1-1-2022

Root exudates impact plant performance under abiotic stress

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Root exudates impact plant performance under abiotic stress

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Abstract

Plant root exudates serve pivotal roles in supporting plant development and interactions with the physicochemical and biological factors in the rhizosphere. Under stress conditions, root exudation is involved in enhancing plant resource-use efficiency and facilitating the crosstalk between plant and soil microbes to ameliorate stress. Although there are a large number of root exudates that remain to be characterized, recent technological advancements have allowed for the function of many exudate compounds to be elucidated. In this review, we discuss current knowledge about the key root exudates that modulate plant resource-use efficiency under various abiotic stresses including drought, aluminum toxicity, phosphorus, nitrogen, and iron deficiency. The role that key root exudates play in shaping microbial communities in the rhizosphere under stress conditions is also an important consideration addressed in this review.

Published in *Trends in Plant Science,* January 2022, Vol. 27, No. 1, pp. 80-91
doi.org/10.1016/j.tplants.2021.08.003

Highlights

- Root exudation plays important roles in plant interactions with soil chemical factors and belowground microorganisms that impact abiotic stresses.
- Carboxylate efflux is a key mechanism for plant phosphorus acquisition and tolerance to aluminum.
- Phenolics are utilized by dicots and non-graminaceous monocots while phytosiderophore exudation is used by grasses to mobilize iron for root uptake.
- Secondary metabolites such as flavonoids and strigolactones facilitate plant association with mycorrhizal fungi under drought and phosphorus-deficient conditions.
- Under nitrogen-limited conditions root exudates facilitate plant associations with nitrogen-fixing microbes and may reduce losses of soil nitrogen through inhibition of the bacteria involved in nitrification and denitrification.

Glossary

Primary metabolites: metabolites that are essential for cell growth and functioning.
Rhizosheath: the region around the root that is characterized by the firm adhesion of soil aggregates to the root tissue that remains attached even after vigorous agitation.
Rhizosphere: the layer of root-surrounding soil that is under the direct influence of root exudates.
Root exudation: the process in which plants release carbon and other compounds from the roots to the surrounding soil.
Secondary metabolites: metabolites that are not directly involved in cell growth and maintenance.

What are root exudates?

Root exudation (see Glossary) processes are a strong carbon sink for plants, accounting for up to 21% of plant net photosynthates depending on the plant species, growth stage, and nutrient status [1]. A large

proportion of plant root exudates are comprised of high-molecular-weight compounds such as proteins and mucilage while the lower molecular weight compounds are more diverse. These low-molecular-weight compounds encompass a wide range of **primary metabolites** (amino acids, sugars, carboxylates, etc.) and **secondary metabolites** (sorgoleone, flavonoids, coumarin, etc.) [2]. Plant root exudates have a multitude of functions that may affect plant performance under stress conditions [2]. For example, carboxylates in the root exudates are well-known chelating agents that solubilize phosphorus (P) for plant uptake [3]. In addition to altering soil nutrient availability, root exudates have crucial roles in facilitating root interactions with beneficial microbes while also suppressing pathogens [4]. For example, a major secondary metabolite in maize root exudates, 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA), not only acts as an allelochemical against soil microbes and nearby plants, but is also a chemoattractant for the plant growth-promoting bacterium *Pseudomonas putida KT2440* [5]. In this review, we compile the most recent information about root exudates that confer plant resource-use efficiency under various abiotic stresses including deficits in water, P, nitrogen (N), and iron (Fe), and the toxicity of aluminum (Al). We also address the complex interplay between root exudates and soil microbes that enhance plant resource-use efficiency under these stress conditions. Since root exudate composition can vary significantly across different plant species, we compare some of the belowground strategies that different plant species use for coping with these stresses.

Rhizosheath, mycorrhizal fungi, abscisic acid, and osmolytes influence plant performance under drought stress

Drought has many deleterious effects on plants including but not limited to decreasing growth and increasing oxidative stress [6]. The formation of a **rhizosheath** around roots helps plants cope with drought and has been reported in desert plants and many crops including maize, sorghum, oat, and barley [7]. Although the exact mechanism by which the rhizosheath ameliorates plant drought stress is unknown, it has been suggested to reduce root water loss by increasing the

contact between soil and roots while minimizing direct root exposure to drought-induced air gaps in soil which have high hydraulic resistance [8]. The formation of a rhizosheath is enhanced under drought conditions when mucilage is exuded by roots, which is responsible for assembling soil particles and stabilizing the resulting soil aggregates around roots [7]. In addition to mucilage secreted from plants, many bacteria are also involved in rhizosheath formation by producing exopolysaccharides [9,10]. For example, inoculation on wheat of *Paenibacillus polymyxa* CF43 with impaired ability to produce levan was shown to significantly decrease the rhizosheath size [9]. Together, these results demonstrate the interplay between plant root exudates and soil microbes in facilitating the formation of a rhizosheath.

