (pH ~10) on root exudation of both these species is studied in a hydroponic set up. Both species, when exposed to stress conditions, were able to neutralize the pH in the collection solution, with a reduction of ~2 pH units. For both yorkshire fog and red clover, the net exudation had no statistical difference between non-stressed and pH-stressed conditions (263 vs 220 μg DOC $(\text{g-root})^{-1}(\text{hrs})^{-1}$ for yorkshire fog and 158 vs 118 μg DOC $(\text{g-root})^{-1}(\text{hrs})^{-1}$ for red clover). GC-MS analysis of the exudate solutions, however, shows a shift from a sugars and sugar alcohols-dominated exudate solution in non-stressed conditions, to an exudate solution with upregulated organic acids in pH stressed yorkshire fog and organic acids plus amino acids in red clover. These results show a similar stress response for these two species, contrary to the general assumption that grass species are less efficient than eudicots in producing organic acids in response to stress. The prevalence of organic acids in exudates under stress conditions, could favour metal and nutrient mobilization in growth substrates, though the lack of an increase in overall exudation limits the extent of their potential impact.

## 1. Introduction

Development of vegetative cover on the surface of polluted waste deposits provides not only a physical protective layer, but may also have other effects on the material, as plants release organic compounds through their roots (Potysz et al., 2017). Of these compounds, primary metabolites, namely sugars, amino acids and organic acids, generally have higher concentrations (Adeleke et al., 2017; Canarini et al., 2019; Potysz et al., 2017; Maurer et al., 2021; Vives-Peris et al., 2020).

The concentration of plant exudates are highly variable, depending on the plant species and environmental conditions (e.g. moisture content; Potysz et al., 2017; Adeleke et al., 2017; Maurer et al., 2021). Stress conditions generated by the substrate (e.g. extreme pH conditions, metal toxicity, nutrient deficiency) on plant growth can greatly influence root exudation, altering the type and quantity of compounds exuded (Adeleke et al., 2017; Canarini et al., 2019; Amezcua-Allieri et al., 2010; Maurer et al., 2021). Substrate pH in particular plays a crucial role in determining exudation, as it affects other properties such as nutrient availability (e.g. Fe, Mn and P), and plants exploit root exudation to moderate pH and overcome these limitations (Chen and Liu, 2024; Vives-Peris et al., 2020; Tsai and Schmidt, 2021; López-Bucio et al., 2000). Thus, plant stress responses can alter the geochemistry of the substrate (such as a waste material), by input of plant exudates and complexation of metals with organic compounds (particularly organic acids) which can increase metal leaching (Adeleke et al., 2017; Potysz et al., 2017; Alasmary, 2020; Debela et al., 2010; Padmavathiamma and Li, 2012; Hashimoto et al., 2011; Gupta and Kumar, 2016).

Grassland species have repeatedly been found to be early colonisers

---

* Corresponding author.
*E-mail addresses:* f.sepulvedaolea@leeds.ac.uk (F.E. Sepúlveda Olea), i.t.burke@leeds.ac.uk (I.T. Burke), rob.hancock@hutton.ac.uk (R.D. Hancock), simon.pont@hutton.ac.uk (S.D.A. Pont), d.i.stewart@leeds.ac.uk (D.I. Stewart).

https://doi.org/10.1016/j.rhisph.2025.101115
Received 14 March 2025; Received in revised form 30 May 2025; Accepted 30 May 2025

of industrial wastes in the process of primary succession at waste disposal sites (Gomes et al., 2016; Tischew et al., 2014; Kiehl et al., 2010). This is thought to be due to their tolerance to metal stress and capacity to develop a dense and strong root system (Kucharski et al., 2005; Hashimoto et al., 2008; Wasilkowski et al., 2014). The latter, along with an observed low translocation of contaminant metals to above ground tissues (Hashimoto et al., 2008), has led to grassland species being widely used for industrial and mining waste management through phytostabilization.

Alkaline wastes such as red mud (bauxite residue), steelworks slag, and chromite ore processing residue, have been widely produced in large volumes and pose a challenge in terms of management and ecological restoration (Gomes et al., 2016). Both planned management with vegetative cover and spontaneous revegetation have been studied in these type of wastes, with varying results and effects on the material (Gomes et al., 2016). Bray et al. (2018) observed resilient vegetative cover by grassland species (*Agrostis stolonifera, Holcus lanatus, Lolium perenne, Trifolium repens,* and *Trifolium pratense*) at a restored bauxite waste site 16 years after seeding and concluded that development of an active rhizosphere contributes to pH neutralization at depth, via secretion of exudates and flow of $CO_2$.

The grassland species yorkshire fog grass (*Holcus lanatus*) and red clover (*Trifolium pratense*), in particular, have been observed to grow well at alkaline waste sites (Courtney et al., 2009; Santini and Fey, 2013; Ash et al., 1994; Lee and Greenwood, 1976). Yorkshire fog grass and red clover are respectively a monocot and eudicot. Monocots and eudicots are the two major classes of angiosperms which diverged in their evolution between the Jurassic and Cretaceous periods (Chaw et al., 2004). Monocots and eudicots have differences in morphology and physiology, such as the structural features and storage compounds in seeds (Wang et al., 2016). Differences at the physiological level include exudation patterns. For example, the response to Fe deficiency in eudicot species can involve enhanced mobilization of $Fe^{3+}$ via acidification of the rhizosphere, by releasing phenolic and organic acids (known as Strategy I), whereas grasses solubilize and mobilize Fe by releasing Fe chelating compounds (phytosiderophores; known as Strategy II) (Tiziani et al., 2023; Hiradate et al., 2007; Mimmo et al., 2014).

The evolution of monocot and eudicot plants diverged about 150 Myr ago (Chaw et al., 2004), and they have developed many distinct physiological characteristics (Scarpella and Meijer, 2004), yet research comparing their root exudation strategies in response to stress is very limited. Therefore, this study directly compares the alkaline-stress response of yorkshire fog (monocot) and the red clover (eudicot) in a hydroponic growth set up. These species were chosen because they are commonly found in calcareous grassland. Yorkshire fog has been observed to be a persistent species on revegetated alkaline waste (Courtney et al., 2009; Courtney and Harrington, 2012; Rahman and Khan, 2023; Young et al., 2018), and red clover is frequently an important species on revegetated wastes and contaminated land, where it plays an important role in alleviating nitrogen deficiency (Di Carlo et al., 2019; Courtney et al., 2009; Courtney et al., 2003; Courtney et al., 2013; Rybak et al., 2018; Davin et al., 2019). The impact of pH stress on the net exudation rate and the exudate composition were studied over a photoperiod in a hydroponic set up. The effects that pH-driven changes on root exudation may have in polluted alkaline wastes is discussed.

## 2. Material and methods

Yorkshire fog (*Holcus lanatus*) and red clover (*Trifolium pratense*) seeds were purchased from a commercial seed provider (Emorsgate Seeds, Cambridgeshire, UK).

