# Impacts of elevated $CO_2$ on plant resistance to nutrient deficiency and toxic ions via root exudates: A review

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24 **Abstract**

Elevated atmospheric $CO_2$ (e$CO_2$) concentration can increase root exudation into soils, which
26 improves plant tolerance to abiotic stresses. This review used a meta-analysis to assess effect
sizes of e$CO_2$ on both efflux rates and total amounts of some specific root exudates, and
28 dissected whether e$CO_2$ enhances plant's resistance to nutrient deficiency and ion toxicity via
root exudates. Elevated $CO_2$ did not affect efflux rates of total dissolved organic carbon, a
30 measure of combined root exudates per unit of root biomass or length, but increased the
efflux amount of root systems per plant by 31% which is likely attributed to increased root
32 biomass (29%). Elevated $CO_2$ increased efflux rates of soluble-sugars, carboxylates, and
citrate by 47%, 111%, and 16%, respectively, but did not affect those of amino acids and
34 malate. The increased carbon allocation to roots, increased plant requirements of mineral
nutrients, and heightened detoxification responses to toxic ions under e$CO_2$ collectively
36 contribute to the increased efflux rates despite lacking molecular evidence. The increased
efflux rates of root exudates under e$CO_2$ were closely associated with improved nutrient
38 uptake whilst less studies have validated the associations between root exudates and
resistance to toxic ions of plants when grown under e$CO_2$. Future studies are required to
40 reveal how climate change (e$CO_2$) affect the efflux of specific root exudates, particularly
organic anions, the corresponding nutrient uptake and toxic ion resistance from plant
42 molecular biology and soil microbial ecology perspectives.


44 **Key words:** Carbon availability, carboxylates, climate change, $CO_2$ enrichment, heavy-metal
remediation, nutrient mobilization, organic anions

## 1. Introduction

Living plant roots exude soluble compounds into the rhizosphere referred to as root exudates (Oburger and Jones, 2018). Root exudates consist mainly of various carbon-based compounds, including low-molecular-weight (such as soluble sugars, amino acids and organic acid anions) and high-molecular-weight (such as proteins and mucilage) molecules (Jones et al., 2009). Organic anions in particular are secreted and can form complexes with nutrients (such as Mn and Zn) to enhance mobilization, or form complexes with toxic ions (such as $Al^{3+}$ or heavy metals) to render them non-phyotoxic (Jones et al., 2009; Marschner, 2012).

On the other hand, the concentration of atmospheric $CO_2$ is currently 414 μmol $mol^{-1}$ (https://www.esrl.noaa.gov/gmd/ccgg/trends/, July, 2020), and is predicted to reach 720 μmol $mol^{-1}$ by the end of this century due to combustion of fossil fuels, deforestation and land-use changes (IPCC, 2014). Elevated $CO_2$ (e$CO_2$) promotes photosynthetic $CO_2$ fixation and thus plant productivity and yield (Leakey et al., 2009; Norby and Zak, 2011). In some cases, e$CO_2$ enhances the tolerance of plants to abiotic stresses, such as nutrient deficiency and ion toxicity through increased carbon availability of plants (AbdElgawad et al., 2016; Huang and Xu, 2015). In recent decades, a great attention has been paid to the effect of e$CO_2$ on root exudates. It has been found that e$CO_2$ more frequently increases the efflux of root exudates (Freeman et al., 2004; Nie et al., 2013; Phillips et al., 2006). Firstly, the increased carbon fixation under e$CO_2$ tends to increase carbon allocation including root exudates to soils (Bhattacharyya et al., 2016; Jin et al. 2014; Rogers et al., 1995), which becomes a driving force for carbon sequestration in soils and thus contributes to the mitigation of climate change (Groenigen Kees et al., 2017; Pausch and Kuzyakov, 2017). In addition, e$CO_2$ might promote plant performance by enhancing the plant's ability to resist abiotic stresses, to mobilize nutrients or to remediate toxic ions through increased root exudates (Jia et al., 2015; Phillips et al., 2011).

Previous studies have briefly summarized that e$CO_2$ can increase the total efflux amount of root exudates (Nie and Pendall, 2016; Pausch and Kuzyakov, 2017). However, the collected references were insufficient to conclude the effect of e$CO_2$ on specific compounds that are considered to be important to nutrient uptake and toxic-ion resistance. In addition, the underlying mechanisms are more frequently speculative and not well understood (Bhattacharyya et al., 2014).

In this review, we collate the findings of studies investigating the effect of e$CO_2$ on root exudates of various plant species. Our primary aims are to determine (1) the effect size of e$CO_2$ on efflux rates and amount of root exudates, (2) the mechanisms by which e$CO_2$ can increase root exudates, and (3) the association of root exudates with the uptake of some specific nutrients (N, P and Fe) and resistance to toxic ions (Al and Cd) under e$CO_2$.

## 2. Methodology

*2.1. Data collection*

A literature survey was carried out using the databases of Scopus, Web of Knowledge, Google Scholar and ScienceDirect. The keywords "elevated $CO_2$", "elevated carbon dioxide", "$CO_2$ enrichment", "climate change", "FACE", "rhizodeposition", "root exudate", "total organic carbon", "dissolved organic carbon", "soluble sugar", "amino acid", "organic acid/carboxylic acid/carboxylate", "phenolic acid", "citrate", "malate" and "oxalate" were used. Dissolved organic carbon is assumed to be a measure of total root exudates.

