# Phosphate, Fe and Mn uptake of $N_2$ fixing red clover and ryegrass from an Oxisol as affected by P and model humic substances application.
# 1. Plant parameters and soil solution composition

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Dedicated to Prof. Dr. *Albrecht Jungk* on the occasion of his 65th birthday

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## Summary - Zusammenfassung

The effect of humic substances on P-availability in soil is still debated. Therefore, the effect of model humic substances synthesized from hydroquinone on P, Fe, and Al solubility in a strong P fixing Oxisol and on P acquisition by red clover and ryegrass was investigated.

After 4 months of incubation, P concentration of soil solution had increased by a factor of > 10 at the highest humic level (50 g humics $kg^{-1}$ soil), accompanied by a similar increase in Fe and Al concentrations.

Soil samples with 0, 10, 30, 50 g humics $kg^{-1}$ soil were planted with red clover and ryegrass. Red clover showed a small increase of shoot yield and a moderate increase of P uptake after humics addition. High humics levels increased slightly Fe concentration in the shoots but strongly that of Mn leading to Mn toxicity. Ryegrass showed a strong increase in shoot yield after humics addition of about 150 % at the highest humics level compared to the control without humics. At each humic level, P application (100 mg $kg^{-1}$ soil) had no effect on P uptake of red clover and a small effect on P uptake by ryegrass.

The relatively small effect of humics and P application on shoot yield of clover compared to grass can be explained by chemical P mobilization of red clover via exudation of citrate (about 12 µmol citrate $g^{-1}$ soil). This agrees with the finding that P solubility increased in the soil under red clover but not under ryegrass from the first to the second harvest, indicating that red clover mobilized P.

## Phosphat-, Fe- und Mn-Aufnahme von $N_2$-fixierendem Rotklee und Weidelgras aus einem Oxisol in Abhängigkeit von der P-Düngung und der Zugabe von Modellhuminstoffen. 1. Pflanzenparameter und Zusammensetzung der Bodenlösung

Die Bedeutung von Huminstoffen für die Phosphatverfügbarkeit im Boden wird kontrovers diskutiert. Deswegen wurde der Einfluß der Zugabe eines Modellhuminstoffes aus Hydrochinon auf die Löslichkeit dieser Elemente in einem P-fixierenden Oxisol untersucht und die Bedeutung für die P-, Fe- und Mn-Aufnahme durch Rotklee und Weidelgras ermittelt.

Nach 4 Monaten Inkubationszeit stieg die P-Konzentration in der Bodenlösung nach Huminstoffzusatz (0, 10, 30, 50 g $kg^{-1}$ Boden) um mehr als das zehnfache in der höchsten Huminstoffstufe an. Der Einfluß der Huminstoffe auf die P-Löslichkeit war damit sehr viel größer, als eine P-Gabe von 100 mg $kg^{-1}$ Boden. Die Löslichkeit von Fe und Al stieg nach Huminstoffzusatz in ähnlicher Weise wie die von Phosphat an.

Im Gefäßversuch wurde bei Rotklee durch Huminstoffe der Sproßertrag fast nicht und der P-Entzug nur in geringem Maße erhöht. Höhere Huminstoffkonzentrationen führten zu leicht ansteigenden Fe-Konzentrationen im Sproß, aber erhöhten die Mn-Konzentrationen so stark, daß Symptome von Mn-Toxizität zu beobachten waren.

Weidelgras zeigte einen starken Ertragsanstieg mit zunehmender Huminstoffgabe. Dieser betrug in der höchsten Huminstoffstufe rund 150 % im Vergleich zur Kontrolle ohne Huminstoff.

Die P-Düngung hatte einen geringen Einfluß auf den Sproßertrag von Gras und keinen Effekt auf den von Rotklee.

Der geringe Einfluß der Huminstoffe und des gedüngten P auf den Sproßertrag von Rotklee im Vergleich zum Weidelgras kann mit der chemischen Mobilisierung von P durch die Ausscheidung von Citronensäure (etwa 12 µmol $g^{-1}$ Boden) erklärt werden. In Einklang damit stiegen die P-Lösungskonzentrationen im Boden unter Rotklee von der 1. zur 2. Ernte an, nicht jedoch im Boden unter Weidelgras.

## Introduction

Phosphate deficiency of crops is common in highly weathered soils, rich in variable charge colloids (*Sanchez*, 1976) often caused by P fixation and not by absolute low P contents of the soils (*Pagel* et al., 1982).

Humic substances as the main constituent of stabile soil organic matter can affect P availability in soils by different mechanisms. They can desorb P by ligand exchange (*Yuan*, 1980; *Sibanda* and *Young*, 1986) and solubilize P as humic-Fe(Al)-P complexes (*Gerke*, 1992), thereby reducing P adsorption. On the other hand, humics can increase P adsorption by inhibition of the crystallization of poorly ordered oxides (*Schwertmann* et al., 1986) and also by complexing Fe and Al. Humic-Fe(Al) complexes possess a higher P sorption capacity compared to poorly ordered Fe-and Al-oxides (*Gunijgake* and *Wada*, 1981; *Gerke* and *Hermann*, 1992).

Plant roots can increase nutrient availability by excretion of $H^+$ or $HCO_3^-/OH^-$ (*Smiley*, 1974), the excretion of complexing agents, e.g. di- or tricarboxylic acids (*Gardner* et al., 1983) and nonproteinogenic amino acids (*Marschner* et al., 1989), or by lowering the redox potential (*Fischer* et al., 1989).