### 2.1. Hydroponic set up

Plants were germinated on perlite in starter trays placed within a second containment tray (SI Figure S1). For yorkshire fog 20 mg of seeds (~60 seeds, germination rate ~20 %, tested on the same batch of seeds) were placed in each well (24 wells), and for red clover, which has larger seeds and a higher germination rate (~100 %), 14 seeds were placed in each well (24 wells). 10 ml of full concentration Hoagland's solution (Sigma-Aldrich, Hoagland's No. 2 basal salt mixture) was pipetted into each well, and a further 200 ml of Milli-Q water was added at the base of the containment trays. Trays were placed in a growth chamber set at 25 °C with a 14 h photoperiod ($54 \pm 9$ μmol $s^{-1}$ $m^{-2}$ of photosynthetically active radiation, measured at the level of the top of the tray). After two weeks, trays were moved to a larger growth "tent" with a 14 h photoperiod ($221 \pm 48$ μmol $s^{-1}$ $m^{-2}$ of photosynthetically active radiation, measured at the level of the top of the tray), at room temperature. Milli-Q water was regularly topped up in the containment trays to maintain moisture and Hoagland's solution was added every week (10 ml per well). On week 6, plants were transferred from the starter tray to a hydroponic set up. Plants from each well were taken out, washed to remove perlite particles and transferred to a 50 mm (~70 $cm^3$) net pot. Groups of three net pots were placed in 2 l polypropylene bowls through holes in the polystyrene lid (SI Figure S1). 1 l of 1/10 Hoagland's solution was added in each bowl (pH $5.9 \pm 0.3$). Milli-Q water was regularly added to maintain the volume of media. The growth media was changed weekly with fresh 1/10 Hoagland's solution and replicate net pots were moved position within the set up weekly, to compensate for the variability caused by the specific positioning within the chamber. On week 12, pH stress conditions were started in half of the bowls (4 bowls containing 12 replicate net cups per plant species) by adding 1M KOH until a pH of $10 \pm 0.5$ was achieved. pH was adjusted daily in "stressed" bowls until harvesting time (with an average daily 1M KOH addition of ~1000 μl ≈ 1 ml, and a maximum of 7 consecutive days adjusting pH without media change, the initial K concentration of 0.6 mM K was cumulatively altered to 7.5 mM K on day 7). For yorkshire fog the pH was $8.1 \pm 0.5$ before daily addition of KOH and $10.2 \pm 0.2$ afterwards. For red clover the pH was $7.8 \pm 0.6$ before and $10.4 \pm 0.3$ afterwards. The average temperature throughout the growth period was of 25.6 °C, with the lowest daily average being 24.8 °C and highest daily average 26.2 °C. Daily temperature fluctuations ranged from 0 to 2.5 °C (SI Figure S2 shows the temperature log for the growth period).

### 2.2. Root exudates collection and analysis

Exudates were collected in week 15 and the plants were harvested immediately afterwards. The decision to harvest exudates at this time point was driven by practical considerations. The plants were initially grown to the point where they were producing sufficient exudates for harvesting and analysis, and then half the plants were stressed for two weeks to ensure that the plants were acclimatised to the alkaline conditions before their response in a quasi-steady state was determined. To detect diurnal variations of the overall exudation as well as that of specific metabolites (de Barros Dantas et al., 2023), collection took place over four 3 h collection periods that covered the 14 h photoperiod (with ~40 min between collection periods). 3 h was selected as an optimal length that allowed for sufficient accumulation of exudates in solution for detection with the analytical method, while avoiding saturation or re-uptake of exudates by plants, which can occur in longer collection periods (McLaughlin et al., 2025).12 replicate net pots were used for each treatment (yorkshire fog pH-stressed, yorkshire fog non-stressed, red clover pH-stressed, red clover non-stressed). Roots were washed 3x before collection to remove debris, by submersion of the net pots in fresh Milli-Q water for ~20 s with gentle circular motion.

The net pots were placed in 150 ml glass beakers containing 75 ml of collection solution. The beaker was covered with aluminium foil to prevent direct incidence of light on the roots. The collection solution contained the major components of 1/10 strength Hoagland's solution ($NH_4H_2PO_4$, $KNO_3$, $Ca(NO_3)_2$, $MgSO_4$), to maintain conditions as close to those of the hydroponic media, while reducing interference with chromatographic analysis (Vives-Peris et al., 2020), and avoiding exudation alterations caused by osmotic stress, which can occur when using distilled water for collection (Vranova et al., 2013). For pH-stressed plants, the pH of the collection solution was adjusted with 1M KOH to a 10.5 ± 0.2. Beakers with plants were then returned to the growth tent. After 3 h each beaker was gently stirred manually for 40 s and then the net pot was removed and transferred to a new beaker for the consecutive collection period. A control-blank beaker with 75 ml of collection solution was included in each period. After completion of the 4 collection periods, the plant shoot and root biomass were separated using scissors. Both were dried in an oven at 70 °C for 48 h, after which the dry weight was recorded.

After removing the net pots, 50 ml of each collection solution containing the plant exudates was filtered with nylon syringe filters (0.45 μm), transferred to a 50 ml polypropylene centrifuge tube and stored at −20 °C. pH and electrical conductivity (EC) were measured in the remaining solution (HACH HQ40D pH meter with HACH Intellical pH and EC probes). The filtered exudate solutions were freeze dried until complete dryness. Half of the replicates (6 reps per treatment per collection period) were re-dissolved in 5 ml of a 5 % $HNO_3$ in Milli-Q water solution for dissolved organic carbon (DOC) analysis. Care was taken to make sure all of the dried material in the tube walls was dissolved, by vortexing the tubes before and after placing them in a roller mixer for 1 h. DOC was analysed with an Analytik Jena Multi N/C 2100 combustion analysers calibrated using commercially prepared stocks of organic and inorganic carbon.

The 6 remaining freeze-dried replicates for each condition were re-suspended in 5 ml of 20 % methanol and combined into one composite sample. The 16 resulting composite samples (four treatments, four collection periods) were then evaporated to dryness under reduced pressure. These were then prepared and analysed by GC-MS as previously described in Karpinska et al. (2018). Briefly, the combined samples were re-dissolved in 1.5 ml 100 % methanol at 30 °C with agitation at 1500 rpm for 30 min. 50 μl of internal standard (ribitol, 2 mg $ml^{-1}$) was added along with 375 μl of water and agitation continued for a further 30 min at 2500 rpm. 3 mL of chloroform was added and agitation repeated for a further 30 min. Finally, 750 μl of water was added and the tubes were briefly shaken. Samples were separated into polar and non-polar phases by centrifugation (1000 g, 10 min). The non-polar phase was discarded and the polar phase was converted to trimethylsilyl derivatives following oximation as previously described by Foito et al. (2013, 2013). Metabolite profiles were acquired following separation of compounds on a DB5-MSTM column (15m × 0.25 mm × 0.25 μm; J&W, Folsom, CA, USA) using a Thermo Finnigan (San Jose, CA, USA) DSQII GC-MS system as described in (Foito et al., 2013). GC-MS data were normalized by peak area relative to the ribitol internal standard and compounds were identified based on equivalent retention time and mass spectra obtained from a custom library built using authenticated standards.

### 2.3. Statistical analysis

Statistical analysis was carried out in Microsoft Excel (2016) using the data analysis package. ANOVA was conducted to assess significant variations of parameters (pH and DOC) between collection periods for each experimental condition (Table S.3 and S.4). This was supported with pairwise *t*-Test analysis ($\alpha = 0.05$). Pairwise *t*-Test analysis was also used to compare across experimental conditions, one variable at a time (e.g. [1] compare DOC data for the same species, in the same collection period, with varying pH conditions; [2] compare root biomass for the same species, with and without alkaline stress). The "F-Test Two-samples for variance" (with $\alpha = 0.05$), was applied to each pair to determine whether "*t*-Test: Two samples assuming equal variance" or "*t*-Test: Two samples assuming unequal variance" was used. Normality was assessed by visual inspection of a Normal Q-Q plot. In specific cases where normality was not likely, the non-parametric Mann–Whitney *U* test was used in replacement of the paired *t*-Test.

## 3. Results

### 3.1. Plant biomass

There was no statistical difference in the amount of either above ground (p-value of 0.3 and 0.5 for red clover and yorkshire fog respectively) or root biomass (p-values of 0.9 for both red clover and yorkshire fog) between stressed and non-stressed plants (Fig. 1). For yorkshire fog the ratio of above ground biomass to root biomass averaged 1.58 ± 0.18, with a good correlation between the two ($R^2 = 0.82$). For red clover the ratio of above ground biomass to root biomass was higher (2.88 ± 0.31), but the correlation was lower ($R^2 = 0.61$).