96    Only references published before 2020 that had measurements of root exudates under $eCO_2$ were selected. In summary, the $eCO_2$ concentrations of plant growth in the thirty-four

98    references collected were between 550 and 1200 $\mu mol\ mol^{-1}$ whilst the concentrations of ambient $CO_2$ as the control were between 350 and 425 $\mu mol\ mol^{-1}$ (Table 1). The amounts of

100    root exudates are defined as total amount per plant whilst the efflux rates of root exudates are expressed as per unit time, per unit root biomass or per unit root length. The data in collection

102    were means and replicate number of the measurements under both ambient and elevated $CO_2$. The data presented in figures of the references were extracted and estimated by using ImageJ

104    (Version 1.51a, National Institutes of Health, USA). If the study had included more than one species or cultivar, development stage, or was carried out for multiple years or under several

106    $CO_2$ concentrations, these observations were taken as independent and included in our databases.

108

*2.2. Meta-analysis*

110    A meta-analysis was carried out to assess the effect size of $CO_2$ on root biomass, efflux amount and efflux rate of root exudates, including dissolved organic C, total soluble sugars,

112    total amino acids, total carboxylates, malate and citrate expressed as percentages using methods described by van Groenigen et al. (2011. These compounds were selected as there

114    were more than two studies involved, which reduces the inaccuracy of current meta-analysis due to insufficient available data. The metric of effect size was determined as the response

116    ratio R:

$$R = E/A$$

118    where *E* and *A* represent for the mean values of given parameters under elevated and ambient $CO_2$, respectively. Meta-analytic studies statistically weight the effect size by the reciprocal

120    of corresponding variances, which give greater weights to these studies of greater precision (van Groenigen et al., 2011). Since these variances were not frequently presented in the

122    literatures, the effect sizes were alternatively weighted by the replicate number (Dong et al., 2020; Loladze, 2014):

124    $$Weight = (n_a \times n_e)/ (n_a + n_e)$$

where $n_a$ and $n_e$ stand for the number of replicates of ambient and elevated $CO_2$ treatments,

126    respectively. Both weighted and unweighted effect sizes were calculated in this study and there were no large differences between the two calculations on all parameters, and thus only

128    the weighted effect sizes for all compounds are presented here (Loladze, 2014). The ratio (R) was natural logarithm-transformed for analysis to reduce biases towards increases (Hedges et

130    al., 1999; Jablonski et al., 2002). The means and 95% confidence intervals of these effect sizes were determined by a non-parametric bootstrap method (5000 iterations) using package

132    *BootES* in R user surface (Version 3.3.2). The effect sizes were regarded as significant when 95% confidence intervals were not overlapping with zero. The effect size was back-

134    transformed for the presentation of the results.

136    **3.  Results and discussion**

*3.1. Effect size of $eCO_2$ on root exudates*

138    Elevated $CO_2$ did not affect efflux rate of dissolved organic C (DOC) but increased its efflux amount (Fig. 1). Despite there being no mean increase in efflux rates of DOC, some studies

140    found that $eCO_2$ increased efflux rates (Johansson et al., 2009; Phillips et al., 2011; van Ginkel et al., 1997), whilst others found no significant increase (Kogawara et al., 2006;

142    Norby et al., 1987), or even decrease (Augustine et al., 2011; Hodge and Millard, 1998; Hodge et al., 1998). These indicate that the impacts of $eCO_2$ on DOC could be context-

144    dependent and vary with species or environmental variables e.g. water availability (Augustine et al., 2011). We observed that $eCO_2$ increased total amount of DOC (31%) to a similar

146     extent of root biomass, indicating that the increased total amount of DOC is more likely due to the increased root growth rather than increases in the efflux rates, as verified by several

148     studies (Fenner et al., 2007; Haasea et al., 2006; van Ginkel et al., 2000). Another meta-analysis found that $eCO_2$ increased total amounts of rhizodeposits (all carbon release from

150     roots including root exudates) and total root biomass by 38% (n=7) and 29% (n=133), respectively (Nie et al., 2013), which are similar estimates to our study.

152

Elevated $CO_2$ increased efflux rates of some specific compounds, including total soluble

154     sugars by 47%, total carboxylates by 111%, and citrate by 16% (Fig. 1). These results indicate that $eCO_2$ under some circumstances increases plant requirement or ability to alter

156     the rhizosphere properties by increasing root exudates. Because carboxylates are thought to be the major compounds responsible for mobilising nutrients and detoxifying harmful ions

158     (Chen and Liao, 2016; Jones, 1998), the larger increases in efflux rates of these compounds under $eCO_2$ indicate that $eCO_2$ potentially affects the compositions of root exudates that

160     allow adaptation to nutrient deficiency or toxic environments.