Plant species which form clusters of rootlets, called "proteoid roots" accumulate citric and malic acid in the rhizosphere (*Dinkelaker* et al., 1989; *Grierson*, 1992; *Gerke* et al., 1994). *Scheffer* et al. (1962) demonstrated that red clover excreted malic acid into solution. *Hoffland* et al. (1989) found that rape excreted citric and malic acid as a result of P starvation. Experiments with red clover showed that P-starving plants strongly increased the efflux of citric acid compared to plants with sufficient P supply (*Gerke*, unpublished results). Graminaceous species are known to react to Fe deficiency by excreting nonproteinogenic amino acids which form stable complexes with Fe (*Marschner* et al., 1989). The simultaneous mobilization of Fe associated P has been shown by *Jayachandran* et al. (1989) who used synthetic complexants. At low P coverage of the soil colloids this process was of minor importance compared to higher P coverage. However, *Föhse* et al. (1988) showed that wheat and ryegrass increased the root/shoot ratio at low P-level, thereby increasing the root surface which supplies one unit of shoot with nutrients. Thus, the acquisition of P by red clover and ryegrass from P fixing soils include different strategies.

The P and Fe mobilizing effect of plant roots by changing chemical parameters in the rhizosphere depends on the P and Fe forms in soil. The superior effect of citrate on solubilization of Fe and reduction of P adsorption to humic-Fe complexes and Fe-oxide compared to malate, tartrate, and phthalate was recently shown (*Gerke*, 1993). *Fox* et al. (1990) demonstrated a strong increase of P solubility after adding citric or oxalic acid to samples of a B horizon of a Spodosol. In this horizon, phosphate is mainly bound to Al sites which are probably associated with humics as shown by the approximately same concentrations of oxalate and pyrophosphate extractable Al. Consistent with these results, *Gerke* (1992) found a much stronger P mobilization by citrate in a Podzol with humic-Al-P as the main fraction compared to an Oxisol low in $C_{org}$. These results indicate that P mobilization by organic acids is stronger from humic-Al(Fe)-P complexes than from Fe/Al-oxide-P complexes. However, verification of this hypothesis in the soil/plant system is still missing.

One of the main problems associated with an experimental approach for this hypothesis is the selection of humic substances for the addition to soils. Humics extracted from soils inevitably have P, Al and Fe as constituents (*Dormaar*, 1972; *Swift* and *Posner*, 1972; *Kodama* et al., 1988). Thus, adding extracted humic substances to soil is necessarily accompanied by the addition of P, Al and Fe which will modify the effect of humics on P-availability. For this reason we used model humic substances synthesized by polymerization of hydroquinone in diluted NaOH (as a model for soil humic substances) to avoid the additional introduction of these elements into the soil. Such polymers were extensively investigated by *Flaig* et al. (1975) and *Ziechmann* (1980).

The objectives of the present paper are:

(i) to investigate the effect of humic substances on soil solution concentrations of P, Fe, and Al in samples of an Oxisol low in $C_{org}$ and

(ii) to investigate the acquisition of P and Fe by ryegrass and red clover from an Oxisol incubated with different concentrations of humic substances.

In a subsequent paper the effects of ryegrass or red clover cultivation in combination with the addition of humics on P and Al species in the soil solution will be reported.

## Materials and Methods

### Incubation of humic substances in an Oxisol

The synthetic humic acids were prepared from hydroquinone in diluted NaOH, as described by *Scheffer* et al. (1958). These synthetic polymers mainly consist of aromatic subunits with low proportions of alkyl groups (*Hermann*, 1993). The total acidity of the polymers (determined by the $Ba(OH)_2$ method) was 9.80 meq $g^{-1}$ and the COOH-content (determined by the Ca-acetate method) was 3.20 meq $g^{-1}$.

A sample of 1.2 kg of an Oxisol top soil of which characteristics are presented in table 1 were mixed with 2.4 kg quartz sand. Model humic substances were added to reach concentrations of 10, 30, or 50 g humics $kg^{-1}$ soil. In a subsequent step, the soil/sand mixtures with different concentrations of humics were divided into two portions from which one received 33,3 mg P $kg^{-1}$ soil-sand mixture (as $Na_2HPO_4 \cdot H_2O$ and $KH_2PO_4$ in a ratio of 5:95).

The soil-sand mixtures were filled into 3 L PVC pots, watered to approximately field capacity (16 % w/w), covered with metal plates and were incubated for 3 months at 20 °C.

**Table 1:** Characteristics of the relictic Oxisol (Lich/Vogelsberg, Hessen)
**Tabelle 1:** Bodenkenndaten des reliktischen Oxisols (Lich/Vogelsberg, Hessen)

| pH | Corg | $Al_o$ | $Fe_o$ | CAL | $H_2O$ |
|---|---|---|---|---|---|
| 0.01 M $CaCl_2$ | (%) | —— mg/kg | —— | —— mg P/100 g | —— |
| 5.5 | 0.3 | 3.544 | 2.925 | 0.7 | 0.15 |

$Al_o$, $Fe_o$ = oxalate extractable Fe and Al according to *Schwertmann* (1964)

To investigate the effect of humics addition on P, Al, and Fe solubility we recovered soil solution in time intervals by displacement with $H_2O_{demin}$ according to the method of *Adams* (1974) and analyzed it as described below. The soil used for the recovery of the soil solution was afterwards refilled into the PVC pots to avoid losses of soil. The soil was allowed to evaporate until field capacity was reached again and was then further incubated.

### Cultivation of plants

After incubation, the soil-sand mixtures were air dried and fertilized with 100 mg K as $K_2SO_4$, 50 mg Mg as $MgSO_4 \cdot 7\ H_2O$, 2 mg B as

$H_3BO_3$, and 10 mg Mo as $(NH_4)_6Mo_7O_{24} \cdot 4\ H_2O$ per kg soil-sand mixture.

The mixtures were air dried again and 230 g were filled into PVC vessels of 14.8 cm height and 2.1 cm radius after closing the vessels with two sheets of parafilm at the bottom. We used such small vessels for plant cultivation in order to reach a high root density and, consequently, a possible strong modification of cultivated soil as a result of root excretion.