### 3.2. pH and EC in the collection solution

Under alkali stressed conditions (initial pH = 10.5), the pH of the collection solutions from both yorkshire fog and red clover were around 2 pH units lower than the control solution (control pH of 9.8 ± 0.2, Fig. 2). For both plants, the average pH in the collection solution decreased very slightly over the photoperiod, although in both cases only in period 4 was the pH of the collection solution (pH ~7.4 for yorkshire fog and ~7.2 for red clover) statistically lower than the other collection periods (pH ~7.6 and ~7.4–7.5 respectively) (see SI Table S3).

Under non-stressed conditions (initial pH = 5.9), the pH of the collection solution from yorkshire fog was ~1 pH unit higher than the corresponding control (control pH of 5.5 ± 0.2), whereas the pH of the collection solution from red clover was very similar to the corresponding control. For unstressed yorkshire fog, the pH during the first collection period (pH ~6.4) was slightly lower than the other collection periods (pH ~6.7–6.9; significant at $p < 0.05$, see Table S3), whereas for unstressed red clover, there was a gradual increase in pH over the collection period from ~4.8 to ~5.7 (although the difference between period 1 and 2 is not significant, see Table S3)

The electrical conductivity (EC) of the collection solution from the pH stressed plants tended to be slightly higher than in the respective controls (Figure S.3). In contrast, the EC of the collection solution from the unstressed plants tended to be slightly lower than in the respective controls.

### 3.3. Exudation rate

Under pH-stress yorkshire fog exhibited no statistical variation in

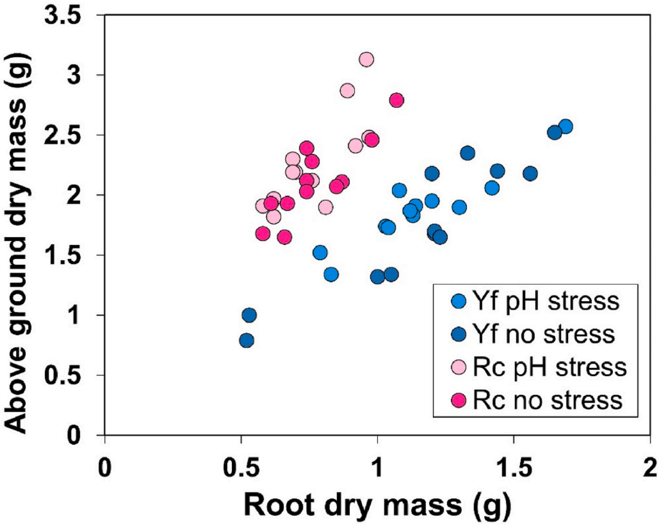

**Fig. 1.** Above ground and root dry mass of yorkshire fog (Yf) and red clover (Rc) experimental plants.

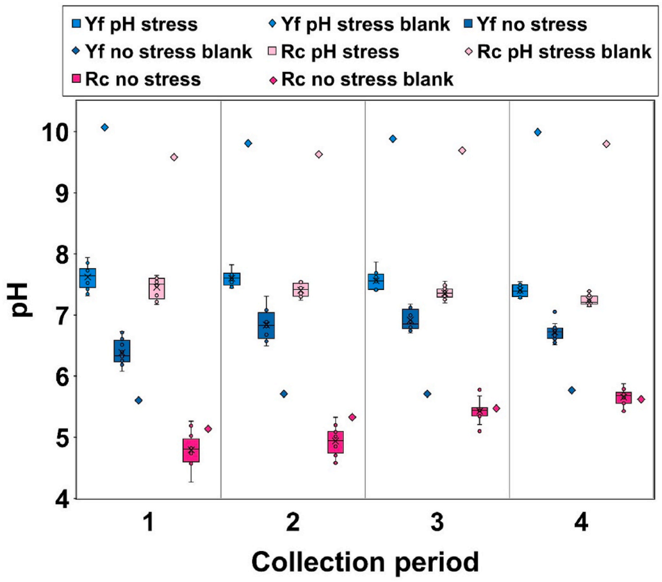

**Fig. 2.** pH in exudate collection solution of pH-stressed and non-stressed yorkshire fog (Yf) and red clover (Rc) along with experimental blanks ( × mean values; boxes show median, and 25th and 75th percentiles). Sampling was conducted over a single 14hr photoperiod (period 1: 0hrs–3hrs, period 2: 3hrs40min-6hrs40mins, period 3: 7hrs20mins-10hrs20mins, period 4: 11hrs–14hrs).

exudation rate between the collection periods, with an average rate of 220 µg DOC (g-root dry mass)$^{-1}$(hrs)$^{-1}$ (Fig. 3 and SI Table S.4). Similarly, non-stressed yorkshire fog exhibited no statistical variation in exudation rate between the collection periods (Table S.4), with an average rate of 263 µg DOC (g-root dry mass)$^{-1}$(hrs)$^{-1}$. The exudation rate for stressed and unstressed plants were compared for each respective collection period (*t*-test, SI Table S.5), as were the total amounts of DOC produced over the photoperiod (Figure S.6, compared with the Mann–Whitney *U* test), and there were no statistically significant differences in exudation rate between stress and unstressed yorkshire fog (p-values >0.05 and U-value > critical value).

Likewise, there was no significant difference (Table S.4) in the exudation rate of pH-stressed red clover over the photoperiod (average exudation rate over the photoperiod 118 µg DOC (g-root dry mass)$^{-1}$(hrs)$^{-1}$). In contrast, the average exudation rate of non-stressed red clover was lower in the 3rd and 4th collection periods (93 and 91 µg DOC (g-root dry mass)$^{-1}$(hrs)$^{-1}$, respectively) than in the 1st collection period (282 µg DOC (g-root dry mass)$^{-1}$(hrs)$^{-1}$) (note: one unstressed replicate was excluded from this comparison as an outlier; see Figures S.5 and S.6). However, there was no significant difference between exudation rate of red clover between the pH-stressed and unstressed condition when compared by collection period (Table S.5) or by total exudation with the Mann–Whitney *U* test (average exudation rate in the unstressed system over the photoperiod was 158 µg DOC (g-root dry mass)$^{-1}$(hrs)$^{-1}$).

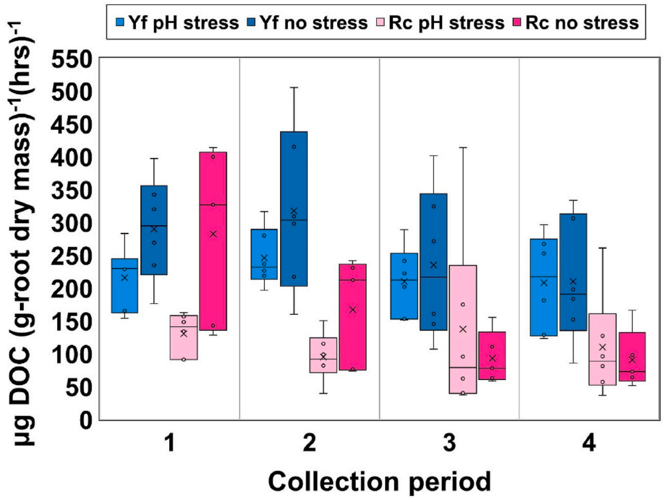

**Fig. 3.** Exudation measured in the exudate collection solution of pH-stressed and non-stressed yorkshire fog (Yf) and red clover (Rc) during four consecutive collection periods over a single photoperiod (○ data points; × mean values; boxes show median, and 25th and 75th percentiles). Sampling was conducted over a single 14hr photoperiod (period 1: 0hrs–3hrs, period 2: 3hrs40min-6hrs40mins, period 3: 7hrs20mins-10hrs20mins, period 4: 11hrs–14hrs).

