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The Excretion of Di- and Tricarboxylic Acids by Roots of Higher Plants Can Strongly Improve the Acquisition of Phosphate (P) by Plants in P-Fixing Soils

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Abstract

The excretion of di- and tricarboxylates by roots of higher plants represents a very efficient way to acquire phosphate (P) from soils, which are low in available P. Despite the extensive experimental work in evaluating the effect of carboxylates on the acquisition of soil phosphate in the fields of plant physiology, plant nutrition and soil chemistry in the last three decades, the effect of root excreted carboxylates on soil P acquisition by higher plants is still a matter of debate. This still ongoing debate has its origin in methodological deficits in experiments and misconceptions considering the role of carboxylates in P-fixing soils on P acquisition. The main, often found misconception is to assume that the parameter carboxylates in the rhizosphere soil solution is the most important parameter driving the P mobilization by carboxylates. Carboxylates in the soil solution are an easily degradable source of rhizosphere microorganisms, and carboxylates will induce P mobilization if the carboxylate is adsorbed onto the soil solid phase, a step which also will reduce microbial carboxylate degradation. Thus, often realistic concepts to quantify the effect of carboxylates on P mobilization are still missing. Second, the very important parameter of carboxylate accumulation at the rhizosphere soil solid is often ignored or is measured by weak extractants, which do not allow the quantitative determination of carboxylates bound to the soil solid phase. Both shortcomings are critically discussed in this chapter, and a experimental and mathematical procedure is presented to evaluate the effect of carboxylate excretion on P acquisition by higher plants.

Keywords: di- and tricarboxylic acids, adsorbed soil phosphate, humic-P complexes, P mobilization, acquisition of mobilized P, model calculations, strategies of P acquisition, rhizosphere of higher plants

1. Introduction

Since the publication of the paper of Dinkelaker et al. [1], increasing attention has been paid to the release of carboxylates by roots of higher plants and to its function in phosphate (P) mobilization in soil and P acquisition in P-fixing soils. Dinkelaker et al. [1] found in the rhizosphere of white lupin (*Lupinus albus* L.) grown in a calcareous soil, a high accumulation of macroscopic visible Ca-citrate precipitates as a result of selective citrate excretion by cluster roots of P-deficient white lupin plants.

From the viewpoint of soil chemistry, it has been relatively shown earlier that di- and tri-carboxylates such as citrate, malate, oxalate or oxaloacetate can mobilize/dissolve strongly bound P in soil, which may contribute to the acquisition of P in P-deficient soils (Earl et al. [2]; Lopez- Hernandez et al. [3]).

Among the macronutrients of higher plants, N, K, P, Ca, Mg, S and P, phosphate is the nutrient which is strongly bound to the soil solid phase in many agricultural soils.

Worldwide, P deficiency of cultivated plants is a main, probably the main factor limiting plant yield [4–6]. The reserves for P fertilizer production are restricted compared to the reserves of other macronutrients [6].

In this chapter, the author considers the potential of the release of carboxylates for the improvement of the acquisition of P in P-fixing soils beginning with the potential of carboxylates excretion by the roots as a result of P deficiency, increasing the complexity of influencing parameters with each chapter of this contribution and finally, considering the acquisition of mobilized P by higher plants using mathematical modeling.

It will be shown that the adaptive excretion of carboxylates, especially citrate, and, to a lesser extent, oxalate is a very efficient mechanism to improve P acquisition by higher plants grown in P-fixing soils.

1.1. Adaptions of higher plants to P deficiency

Plants have developed several adaption mechanisms to P deficiency, morphological and physiological adaptations.

Morphological adaptations include an increasing root:shoot ratio with increasing P deficiency [7, 8], a higher number and length of root hairs [9, 10]. Both adaptations induce the same result, and the root surface is increased in relation to the shoot biomass, which may increase the acquisition of P from soils that are low in available P.

Physiological adaptations to P deficiency include, among others, the release of root exudates by P-starving plants, H^+ or OH^- equivalents (see for a review Hinsinger [11]), reducing equivalents [12, 13] or the release of di- or tricarboxylic acid anions.

Plaxton and Tran [14] have recently provided an overview on the physiological adaptations of higher plants to P deficiency. In our context, the adaptations leading to an increased carboxylate efflux are of central importance.

An increased excretion of carboxylates during P deficiency requires two different simultaneous physiological adaptations, which are as follows: (1) an increase in the synthesis of carboxylates and (2) an increased rate of excretion (efflux) of the carboxylates.

- a. An enhanced synthesis of carboxylate anions within the plants is correlated with the upregulation of the phosphoenol pyruvate carboxylase (PEPC), leading to an increased synthesis of oxaloacetate, an increased activity of malate dehydrogenase, leading to an increased reduction of oxaloacetate to malate, an increased activity of citrate synthase leading to an increased synthesis of citrate [14].
- b. Organic acid anions are dissociated at the cytoplasmic pH within the roots. It was shown by Yan et al. [15] and Zhu et al. [16] that H⁺-pumping ATPases in the plasma membrane of the roots are involved in an increased carboxylate efflux. At present, two separate transport processes for the release of carboxylates into the rhizosphere soil are assumed, an active H⁺-efflux-driven carboxylate excretion process and a passive efflux of organic anions [14, 15].