162     By contrast, $eCO_2$ had a near-significant ($p<0.08$) effect on the efflux rate of total amino acids of 25% whilst it did not affect the efflux rate of malate. The environmental and plant

164     species variation between studies may contribute to the lack of significant differences of the efflux rates of these compounds between the $CO_2$ treatments. Elevated $CO_2$ increased efflux

166     rate of total amino acids in seedlings of Scots pine (*Pinus sylvestris*) (Johansson et al., 2009) and maize (*Zea mays*) (Phillips et al., 2006) but did not affect them in medic (*Medicago*

168     *truncatula*) and annual rye grass (*Lolium multiflorum*) (Phillips et al., 2006) and common bean (*Phaseolus vulgaris*) (Haase et al., 2007). The lack of increase in malate efflux rate

170     might be attributed to variation in management or environment across the studies such as N supply or plant species. Specifically, $eCO_2$ increased malate efflux rate of common bean at

172     high N supply but not at low N supply (Haase et al., 2007). Elevated $CO_2$ decreased malate efflux rate of the grass *Stipa krylovii* but did not affect that of the subshrub *Artemisia frigida*

174     (Liu et al., 2016).

176     Since there have been fewer studies on the effect of $CO_2$ on efflux rates of oxalate and phenolics compared to other compounds, meta-analysis of these two compounds was not

178     done. As a general, there lacks effect of $eCO_2$ on efflux rates of oxalate and phenolics possibly attributed to species variation between studies. Elevated $CO_2$ greatly increased

180     oxalate efflux rate of Ponderosa pine (*Pinus ponderosa*) (Delucia et al., 1997) but did not affect that of white lupin (*Lupinus albus*) (Watt and Evans, 1999). Similarly, $eCO_2$ increased

182     efflux rates of phenolics as indicated by a greater increase in total phenolic acids than below-ground biomass in a peatland dominated by a genus of *Sphagnum* whilst $eCO_2$ decreased

184     efflux rates of phenolic acids in perennial ryegrass *Lolium perenne* (Hodge et al., 1998).

186     Due to increased root growth under $eCO_2$, the effect sizes of the efflux amounts of some specific compounds were greater than those of the rates. Elevated $CO_2$ increased efflux

188     amounts of total soluble sugars by 84%, total carboxylic acids by 164%, total amino acids by 74%, citrate by 36% and malate by 29% (Fig. 1). Given that $eCO_2$ improved root growth and

190     potentially increased root density and the likelihood of root overlap within the rhizosphere (Cardon, 1995), we speculate that root overlapping would concentrate root exudates within a

192     given unit of rhizosphere. Hence, the increased amount of root exudates potentially strengthens the reactions of root exudates with soils and thus facilitates nutrient mobilization

194     or ion detoxification despite the lack of effect on efflux rates. However, there is little empirical evidence confirming this hypothesis.

196

*3.2. Mechanisms of eCO₂ driving root exudates*

198 As reviewed by Oburger and Jones (2018 and Badri and Vivanco (2009, the efflux rates of root exudates depend on the following metabolic processes: (1) passive diffusion of low-
200 molecular-weight or uncharged compounds across plasma membranes, which depends on membrane permeability and electrochemical gradients as their concentrations in soils (<1
202 µM) are lower than that in plant cell cytoplasm (0.1 to 10 mM) and vacuoles (*ca*. 50 mM), (2) passive diffusion across ion channels which mainly depends on channel permeability and
204 electrochemical gradients. For example, the channel controlling malate efflux in wheat is specifically activated by $Al^{3+}$ with the malate diffusing out of cells of the root tip able to
206 detoxify $Al^{3+}$, (3) active transport through specifically-regulated transporters, or (4) exocytosis release of high-molecular-weight compounds, e.g. mucilage to ease root
208 penetration.

210 To date, despite there being studies focusing on the effect of eCO₂ on gene expressions corresponding with nutrient uptake (Jin et al., 2009; Stitt and Krapp, 1999), none of these
212 genes specifically regulate the efflux of root exudates. In addition, there is little physiological evidence showing how eCO₂ affects the efflux of root exudates. Therefore, we summarize the
214 findings and speculations, and proposes three possible reasons for increased efflux rates under eCO₂ in Figure 2.
216
Firstly, increased carbon allocation to roots can promote the efflux rates of root exudates.
218 Elevated CO₂ can increase carbon availability via the increased net photosynthetic rates in shoots of most plants (Long et al., 2004). The increased carbon availability under eCO₂
220 promotes carbon allocation to plant roots when grown as indicated by increased root-to-shoot biomass ratios (Nie et al., 2013; Rogers et al., 1995). It has been reported that root exudation
222 depends on the rate of carbon translocation into roots rather than the rate of photosynthesis (Dilkes et al., 2004), which builds up a greater gradient between roots and rhizosphere, and
224 thus facilitates passive diffusion of root exudates. Hence, eCO₂ can increase translocation activity of carbon from source to sink in the form of root exudates (Dilkes et al., 2004;
226 Nikinmaa et al., 2013).

228 Secondly, increased nutrient requirement under eCO₂ would contribute to the increased efflux rates. As a result of greater carbon fixation under eCO₂, more nutrients are required to meet
230 the demands of increased shoot growth (Jin et al., 2015; Kogawara et al., 2006; Stitt and Krapp, 1999). Low nutrient availability can specifically activate efflux of root exudates,
232 particularly organic anions that mobilise nutrients into bioavailable forms (Chen and Liao, 2016; Dakora and Phillips, 2002; Marschner, 2012). White lupin is well known to mobilise
234 insoluble P by exuding citrate, which can comprise 20% of total fixed carbon under P-scarce conditions (Johnson et al., 1994). Hence, when plants demand more nutrients under eCO₂ and
236 sense that nutrient availability is low, more root exudates would be induced.