The soil was compacted to a bulk density of about 1.2 g dry soil $cm^{-3}$. Seven replicates were prepared for the two P and four humic substances levels, resulting in 56 pots. 24 pots were fertilized with 50 mg N $pot^{-1}$ as $Ca(NO_3)_2$, further 24 pots were inocculated with a suspension of *Rhizobium trifolii* (30 g Radicin $L^{-1}$) in 0.05 M $CaCl_2$. The pots were then watered with $H_2O_{demin.}$ to reach field capacity. One reference pot for each humics and P level without inocculation and nitrate was additionally prepared for the recovery of soil solution without plants.

Seeds of *Trifolium pratense* L. (cv. Mekra) and *Lolium multiflorum gaudinii* L. (cv. Lirasand) were pregerminated for 48 h on filter paper moistened with 0.01 M $CaCl_2$. Ryegrass was sown in the nitrate fertilized soil and red clover in the inocculated soil with 80 seeds per pot. The pots were covered with quartz sand moistened with a small volume of $H_2O_{demin.}$. The plants were cultivated in a growth chamber at 250 µE $m^{-2}s^{-1}$, a day night regime of 16/8 h, a relative humidity of 60 %/80 % and a temperature of 22 °/15 °C. The time of cultivation was 30 days for both species. The moisture content was adjusted daily by weight in the first 3 weeks and afterwards adjusted twice daily. For the first harvest (after 20 days), one of the three replicates was used, for the second harvest (ten days later) the two remaining replicates. The limited quantity of humic substances allowed no further replicates.

## Analytical procedure

Displaced soil solution of the harvested soils was obtained after preparing a hole of about 2 mm diameter into the two parafilm layers on the bottom and pipetting 20 ml of $H_2O_{demin.}$ to the top of the pots. About 15 ml of displaced soil solution were obtained within 2 hours and filtered through a 0.45 µm cellulose acetate membrane (Sartorius, Göttingen, Germany).

Afterwards, the roots were separated from the soil by washing with $H_2O_{demin.}$. Root lengths were determined by the line intersect method according to *Newman* (1966). The remaining soil suspension was 0.5 mm sieved to remove the quartz particles and dried at 80 °C.

Shoots were dried at 80 °C, ground and digested in concentrated $HNO_3$ at 180 °C in teflon bombs. Phosphate, Al, Fe, and Mn in the soil solution and in the shoot digests were determined by ICP-OES. Humics solubility was determined by photometry at 400 nm as described by *Gerke* (1993).

## Phosphate influx

Phosphate influx was calculated between the first and the second harvest according to equation 1. In this equation linear root growth is assumed.

$$\text{Influx} = \frac{U_2 - U_1}{t_2 - t_1} \cdot \frac{2}{WL_2 + WL_1} \qquad \text{eq. 1}$$

$t_2, t_1$ time interval from sowing to the second resp. first harvest

$U_2, U_1$ P in the shoots at the second resp. first harvest

$WL_2, WL_1$ root length at the second resp. first harvest

## Experiment for the quantification of organic acids in the rhizosphere of red clover

To investigate the accumulation of organic acids in the rhizosphere of red clover, a further pot experiment was conducted. Two PVC cylinders (5.0 cm height and 3.25 cm radius) were filled with a calcareous loess subsoil poor in available P (pH of 7.7 in 0.01 M $CaCl_2$ and 8.5 % $CaCO_3$). This soil and not the Oxisol was used because citrate and malate added to this soil are extracted by water with more than 90 % recovery (*Gerke* et al., 1994). Organic acids are not quantitatively extractable from the Oxisol even with strong extractants.

On one site the cylinders were closed with parafilm and on the other with a nylon net (net size 30 µm) to allow only root hairs to penetrate into the soil. The two PVC cylinders were arranged that the two nylon nets were facing each other (Fig. 1).

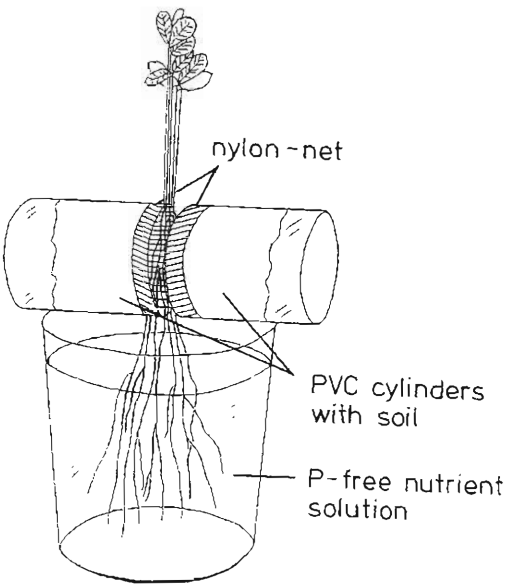

**Figure 1:** Experimental design for the investigation of organic acid accumulation in the rhizosphere soil of red clover.
**Abbildung 1:** Schematische Darstellung der Gefäßversuche zur Ermittlung der Konzentrationen an organischen Säuren in der Rhizosphäre von Rotklee.

Pregerminated clover plants were fixed in the gap with a distance of about 3 mm between the two PVC cylinders. During the growth period of six weeks the roots covered densely the interior space between the cylinders. Plant nutrition was ensured by roots growing between the two cylinders into the P-free nutrient solution which was prepared according to *Jungk* and *Barber* (1974). At the end of the experiment, rhizosphere soil from different proximity to the roots was collected by cutting thin layers of soil behind the nylon net.