1.2. Soil P forms, P availability and the interaction of soil P with carboxylates

Among the macronutrients, phosphorus, which is present in soil and plants nearly exclusively as orthophosphate anion (P) and its esters with organic alcohols, has, by far, the strongest affinity to the soil solid phase and consequently, the lowest solubility in the soil solution. In soil chemistry, the term buffering or buffer power is used to describe the intensity of bonding to the soil solid phase, which is described by the adsorption or desorption curve. High buffering or buffer power indicates that most of the added nutrient is bound to the solid phase and that the equilibrium soil solution concentration is low. Soils with high P buffering are defined as P-fixing soils. Such soils may contain high quantities of P; however, because of high concentration of P-sorbing sites, the P equilibrium soil solution concentration is low or even very low, sometimes near zero. The concentration or precisely the activity of P in the soil solution is important for the P acquisition of higher plants from soil because P is transported to the roots almost exclusively within the soil solution via diffusion [17, 18]. The diffusive flux strongly depends on the initial P concentration of the soil solution and consequently, the P concentration gradient within the rhizosphere soil solution (see for details [17–19]). Often, for maximum yield, higher plants need P soil solution concentrations above 1–5 μM P depending on the rooting density, the formation of root hairs, the root:shoot ratio and the formation of mycorrhiza. However, at very low P soil solution concentrations, an increase in the P absorbing root surface does not promote the acquisition of P adequately.

Under these conditions, strategies to increase the rhizosphere soil solution concentration of P by root exudates (chemical P mobilization) are promising and the central way to acquire P from strong P.

1.3. Chemical P mobilization in P-fixing soils

Several types of root exudates may increase the P solubility in soil and consequently increase the acquisition of P by the roots of higher plants.

As an adaption to P deficiency, plants can increase the efflux of protons followed by the acidification of the rhizosphere [11], they can increase the release of reducing agents (e.g., Tomasi et al. [13]), and they can increase the efflux of di- and tricarboxylates (see for a review, Gerke [17]).

The release of carboxylates is by far the most important way to acquire P even in P-fixing soils [17, 20].

To support this statement, information on the carboxylate efflux of different plant species and genotypes, the accumulation of carboxylates in the rhizosphere, the effect of carboxylates on the mobilization/dissolution of soil P and the acquisition of mobilized P by higher plants are required.

1.4. Carboxylate efflux by different plant species as affected by the P status

Proton release and carboxylate release are separate mechanisms in higher plants [15, 16]. However, often carboxylate and proton efflux is a coupled reaction to P deficiency.

As a result of P deficiency, many plant species show an increased carboxylate efflux, for example, white lupin [1, 21–23], alfalfa [24, 25], spinach [24, 26], chickpea [23]; red clover [24], yellow lupin [21, 27], radish buckwheat [28] and many members of the Proteaceae [29, 30] and of the Cyperaceae [31].

Many graminaceous species are considered to be ineffective in carboxylate excretion, but some graminaceous species show an increased carboxylate efflux to avoid Al- toxicity [32–35].

In buckwheat, oxalate efflux was increased during Al- toxicity [36].

Whether and to which extent Al- toxicity induced carboxylate excretion is controlled by reduced P acquisition or affects P acquisition is unknown.

The release of carboxylates by dicotyledonous plant species is not uniform along the root but is concentrated on the region behind the root tips [24, 26, 37–39].

The carboxylate release strongly depends on the P status of the plants and may be by a factor of 10–100 higher in P-deficient plants compared to high P plants.

Keerthisinghe et al. [40] found a very high citrate release in fully developed cluster roots of white lupin with a maximum efflux of about $6.1 \text{ [nmol h}^{-1} \text{ cm}^{-1}]$, decreasing in younger and old cluster root segments by a factor of 10 or even more.

Neumann et al. [41] confirmed these results; however, Watts and Evans (1999) measured a maximum citrate in cluster roots of white lupin of $33 \text{ [nmol min}^{-1} \text{ m}^{-1}]$, which is about three times higher than the values reported by Keerthisinghe et al. [40].

Yellow lupin, a plant species that also forms cluster roots [21, 27], exhibits a maximum citrate efflux within the cluster roots of about 70–80% than that of the cluster roots of white lupin [27].

Cluster roots of the Proteaceae, for example, *Hakea prostrata* shows a carboxylate efflux of cluster roots, which is much higher than that of the white lupin [31, 42]. In *Hakea undulate*, malate is the main carboxylate, which is excreted with a root efflux being two times higher

than the citrate efflux in white lupin [43]. Members of the Cyperaceae form “dauciform” roots as an adaption to P deficiency, which exhibit carboxylate efflux rates which are similar to the cluster roots of Proteaceae species [31].

Also, plant species that do not form cluster or dauciform roots can show a high carboxylate efflux during P deficiency. The region of high carboxylate efflux is often restricted to about 1–2 mm behind the root tips. For example, Beißner [37] showed for sugar beet, an increased oxalate efflux during P deficiency, the carboxylate efflux being by a factor of 3–4 higher, 1–2 mm behind the root tips, compared to the overall efflux of the whole root system.

Gerke [24] investigated the carboxylate efflux of several plant species, grown in quartz sand at four levels of P supply (**Figures 1–6**).

As the shoot P concentrations decreased, the efflux of carboxylates increased in all plant species was investigated (**Figures 1–6**).