238 Lastly, heightened detoxification response to toxic ions under eCO₂ might increase efflux rates of root exudates. Plants have developed effective strategies to reduce ion toxicity of
240 soils surrounding the roots by specifically-regulated efflux of root exudates in order to maintain biological fitness in hostile soils, such as to detoxify $Al^{3+}$ by efflux of malate, citrate
242 and oxalate (Kochian et al., 2015). Elevated CO₂ potentially increases the availability of toxic ions in soils and thus heightens the detoxification response of plants. For example, eCO₂ can
244 increase the availability of toxic ions by decreasing soil pH as a result of increasing $NH_4^+$

uptake by plants, and hence increasing $Al^{3+}$ concentration in soils (Dong et al., 2018b).
Therefore, the wheat plants grown under an increased concentration of $Al^{3+}$ under $eCO_2$ are required to respond to toxic $Al^{3+}$ ions by activating efflux of malate to a greater extent than plants grown under ambient $eCO_2$. In this case, the increased root exudates should be taken as a resultant effect rather than an indication of an inherent increased resistance against toxic ions.

*3.3. Root exudates and nutrient mobilization under $eCO_2$*

Elevated $CO_2$ potentially increases the efflux rates of organic carbon as energy sources that results in the enhancement of microbial degradation of soil organic matter. Elevated $CO_2$ is demonstrated to increase the efflux rate of DOC from the roots of loblolly pine (*Pinus taeda*) when grown in low-N soils (Phillips et al., 2011). The increased DOC efflux has hardly been demonstrated to promote plant N uptake directly, whilst it in turn increased microbial activity and secretion of extracellular enzymes that degraded soil organic matter, which mineralized additional N required for greater plant growth (Xu et al., 2018). By using C isotope techniques rather than measuring C efflux from roots, there are numerous studies to show that $eCO_2$ increases efflux of plant-derived carbon for N mobilization (Cheng and Johnson, 1998; Phillips et al., 2012; Xiao et al., 2018). However, $eCO_2$ did not affect efflux rates when grown in high-N soils (Phillips et al., 2011). This study firstly showed that $eCO_2$ increased the efflux rates of DOC from intact root systems that were collected from plants grown in soils. Similarly, $eCO_2$ was thought to promote the efflux rates of sugars, malate and flavonoids from roots, which improved carbon supply to microsymbionts to meet the increased N requirement of common bean under low soil N (Haase et al., 2007). Elevated $CO_2$ increased the efflux rate of DOC of loblolly pine seedlings grown in solution culture under low N but not under high N (Phillips et al., 2009). However, $eCO_2$ did not affect the efflux rates of total dissolved carbon in *Robinia pseudoacacia* plants under either 0 or 10 mM N in solution culture (Uselman et al., 2000), indicating that a more specific compound rather than total dissolved carbon might be required to be measured to reflect the response of N metabolism of this $N_2$-fixing tree.

There is another possibility that $eCO_2$ potentially increases efflux rates of root exudates as a signal to enhance microbial activity. Elevated $CO_2$ can enhance the efflux rates of flavonoids that activate symbiotic $N_2$ fixation as signals of common bean under low N supply (Haase et al., 2007), indicating that legume plants under $eCO_2$ might be physiologically N-deficient and thus enhance flavonoid efflux to stimulate rhizobial activity for $N_2$ fixation.

With respect to P, $eCO_2$ can increase root exudation resulting in an improvement in microbial mineralization of organic P. Elevated $CO_2$ can increase carbon allocation to mycorrhizal fungi to facilitate fungal growth (Drigo et al., 2010; Kogawara et al., 2006), which mobilizes P and enhances P availability to plants grown under P-deficient conditions. Elevated $CO_2$ enhanced the efflux rates of root exudates in wheat after 6 weeks of growth, which transformed mineral P to organic P by activating microorganisms in a Vertosol soil. This increased organic P storage could then be decomposed to meet increased P demands for plant growth at later growth stages (Jin et al., 2014).

Organic anions such as citrate, oxalate and malate have also been associated with P uptake in various species (Carvalho et al., 2016; Sas et al. 2001; Shen et al., 2011; Vance et al., 2003). Elevated $CO_2$ can enhance the efflux rate of citrate in grasses (Gifford et al., 1995) and oxalate in *Pinus ponderosa* (Delucia et al., 1997) and total amount of root exudation of

294  acetate, tartrate, malate and citrate in rice plants when grown under P-deficient conditions (Bhattacharyya et al., 2014). These are thought to enhance complexation with cations of soil
296  metals, hence mobilise P from soils, and to improve P availability and P uptake by plants (Dong et al., 2018a). On average, $eCO_2$ increased the efflux rate of citrate but not malate
298  (Fig. 1), which implies that the increased P requirement under $eCO_2$ might activate the efflux of citrate that has a greater ability to complex with Fe, Al and Ca in soils (Jones, 1998), and
300  thus facilitates P mobilization to a greater extent when compared to malate. By contrast, $eCO_2$ did not affect the rate of citrate efflux in several species, including white lupin
302  (Campbell and Sage, 2006; Wasaki et al., 2005; Watt and Evans, 1999), needlegrass *Stipa krylovii* and a subshrub *Artemisia frigida* in P-deficient conditions (Liu et al., 2016),
304  indicating that $eCO_2$ might not improve P mobilisation in these species. Elevated $CO_2$ even decreased malate efflux rate in *Stipa krylovii* plants (Liu et al., 2016), indicating that $eCO_2$
306  might inhibit plant ability to mobilise P in some species.