Organic acids were determined by a combination of water extraction and quantitative thin layer chromatography as described by *Gerke* et al. (1994). To the rhizosphere soil $H_2O_{demin.}$ was added to reach a soil/water ratio of 1:10. The pH of the suspension was adjusted with HCl to 4 and readjusted several times during the extraction procedure. Extraction was performed on an end over end shaker for 4 hours. The suspension was then filtered through a paper filter and subsequently through a 0.45 µm membrane filter. Citrate in the filtrate was purified and preconcentrated by solid phase extraction (SPE) using Bakerbond -7091-03- columns (Baker, Groß-Gerau, Germany). The elution mixture resulting from SPE was directly

**Table 2:** Shoot yield, P uptake, Fe- and Mn-concentration of the shoots of ryegrass and red clover after 30 days of growth.
**Tabelle 2:** Sproßertrag, P-Entzug sowie Fe- und Mn-Konzentrationen im Sproß von Weidelgras und Rotklee nach 30 Tagen Wachstumszeit.

| | **rye grass** | | | | | | **red clover** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Yield | $cv^x$ [%] | P uptake | P | Fe | Mn | Yield | $cv^x$ [%] | P uptake | P | Fe | Mn |
| | g DM $pot^{-1}$ | | mg P $pot^{-1}$ | g kg $DM^{-1}$ | mg $kg^{-1}$ | | g DM $pot^{-1}$ | | mg P $pot^{-1}$ | g kg $DM^{-1}$ | mg $kg^{-1}$ | |
| $H_0P_0$* | 0.50 | 7.4 | 0.40 | 0.81 | 150 | 210 | 0.95 | 10.9 | 0.81 | 0.85 | 139 | 450 |
| $H_0P_1$ | 0.71 | 10.6 | 0.81 | 1.11 | 102 | 180 | 1.00 | 21.3 | 0.95 | 0.95 | 107 | 223 |
| $H_{10}P_0$ | 1.01 | 12.3 | 0.83 | 0.80 | 177 | 780 | 1.12 | 14.8 | 1.11 | 0.99 | 205 | 2240 |
| $H_{10}P_1$ | 1.20 | 10.4 | 1.12 | 0.91 | 103 | 630 | 0.99 | 8.9 | 1.11 | 1.12 | 133 | 2273 |
| $H_{30}P_0$ | 1.12 | 24.6 | 1.05 | 0.90 | 235 | 1240 | 1.07 | 17.9 | 1.35 | 1.26 | 327 | 2997 |
| $H_{30}P_1$ | 1.38 | 14.1 | 1.38 | 1.03 | 229 | 945 | 1.08 | 13.8 | 1.41 | 1.31 | 295 | 2415 |
| $H_{50}P_0$ | 1.23 | 23.1 | 1.11 | 0.90 | 248 | 1455 | 1.13 | 20.1 | 1.39 | 1.23 | 333 | 2430 |
| $H_{50}P_1$ | 1.35 | 8.9 | 1.35 | 1.01 | 233 | 1233 | 1.04 | 16.9 | 1.33 | 1.27 | 185 | 2401 |

* control

x coefficient of variation of the DM yield of ryegrass and red clover

$P_0$: without phosphate; $P_1$: with 100 mg P $kg^{-1}$ soil
$H_0$: without humics; $H_{10}$: with 10 g humics $kg^{-1}$ soil
$H_{30}$: with 30 g humics $kg^{-1}$ soil; $H_{50}$: with 50 g humics $kg^{-1}$ soil

used for quantitative thin layer chromatography. The determination of organic acid concentration was performed as described before (*Gerke* et al., 1994).

## Results

The shoot yield of ryegrass increased with increasing humics addition and by the application of P (table 2). For red clover, humics addition had a much smaller positive effect on shoot yield whereas P addition had no effect. Similarly, P uptake of ryegrass increased by humics and P addition, whereas humics but not P addition increased P uptake of red clover. Both plant species had very low P concentrations in the shoots at the second harvest even with phosphate application (table 2). Iron concentrations in the shoots were similar for both species and were slightly raised by increasing concentrations of humic substances in the soil.

In contrast, Mn concentrations in the shoots of both species were strongly raised with humic substances concentrations. Those were twice as high for red clover compared to ryegrass. In the soil with humics, clover showed symptoms of Mn toxicity. The symptoms were similar to those described by *Bergmann* (1986, p. 243) with chlorosis and necrosis beginning at the edges of the older leaves. Phosphate fertilization reduced the Mn concentrations and the observed symptoms. Additional incubation and measurement showed that humics strongly increased Mn solubility in soil (data not presented).

The results demonstrate that humics increased P availability in the Oxisol. 10 g humics $kg^{-1}$ soil was more effective than the addition of 100 mg P $kg^{-1}$ soil. Total P concentrations in the displaced soil solution of the pots without plants further support this observation (table 3).

**Table 3:** Phosphate, Fe, Al and humics concentration and pH in the soil solution of an Oxisol in relation to humics and P addition after 3 months of incubation.
**Tabelle 3:** Phosphat, Fe, Al und Huminstoffkonzentrationen sowie der pH-Wert in der Bodenlösung eines Oxisols nach 3 Monaten Inkubationszeit in Abhängigkeit von der Huminstoff- und Phosphatgabe.

| | P | Al | Fe | humics | pH |
|---|---|---|---|---|---|
| | µmol $L^{-1}$ | | | mg $L^{-1}$ | |
| $H_0P_0$* | 0.35 | 0.29 | 0.10 | 0.00 | 5.61 |
| $H_0P_1$ | 1.35 | 0.31 | 0.40 | 0.00 | 5.69 |
| $H_{10}P_0$ | 1.05 | 2.95 | 0.89 | 1.48 | 5.53 |
| $H_{10}P_1$ | 3.24 | 4.57 | 0.82 | 2.94 | 5.54 |
| $H_{30}P_0$ | 2.59 | 20.59 | 3.54 | 8.38 | 5.48 |
| $H_{30}P_1$ | 5.95 | 18.78 | 6.86 | 7.97 | 5.53 |
| $H_{50}P_0$ | 8.33 | 48.14 | 10.93 | 11.58 | 5.39 |
| $H_{50}P_1$ | 9.91 | 39.40 | 7.47 | 9.59 | 5.58 |