However, the main carboxylates differed between the plant species. In legumes such as white clover, red clover or alfalfa, citrate is the dominant anion, whereas in spinach, oxalate is dominant and in the Brassicaceae species, Chinese cabbage (*Brassica Chinensis*), malate is dominant. Also, in rape, malate is the dominant anion [38].

In graminaceous species such as rye grass, the carboxylate efflux is extremely low, even at strong P deficiency (**Figure 6**), whereas especially in alfalfa and red clover, the overall citrate efflux was very high under P deficiency (**Figures 1 and 2**).

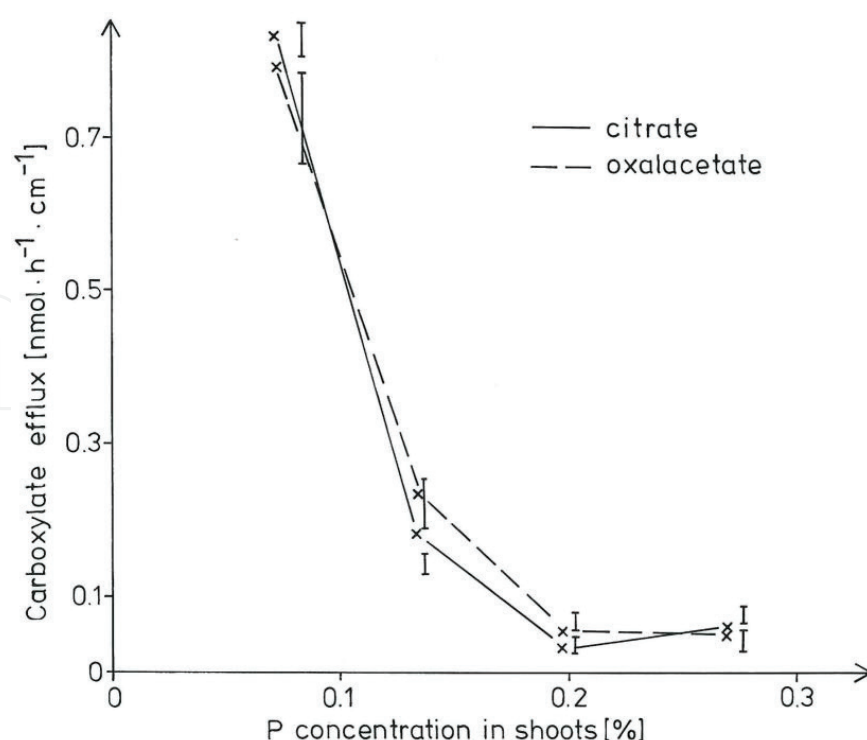


Figure 1. Carboxylate efflux by alfalfa (*Medicago sativa* L.) as affected by shoot P concentrations. (Modified from Gerke [24]).

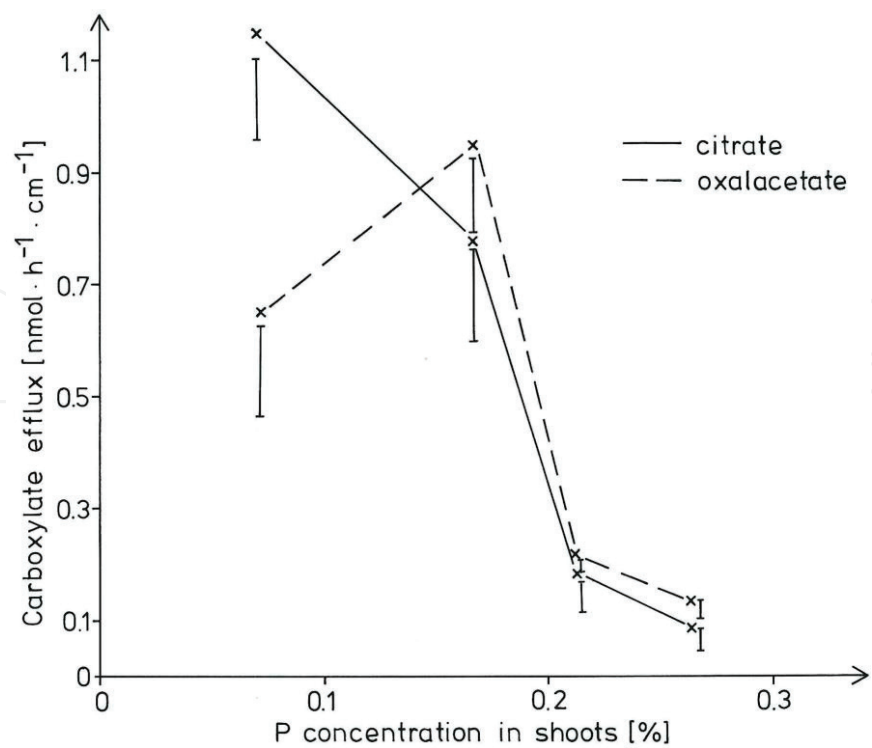


Figure 2. Carboxylate efflux by red clover (*Trifolium pratense* L.) as affected by shoot P concentrations. (Modified from Gerke [24]).