The exudation rate of red clover was statistically lower than that of yorkshire fog in the 1st and 2nd collection periods (p-values of 0.005 and 8 x 10$^{-5}$ respectively, Table S5) under pH-stress and in the 2nd, 3rd and 4th collection periods without pH-stress (p-values of 0.05, 0.03 and 0.03 respectively). Furthermore, the cumulative DOC production by yorkshire fog over the 4 collection periods (Figure S6) was statistically higher (U-value ≤ critical value) than that by red clover for both the stressed (average 1760 vs 666 µg DOC g-root dry mass$^{-1}$) and unstressed conditions (average 2305 vs 803 µg DOC g-root dry mass$^{-1}$).

### *3.4. Root exudate composition (GC-MS)*

The GC-MS data were normalized by peak area relative to the ribitol internal standard, and then corrected for the total root dry mass in each system. This allows quantitative comparison of the relative peak area of each compound between samples, but only qualitative comparison between different compounds. As an approximation, however, compounds were grouped by order of magnitude of the relative peak areas.

16 different organic compounds were identified within the exudate solutions (2 amino acids, 6 other organic acids, 3 sugars and 5 sugar alcohols, see Table S.1 and Fig. 4), along with an unknown compound. All 16 metabolites were detected in the exudates from both pH-stressed and unstressed yorkshire fog during at least one collection period. Twelve metabolites were detected in the exudates from pH-stressed red clover during at least one collection period, while only nine metabolites were detected in the exudates from unstressed red clover.

The compounds with the largest normalized peak areas (on average) in non-stressed yorkshire fog were inositol, fructose and glucose (in the range 0.01–0.1, see Fig. 4 and Table S.2), with organic acids of the tricarboxylic acid cycle (TCA cycle, central metabolism), along with quinic acid, showing smaller normalized peaks (in the range 0.00001–0.01). The sugars have the largest peak areas in the first collection period, decreasing as the photoperiod progresses. In pH-stressed yorkshire fog, the largest normalized peak areas (on average) were citric acid, malic acid and inositol (in the range 0.01–0.1), with an increase of 1–2 orders of magnitude in the TCA-organic acids (21, 32, 139 and 4 times the relative peak area averaged over the 4 photo periods for citric, malic, fumaric and succinic acid, respectively), and reductions of 1–2 orders of magnitude in the sugars (0.2, 0.06 and 0.01 times for glucose, fructose and sucrose, respectively). In this case, none of the compounds appear to have pattern in relation to the photoperiod, with organic acids appearing from the first collection period.

In non-stressed red clover, the largest normalized peak areas (on average) were also inositol (in the range 1–10), glucose (in the range 0.1–1) and fructose (in the range 0.01–0.1), with no detection of amino acids and TCA-organic acids (apart from a very small succinic acid peak) in the exudate solution (see Table S2). Glucose, fructose as well as some of the sugar alcohols, have higher peak area in the first collection period, decreasing or disappearing in later collection periods (Fig. 4). In pH-stressed red clover, in contrast, the largest normalized peaks (on average) included citric acid, γ-aminobutyric acid (GABA, a non-protein forming amino acid; Guo et al., 2023), inositol, glucose and glycine (in the range 0.01–0.1). Other TCA-organic acids (malic, fumaric and

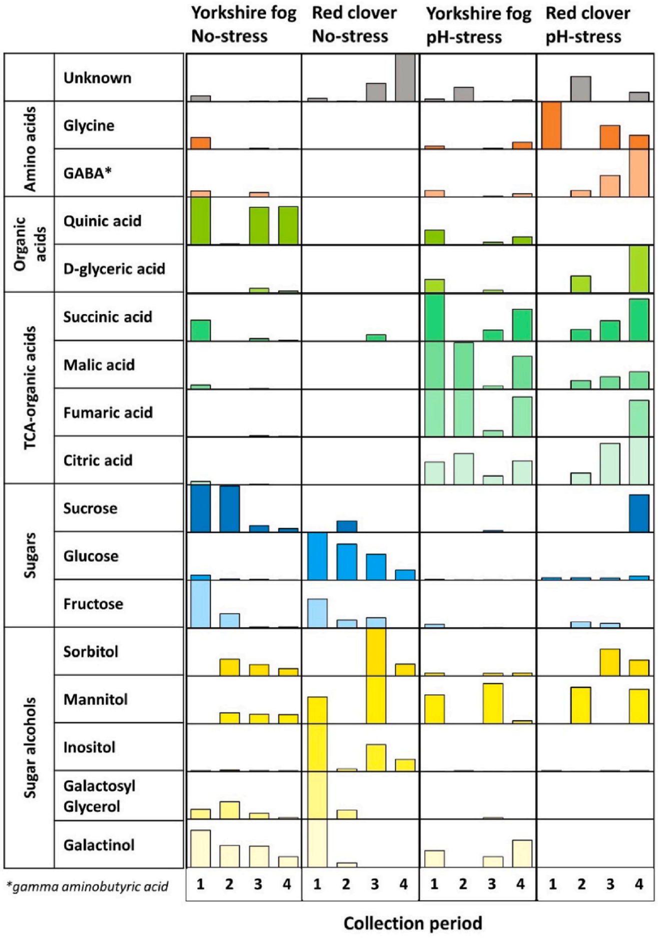

**Fig. 4.** Relative concentration of each compound identified by GC-MS analysis in the exudate solutions from yorkshire fog and red clover under non-stressed and pH-stressed conditions (relative concentration of a compound was determined by peak area ratio relative to the ribitol internal standard; peak heights in this figure should not be compared between compounds). Compounds are grouped and colour coded by functional classes: sugar alcohols (yellows), sugars (blues), TCA-organic acids and other organic acids (greens), amino acids (oranges), unknown (grey).

succinic acid) are also detected in the solution (in the range 0.001–0.01). While still present amongst the main peaks, the normalized areas of fructose, inositol and other sugar alcohols is reduced by 1–2 orders of magnitude (0.02, 0.1, and 0.2 times for inositol, glucose and fructose respectively, and from the order of 0.001–0.01 to non-detected for galactosyl glycerol and galactinol, see Table S2). Citric, malic, succinic acids, as well as GABA, appear from the second collection period, with growing peak areas as the photoperiod progresses. Glycine on the other hand, appears to have the opposite trend.

Comparing non-stressed yorkshire fog and red clover, reveals compositional differences in their exudates, notably the absence of most TCA-organic acids, quinic acid and amino acids in the exudates of red clover. Conversely, red clover has peaks for glucose and inositol that are, respectively, 1 and 2 orders of magnitude larger than yorkshire fog (17 and 29 times the average relative peak area, respectively). Under pH-stress, however, both species show similar compositional shifts. The upregulation of TCA-organic acids occurred in both species, and was accompanied by upregulation of amino acids (GABA and glycine) in red clover. pH-stress also caused downregulation of sugars (fructose and glucose) in both species, along with downregulation of sugar alcohols in red clover (particularly inositol, but galactosyl glycerol and galactinol became undetectable).

## 4. Discussion

### 4.1. Exudate composition in response to stress

Monocot and eudicot evolution diverged about 150 Myr ago (Chaw et al., 2004), so it has been hypothesized that they may have evolved different physiological responses to environmental cues (Wang et al., 2016). However, in this study we observed only modest differences in the exudation patterns of yorkshire fog (monocot) and red clover (eudicot) under unstressed conditions. Sugar alcohols and sugars were dominant in the exudate solutions from both species, although red clover produced larger normalized inositol and glucose peaks, while yorkshire fog produced modest normalized peaks from some organic acids (TCA, amino and quinic acid) which were absent in red clover. Overall, a greater number of compounds were detected in the exudate solutions from yorkshire fog than red clover. These exudation patterns caused a modest increase in the pH of the collection solution with yorkshire fog (∼1 pH unit), whereas red clover caused very little change in pH.