* control

$P_0$: without phosphate; $P_1$: with 100 mg P $kg^{-1}$ soil
$H_0$: without humics; $H_{10}$: with 10 g humics $kg^{-1}$ soil
$H_{30}$: with 30 g humics $kg^{-1}$ soil; $H_{50}$: with 50 g humics $kg^{-1}$ soil

Humics addition raised P solution concentrations up to the 25-fold without P-application and to the 7-fold in the soil with 100 mg P $kg^{-1}$ soil (table 3). Humics and P increased the P concentration in the soil solution up to about 10 µmol $L^{-1}$. With increasing humics concentrations, Fe and Al solubility was strongly raised too, from virtually nil to about 10 and 40 µmol $L^{-1}$, respectively. Humics solubility also increased with increasing humics addition and P application had a small effect on Al, Fe, and humics solubility.

Soil solution concentrations in the soil cultivated with plants were also determined. Because of the high root density (> 100 cm $cm^{-3}$), this solution can be considered to be strongly affected by plant roots. Phosphate concentrations

**Table 4:** Phosphate, Fe, Al and humics concentration and pH in the soil solution of an Oxisol cultivated with ryegrass in relation to humics and P addition. Treatments are the same as in table 2 and 3.

**Tabelle 4:** Phosphat, Fe, Al und Huminstoffkonzentrationen sowie der pH-Wert in der Bodenlösung eines Oxisols unter Weidelgras nach 3 Monaten Inkubationszeit in Abhängigkeit von der Huminstoff- und Phosphatgabe. Die Versuchsglieder sind dieselben wie in Tabelle 2 und 3.

| | 1. harvest | | | | | 2. harvest | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | Al<br>µmol $L^{-1}$ | Fe | humics<br>mg $L^{-1}$ | pH | P | Al<br>µmol $L^{-1}$ | Fe | humics<br>mg $L^{-1}$ | pH | cv[x]<br>[%] |
| $H_0P_0$[*] | 0.46 | 0.73 | 0.21 | 0.00 | 6.21 | 0.31 | 0.10 | 0.43 | 0.00 | 5.95 | 15.4 |
| $H_0P_1$ | 0.59 | 1.11 | 0.19 | 0.00 | 6.28 | 0.89 | 0.51 | 1.19 | 0.00 | 5.87 | 26.8 |
| $H_{10}P_0$ | 2.02 | 0.50 | 1.20 | 2.50 | 6.15 | 1.33 | 0.89 | 1.20 | 2.90 | 5.93 | 10.1 |
| $H_{10}P_1$ | 1.58 | 0.89 | 0.39 | 3.80 | 6.13 | 0.93 | 1.34 | 3.21 | 1.47 | 6.01 | 17.9 |
| $H_{30}P_0$ | 2.05 | 15.70 | 1.23 | 7.93 | 5.73 | 1.40 | 6.50 | 1.52 | 6.83 | 5.43 | 28.1 |
| $H_{30}P_1$ | 2.86 | 11.73 | 0.40 | 6.24 | 5.59 | 2.57 | 5.73 | 2.67 | 5.30 | 5.48 | 10.5 |
| $H_{50}P_0$ | 3.70 | 20.01 | 2.13 | 9.13 | 5.59 | 3.24 | 34.40 | 6.17 | 4.70 | 5.39 | 7.5 |
| $H_{50}P_1$ | 3.93 | 36.30 | 1.03 | 10.20 | 5.47 | 3.94 | 21.31 | 3.38 | 6.93 | 5.56 | 16.1 |

[*] control

[x] coefficient of variation of phosphate concentration in the soil solution at the second harvest

**Table 5:** Phosphate, Fe, Al and humics concentration and pH in the soil solution of an Oxisol cultivated with red clover in relation to humics and P addition. Treatments are the same as in table 2 and 3.

**Tabelle 5:** Phosphat, Fe, Al und Huminstoffkonzentrationen sowie der pH-Wert in der Bodenlösung eines Oxisols unter Rotklee nach 3 Monaten Inkubationszeit in Abhängigkeit von der Huminstoff- und Phosphatgabe. Die Versuchsglieder sind dieselben wie in Tabelle 2 und 3.

| | 1. harvest | | | | | 2. harvest | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | Al<br>µmol $L^{-1}$ | Fe | humics<br>mg $L^{-1}$ | pH | P | Al<br>µmol $L^{-1}$ | Fe | humics<br>mg $L^{-1}$ | pH | cv[x]<br>[%] |
| $H_0P_0$[*] | 0.45 | 5.66 | 1.04 | 0.00 | 5.05 | 1.17 | 5.36 | 0.14 | 0.00 | 4.71 | 7.1 |
| $H_0P_1$ | 0.95 | 2.37 | 0.72 | 0.00 | 5.21 | 1.70 | 2.07 | 0.25 | 0.00 | 4.85 | 14.8 |
| $H_{10}P_0$ | 1.28 | 19.43 | 1.07 | 3.50 | 4.95 | 3.59 | 38.81 | 1.10 | 4.55 | 4.57 | 27.3 |
| $H_{10}P_1$ | 2.21 | 11.02 | 1.03 | 2.81 | 5.04 | 2.41 | 41.41 | 2.35 | 6.01 | 4.69 | 10.9 |
| $H_{30}P_0$ | 1.98 | 11.95 | 1.19 | 8.93 | 4.88 | 3.39 | 53.35 | 2.80 | 9.31 | 4.78 | 36.8 |
| $H_{30}P_1$ | 1.99 | 14.97 | 1.98 | 8.91 | 4.97 | 4.90 | 57.17 | 2.13 | 9.90 | 4.43 | 28.1 |
| $H_{50}P_0$ | 1.61 | 29.55 | 2.61 | 11.51 | 4.83 | 3.45 | 66.51 | 3.20 | 16.90 | 4.65 | 10.4 |
| $H_{50}P_1$ | 2.34 | 43.89 | 2.13 | 14.70 | 4.89 | 4.69 | 70.29 | 2.45 | 15.80 | 4.72 | 17.5 |