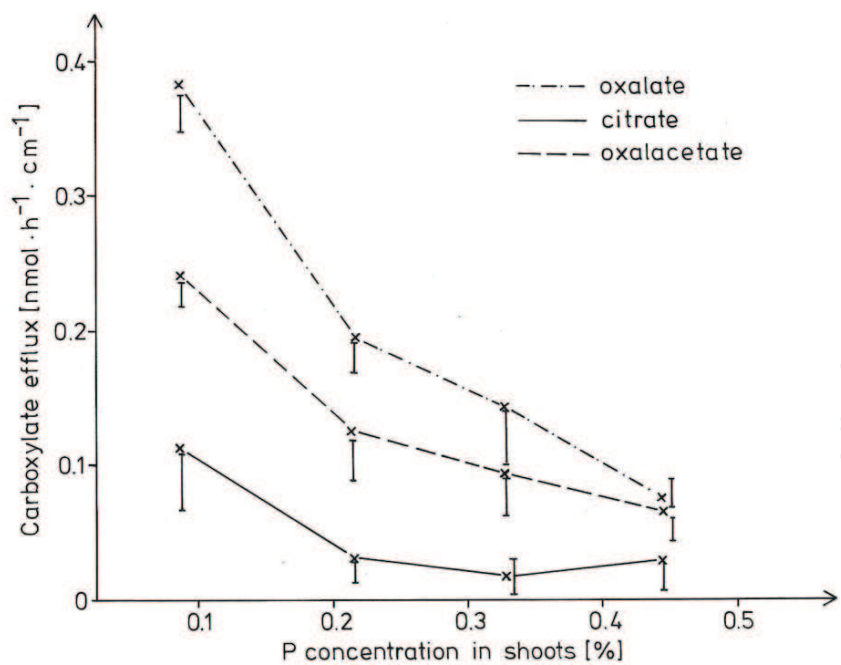


Figure 3. Carboxylate efflux by spinach (*Spinacia oleracea* L.) as affected by shoot P concentrations. (Modified from Gerke [24]).

1.5. Accumulation of carboxylates in the rhizosphere

A high or even very high carboxylate efflux by roots of P-deficient plants is no guarantee for its accumulation within the rhizosphere soil. Carboxylates which are excreted into the soil solution

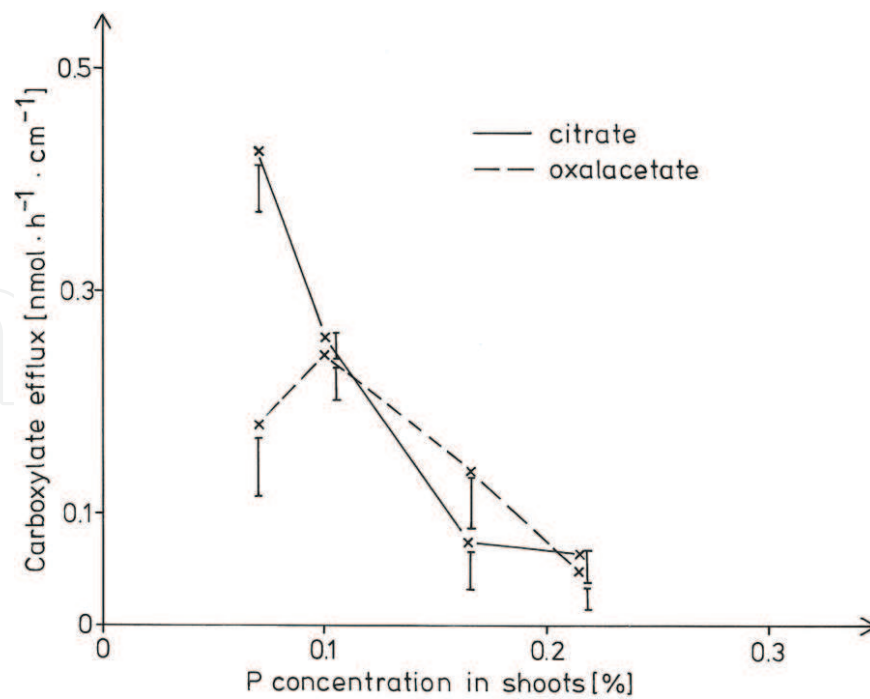


Figure 4. Carboxylate efflux by white clover as affected by shoot P concentrations. (Modified from Gerke [24]).