308  In addition to N and P, $eCO_2$ had been reported to increase the efflux rates of phytosiderophores of barley (*Hordeum vulgare*) (Haasea et al., 2006) and $H^+$ ions of tomato
310  plants (*Lycopersicon esculentum*) (Jin et al., 2009) in Fe-deficient conditions, which is thought to mobilise inorganic Fe and improve plant Fe nutrition.
312

*3.4. Root exudates and bioremediation or resistance to toxic ions under $eCO_2$*

314  It has been speculated that $eCO_2$ increases Cd availability by increased organic anion release to facilitate mobilisation of Cd (Jia et al., 2014). In another case, $eCO_2$ may enhance the
316  efflux rates of $H^+$, acidifies rhizosphere and thus enhances the availability of Cd in soils, which facilitates Cd phytoremediation when hyper-accumulators like *Sedum alfredii* were
318  grown (Guo et al., 2011; Li et al., 2013). Elevated $CO_2$ has increased the efflux amount of DOC, amino acids, soluble phenolic acids and soluble sugars in the rhizosphere of wheat
320  seedlings under Cd stresses (Jia et al., 2016). These compounds are thought to enhance microbial activity that improves nutrient uptake of plants indirectly through alleviating Cd
322  toxicity under $eCO_2$ (Jia et al., 2016; Li et al., 2014b). The increased resistance to heavy metals may further improve plant growth, which facilitates the bio-extraction of heavy metals
324  from soils as a result of increased plant biomass. However, to date, the evidence was insufficient to closely connect the increased root exudates under $eCO_2$ with enhanced
326  bioremediation of heavy metals in polluted soils.

328  By contrast, $eCO_2$ did not affect the efflux rate of malate in wheat plants when grown in an acid soil (Tian et al., 2013) and did not affect the efflux rate of either malate or citrate of
330  wheat plants in $Al^{3+}$-incubated solution (Dong et al., 2018b), whilst it did not affect citrate efflux rate of rice plants grown in $Al^{3+}$-toxic solutions (Zhu et al., 2017), demonstrating that
332  $eCO_2$ might not detoxify $Al^{3+}$ via increasing efflux rates of organic anions. Given that the efflux rate of malate or citrate mainly depends on specific transporters rather than the
334  availability of these compounds within cells (Kochian et al., 2004; Ryan et al., 1995), $eCO_2$ might not promote the transport of compounds to the appoplast or rhizosphere in order to
336  enhance $Al^{3+}$ resistance. In fact, $eCO_2$ did not affect the concentration of malate within root apices of both $Al^{3+}$-sensitive and $Al^{3+}$-resistant wheat lines despite increasing carbon
338  availability indicated by increased total biomass (Tian et al., 2013).

340  *3.5. Limitations of this and previous studies*
Despite the fact that there are studies using sterile solutions or sterilized root apices for the
342  collection of root exudates, previous studies have not succeeded in growing plants in sterile

344 conditions and have not checked whether there are microbial contamination on root exudates except studies of Phillips et al. (2006) and Johansson et al. (2009), and therefore cannot ensure that there was no microbial degradation of root exudates. These microbial

346 degradations might contribute to some of the differences among treatments including the effect of $eCO_2$. Since some compounds of root exudates could be easily degraded, such as

348 malate (Delhaize et al., 1993; Ryan et al., 1995), future studies need to address this issue and improve the methodology to create a sterile environment for the collection of root exudates in

350 order to detect trace compounds of root exudates with greater accuracy.

352 In addition, the current study using meta-analysis might not summarise the effect of $eCO_2$ on the efflux of root exudates with great accuracy due to lack of sufficient data and large

354 environmental and species variations. We expect this review will raise the interests of more scholars leading to a better understanding of how plants adapt to the changing climate and

356 abiotic stresses by root exudates.

358 **4. Conclusions**

This review concludes that $eCO_2$ did not affect efflux rate of total root exudates indicated by

360 total dissolved organic carbon despite increased total amounts of root exudates resulting from increased root growth (root biomass). Elevated $CO_2$ increased both efflux rates and total

362 amounts of soluble sugars, and carboxylates including citrate whilst the impacts on amino acids and malate were insignificant, likely attributed to plant species and environmental

364 variations. We have attributed the increased efflux rates of some of those compounds to the increased carbon allocation to roots, increased nutrient requirement, and increased

366 availability of toxic ions induced by soil acidification frequently observed under $eCO_2$. However, whether $eCO_2$ improves plant resistance to abiotic stresses via an increased efflux

368 rate of a particular compound largely depends on the specific regulation of its transporters rather than carbon availability of plants. Future studies should focus on the effect of $eCO_2$ on

370 efflux rates of some specific root exudates that are closely associated with the availability of nutrients (N, P and Fe) and the resistance to toxic ions (Al in acid soils or heavy metals in

372 contaminated soils), and on connections of root exudates with the corresponding transporters from both plant physiological and molecular perspectives.