[*] control

[x] coefficient of variation of phosphate concentration in the soil solution at the second harvest

in the soil solution under grass were lowered during the growth of plants especially in the $H_{30}$ and $H_{50}$ treatments (table 4) compared to the pots without plants (table 3), probably due to plant uptake. However, in the treatments with red clover P solution concentrations increased from the first to the second harvest (table 5). A strong decrease of pH values in the soil solution of the clover pots was measured from 5.6 (table 3) to about 4.7 (table 5) whereas only small pH changes occurred in the soil under ryegrass. Iron concentrations in the soil solution under red clover and ryegrass decreased in the $H_{30}$ and $H_{50}$ treatments compared to those without plants. At the second harvest of red clover humics and Al concentrations in the soil solution were higher than in soil without plants (table 5). In the soil cultivated with ryegrass, Al and humics solubility was decreased compared to the soil without plants (table 4).

The calculation of the average root length and the differential P uptake between the first and the second harvest allowed the calculation of the P influx between the first and the second harvest assuming linear root growth. Phosphorus influx of ryegrass was increased after adding humics or phosphate (fig. 2). In contrast, P influx of red clover was increased by humics but not by P addition.

Investigations on the concentrations of organic acids in the rhizosphere of red clover showed that mainly citrate was accumulated. Malate was detected in trace amounts.

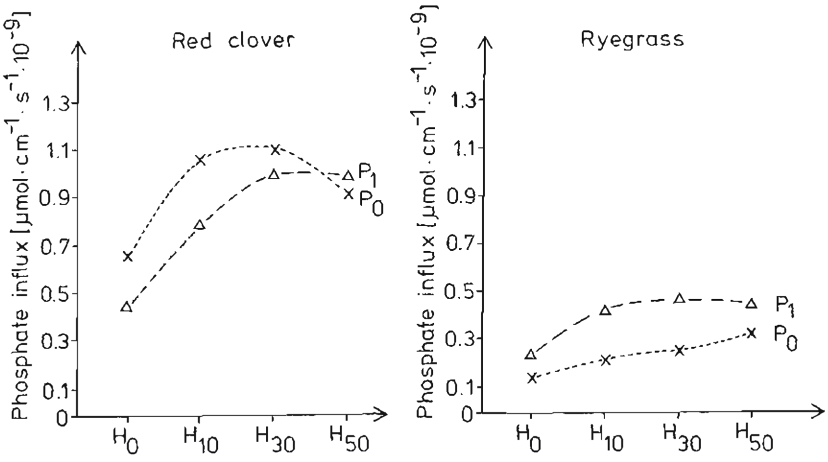

**Figure 2:** Phosphate influx of red clover and ryegrass between 20 and 30 days in relation to the concentration of humic substances and P levels.

**Abbildung 2:** Phosphatinflux von Rotklee und Weidelgras in Abhängigkeit von der Huminstoff- und Phosphatkonzentration im Boden.

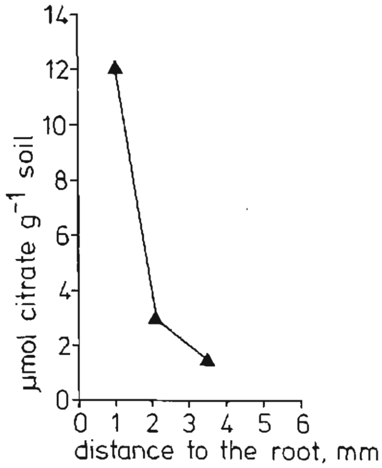

**Figure 3:** Concentration of citrate in the rhizosphere soil of red clover in different proximity to the root surface.

**Abbildung 3:** Citratkonzentration in der Rhizosphäre von Rotklee in Abhängigkeit von der Entfernung zur Wurzeloberfläche.

We observed about 12 µmol citrate $g^{-1}$ soil in the vicinity of roots decreasing with increasing distances from the root surface (fig. 3).

## Discussion

Cultivation of plants in very small pots (205 $cm^3$) leads to high root densities, which can strongly modify the soil due to plant root excretion. This is demonstrated in the present investigation for red clover, which greatly decreased soil solution pH from 5.6 to about 4.7 (Table 3, 5). From the investigation on citrate accumulation in the rhizosphere of a calcareous loess soil, it was shown that citrate ions were excreted by roots of P-starving red clover plants. The measured concentration of citrate in the rhizosphere soil of about 12 µmol $g^{-1}$ soil is an average concentration in about 0.8 mm around the root surface. Considering the citrate concentration profile, it can be assumed, that soil in close proximity to red clover roots will be loaded with higher quantities of citrate, thus will contain more than 12 µmol citrate $g^{-1}$ soil. For methodological reasons we used a calcareous Luvisol to determine citrate concentrations in the rhizosphere of red clover. The investigations on the citrate concentration in the rhizosphere of white lupin in the Oxisol and Luvisol showed that this species accumulated citrate in the same order of magnitude in both soils (*Gerke* et al., 1994). From this result we conclude that red clover may accumulate similar quantities of citrate in close proximity to the root surface in the Oxisol and Luvisol.