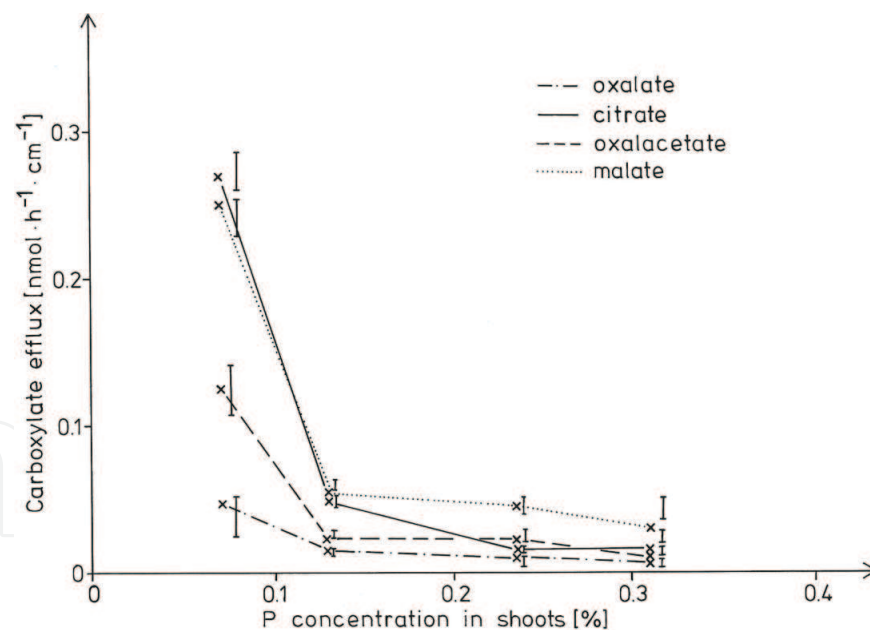


Figure 5. Carboxylate efflux by Chinese cabbage as affected by shoot P concentrations. (Modified from Gerke [24]).

are easily degradable C- source for soil microorganisms, which sometimes has been taken as an argument for the questioning of the relevance of carboxylates for P acquisition in soil (see e.g., the review of Richardson et al. [44]). However, the same review group of Richardson et al. [44] simplified the role of carboxylates in the rhizosphere to the question of carboxylate concentration in the soil solution assuming the soil solution being the main reservoir for carboxylates. However, the interaction of the excreted carboxylates and the soil solid phase is decisive for its effect on soil P solubility and the P acquisition by the plants in P-fixing soils.

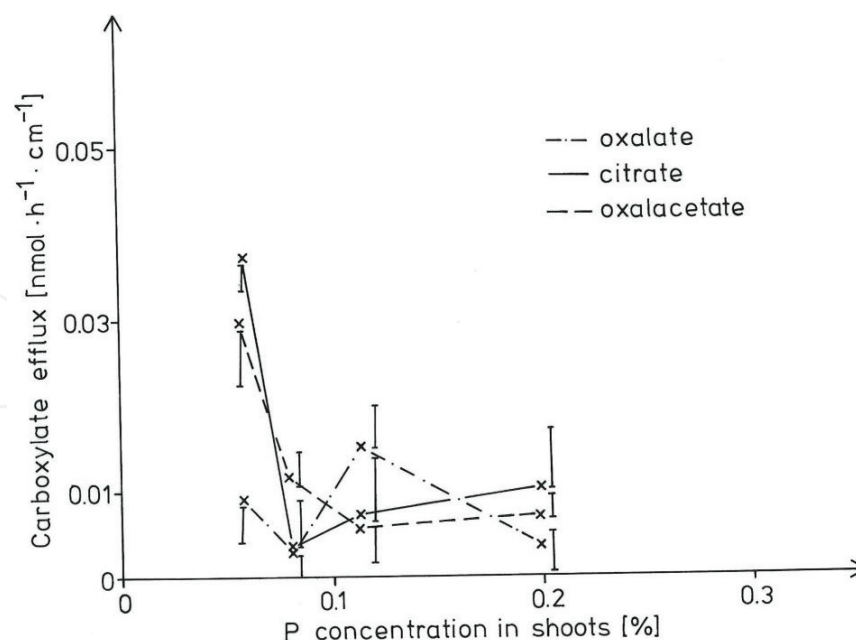


Figure 6. Carboxylate efflux by ryegrass (*Lolium perenne* L.) as affected by shoot P concentrations. (Modified from Gerke [24]).

The carboxylate concentration in the soil solution depends on the carboxylate excretion and the buffering of the carboxylates in soil and is not very relevant. Relevant for P mobilization is the concentration of carboxylates at the soil solid phase. The sorbed carboxylates can desorb P by occupying the P-sorption sites, and they can induce dissolution processes of P-sorbing surfaces such as Al (Fe)-(hydr)oxides or humic-Al(Fe) complexes [45, 46].

The result of these reactions between carboxylates and the soil solid phase is an increase in the P soil solution concentrations as well as an increase in Fe and Al solubility, which was experimentally shown for cluster root rhizosphere soil solution of white lupin [47] and is often reported in model experiments (e.g., Fox et al. [48]; Takeda et al. [49]; Gerke [46]).

Di- and tricarboxylates in the soil solution are easily degraded by microorganisms. If the excreted carboxylate is bound to the rhizosphere soil solid by a fast adsorption reaction, carboxylate degradation is strongly retarded.

Boudot [50] and Jones and Edwards [51] showed that the sorption of carboxylates to aluminosilicates, Al-oxides and Fe-oxides strongly prevented microbial degradation. Boudot [50] investigated the effect of ¹⁴C-citrate adsorption to Al containing soil minerals. Free citrate was mineralized to about 70–80% within 10 days. At a high Al/citrate ratio, adsorption of citrate decreased the mineralization of citrate to zero. Similar results were found by Jones and Edwards [51] in the system citrate/Fe-oxides. Boudot [50] also showed that the formation of Al-citrate complexes in the solution strongly reduced citrate mineralization.

It has sometimes been speculated that acidification of the rhizosphere, for example, by legumes may reduce carboxylate mineralization within the rhizosphere soil (e.g., Lambers et al. [52]). At present, the effect of acidification on carboxylate adsorption and its consequent effect on P mobilization are estimated to be more important (Gerke, 2000a).