In some cases the ability to interact with arbuscular mycorrhizal fungi (AMF) is related to plant tolerance to drought because the extraradical hyphae of AMF aid in acquiring water from the soil pores inaccessible to plant roots and the regions outside the rooting zone [11]. Drought also limits the diffusion of plant exuded carbon to many bacteria and non-mycorrhizal fungi in the **rhizosphere**, hampering potential beneficial plant–microbe interactions [12]. Therefore, plant root exudates under moderate/short-term drought have been suggested to promote plant interactions with AMF and drought-resistant plant growth-promoting bacteria (PGPB) that may improve plant uptake of water and nutrients [13]. While the mechanisms underpinning the crosstalk between root exudates and AMF are complex, evidence suggests that root exudation of strigolactones serves important signaling functions during the early stages of AMF root colonization by promoting AMF hyphal proliferation [14,15]. Further supporting evidence highlights a positive correlation between strigolactones exuded and AMF colonization in tomato and lettuce under drought [16]. Many flavonoids are also involved in plant–AMF interactions, but unlike strigolactones, they are not essential in mediating plant symbiosis with AMF [17]. These findings demonstrate that strigolactone and flavonoid exudates are involved in plant–AMF interactions, which aid in ameliorating drought stress in plants.

Intriguingly, drought also enhances root exudation abscisic acid (ABA) [18–20] and organic osmolytes such as proline, betaine, trehalose, and pinitol [18,20,21]. ABA is a phytohormone that regulates plant responses to various osmotic stresses including drought and

salinity [22]. The effect of ABA on root exudates in plants experiencing drought is still unclear but exogenous application of ABA to the root tissues has been reported to alter root hydraulic conductivity and the expression of water channel aquaporins in rice and barley [23,24]. Intracellular accumulation of osmolytes is another mechanism by which plants and microbes maintain cell turgor to reduce water loss under dry conditions [25,26]. Although the role of osmolytes in the rhizosphere is not clear, they may play important roles in facilitating plant–microbe interactions under drought. For example, root exudation of proline can stimulate the movement of symbiotic *Sinorhizobium meliloti* towards the alfalfa roots [27] and when *Medicago truncatula* is colonized by this strain, a delay in the leaf senescence under drought conditions has been linked to increases in the production of osmolytes and drought-responsive proteins in plants [28]. Root exudation of ABA and osmolytes may also just be the result of the leakage of these compounds since their increased accumulation in roots can be triggered by drought [29,30]. Additional studies will be needed to unravel how these root exudates affect plant performance under drought.

Mycorrhiza, carboxylates, and acid phosphatases enhance plant P acquisition

P deficiency can severely impair plant development because it is essential in many biological molecules (phospholipid, ATP, and nucleic acid) and cellular activities such as protein regulation and energy transduction [31]. While many soils contain a tremendous amount of P, the majority of soil P is immobilized in organic and inorganic forms that are not available for plant uptake. The important mechanisms that plants use to cope with P deficiency include establishing symbiosis with mycorrhizal fungi and exuding P-solubilizing root exudates [32–34]. Given that both of these mechanisms involve a significant carbon investment from plants, they are not often deployed simultaneously but rather mycorrhizal colonization often results in a reduction of carboxylate exudation [32,33]. As symbionts to about 80% of terrestrial plants, AMF can enhance plant P uptake because their hyphae can access labile inorganic P beyond the P-depletion zone with

their specialized high-affinity P transporters on the mycelia [33,35]. Plants growing in nonagricultural ecosystems without P fertilizer addition derive most of their P from AMF because the P that can be accessed by roots is quickly depleted [35]. In contrast to AMF, ectomycorrhiza which dominate forest ecosystems are able to solubilize inorganic P and also play critical roles in mobilizing organic P sequestered in the forest soils [35]. In extremely P impoverished soils, plants often do not form symbiotic associations with mycorrhiza [36]. *Lupinus* and Proteaceae species are some examples of non-mycorrhizal plants that thrive under conditions of extremely low P availability. To acquire P under such conditions, these plants have evolved specialized root structures consisting of densely clustered lateral roots that have the ability to exude large amount of carboxylates to solubilize soil P [37].

Root exudation of low-molecular-weight carboxylates (≤ 3 carboxyl groups) such as malate, citrate, and oxalate is an important plant strategy to increase P uptake because carboxylates can displace immobilized P from P-containing inorganic and organic compounds in soil to make it more available to roots [37] (**Figure 1**). The ability of carboxylates to desorb P is proportional to the concentration exuded and the number of carboxyl groups, with tricarboxylates (citrate, aconitate, etc.) being the strongest while monocarboxylates are the weakest (acetate, pyruvate, etc.) at displacing P [3]. Carboxylates play indispensable roles in enhancing P nutrition in cluster root forming species in Proteaceae and Fabaceae because their densely packed lateral roots can exude a large quantity of carboxylates at a higher rate compared with other plant species in which carboxylate exudation occurs primarily in the region of root apices [38]. The cluster root forming species in Proteaceae and Fabaceae can exude up to 3000 nmol (g fresh root)$^{-1}$ h^{-1} of citrate [38], which is significantly higher than 0.15 and 216 nmol (g fresh root)$^{-1}$ h^{-1} reported in maize [39] and rice [40], respectively. These findings suggest that carboxylate exudation may not be a major P-acquisition strategy in plant species that do not form cluster roots [38,41]. Although greater citrate efflux has been reported in wheat and arabidopsis (*Arabidopsis thaliana)* genotypes that are relatively more tolerant to P-stress than their susceptible counterparts [38,42], the concentration of citrate that is exuded is still too low to effectively mobilize P [41]. Therefore, these P-stress tolerant genotypes may have other attributes that confer their enhanced P uptake