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**Table 1.** Studies examining root growth and root exudates in response to elevated $CO_2$ used for the meta-analysis.

| Species | $CO_2$ | Focused element | Root growth | Amount or rate of root exudates | Experimental conditions | Reference |
|---|---|---|---|---|---|---|
| *Allium cepa* | +600 | C | Biomass NS. | Rate: DOC ↓. | Solution culture CEU | (Jasoni et al., 2004) |
| *Artemisia frigida* | +386 | P | Biomass and length↑. | Rate: malate NS, citrate NS. | Soil culture OTC | (Liu et al., 2016) |
| *Bouteloua gracilis* | +380 | N | Biomass NS. | Amount: DOC ↓. | Soil culture CEU | (Augustine et al., 2011) |
| *Cucumis sativus* | +245/+820 | C | +245: biomass NS both 17 d and 55 d; +820, both ↑. | Amount: fructose, glucose and total sugars (17 d) ↑; fructose and total sugars (55 d) NS, glucose (55 d) ↑; sucrose and maltose NS (both 17 d and 55 d). | Solution culture OTC | (Li et al., 2014a) |
| Forest community | +200 | - | - | Amount: total sugars, phenolic acid and aspartic acid ↑; arginine, valine and methionine, NS. | Soil culture FACE | (Johnson and Pregitzer, 2007) |
| *Danthonia richardsonii* | +319 | P | - | Rate: citrate, NS. | Solution culture CEU | (Gifford et al., 1995) |
| *Hordeum vulgare* | +170 | - | Biomass 71 d, ↑; 91 d, NS. | Rate:71 d: total free amino acids and isopentenyladenine, NS; proline, abscisic acid and K ↓; 91 d: total free amino acids ↓; proline ↓; abscisic acid and K, NS; auxin↑/↓. | Sand culture CEU | (Calvo et al., 2017) |
| | +400 | Fe | Biomass ↑. | Rate: phytosiderophores ↑ in low Fe, NS in high Fe. | Soil culture CEU | (Haasea et al., 2006) |
| *Lolium multiflorum* | +425 | N | Biomass ↑. | Rate: total amino acids NS. | Solution culture CEU | (Phillips et al., 2006) |
| *Lolium perenne* | +350 | C | Biomass ↑. | Amount: DOC NS. | Soil culture CEU | (van Ginkel et al., 2000) |
| | +270 | C | Biomass ↑. | Amount: DOC ↑, total sugars and phenolic acid NS in 1-6 d and ↓ in 14-18 d; total protein and free amino acids NS (both stages). | Sand culture CEU | (Hodge et al., 1998) |
| *Lupinus albus* | +330 | P | Biomass of proteoid roots NS | Citrate: amount ↑ and rate NS in low P; malate: amount and rate NS in low P, and ↑ in high P. | Solution culture CEU | (Campbell and Sage, 2002) |
| | +400 | P | Biomass, NS; no. of cluster root ↑. | Rate: citrate, NS. | Soil culture CEU | (Wasaki et al., 2005) |
| | +350 | P | Earlier onset of rootlet. | Rate: citrate, malate, oxalate and other organic anions, NS. | Solution culture CEU | (Watt and Evans, 1999) |
| *Lycopersicon esculentum* | +450 | Fe | Biomass ↑, root hair ↑. | Rate: averaged of growth days $H^+$ ↑ | Solution culture CEU | (Jin et al., 2009) |
| *Medicago truncatula* | +425 | N | Biomass ↑. | Rate: total amino acids NS. | Solution culture CEU | (Phillips et al., 2006) |
| *Oryza sativa* | +156 | P | Biomass ↑. | Amount: averaged of growth stages and years DOC, acetate, malate, tartrate and citrate all ↑. | Soil culture OTC | (Bhattacharyya et al., 2014) |
| | +180 | C | Biomass ↑. | Amount: DOC↑ across various growth stages. | Soil culture OTC | (Bhattacharyya et al., 2013) |
| | +200 | Al | Length ↑. | Rate: citrate NS. | Solution culture CEU | (Zhu et al., 2017) |
| *Pascopyrum smithii* | +380 | N | Biomass ↑. | Amount: DOC ↓. | Soil culture CEU | (Augustine et al., 2011) |