The accumulation of carboxylates in the rhizosphere is remarkable. Dinkelaker et al. [1] found more than 50 μmol citrate/g soil in the cluster root rhizosphere of white lupin. Gerke [17, 24] found between 12 and 88 μmol citrate/g soil in the cluster root rhizosphere of white lupin grown in different soils at different P levels depending on the method of citrate determination.

Often, carboxylates in the rhizosphere soil of different plant species are quantified after extraction with mild extractants, such as distilled water [53, 54], dilute salt solutions [23, 55, 56] or dilute acid [57], leading to a low recovery of the carboxylates. Gerke et al. [47] found citrate concentrations in the cluster root rhizosphere of between 66 and 88 [μmol citrate/g soil] determined by direct infrared spectroscopy (DRIFT spectroscopy), whereas the quantity of citrate extracted with water was below the detection limit.

Cluster roots are a peculiar adaption of relatively few plant species, among them, both white lupin and yellow lupin are cultivated plant species.

In the noncluster root forming plant species, red clover, Gerke and Meyer [58] showed a citrate accumulation of more than 12 [μmol citrate/g soil] in close proximity to the roots of P-deficient plants.

1.6. The effect of carboxylates on the mobilization/dissolution of soil phosphate

Several carboxylates can strongly increase the P soil solution concentration.

This was relatively shown earlier, for example, by Earl et al. [2] and Lopez-Hernandez et al. [3].

More detailed results were reported by Gerke [24] and Gerke et al. [59].

Often, with increasing carboxylate application and accumulation at the soil solid phase, the P concentrations in the soil solution increase exponentially (**Figures 7 and 8**) [59].

From **Figures 7 and 8** it can be seen that changes in soil pH alone have only a minor impact on the soil P solubility but pH changes strongly affect the effectiveness of citrate to mobilize soil P. The exponential relation can also be shown, for example, for oxalate [24, 37].

Table 1 shows that citrate and, to some extent, oxalate are very efficient in mobilizing soil P, whereas malate and oxaloacetate are relatively inefficient.

1.7. The acquisition of mobilized P by higher plants

Many experimental results demonstrate that P deficiency in higher plants increases the carboxylate efflux. Also, many experimental results show that citrate, oxalate, to some extent, malate and other carboxylates can mobilize P in P-fixing soils.

The separate views on the physiology of carboxylate excretion and root morphology or, on the other hand, the rhizosphere chemistry of carboxylates and its impact on the P solubility in soil does not prove the relevance of carboxylate excretion on the P acquisition of higher plants.

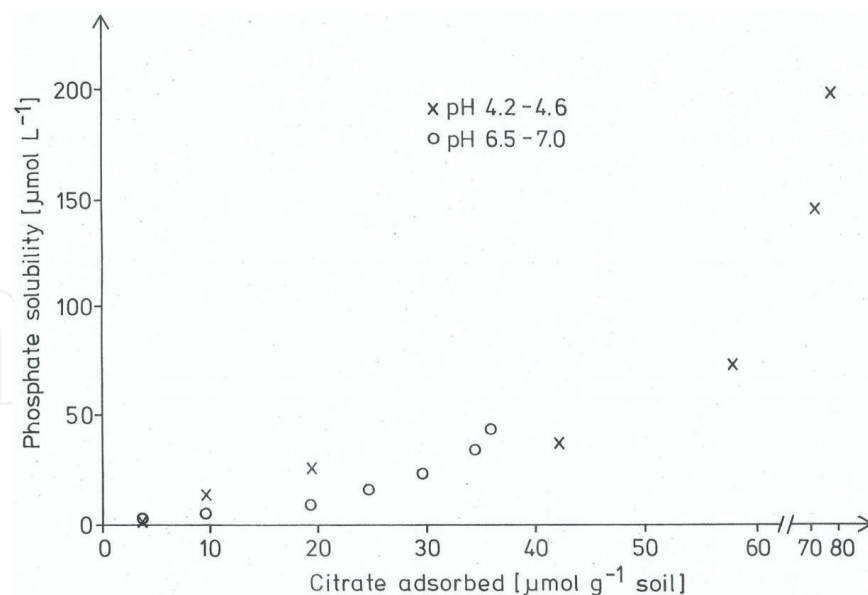


Figure 7. Relation between the citrate concentration in soil and the P solubility at different pH in a Ferralsol. (Modified from Gerke [24]).

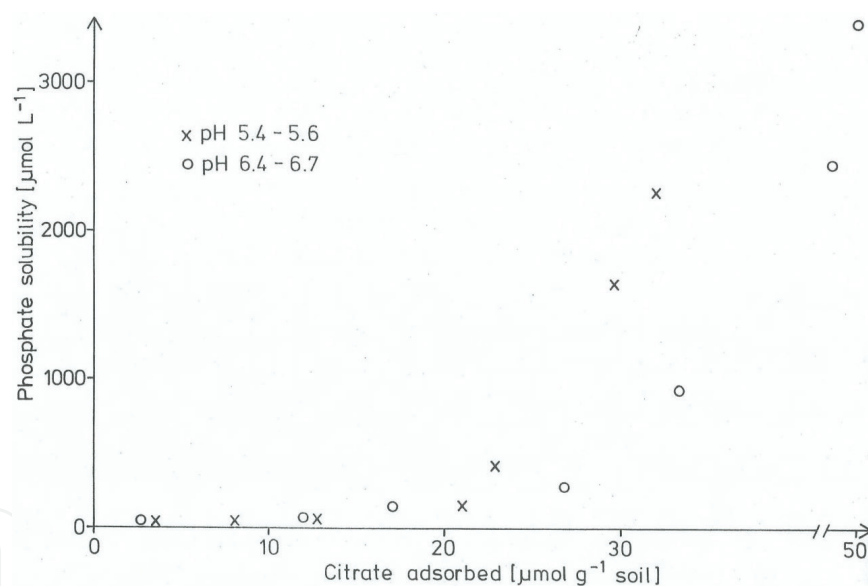


Figure 8. Relation between the citrate concentration in soil and the P solubility at different pH in a humic Podzol. (Modified from Gerke [24]).