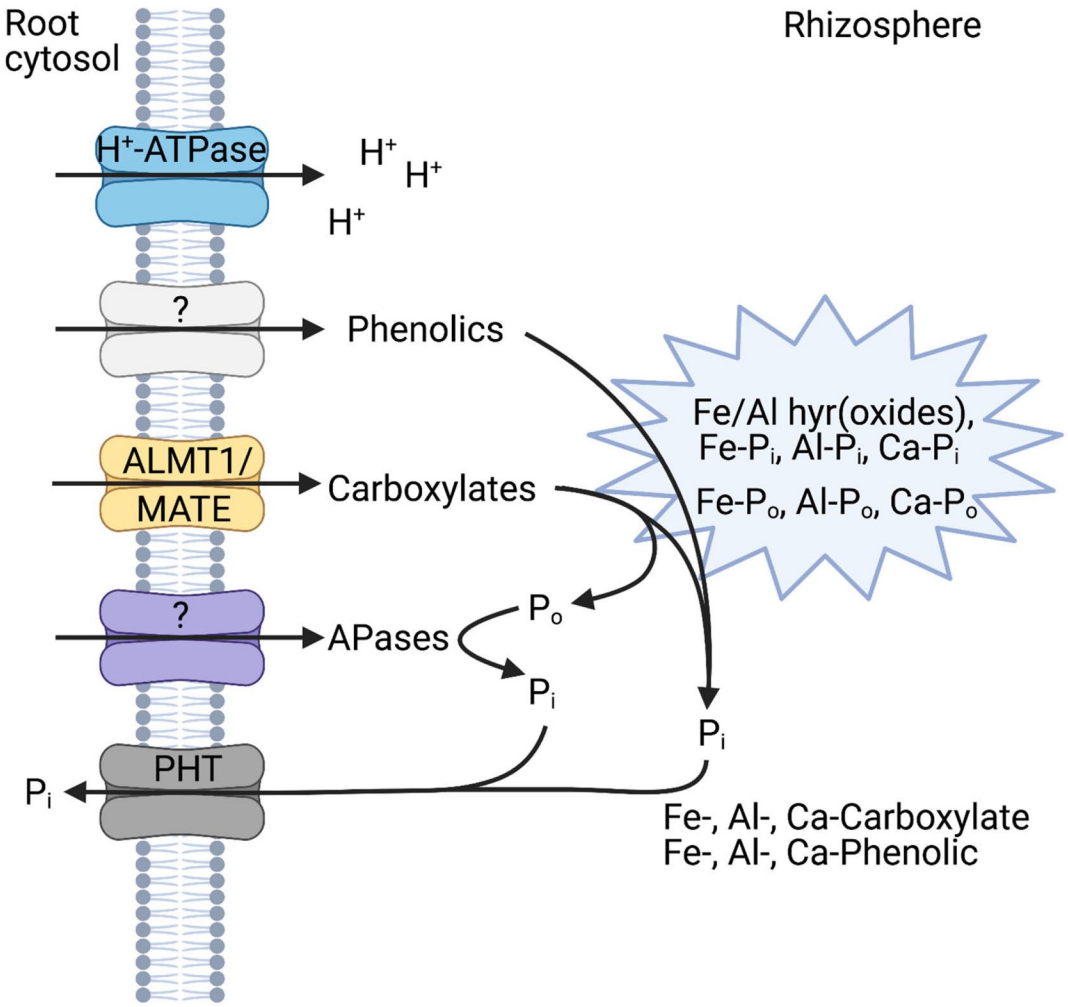

Figure 1. Root exudates facilitate plant phosphorous (P) uptake. Rhizosphere acidification acts synergistically with carboxylates to solubilize the inorganic (Pi) and organic P (Po) pools. Phenolics release P from the inorganic P pool by chelating with P-containing minerals predominantly Fe/Al-hyd(oxides). Acid phosphatases (APases) liberate P from P-containing organic matter at low pH. The transporters responsible for phenolics and acid phosphatase efflux are not known. This figure was created using BioRender (https://biorender.com/).Abbreviations: Al, aluminum; ALMT, aluminum-activated malate transporter; Ca, calcium; Fe, iron; MATE, multidrug and toxic compound extrusion; PHT, proton-phosphate cotransporter.

efficiency, in addition to enhanced carboxylate efflux [38,42]. In order to maintain charge balance, root exudation of carboxylate anions is concomitant with cation extrusions or uptake of another anions [43]. Cation efflux as K^+ has been reported in Proteaceae and wheat [40,44]

while proton extrusion was demonstrated in *Lupinus* and chickpea [45]. When protons are released, acidification in the rhizosphere can occur. The rhizosphere acidification in alkaline soil is also beneficial for P solubilization but over-acidification may trigger P to form complexes with Fe and Al [37]. Alkalinization in the rhizosphere can also happen when carboxylate anions are exuded into acidic environments where they tend to get protonated and this may reduce their ability to solubilize P [37]. As for the soil microbial communities, rhizosphere acidification has been reported to suppress bacterial uptake of carboxylates so that more of these compounds can be retained in soil to solubilize P [46]. Nevertheless, some PGPBs are able to persist in the acidified rhizosphere [47]. For example, acidotolerant *Burkholderia* strains dominate the root surface of white lupin under P-stress and many of these *Burkholderia* strains could utilize carboxylates as carbon sources while promoting P solubilization as well as exhibiting other plant growth promoting characteristics [47]. Therefore, root exudation of carboxylates and rhizosphere acidification are both important in solubilizing P and indirectly enhancing the abundance of P-solubilizing bacteria. This highlights the complex interactions in the rhizosphere that directly and indirectly enhance root uptake of P.