Under pH stress, both species caused a decrease in the pH of the collection solution by ∼2 pH units over each collection period (the final pH value of the collection solution was around pH 7.5 at the end of each collection period with both species). Furthermore, both species showed similar compositional responses to pH stress. Exudation of sugar alcohols and sugars by both species decreased, and exudation of the TCA cycle organic acids increased (Fig. 4 and SI Table S2). Red clover also exhibited an increase in the exudation of the amino acids, GABA and glycine. Interestingly, exudation of organic acids by yorkshire fog increased in all four collection periods relative to the unstressed condition, whereas the response from red clover was largely delayed until the second collection period, even though there were similar decreases in the pH of the collection solution in all four collection periods with both species. This delay in organic acid exudation by red clover indicates that other mechanisms also contribute to the buffering of pH. It is speculated that mechanisms could include proton release (possibly through the plasma membrane $H^+$-ATPase (Zhang et al., 2017);) and the conversion of $CO_2$ produced by respiration to bicarbonate in alkaline solutions.

Exudation of carboxylates by eudicot species in response to environmental stress has been widely reported (e.g. red clover, spinach, chickpea, lupin and alfalfa amongst others can exude carboxylate under P-deficient conditions; Gerke, 2015a; Veneklaas et al., 2003; Lipton et al., 1987; Tiziani et al., 2023; López-Bucio et al., 2000). While considered, in general, less efficient in carboxylate exudation, some graminaceous species (e.g. rye, wheat and triticale) have also been observed to respond to P-deficiency by carboxylate exudation (Gerke, 2015a). Guo et al. (2010) report that wheat exhibited similar changes in exudation patterns to those reported here in response to alkaline stress (decreases in carbohydrates and increases in carboxylates). It is also notable that the graminaceous monocot species yorkshire fog exuded moderate concentrations of citric, succinic, malic and fumaric acid even in unstressed conditions, whereas the eudicot species red clover did not.

Carbohydrates, such as sugars and sugar alcohols, are the primary products of photosynthesis (Maurer et al., 2021; Wang et al., 2016; More et al., 2019). These compounds then support other plant metabolic processes. Primary plant metabolism involves various pathways, such as glycolysis, citrate cycle, shikimate pathway, etc., that produce carboxylates and amino acids. Thus, changes in exudation patterns may be the result of up-regulation of some of these pathways in pH-stressed systems, resulting in consumption of a larger proportion of the available carbohydrates.

Many amino acids participate in plant responses to environmental stress (Trovato et al., 2021; Carvalhais et al., 2011). Upregulation of GABA, for example, has been reported in response to both biotic and abiotic stress (Guo et al., 2023; Seifikalhor et al., 2019). This non-proteinogenic amino acid is a plant signalling molecule that may also directly mitigate stress (e.g. by mopping up reactive oxygen species produced in plant cells in response to stress and as a pH buffer) (Fromm, 2020; Ramesh et al., 2017; Kamran et al., 2020). The glycine-rich protein superfamily are also reported to be involved in cellular stress responses and signalling (Czolpinska and Rurek, 2018). Moreover, GABA is synthesised from 2-oxoglutarate in a series of reactions that form a part of the GABA shunt in the TCA cycle (Bown and Shelp, 2020) and it is proposed that this can support a non-cyclic TCA flux mode that can also enhance the flux of fumarate and malate from the mitochondria to the cytosol (Sweetlove et al., 2010), making them available for export. GABA negatively regulates the activity of aluminium-activated malate transporters which mediate root exudation of malate (Palmer et al., 2016) hence GABA exudation may be enhanced to prevent its accumulation in the cytosol. Thus, it is interesting that increases in the concentration of GABA and glycine in root exudates in response to alkaline stress were only observed with red clover.

### 4.2. Net exudation in response to stress

While the exudates of yorkshire fog and red clover showed clear compositional differences between the pH-stressed and unstressed conditions, there was no significant difference in the net exudation rate of either species due to pH-stress. This suggests that there is a trade-off within the plant carbon budget between organic acid exudation and other plant functions. Research indicates that, to sustain stress-response strategies, plants need to redeploy energy reserves and redistribute carbon and nitrogen budgets. This in turn can produce physiological alterations such as reduction in above-ground growth (Blicharz et al., 2021; Tixier et al., 2024; Williams and de Vries, 2020). While in our experiment two weeks of alkaline stress produced no statistical differences in biomass or overall exudation rate (DOC in μg (g-root dry mass)$^{-1}$ (hrs)$^{-1}$) between pH-stressed and unstressed plants, the shift in exudate composition suggests that metabolites have been redirected from fulfilling metabolic functions in other organs, or that the down-regulation in exudation of certain metabolites (particularly carbohydrates) in favour of the stress response has occurred. However, evidence has shown that the modulation of stress by plants accumulates, producing less stressful conditions for subsequent plant growth, reducing their need for reallocation of resources to stress-response over time (Williams and de Vries, 2020; Bray et al., 2018). Nevertheless, further research is needed to fully understand the longer-term ecological implications.

Not all plant species have both quantitative and compositional responses to stress. For example, Canarini et al. (2016, Canarini et al., 2016) showed that in response to drought stress, soybean showed mostly increased exudation, whereas sunflower maintained root exudation rate but modified exudate composition. Similarly, Tiziani et al. (2023, 2023) showed that tomato exhibited an increase in overall exudation in response to P and Fe deficiency, while barley did not. Further, while there was a significant difference in the average net exudation rate between the two species in this study (combining stressed and unstressed data, the net exudation of red clover was only 60 % that of yorkshire fog), this difference may not be important. Williams et al. (2021, 2021) collected the exudates from similarly aged yorkshire fog and white clover using hydroponics and found that exudation rate for white clover was about 4x higher than that of yorkshire fog.

### 4.3. Potential impacts in the context of revegetation of polluted alkaline wastes

Vegetative cover is a cost-effective and environmentally sustainable method of stabilising and reclaiming industrial and mining sites where high-volume mineral-rich wastes have been dumped (Tordoff et al., 2000; Sapsford et al., 2022). This is because the primary hazards from such industrial residues tend to result from either direct contact with the residue, ingestion or inhalation of dusts, or ingestion of rain-water run-off (Nathanail and Earl, 2001; Strange et al., 2016). However, the properties of such waste material (e.g. high/low pH, low nutrient status, poor physical structure, and lack of rhizosphere microorganism) are often unfavourable to successful vegetation establishment. So, root exudates play an important role in changing the rhizosphere environment to better support plant growth.

When revegetating alkaline wastes (aluminium extraction wastes, iron and steel slags, coal fly ash and cementitious wastes are produced in enormous volumes worldwide; Gomes et al., 2016), the immediate challenge is to buffer the rhizosphere soil to a pH value that better supports plant growth. This work has shown that two typical pioneer species used to revegetate industrial sites (Bray et al., 2018; Bradshaw, 1997) exude sugars and sugar alcohols which can improve soil texture, and organic acids that improve nutrient recovery (Naveed et al., 2017; López-Bucio et al., 2000; Molnár et al., 2023). Further, it has showed that under alkaline stress, both yorkshire fog and red clover increased the exudation of substances that can buffer the pH downwards, such as citric, malic, fumaric and succinic acid. Buffering the pH of alkaline wastes downwards has other benefits in addition to reducing root stress, as high pH is very detrimental the survival and growth of rhizobacteria (Chen and Liu, 2024). Increasing microbial metabolism has further benefits as their metabolites will also buffer alkaline pH (particularly $CO_2$).