The combination of both views, including carboxylate efflux, the accumulation of carboxylates in the rhizosphere soil, the chemistry of P mobilization and the uptake of mobilized P by the roots may help to evaluate the contribution of carboxylate excretion to P acquisition by higher plants.

For this purpose, mathematical models are a useful tool. Also, such models allow, by the aid of sensitivity analysis, to evaluate the contribution of different parameters to the acquisition of P by higher plants.

| | | Phosphate Fe + Al (μmol/l) | |
|-------------------------|--------------|----------------------------|--------|
| Luvisol pH 7.7–8.1 | Citrate | 85.6 | 2340.5 |
| | Oxalate | 70.4 | 1234.3 |
| | Malate | 36.4 | 812.3 |
| | Oxaloacetate | 32.2 | 972.4 |
| Ferralsol pH 4.1–4.7 | Citrate | 198.5 | 9420.1 |
| | Oxalate | 28.3 | 1314.4 |
| | Malate | 25.2 | 2810.4 |
| | Oxaloacetate | 7.5 | 71.5 |

Table 1. Maximum phosphate and iron + aluminum mobilization by organic anions.

Nye [60, 61] developed a mathematical model to quantitatively describe the influx of P as affected by the efflux of mobilizing agents.

Gerke [24] was the first who used the mathematical framework of Nye [60, 61] to quantify the influx of mobilized P as a result of a definitive carboxylate efflux. Some of the results of the calculations are shown in **Figures 9** and **10**.

For the three plant species such as rye grass, white clover and red clover, the maximum citrate efflux was used in the calculations (from **Figures 2, 4, and 6**). For the accumulation of citrate in soil, we assumed two strongly differing values in order to include a possible range of accumulation (see in detail Gerke [24]).

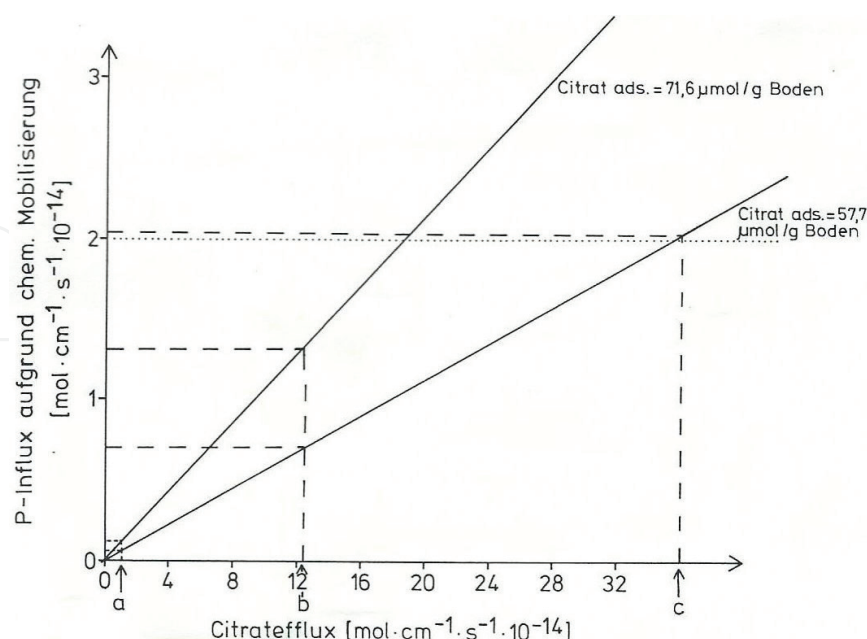


Figure 9. Influx of mobilized P as related to maximum citrate efflux of plants grown in a Ferralsol by (a) rye grass, (b) white clover and (c) red clover. (Modified from Gerke [24]).