P deficiency also induces the exudation of phenolics into the rhizosphere [46,48]. The release of phenolics such as caffeic and protocatechuic acid can facilitate the desorption of P by binding with P-containing minerals in soils to release P for plant uptake [49] (Figure 1). The exudation of phenolics may also function to suppress microbial communities in the rhizosphere that compete with plant roots for limited available P [46]. Furthermore, P deficiency has been shown to induce the secretion of acid phosphatases (APases) into the rhizosphere [50]. APases liberate P from both extracellular and intracellular organic P pool at low pH [51]. When exuded into the rhizosphere, APases hydrolyze organic P including those mobilized by carboxylates into orthophosphate before they can be absorbed by roots (Figure 1). The amount of APases secreted varies greatly among different plant species, with greater quantities being observed in tomato and white lupin, and lesser amounts in wheat, maize, and rice [50,52,53]. These findings highlight the exudate strategies used by different plant species for the acquisition of P.

Root exudates mediate belowground N cycle for plant N acquisition

N is an essential constituent in chlorophyll, nucleic acids, and proteins. N stress affects many fundamental processes in plants such as amino acid biosynthesis, photosynthesis, and the tricarboxylic acid cycle [54]. As a result, the root exudation profiles of N-stressed plants commonly display a marked decrease in amino acids, carboxylates, and sugars [39,55]. However, many secondary metabolites have been shown to be induced by N stress and function to modulate microbial activity involved in the N cycle to enhance plant N acquisition [56]. Increased exudation of flavonoids is generally observed in both legumes [57] and non-legumes [58,59] under N stress, to facilitate root interactions with N-fixing bacteria. In legumes, flavonoids facilitate rhizobial symbiosis by activating the rhizobial nodulation genes (*nod*), thereby modulating the bacterial release of *nod* factors leading to plant nodule development essential for endosymbiotic bacteria and N fixation [57]. Only a subset of flavonoids are *nod* gene inducers and are host-specific [57]. In non-legumes, flavonoids naringenin and daidzein were found to increase root colonization of the diazotrophic *Azorhizobium caulinodans* independent of *nod* factors [58,59]. In both of these interactions, diazotrophic bacteria help reduce the N stress in host plants by providing them with fixed N in exchange for carbon.

In addition to interacting with diazotrophs, certain plant species modulate the N cycle in soils by either suppressing biological nitrification or denitrification via root exudation so that more N may be retained in the soil for plant uptake [56]. Although nitrification inhibition by plant root exudates has been demonstrated in various plant species such groundnut and pearl millet, the compounds responsible for this inhibition are largely unknown [60]. To date, most biological nitrification inhibitors (BNI) discovered are derived from the root exudates of sorghum, including sorgoleone, sakuranetin, and methyl 3-(4-hydroxyphenyl) propionate [61–63]. Other identified BNIs include brachialactone isolated from a forage grass [64] and 1,9-decanediol isolated from rice [65]. These BNIs inhibit the bacterial ammonia monooxygenase (AMO) that catalyzes the oxidation of ammonia into hydroxylamine which is an essential step in nitrification. Sorgoleone, sakuranetin, and brachialactone can also suppress the hydroxylamine

oxidoreductase (HAO), which oxidizes hydroxylamine into nitrite [56] (**Figure 2**). Compared with BNI, biological denitrification inhibitors (BDNI) in root exudates have not been widely characterized. Procyanidin, from the root exudates of *Fallopia* spp. is the only naturally occurring BDNI that has been characterized to our knowledge [66]. *Fallopia* spp. is an invasive grass species that thrives on infertile soils. The procyanidin produced can effectively suppress denitrification by inhibiting the membrane-bound nitrate reductase of denitrifying bacteria [67] (Figure 2). Together, these examples highlight the importance of root exudates in modulating the belowground N cycle to enhance plant N acquisition under N-deprived conditions.

Coumarins, flavins, and phytosiderophores in plant Fe acquisition

Fe is an essential micronutrient involved in many cellular activities including photosynthesis and DNA biosynthesis [68]. Despite being very abundant in the soil, Fe largely exists in the ferric form (Fe^{3+}), which forms highly insoluble oxide and hydroxide minerals, making Fe unavailable for plant uptake. Lower soil pH helps reduce Fe^{3+} to ferrous forms (Fe^{2+}) that are more available for plant uptake, but the Fe^{2+} is prone to oxidation to Fe^{3+} in the presence of oxygen. There are two strategies that plants use to make Fe available to roots. Strategy I is an acidification-reduction approach that involves rhizosphere acidification through proton efflux and the reduction of Fe^{3+} to Fe^{2+} by a plasma membrane-bound ferric chelate reductase with the resulting Fe^{2+} being taken up by plant roots [69] (**Figure 3**). Strategy II is a chelation-based approach that involves the exudation of phytosiderophores that bind and form complexes directly with Fe^{3+}, followed by an uptake of the Fe^{3+}–phytosiderophore complexes and then the chelator is released from the Fe inside cells (Figure 2) [69]. Strategy II is utilized exclusively by the Poaceae family (monocot grasses), whereas Strategy I is used by all non-Poaceae plant species for Fe acquisition with one exception [70]. To date, rice is the only Strategy II plant found to be capable of taking up Fe^{2+}, but does not have inducible ferric chelate reductase [70]. The ability of rice to take up Fe^{2+} may be an adaptation to flooded soils where Fe^{2+} is abundant [71]. However, rice is