In an incubation experiment it was shown that 20 µmol citric acid $g^{-1}$ soil increased P soil solution concentration of the Oxisol by about two orders of magnitude (*Gerke*, 1992). In agreement with these experiments, P mobilization is probably the reason for the increased P solubility under red clover in the Oxisol without humics compared to the treatments without plants (Table 3, 5). Phosphate mobilization may further explain the increased P concentrations in soil solution under red clover from the first to the second harvest. In contrast, P concentration in soil solution under ryegrass decreased at the same time. Preliminary investigations show that the carboxylate excretion of P starving ryegrass is 1-2 orders of magnitude lower than that of red clover indicating that P mobilization by root exudates does not contribute to the P acquisition by ryegrass.

The smaller effect of humics on P uptake of red clover compared to ryegrass may also be due to P mobilization by red clover. Additionally, chemical mobilization probably is the reason for the lack of increased P uptake by red clover after addition of P. Phosphate influx was, in agreement with the explanation of chemical mobilization, 2-3 times higher for red clover compared to ryegrass. The marked increase of Al and humics concentrations in soil solution under red clover supports this view, too (Table 5). *Earl* et al. (1979) showed that P mobilization by citrate and tartrate was accompanied by a much higher release of Fe and Al. *Fox* et al. (1990) investigated the release of P and Al by 16 organic acids and found a close correlation between the P and Al release, the Al release being two orders of magnitude higher. The increased concentration of humics in soil under red clover suggests that humics are mobilized by citric acid.

The importance of mobilization of humic-P complexes by citrate was shown in an incubation experiment with soils (*Gerke*, 1992) and was recently confirmed in experiments with humic-Fe complexes (*Gerke*, 1993). The initial reaction of citrate with humic-Al-P complexes is shown in figure 4. Citrate competes with phosphate for the same sorption sites. Furthermore, citrate introduces negative charges to the P-sorbing surfaces, thereby reducing P-sorption. The introduction of hydrophilic citrate ions into the humic molecules and the possible desorption of Fe and Al from the humic surface increase the humics solubility thereby solubilizing humic associated P. Model humic polymers may differ from humic substances extracted from soil and may cause different effects on metal and phosphate solubility in soil compared to native humic substances. However, contents of strong acidic groups and stability of model humic-Al complexes are very similar to those of soil humic substances indicating that these model polymers are appropriate for this study.

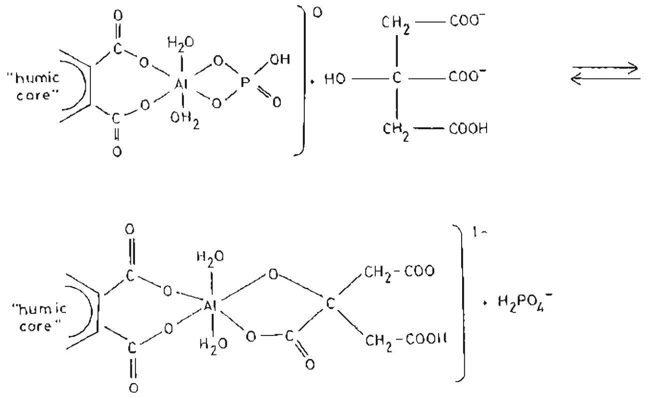

**Figure 4:** Schematic description of the desorption reaction of phosphate by citrate from humic-Al complexes.
**Abbildung 4:** Schematische Darstellung der Desorption von Phosphat durch Citrat von Huminstoff-Al-Komplexen.

The decrease in pH in the soil solution under red clover may cause an increased concentration of monomeric Al species which are toxic to plant roots. *Alva* et al. (1986) showed that activities of monomeric Al $> 10^{-6}$ M cause Al toxicity. The high concentrations of total Al in the soil solution under red clover may indicate toxicity by monomeric Al. However, monomeric Al in the soil solution is complexed by humics resulting in a decrease of Al-toxicity. Aluminum species distribution will be presented in a second paper. The P species distribution in soil solution is important for the availability of solubilized P to plant roots. Humic-(Al)-P-complexes may account for a great proportion of solubilized P in the soil solution. Information on the availability of humic-P to plant roots are rare.

Iron concentrations in the shoots are slightly increased by the addition of humic substances but Mn concentrations are drastically increased (table 2) leading to symptoms of Mn toxicity on red clover leaves. The Mn concentrations in the shoots of red clover are in the range of those reported by *Bergmann* (1986) for plants with Mn toxicity symptoms. Possibly Mn toxicity might have been one reason for the poor increase in shoot yield of red clover with increasing humics addition.

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## References

*Adams, F.* (1974): Soil solution. In: Carsons, E. W. (ed): The plant root and its environment. Univ. press, Charlottesville, U. S. A.

*Alva, A., D. Edwards, C. Asher* and *F. Blamey* (1986): Effect of phosphorus/aluminum molar ratio and calcium concentration on plant response to aluminum toxicity. Soil Sci. Soc. Am. J. 50, 133-137.

*Bergmann, W.* (1986): Farbatlas Ernährungsstörungen bei Kulturpflanzen - visuelle und analytische Pflanzendiagnose. Gustav Fischer Verlag, Jena.

*Dinkelaker, B., V. Römheld* and *H. Marschner* (1989): Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ. 12, 285-292.

*Dormaar, J. F.* (1972): Chemical properties of organic matter extraction from a number of $A_h$-horizons by a number of methods. Can. J. Soil Sci. 52, 67-72.

*Earl, K., J. Syers* and *R. McLaughlin* (1979): Origin of the effect of citrate, tartrate and acetate on phosphate sorption by soils and synthetic gels. Soil Sci. Soc. Am. J. 43, 674-678.

*Fischer, W. R., H. Flessa* and *G. Schaller* (1989): pH-values and redox potentials in microsites of the rhizosphere. Z. Pflanzenernähr. Bodenkde. 152, 191-195.