There may be concern that plant exudation could mobilize heavy metals from alkaline waste, as carboxylic acids can enhance cation mobilization in the substrate both by acidification and chelation (López-Bucio et al., 2000; Molnár et al., 2023; Kamran et al., 2020; Gerke, 2015b; More et al., 2019). Carboxylic organic acids have a negative charge and are strong chelators for metal and heavy metal cations like Cd, Cu, Pb, and Zn; and citrate in particular is one of the most powerful cation chelators in the organic acid group (Yan et al., 2022; Furukawa and Tokunaga, 2004; Jean-Soro et al., 2012; Gao et al., 2003; Shahid et al., 2012). Chelation plays a critical role in sorption reactions at the mineral–water interface, as both ternary surface complexes (which increase sorption) and aqueous complexes (which increase desorption) can be formed (Yan et al., 2022; Furukawa and Tokunaga, 2004; Kim and Duquette, 2006; Yang et al., 2020). Typically, organic acids tend to sorb to soil organic matter and clay minerals at lower concentrations inhibiting desorption of metals, whereas higher concentrations are required to promote desorption (Gao et al., 2003; Yan et al., 2022). It is not possible to translate the exudation rates observed here into estimates of the likely organic acid concentrations at contaminated sites as both the rate of diffusion away from root surfaces and the rate microbial breakdown in rhizosphere are unknown. Nevertheless, it indicates that further research with yorkshire fog and red clover directly grown on alkaline waste is needed to understand how the quantities of compounds released might impact substrate physiochemistry.

When comparing this hydroponic laboratory study to field settings (e.g. plants growing in alkaline waste management facilities) it is important to recognise that additional factors to pH, can affect rhizosphere dynamics and plant physiology. For example, low availability of nutrients, such as K, may interfere in the efficiency of targeted stress response mechanisms (i.e. organic acid upregulation; Miller et al., 1990). For this reason, planned waste revegetation schemes should take into account nutrient availability, and amend it when necessary. Monitoring of rhizosphere pH over time, in the field, would also allow to inform the efficiency of root exudates-mediated pH-buffering, as compared to the results obtained here in a hydroponic set up. Although the complexity added by the activity of other soil compartments would have to be considered.

## 5. Conclusions

Under unstressed conditions net root exudation of DOC by the monocot yorkshire fog (*Holcus lanatus*) was higher (up to ~ x2.5) than that of the eudicot red clover (*Trifolium pratense*), and had a greater number of detectable compounds. Root exudates from both species, however, were dominated by sugar alcohols and sugars. Exudation by unstressed yorkshire fog caused a modest increase in the pH of the collection solution, while unstressed red clover caused very little change in pH.

In response to pH stress (pH ~10), neither species showed a significant change in the net exudation rate when compared to the unstressed condition. However, both species reduced the pH of the collection solution. With both species the exudation of sugar alcohols and sugars decreased in response to stress, and exudation of the TCA cycle organic acids increased, contrary to previous research indicating that graminaceous monocots are less efficient in exudation of carboxylates. Red clover also exhibited an increase in the exudation of the amino acids, GABA and glycine.

Both yorkshire fog and red clover are grown as vegetative cover on alkaline waste repositories to reduce the environmental risk posed by such facilities. Successful establishment of vegetation depends on the plants ability to buffer the pH of an alkaline waste to a more benign value. In the short-term both plants respond to alkaline stress by increased exudation of organic acids, which reduce the ambient pH, without change in the overall exudation rate. While this stress response is likely to produce a trade-off within the plant carbon budget, that may impact upon plant growth, in the long-term, the investment in stress response should decrease as the rhizosphere becomes more benign for subsequent plants and vegetation density increases. Thus, early interventions that increase rapid establishment of vegetation will increase likelihood of enduring plant cover at these sites.

## CRediT authorship contribution statement

**Felipe E. Sepúlveda Olea:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Conceptualization. **Ian T. Burke:** Writing – review & editing, Supervision, Methodology, Investigation, Funding acquisition, Conceptualization. **Robert D. Hancock:** Writing – review & editing, Methodology, Formal analysis. **Simon D.A. Pont:** Investigation. **Douglas I. Stewart:** Writing – review & editing, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## 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.

## 8. Acknowledgements

We acknowledge support from Engineering and Physical Science Research Council grant EP/T031166/1. We would like to thank Emma Tidswell, David Elliot, Morgan McGowan, Rachel Gasior, Andrew Hobson and G Nasir Khan at the University of Leeds for their support during this research project.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rhisph.2025.101115.

## Data availability

Data will be made available on request.

## References

Adeleke, R., Nwangburuka, C., Oboirien, B., 2017. Origins, roles and fate of organic acids in soils: a review. South Afr. J. Bot. 108, 393–406.

Alasmary, Z.S., 2020. *Laboratory- to Field-Scale Investigations to Evaluate Phosphate Amendments and Miscanthus for Phytostabilization of Lead-Contaminated Military Sites.* Doctor of Philosophy. Kansas State University.

Amezcua-Allieri, M.A., Rodriguez-Dorantes, A., Melendez-Estrada, J., 2010. The use of biostimulation and bioaugmentation to remove phenanthrene from soil. Int. J. Oil Gas Coal Technol. 3, 39–59.

Ash, H.J., Gemmell, R.P., Bradshaw, A.D., 1994. The introduction of native plant species on industrial waste heaps: a test of immigration and other factors affecting primary succession. J. Appl. Ecol. 31, 74–84.

Blicharz, S., Beemster, G.T.S., Ragni, L., De Diego, N., Spíchal, L., Hernándiz, A.E., Marczak, Ł., Olszak, M., Perlikowski, D., Kosmala, A., Malinowski, R., 2021. Phloem exudate metabolic content reflects the response to water-deficit stress in pea plants (Pisum sativum L.). Plant J. 106, 1338–1355.

Bown, A.W., Shelp, B.J., 2020. Does the GABA shunt regulate cytosolic GABA? Trends Plant Sci. 25, 422–424.

Bradshaw, A., 1997. Restoration of mined lands—using natural processes. Ecol. Eng. 8, 255–269.

Bray, A.W., Stewart, D.I., Courtney, R., Rout, S.P., Humphreys, P.N., Mayes, W.M., Burke, I.T., 2018. Sustained bauxite residue rehabilitation with gypsum and organic matter 16 years after initial treatment. Environ. Sci. Technol. 52, 152–161.

Canarini, A., Kaiser, C., Merchant, A., Richter, A., Wanek, W., 2019. Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. Front. Plant Sci. 10.

Canarini, A., Merchant, A., Dijkstra, F.A., 2016. Drought effects on Helianthus annuus and Glycine max metabolites: from phloem to root exudates. Rhizosphere 2, 85–97.

Carvalhais, L.C., Dennis, P.G., Fedoseyenko, D., Hajirezaei, M.-R., Borriss, R., von Wirén, N., 2011. Root exudation of sugars, amino acids, and organic acids by maize as affected by nitrogen, phosphorus, potassium, and iron deficiency. J. Plant Nutr. Soil Sci. 174, 3–11.

Chaw, S.-M., Chang, C.-C., Chen, H.-L., Li, W.-H., 2004. Dating the monocot–dicot divergence and the origin of core eudicots using whole chloroplast genomes. J. Mol. Evol. 58, 424–441.

Chen, L., Liu, Y., 2024. The function of root exudates in the root colonization by beneficial soil rhizobacteria. Biology (Basel) 13.

Courtney, R., Harrington, T., 2012. Growth and nutrition of Holcus lanatus in bauxite residue amended with combinations of spent mushroom compost and gypsum. Land Degrad. Dev. 23, 144–149.

Courtney, R., Harrington, T., Byrne, K.A., 2013. Indicators of soil formation in restored bauxite residues. Ecol. Eng. 58, 63–68.

Courtney, R., Mullen, G., Harrington, T., 2009. An evaluation of revegetation success on bauxite residue. Restor. Ecol. 17, 350–358.