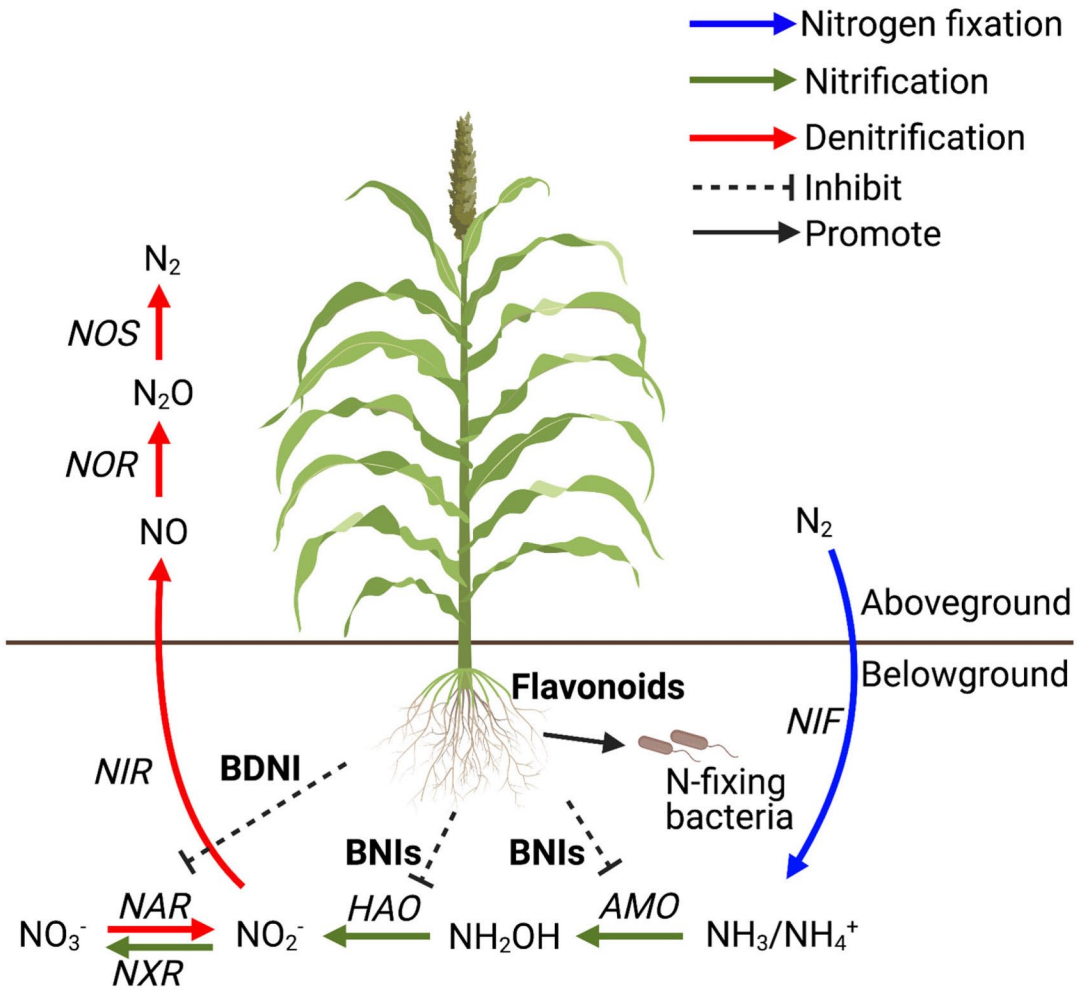

Figure 2. Root exudates as mediators of the belowground nitrogen (N) cycle. Flavonoids facilitate plant interactions with N-fixing bacteria to promote N acquisition. Biological nitrification inhibitors (BNIs) suppress nitrification by inhibiting the AMO and/or HAO catalyzing the first and second step in nitrification, respectively. Biological denitrification inhibitor (BDNI) procyanidin suppresses N loss to nitrous oxide by inhibiting the NAR enzyme catalyzing nitrate reduction to nitrite in the denitrification step. Red unbroken arrows denote denitrification and green unbroken arrows denote nitrification. The enzyme catalyzing each reaction is illustrated next to the corresponding solid arrow. Blunt-ended lines denote inhibitory effect while unbroken arrows illustrate the promoting effect on the enzymes catalyzing the N transformation pathways by root exudates. This figure was created using BioRender (https://biorender.com/). Abbreviations: AMO, ammonia monooxygenase; HAO, hydroxylamine oxidoreductase; NAR, nitrate reductase; NIF, nitrogenase; NIR, nitrite reductase; NOR, nitric oxidase reductase; NOS, nitrous oxide reductase; NXR, nitrite oxidoreductase.