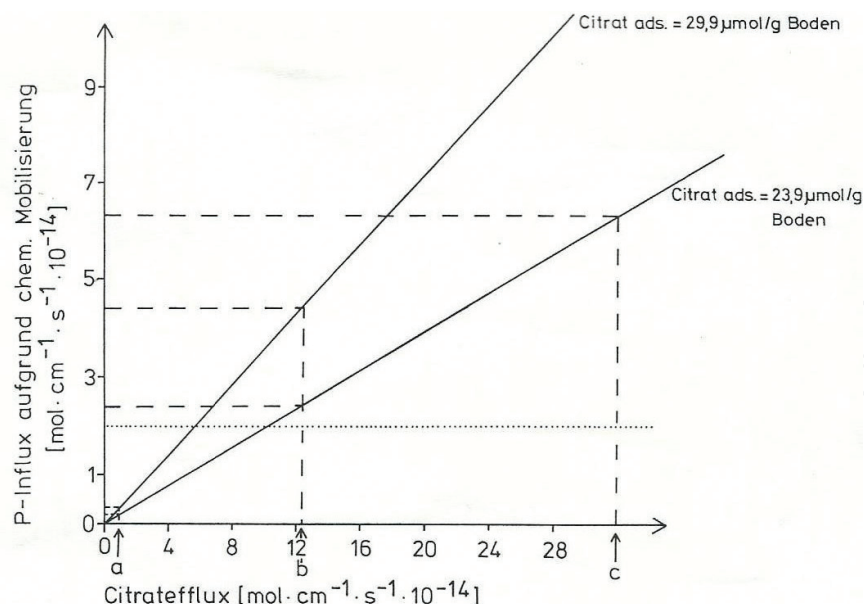


Figure 10. Influx of mobilized P as related to citrate efflux of plants grown in a humic Podzol by (a) rye grass, (b) white clover and (c) red clover. (Modified from Gerke [24]).

In none of the soils at either citrate accumulation level did the maximum citrate efflux of rye grass have any effect on the acquisition of P. The high overall citrate efflux of P-deficient red clover strongly increased the influx of mobilized P over the required level in all cases. White clover with a medium citrate efflux strongly increased the P influx of mobilized P in a soil with humic-Al(Fe)-P complexes as dominant P fraction. Compared to soils with mainly Al(Fe)-oxide-P complexes, humic-associated P is much easier to mobilize by carboxylates [46, 58].

The results shown in **Figures 9** and **10** show that citrate excretion by P-deficient roots is an exceptional efficient way to improve P acquisition in P-fixing soils.

In their review on P efficiency, Richardson et al. [44] presented a separate chapter titled: “Can the release of organic anions from roots mobilize phosphorus?”

In this chapter, Richardson et al. [44] related [5–50 μmol citrate/g rhizosphere soil] to 1–10 [mM] citrate in the soil solution and then stated that 1 [mM] citrate or more is effective for P mobilization in some soils. This view on the rhizosphere chemistry of carboxylates and phosphate is misleading for at least two reasons.

First, the buffering of carboxylates in soil is a strongly varying soil parameter and cannot be generalized to a relation of 1–10 [mM] citrate versus 5–50 [μmol citrate/g soil], suggesting this as the general range of citrate buffering in all soils. Gerke [17, 24] showed for a strongly anion-fixing Ferralsol, that citrate concentration in the soil solution of less than 10 μM corresponds to more than 60 [μmol citrate/g soil] at the soil solid phase suggesting a much wider range of citrate buffering.

Second, it is a misleading view that carboxylates in the soil solution are responsible for the mobilization of P. The initial or essential step in P mobilization is the adsorption of the carboxylates to the soil solid phase where the carboxylate-mediated P desorption or dissolution

of P-sorbing surfaces is initiated. In this context, simultaneous acidification and carboxylate excretion may increase the dissolution of P-sorbing sites more efficient than carboxylate excretion alone [21].

Finally, the role of root-derived carboxylates on the acquisition of P esters should be considered. This topic has been reviewed by Gerke [20]. In general, phosphate monoesters may accumulate in soils among which higher phosphorylated inositol phosphates account for the dominant proportion of ester-P in many soils [20].

Worldwide, more than 51 millions of tons of phytate (myoinositol hexakisphosphate) are annually produced in crops, seeds and fruits, which is equivalent to about 66% of the P annually applied to agricultural land [62]. Higher phosphorylated inositol phosphates are strongly bound to the soil solid phase often much stronger than the orthophosphate anion [20, 63]. The initial step of P acquisition from inositol phosphates is its mobilization.

Adams and Pate [64] showed that in soil, white lupin but not narrow leaf lupin can acquire P from phytate. White lupin forms root clusters with extensive citrate excretion, but narrow leaf lupin forms no root clusters [27].

The mobilization by carboxylates is the rate limiting step in acquisition of P from phytate and similar molecules and not the reduced activity of phytases. For a detailed discussion, see Gerke [20].

2. Conclusions

The partly contrasting results concerning the role of root-released carboxylates on P acquisition by higher plants are mainly due to deficits in the concepts of research and experimental methods.

Carboxylates will affect the soil P solubility after adsorption to the soil solid phase and not dissolved in the soil solution. It is a misconception to consider exclusively the carboxylate concentration in the rhizosphere soil solution and not the carboxylate concentration in the soil solid phase.

The determination of carboxylate bound to the soil solid phase is often ignored or is determined by deficient methods. Often, weak extractants such as dilute acids or dilute salt solutions are used, which extract a very low proportion of rhizosphere soil-bound carboxylates.

Considering the range of carboxylate efflux of P-deficient plants, especially cluster root forming plants such as white and yellow lupin, and species such as red clover, alfalfa, sugar beet and spinach may strongly mobilize the soil P and acquire the mobilized P.

Citrate and, to some extent, oxalate are the anions, which are most efficient with respect to P mobilization.

The most important organic P forms in soil, higher phosphorylated inositol phosphates are strongly bound to the soil solid phase and can be mobilized by carboxylates similar to the orthophosphate anion.

The quantitative effect of carboxylate excretion on the acquisition of soil P by higher plants requires an integrative concept, including all relevant soil and plant parameters. Such a framework may be a mathematical model based on experimentally determined parameters.

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