Courtney Timpson, J.P., R., Grennan, E., 2003. Growth of Trifolium pratense in red mud amended with process sand, gypsum and thermally dried sewage sludge. Int. J. Surf. Min. Reclamat. Environ. 17, 227–233.

Czolpinska, M., Rurek, M., 2018. Plant glycine-rich proteins in stress response: an emerging, still prospective story. Front. Plant Sci. 9.

Davin, M., Starren, A., Marit, E., Lefébure, K., Fauconnier, M.-L., Colinet, G., 2019. Investigating the effect of medicago sativa L. And Trifolium pratense L. Root exudates on PAHs bioremediation in an aged-contaminated soil. Water, Air, Soil Pollut. 230, 296.

de Barros Dantas, L.L., Eldridge, B.M., Dorling, J., Dekeya, R., Lynch, D.A., Dodd, A.N., 2023. Circadian regulation of metabolism across photosynthetic organisms. Plant J. 116, 650–668.

Debela, F., Arocena, J.M., Thring, R.W., Whitcombe, T., 2010. Organic acid-induced release of lead from pyromorphite and its relevance to reclamation of Pb-contaminated soils. Chemosphere 80, 450–456.

Di Carlo, E., Chen, C.R., Haynes, R.J., Phillips, I.R., Courtney, R., 2019. Soil quality and vegetation performance indicators for sustainable rehabilitation of bauxite residue disposal areas: a review. Soil Res. 57, 419–446.

Foito, A., Byrne, S.L., Hackett, C.A., Hancock, R.D., Stewart, D., Barth, S., 2013. Short-term response in leaf metabolism of perennial ryegrass (Lolium perenne) to alterations in nitrogen supply. Metabolomics (Dordr.) 9, 145–156.

Fromm, H., 2020. GABA signaling in plants: targeting the missing pieces of the puzzle. J. Exp. Bot. 71, 6238–6245.

Furukawa, M., Tokunaga, S., 2004. Extraction of heavy metals from a contaminated soil using citrate-enhancing extraction by pH control and ultrasound application. J. Environ. Sci. Health Part A 39, 627–638.

Gao, Y., He, J., Ling, W., Hu, H., Liu, F., 2003. Effects of organic acids on copper and cadmium desorption from contaminated soils. Environ. Int. 29, 613–618.

Gerke, J., 2015a. The acquisition of phosphate by higher plants: effect of carboxylate release by the roots. A critical review. J. Plant Nutr. Soil Sci. 178, 351–364.

Gerke, J., 2015b. Phytate (inositol hexakisphosphate) in soil and phosphate acquisition from inositol phosphates by higher plants. A review. Plants 4, 253–266.

Gomes, H.I., Mayes, W.M., Rogerson, M., Stewart, D.I., Burke, I.T., 2016. Alkaline residues and the environment: a review of impacts, management practices and opportunities. J. Clean. Prod. 112, 3571–3582.

Guo, R., Shi, L., Ding, X., Hu, Y., Tian, S., Yan, D., Shao, S., Gao, Y., Liu, R., Yang, Y., 2010. Effects of saline and alkaline stress on germination, seedling growth, and ion balance in wheat. Agron. J. 102, 1252–1260.

Guo, Z., Gong, J., Luo, S., Zuo, Y., Shen, Y., 2023. Role of gamma-aminobutyric acid in plant defense response. Metabolites 13.

Gupta, P., Kumar, V., 2016. Value added phytoremediation of metal stressed soils using phosphate solubilizing microbial consortium. World J. Microbiol. Biotechnol. 33, 9.

Hashimoto, Y., Matsufuru, H., Sato, T., 2008. Attenuation of lead leachability in shooting range soils using poultry waste amendments in combination with indigenous plant species. Chemosphere 73, 643–649.

Hashimoto, Y., Takaoka, M., Shiota, K., 2011. Enhanced transformation of lead speciation in rhizosphere soils using phosphorus amendments and phytostabilization: an X-ray absorption fine structure spectroscopy investigation. J. Environ. Qual. 40, 696–703.

Hiradate, S., Ma, J.F., Matsumoto, H., 2007. Strategies of plants to adapt to mineral stresses in problem soils. Advances in Agronomy. Academic Press.

Jean-Soro, L., Bordas, F., Bollinger, J.-C., 2012. Column leaching of chromium and nickel from a contaminated soil using EDTA and citric acid. Environ. Pollut. (Amsterdam, Neth.) 164, 175–181.

Kamran, M., Ramesh, S.A., Gilliham, M., Tyerman, S.D., Bose, J., 2020. Role of TaALMT1 malate-GABA transporter in alkaline pH tolerance of wheat. Plant Cell Environ. 43, 2443–2459.

Karpinska, B., Zhang, K., Rasool, B., Pastok, D., Morris, J., Verrall, S.R., Hedley, P.E., Hancock, R.D., Foyer, C.H., 2018. The redox state of the apoplast influences the acclimation of photosynthesis and leaf metabolism to changing irradiance. Plant Cell Environ. 41, 1083–1097.

Kiehl, K., Kirmer, A., Donath, T.W., Rasran, L., Hölzel, N., 2010. Species introduction in restoration projects – evaluation of different techniques for the establishment of semi-natural grasslands in Central and Northwestern Europe. Basic Appl. Ecol. 11, 285–299.

Kim, S., Duquette, D.J., 2006. Effect of chemical composition on adhesion of directly electrodeposited copper film on TiN. J. Electrochem. Soc. 153, C417.

Kucharski, R., Sas-Nowosielska, A., Małkowski, E., Japenga, J., Kuperberg, J.M., Pogrzeba, M., Krzyżak, J., 2005. The use of indigenous plant species and calcium phosphate for the stabilization of highly metal-polluted sites in southern Poland. Plant Soil 273, 291–305.

Lee, J.A., Greenwood, B., 1976. The colonisation by plants of calcareous wastes from the salt and alkali industry in Cheshire, England. Biol. Conserv. 10, 131–149.

Lipton, D.S., Blanchar, R.W., Blevins, D.G., 1987. Citrate, malate, and succinate concentration in exudates from P-sufficient and P-stressed medicago sativa L. Seedlings 1. Plant Physiol. 85, 315–317.

López-Bucio, J., Nieto-Jacobo, M.a.F., Ramırez-Rodrıguez, V., Herrera-Estrella, L., 2000. Organic acid metabolism in plants: from adaptive physiology to transgenic varieties for cultivation in extreme soils. Plant Sci. 160, 1–13.

Maurer, D., Malique, F., Alfarraj, S., Albasher, G., Horn, M.A., Butterbach-Bahl, K., Dannenmann, M., Rennenberg, H., 2021. Interactive regulation of root exudation and rhizosphere denitrification by plant metabolite content and soil properties. Plant Soil 467, 107–127.

McLaughlin, S., Himmighofen, P., Khan, S.A., Siffert, A., Robert, C.A.M., Sasse, J., 2025. Root exudation: an in-depth experimental guide. Plant Cell Environ. 48, 3052–3065.

Miller, G.W., Shigematsu, A., Welkie, G.W., Motoji, N., Szlek, M., 1990. Potassium effect on iron stress in tomato: : II. The effects on root CO2-fixation and organic acid formation. J. Plant Nutr. 13, 1355–1370.

Mimmo, T., Del Buono, D., Terzano, R., Tomasi, N., Vigani, G., Crecchio, C., Pinton, R., Zocchi, G., Cesco, S., 2014. Rhizospheric organic compounds in the soil–microorganism–plant system: their role in iron availability. Eur. J. Soil Sci. 65, 629–642.

Molnár, Z., Solomon, W., Mutum, L., Janda, T., 2023. Understanding the mechanisms of Fe deficiency in the rhizosphere to promote plant resilience. Plants 12.

More, S., Shinde, S., Kasture, M., 2019. Root exudates a key factor for soil and plant: an overview. Pharm. Innov. 8 (6), 449–459.