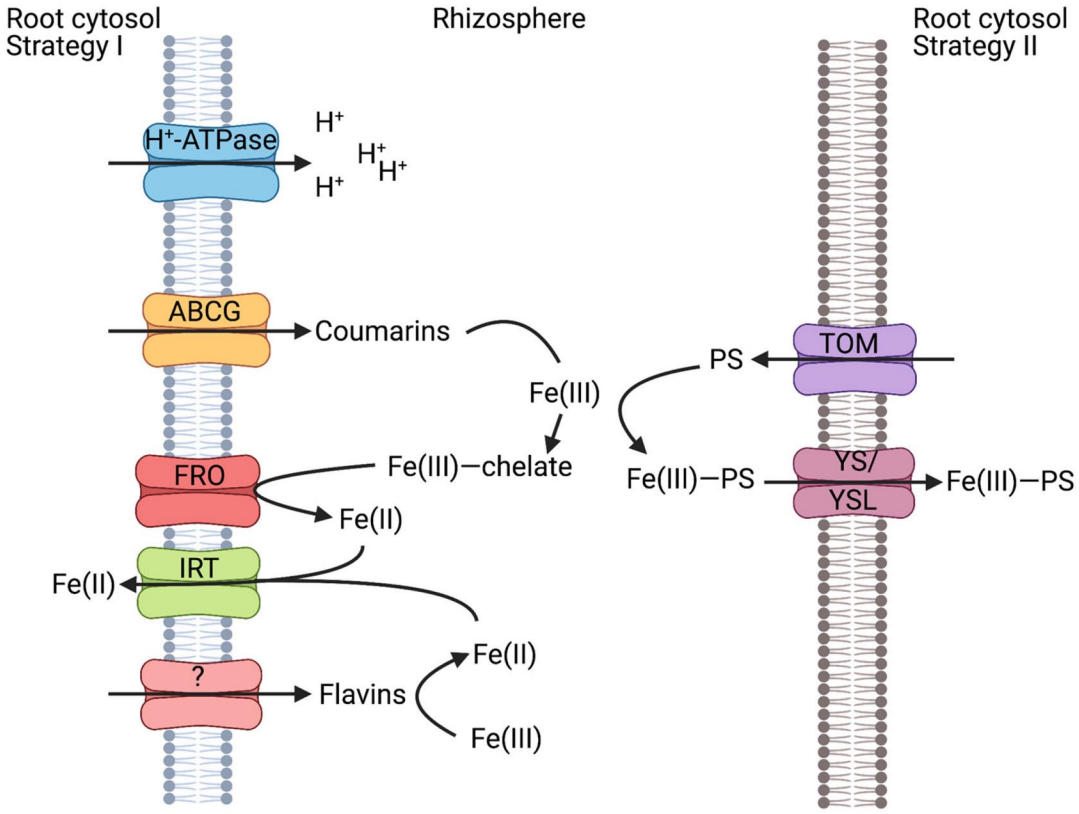

Figure 3. The iron (Fe)-acquiring strategies used by non-Poaceae (Strategy I) and Poaceae plant species (Strategy II). Strategy I involves rhizosphere acidification through proton efflux and a subsequent ferric-chelate reduction by a plasma membrane-bound ferric chelate reductase. Strategy I plants also produce coumarin that chelates Fe^{3+} and flavin that reduces Fe^{3+} into Fe^{2+}. Strategy II is characterized by the excretion of phytosiderophores. Phytosiderophores binds ferric ions directly followed by the reuptake of the ferric-PS complex. This figure was created using BioRender (https:// biorender.com/). Abbreviations: ABCG, ATP-binding cassette G subfamily; FRO, ferric chelate reductase; IRT, iron-regulated transporter; PS, phytosiderophores; TOM, transporter of mugineic acids; YS/YSL, yellow stripe or yellow stripe-like transporter.

more susceptible to Fe stress relative to other Poaceae species. This may be due to the fact that rice exudes significantly less phytosiderophores compared with others [72].

Fe solubilization mediated by phytosiderophores (Strategy II) which bypasses Fe^{3+} reduction to Fe^{2+} is more effective under alkaline conditions compared with the acidification-reduction approach (Strategy I) [73]. Under alkaline conditions, Fe acquisition of certain

Strategy I plants is dependent on phenolic exudates because high pH has little effect on their ability to chelate Fe [74,75]. The phenolics with this function that are most thoroughly characterized are coumarins. In arabidopsis, higher pH strongly increased the exudation of the coumarin fraxetin, and the Fe solubilized by fraxetin was significantly greater at higher pH (7.5) than at a lower pH (5.5) [74]. The Fe solubilizing ability of coumarins is structure-dependent, with the coumarins containing catechol moieties such as flaxetin, sideretin, and esculetin being more effective at chelating Fe than those without catechol such as scopoletin and isofraxidin [74]. Despite having weak Fe solubilizing ability, scopoletin has been characterized as a phytoalexin that has antibacterial and antifungal functions [76]. Flaxetin and sideretin were also found to shape arabidopsis root bacterial communities by inhibiting the proliferating pseudomonads by generating hydrogen peroxide [77]. These findings suggest that the exudation of coumarins by Fe-stressed plants may go beyond Fe solubilization and suppress the heterotrophic competitive activities of soil microbes. In addition to coumarins, certain Strategy I plants like Chinese milkvetch and sugar beet can solubilize Fe by exuding vitamin flavins (e.g., riboflavin) which mobilize ferric ions by donating electrons to reduce Fe^{3+} to Fe^{2+} in the presence of NADH [78,79]. Interestingly, the synthesis of coumarins and flavins are host-specific and mutually exclusive [80], highlighting the diverse mechanisms of plant Fe acquisition.