| Species | $CO_2$ | Focused element | Root growth | Amount or rate of root exudates | Experimental conditions | Reference |
|---------|--------|-----------------|-------------|--------------------------------|-------------------------|-----------|
| Peatland | +235 | C | Belowground biomass ↑. | Amount: DOC ↑; phenolic compounds ↑. | Wetland solardrome | (Fenner et al., 2007) |
| | +235 | C | - | Amount: DOC ↑ greater under rich nutrient. | Wetland solardrome | (Freeman et al., 2004) |
| *Phaseolus vulgaris* | +400 | N | Biomass ↑ in 12 d; NS in 15,18 and 21 d. | Rate on average: total sugars ↑; malonate, citrate and total amino acids, NS; coumestrol, genistein, daidzein and total phenolics ↑. | Soil culture CEU | (Haase et al., 2007) |
| *Plantago lanceolata* | +400 | C | Biomass ↑. | Amount: DOC NS. | Solution culture CEU | (Hodge and Millard, 1998) |
| *Phragmites australis* | +183 | C | - | Amount: DOC ↑. | Wetland FACE | (Sánchez-Carrillo et al., 2018) |
| *Pinus densiflora* | +350 | P | - | Amount and rate: DOC NS. | Solution culture CEU | (Kogawara et al., 2006) |
| *Pinus echinata* | +327 | C | Fine root biomass ↑ 34 weeks. | Amount: DOC 34 weeks ↑, DOC, 41 weeks NS; rate: DOC NS both harvests. | Soil culture CEU | (Norby et al., 1987) |
| *Pinus ponderosa* | +350 | P | Biomass ↑. | Amount: oxalate ↑. | Sand culture CEU | (Delucia et al., 1997) |
| *Pinus sylvestris* | +350 | C | Biomass NS. | Rate: total carboxylates ↑, amino acids ↑, soluble monosaccharides ↑, DOC ↑. | Substrate culture CEU | (Johansson et al., 2009) |
| *Pinus taeda* | +200 | N | Biomass ↑. | Amount: DOC ↑. | Soil culture FACE | (Drake et al., 2011) |
| | +200/+350 | N | Fine root biomass↑ in high N | Amount and rate: DOC ↑ in low N, NS in high N. | Solution and glass beads culture CEU | (Phillips et al., 2009) |
| | +200 | N | Fine root biomass ↑. | Amount: DOC ↑; rate: DOC ↑ in low N, NS in high N. | Soil culture FACE | (Phillips et al., 2011) |
| *Robinia pseudoacacia* | +350 | N | Biomass NS. | Amount and rate: DOC NS. | Sand culture CEU | (Uselman et al., 2000) |
| *Stipa krylovii* | +386 | P | Biomass ↑, total root length NS. | Rate: malate ↓, citrate NS. | Soil culture, OTC | (Liu et al., 2016) |
| *Triticum aestivum* | +350 | Cd | - | Amount: total soluble sugar↑, total free amino acids↑, total phenolic acids↑, total carboxylates↑. | Soil culture OTC | (Jia et al., 2014) |
| | +400 | Al | Biomass and length, ↑ | Rate: malate and citrate NS. | Solution culture CEU | (Dong et al., 2018b) |
| | +386 | Al | Biomass and length, ↑ in ET8, NS in ES8. | Rate: malate NS in both lines. | Soil culture OTC | (Tian et al., 2013) |
| *Zea mays* | +425 | N | Biomass NS. | Rate: total amino acids, arginine, alanine, proline, tyrosine, lysine and leucine ↑, other amino acids NS. | Solution culture CEU | (Phillips et al., 2006) |