Nathanail, C.P., Earl, N., 2001. Human health risk assessment: guideline values and magic numbers. In: HESTER, R.E., HARRISON, R.M., HARRISON, R.M., HESTER, R. E. (Eds.), Assessment and Reclamation of Contaminated Land. The Royal Society of Chemistry.

Naveed, M., Brown, L.K., Raffan, A.C., George, T.S., Bengough, A.G., Roose, T., Sinclair, I., Koebernick, N., Cooper, L., Hackett, C.A., Hallett, P.D., 2017. Plant exudates may stabilize or weaken soil depending on species, origin and time. Eur. J. Soil Sci. 68, 806–816.

Padmavathiamma, P.K., Li, L.Y., 2012. Rhizosphere influence and seasonal impact on phytostabilisation of metals—a field study. Water, Air, Soil Pollut. 223, 107–124.

Palmer, Antony J., Baker, A., Muench, Stephen P., 2016. The varied functions of aluminium-activated malate transporters–much more than aluminium resistance. Biochem. Soc. Trans. 44, 856–862.

Potysz, A., Grybos, M., Kierczak, J., Guibaud, G., Fondaneche, P., Lens, P.N.L., van Hullebusch, E.D., 2017. Metal mobilization from metallurgical wastes by soil organic acids. Chemosphere 178, 197–211.

Rahman, I.M.M., Khan, B.M., 2023. Physiological responses of wild grass Holcus lanatus L. to potentially toxic elements in soils: a review. Environ. Sci. Pollut. Control Ser. 30, 54470–54482.

Ramesh, S.A., Tyerman, S.D., Gilliham, M., Xu, B., 2017. γ-Aminobutyric acid (GABA) signalling in plants. Cell. Mol. Life Sci. 74, 1577–1603.

Rybak, A.V., Belykh, E.S., Maystrenko, T.A., Velegzhaninov, I.O., 2018. Microsatellite polymorphism of Trifolium pratense population at the conditions of radioactive and chemical contamination of soil (Komi republic, Russia). Environ. Sci. Pollut. Control Ser. 25, 34701–34710.

Santini, T.C., Fey, M.V., 2013. Spontaneous vegetation encroachment upon bauxite residue (red mud) as an indicator and facilitator of in situ remediation processes. Environ. Sci. Technol. 47, 12089–12096.

Sapsford, D.J., Stewart, D.I., Sinnett, D.E., Burke, I.T., Cleall, P.J., Harbottle, M.J., Mayes, W., Owen, N.E., Sardo, A.M., Weightman, A., 2022. Circular economy landfills for temporary storage and treatment of mineral-rich wastes. Proc. Inst. Civil Eng. Waste Resour. Manag. 176, 77–93.

Scarpella, E., Meijer, A.H., 2004. Pattern formation in the vascular system of monocot and dicot plant species. New Phytol. 164, 209–242.

Seifikalhor, M., Aliniaeifard, S., Hassani, B., Niknam, V., Lastochkina, O., 2019. Diverse role of γ-aminobutyric acid in dynamic plant cell responses. Plant Cell Rep. 38, 847–867.

Shahid, M., Pinelli, E., Dumat, C., 2012. Review of Pb availability and toxicity to plants in relation with metal speciation; role of synthetic and natural organic ligands. J. Hazard Mater. 219–220, 1–12.

Strange, J., Langdon, N., Large, A., 2016. Contaminated Land Guidance: the Route to Sustainable Economic Solutions. ICE Publishing, London.

Sweetlove, L.J., Beard, K.F.M., Nunes-Nesi, A., Fernie, A.R., Ratcliffe, R.G., 2010. Not just a circle: flux modes in the plant TCA cycle. Trends Plant Sci. 15, 462–470.

Tischew, S., Baasch, A., Grunert, H., Kirmer, A., 2014. How to develop native plant communities in heavily altered ecosystems: examples from large-scale surface mining in Germany. Appl. Veg. Sci. 17, 288–301.

Tixier, A., Barnard, R.L., Jeudy, C., Prudent, M., 2024. Sugar and amino acid exhibit different spatial patterns of root exudation in response to water stress and n limitation in pea. Plant Soil. https://doi.org/10.1007/s11104-024-07139-y.

Tiziani, R., Pranter, M., Valentinuzzi, F., Pii, Y., Luigimaria, B., Cesco, S., Mimmo, T., 2023. Unraveling plant adaptation to single and combined nutrient deficiencies in a dicotyledonous and a monocotyledonous plant species. Plant Sci. 335, 111793.

Tordoff, G.M., Baker, A.J.M., Willis, A.J., 2000. Current approaches to the revegetation and reclamation of metalliferous mine wastes. Chemosphere 41, 219–228.

Trovato, M., Funck, D., Forlani, G., Okumoto, S., Amir, R., 2021. Editorial: amino acids in plants: regulation and functions in development and stress defense. Front. Plant Sci. 12.

Tsai, H.-H., Schmidt, W., 2021. The enigma of environmental pH sensing in plants. Nat. Plants 7, 106–115.

Veneklaas, E.J., Stevens, J., Cawthray, G.R., Turner, S., Grigg, A.M., Lambers, H., 2003. Chickpea and white lupin rhizosphere carboxylates vary with soil properties and enhance phosphorus uptake. Plant Soil 248, 187–197.

Vives-Peris, V., de Ollas, C., Gómez-Cadenas, A., Pérez-Clemente, R.M., 2020. Root exudates: from plant to rhizosphere and beyond. Plant Cell Rep. 39, 3–17.

Vranova, V., Rejsek, K., Skene, K.R., Janous, D., Formanek, P., 2013. Methods of collection of plant root exudates in relation to plant metabolism and purpose: a review. J. Plant Nutr. Soil Sci. 176, 175–199.

Wang, W., Zhou, H., Ma, B., Owiti, A., Korban, S.S., Han, Y., 2016. Divergent evolutionary pattern of sugar transporter genes is associated with the difference in sugar accumulation between grasses and eudicots. Sci. Rep. 6, 29153.

Wasilkowski, D., Mrozik, A., Piotrowska-Seget, Z., Krzyżak, J., Pogrzeba, M., Płaza, G., 2014. Changes in enzyme activities and microbial community structure in heavy metal-contaminated soil under in situ aided phytostabilization. Clean: Soil, Air, Water 42, 1618–1625.

Williams, A., de Vries, F.T., 2020. Plant root exudation under drought: implications for ecosystem functioning. New Phytol. 225, 1899–1905.

Williams, A., Langridge, H., Straathof, A.L., Fox, G., Muhammadali, H., Hollywood, K.A., Xu, Y., Goodacre, R., de Vries, F.T., 2021. Comparing root exudate collection techniques: an improved hybrid method. Soil Biol. Biochem. 161, 108391.

Yan, Y., Wan, B., Mansor, M., Wang, X., Zhang, Q., Kappler, A., Feng, X., 2022. Co-sorption of metal ions and inorganic anions/organic ligands on environmental minerals: a review. Sci. Total Environ. 803, 149918.

Yang, X., Liu, L., Tan, W., Liu, C., Dang, Z., Qiu, G., 2020. Remediation of heavy metal contaminated soils by organic acid extraction and electrochemical adsorption. Environ. Pollut. (Amsterdam, Neth.) 264, 114745.

Young, E., Carey, M., Meharg, A.A., Meharg, C., 2018. Microbiome and ecotypic adaption of Holcus lanatus (L.) to extremes of its soil pH range, investigated through transcriptome sequencing. Microbiome 6, 48.

Zhang, J., Wei, J., Li, D., Kong, X., Rengel, Z., Chen, L., Yang, Y., Cui, X., Chen, Q., 2017. The role of the plasma membrane H+-ATPase in plant responses to aluminum toxicity. Front. Plant Sci. 8.