Carboxylates confer tolerance to Al toxicity

Due to the abundance and high solubility of Al at low pH, Al toxicity has become a major constraint for crop production, especially in tropical countries where acidic soils are prevalent. Upon exposure to Al, root growth is rapidly inhibited [81]. Long term exposure to Al not only puts plants under significant amounts of oxidative stress, but also damages the root systems, disrupting the uptake of water and nutrients [81]. The secretion of carboxylates plays a crucial role in enhancing plant tolerance to Al toxicity [82]. Upon exposure to Al, the root exudation of carboxylates increases in certain plant genotypes and functions to chelate the toxic Al trivalent cations in

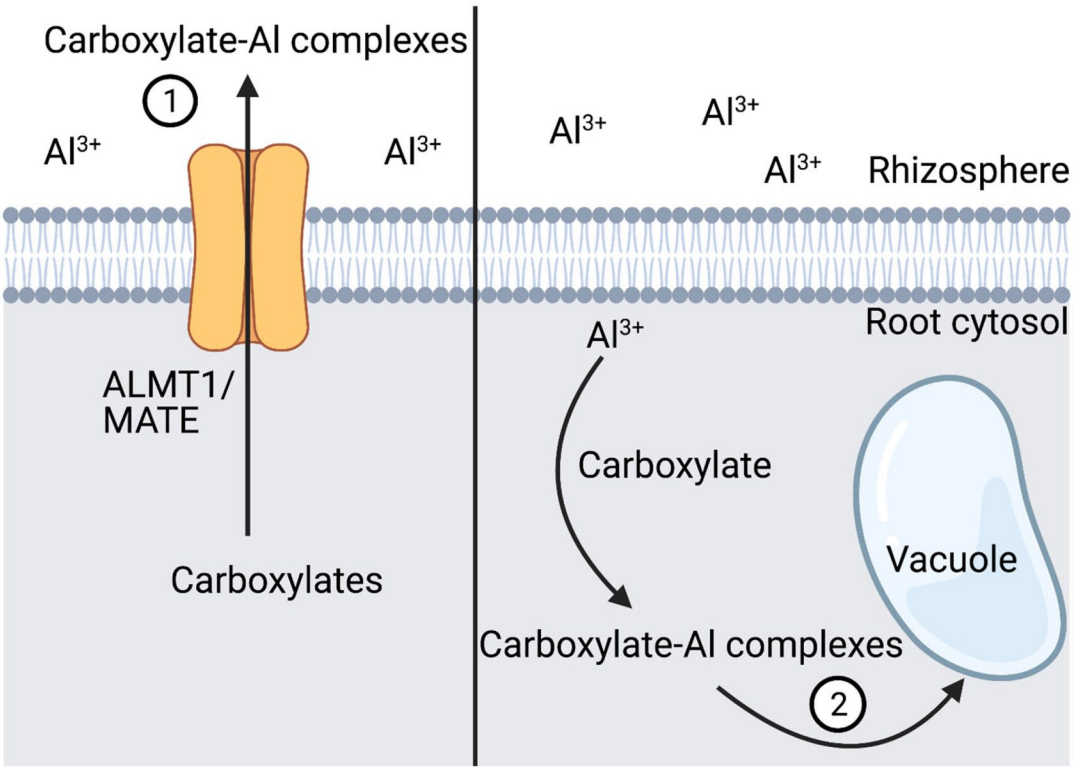

Figure 4. Aluminum (Al) detoxification process mediated by carboxylates via (1) exudation and (2) sequestration in the vacuole. Upon exposure to AL, many plant species enhance the carboxylate exudation especially in the root apex to chelate the toxic trivalent Al cations. When Al enters the cytosol, detoxification by carboxylates involves an initial chelation with Al followed by sequestration of the carboxylate-Al complexes in the vacuole. This figure was created using BioRender (https://biorender.com/). Abbreviations: ALMT1, aluminum-activated malate transporter; MATE, multidrug and toxic compound extrusion.

the rhizosphere to make it insoluble thereby reducing the toxicity (**Figure 4**). The exudation of carboxylates is often restricted to the root apex which is the root region most sensitive to Al toxicity [82]. Malate, citrate, and oxalate are the most common carboxylates used to detoxify Al and the type of carboxylate exuded is dependent on the plant species [81]. Carboxylate secretion has also been demonstrated to mediate the Al tolerance in cereals including barley [83], wheat [84], and rice [85]. The mechanism that confers Al tolerance in barley and wheat is determined by one major gene that mediates citrate efflux in barley [83] and malate efflux wheat [84]. However,

the Al tolerance in rice which is superior to other cereal crops, does not appear to be solely attributed to root carboxylate efflux [86]. For example, the Al resistance transcription factor (*ART1)* and sensitive to Al rhizotoxicity proteins (*STAR1* and *STAR2*), act together to regulate uridine diphosphate (UDP)-glucose transport that masks Al binding sites on the root cell wall conferring rice Al tolerance [87]. Recently, the microbiome associated with Al-tolerant soybean has also been characterized, but there is still a lack of a clear cause and effect relationship showing that the microbiome enriched in Al-tolerant line contributes to Al tolerance [88,89]. In addition to external detoxification, carboxylates play important roles in chelating the Al inside the cytosol and the subsequent compartmentalization of Al in the vacuole, but this is beyond the scope of this review [82] (Figure 4). Overall carboxylates are indispensable for both internal and external Al detoxification in many plant species.