Focused element means the examining element in study. "+" means above the ambient $CO_2$ concentration ($\mu mol\ mol^{-1}$); -, no data; ↑, increase; ↓, decrease; NS, not significant. OTC, open-top chamber; FACE, free-air $CO_2$ enrichment; CEU, controlled-environment unit (chamber or greenhouse).
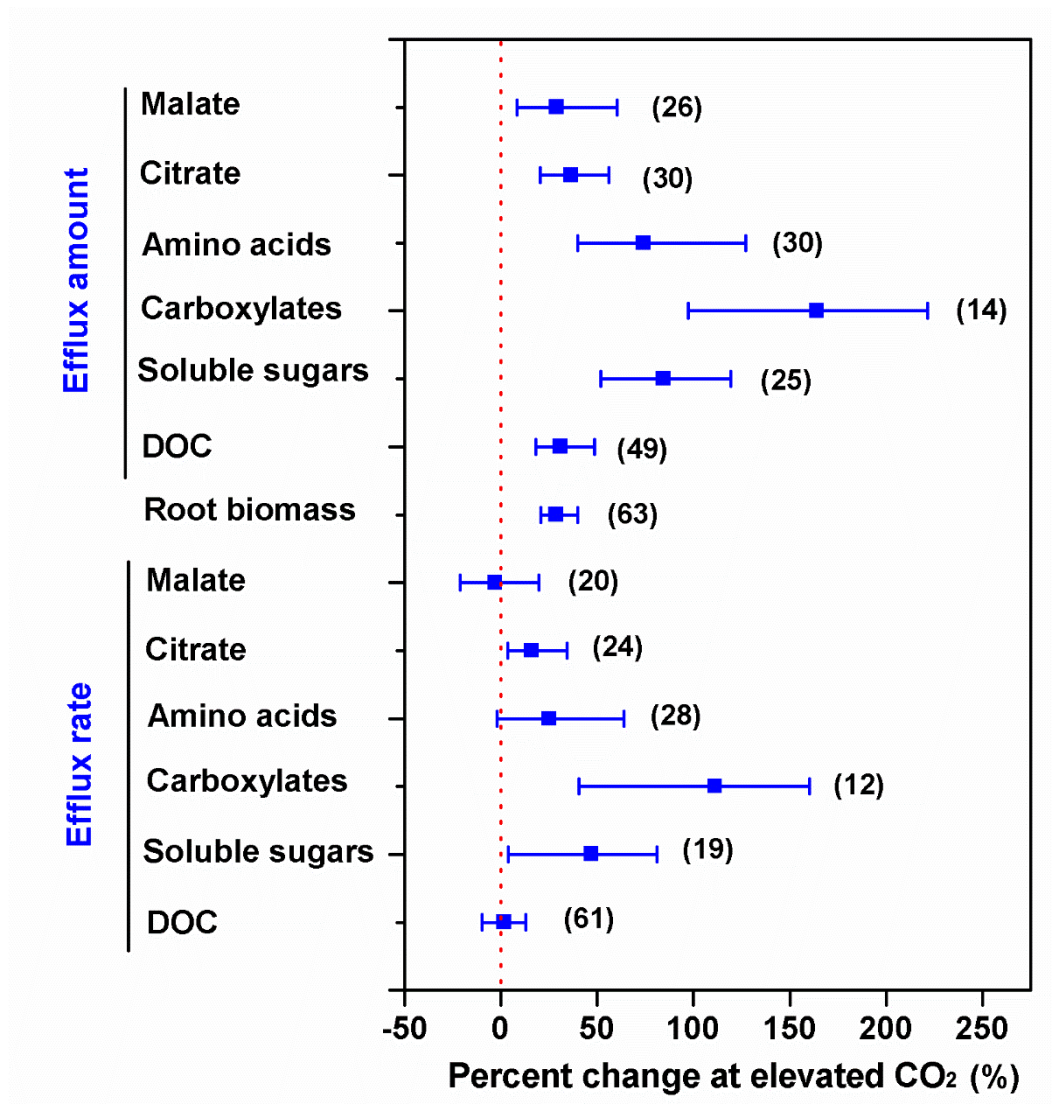
**Figure 1.** Effect of elevated $CO_2$ on efflux rate and amount of root exudates and root biomass of various plant species. Meta-analysis did not include other exudates that were reported by no more than two studies. Data are means of percent change with 95% confidence intervals (indicated with error bars) under elevated $CO_2$ compared to ambient $CO_2$. The number of observations is shown in parentheses. DOC, dissolved organic carbon.
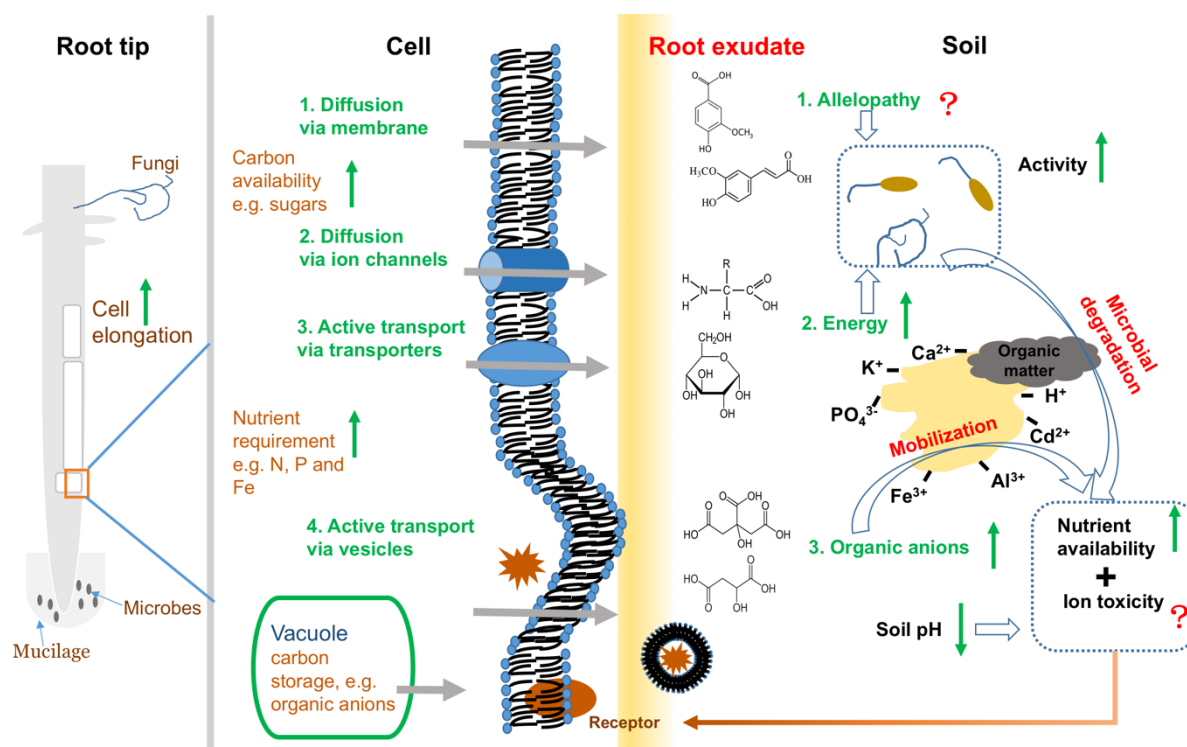
**Figure 2.** The conceptual framework of the effect of elevated $CO_2$ (e$CO_2$) on root growth, root exudates and soil biogeodynamics. ↑, increase; ↓, decrease; ?, remains unclear. This framework is modified according to two reviews (Badri and Vivanco, 2009; Oburger and Jones, 2018) and studies on the effect of e$CO_2$ on root exudates (Table 1). Elevated $CO_2$ increases root exudation via either increased root growth or increased efflux rates of root exudates. The efflux rates of specific root exudates might be increased and activated by increased membrane permeability, increased availability of root exudates within cells, and enhanced activity of transporters and vesicles located in the membrane. The increased root exudates could thereby contribute to the improved nutrient availability and improved tolerance to toxic ions in some cases.