Concluding remarks

The roles of root exudates for enhancing plant performance under drought and various abiotic stresses are critical for plant growth and productivity. It is promising that plant resource-use efficiency can be improved by altering root exudation processes through breeding or genetic engineering. For example, the wheat Al-activated malate transporter (*TaALMT1*) that confers Al-tolerance in wheat genotype CAR3911 has been successfully introgressed into the Al-sensitive, high-yielding winter wheat cultivar Kumpa-INIA using a marker-assisted backcrossing approach [90]. The resulting homozygous offspring exhibits threefold greater Al tolerance compared with the original Kumpa-INIA line [90]. In addition, genetic engineering through enhancing a plant's capacity to synthesize certain root exudates or express transporter proteins, has been successfully incorporated into many important crops to improve their tolerance to abiotic stress [91,92]. For example, increased expression of mitochondrial citrate synthase from arabidopsis has been found to improve canola's tolerance to Al [93] while overexpression of multidrug and toxic compound extrusion (*MATE*) and *ALMT1* transporters has been demonstrated to be efficient in ameliorating Al toxicity in barley, soybean, and wheat

by increasing the efflux of carboxylates [91,94–97]. It is however critical to verify the function and localization of the targeted genes because not all *MATE* and *ALMT1* homologues in different plant species are directly involved in mediating Al tolerance [98]. The functional diversity of the multiple ALMT-type transporters in different plant species could make the genetic modification of root exudation processes challenging.

While it seems straightforward to introduce desirable traits in crops using genetic modification, there are a few limiting factors to take into account. One of the important factors to consider when introducing targeted traits in crops is the concentration of root exudates necessary for eliciting a desirable outcome for plant growth, because more is not always better. Camalexin, a secondary metabolite important in facilitating arabidopsis interaction with beneficial bacteria can suppress the plant-growth promoting bacteria when exuded in large quantities [99]. Too much exudation of a certain compound may also exert a significant carbon drain on a plant thereby decreasing the yield. One approach to reducing the carbon cost to plants is to specify the precise localization of the root exudation process. Since the root apex is critical to root growth and highly sensitive to Al toxicity, targeting expression to this region during the genetic modification of the *ALMT1* transporter may not only decrease the carbon cost of carboxylate exudation by localization to a specific root region, but may also reduce undesirable reductions in growth. In addition, genetic modification of the root exudation processes can sometimes lead to unexpected outcome. For example, introducing the ability to exude the sesquiterpene (E)-β-caryophyllene, which is important for protecting plants against root herbivores in maize that does not produce this compound unintentionally compromised plant defense against pathogenic fungi [100]. In conclusion, a deeper understanding of the underlying mechanisms by which root exudates ameliorate plant stresses as well as the costs associated with the exudation of primary and secondary metabolites, will be advantageous for future crop improvement and the development of stress tolerance strategies for enhanced agricultural sustainability (see Outstanding questions).

Outstanding questions

- Is mycorrhiza-facilitated P uptake affected by soil pH since pH can affect the P-mobilization efficiency of carboxylates?
- How effective will plant exuded biological nitrification or denitrification inhibitors be for reducing loss of nitrogen in conventional agricultural fields?
- Do changes in the microbiome due to carboxylate exudation play a role in enhancing tolerance to Al toxicity?
- Since some soil microbes systemically induce root exudation of certain metabolites, can these microbes or key metabolites they produce be used to enhance root efflux of stress ameliorating compounds such as carboxylates under P stress?
- Is it possible to reduce the loss of carbon through root exudation processes under stressful conditions to improve the overall carbon use efficiency of plants and enhance resilience to stress?
- To what extent can the root exudation process be genetically modified for the improvement of crop productivity under P stress conditions or to mediate beneficial plant–microbe interactions to the extent that chemical fertilizers can be reduced or eliminated?
- What are the interactions between exudates in the rhizosphere and how does this impact plant stress resistance?
- Do plant root exudates contain sufficient amounts of phytohormones to impact microbiome mediated plant response to stress?
- Will modification of root exudation processes ultimately provide a successful approach for enhancing root interactions with plant stress-reducing bioinoculants?

Acknowledgments This work is supported by the Agricultural Research Division (ARD) of the Institute of Agriculture and Natural Resources. We thank the James Schnable, Sydney Everhart, Yufeng Ge, and the anonymous reviewers for their input in this work. Work on this topic in the Schachtman Lab has been supported by United States Department of Agriculture (USDA)-National Institute of Food and Agriculture (NIFA) award 2020-67019-31796 and the National Science Foundation EPSCOR to fund The Center for Root and Rhizobiome Innovation (award OIA-1557417).

Declaration There are no competing interests to declare.

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