*Flaig, W., H. Beutelspacher* and *E. Rietz* (1975): Chemical composition and physical properties of humic substances. In J. E. Gieseking (ed): Soil components, volume I. Springer Verlag, Berlin, Heidelberg, New York.

*Föhse, D., N. Claassen* and *A. Jungk* (1988): Phosphorus efficiency of plants. I. External and internal P requirement and uptake efficiency of different plant species. Plant and Soil 110, 101-109.

*Fox, T., N. Comerford* and *W. McFee* (1990): Phosphorus and aluminum release from a spodic horizon mediated by organic acids. Soil Sci. Soc. Am. J. 54, 1763-1767.

*Gardner, W., D. Barber* and *D. Parbery* (1983): The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil root interface is enhanced. Plant Soil 70, 107-124.

*Gerke, J.* (1992): Phosphate, aluminum and iron in the soil solution of three different soils in relation to varying concentrations of citric acid. Z. Pflanzenernähr. Bodenkde. 155, 339-343.

*Gerke, J.* (1993): Solubilization of Fe (III) from humic-Fe complexes, humic/Fe-oxide mixtures and from poorly ordered Fe-oxide by organic acids - consequences for P adsorption. Z. Pflanzenernähr. Bodenkde. 156, 253-257.

*Gerke, J.* and *R. Hermann* (1992): Adsorption of orthophosphate to humic-Fe complexes and to amorphous Fe-oxide. Z. Pflanzenernähr. Bodenkde. 155, 233-236.

*Gerke, J., W. Römer* and *A. Jungk* (1994): The excretion of citric and malic acid by proteoid roots of *Lupinus albus* L.; effects on solubility of phosphate, iron and aluminum in the proteoid rhizosphere in samples of an oxisol and a luvisol. Z. Pflanzenernähr. Bodenkde. 157, 289-294.

*Grierson, P. F.* (1992): Organic acids in the rhizosphere of *Banksia integrifolia* L. Plant Soil 144, 259-265.

*Gunijgake, N.* and *K. Wada* (1981): Effects of phosphorus concentration and pH on phosphate retention by active aluminum and iron of Ando soils. Soil Sci. 132, 347-352.

*Hermann, R.* (1993): Modifiziertes Reaktionsverhalten von Huminstoffen infolge photochemisch induzierter Prozesse gegenüber ausgewählten organischen Substanzen und anorganischen Ionen. Dissertation, Universität Göttingen.

*Hoffland, E., G. F. Findenegg* and *J. A. Nelemans* (1989): Solubilization of rock phosphate by rape. II. Local exudation of organic acids as response to P-starvation. Plant Soil 113, 161-165.

*Jayachandran, K., A. P. Schwab* and *B. A. Hetrick* (1989): Mycorrhizal mediation of phosphorus availability: Synthetic iron chelate effects on phosphorus solubility. Soil Sci. Soc. Am. J. 53, 1701-1706.

*Jungk, A.* and *S. A. Barber* (1974): Phosphate uptake rate of corn roots as related to the proportion of the roots exposed to phosphate. Agron. J. 66, 554-557.

*Kodama, H., M. Schnitzer* and *E. Murad* (1988): An investigation of iron (III)-fulvic acid complexes by Mössbauer spectroscopy and chemical methods. Soil Sci. Soc. Am. J. 52, 994-998.

*Marschner, H., M. Treeby* and *V. Römheld* (1989): Role of root induced changes in the rhizosphere for iron acquisition in higher plants. Z. Pflanzenernähr. Bodenkde. 152, 197-204.

*Newman, E.* (1966): A method of estimation the total length of root in a sample. J. Appl. Ecol. 3, 133-145.

*Pagel, H., J. Enzmann* and *H. Mutscher* (1982): Pflanzennährstoffe in tropischen Böden - ihre Bestimmung und Bewertung. VEB Deutscher Landwirtschaftsverlag, Berlin.

*Sanchez, P. A.* (1976): Properties and Management of Soils in the Tropics. John Wiley and Sons, New York.

*Scheffer, F., R. Kickhut* and *J. Visser* (1962): Wurzelausscheidungen höherer Pflanzen. I. Isolierung und Identifizierung der Wurzelausscheidungen von *Trifolium pratense* L., *Medicago sativa* L. und *Eragostis curvula* (Schrad.). Z. Pflanzenernähr. Bodenkde. 97, 26-40.

*Scheffer, F., W. Ziechmann* and *H. Scholz* (1958): Über Synthesehuminsäuren aus Polyoxybenzolen. 2. Quantitative Untersuchung zur Oxidation von Hydrochinon im homogenen System mit alkalischer Reaktion. Z. Pflanzenernähr. Bodenkde. 80, 134-149.

*Schwertmann, U.* (1964): Differenzierung der Eisenoxide des Bodens durch Extraktion mit Ammoniumoxalat-Lösung. Z. Pflanzenernähr. Bodenkde. 105, 194-202.

*Schwertmann, U., H. Kodama* and *W. R. Fischer* (1986): Mutual interactions between organics and iron oxides. In: P. M. Huang and M. Schnitzer (ed): Interactions of soil minerals with natural organics and microbes. SSSA. Spec. publ. 17, Madison.

*Sibanda, H. M.* and *S. D. Young* (1986): Competitive adsorption of humic acids and phosphate on goethite, gibbsite and two tropical soils. J. Soil Sci. 37, 197-204.

*Smiley, R. W.* (1974): Rhizosphere pH as influenced by plants, soils and nitrogen fertilizers. Soil Sci. Soc. Amer. Proc. 38, 795-799.

*Swift, R. S.* and *A. M. Posner* (1972): Nitrogen, phosphorus and sulfur contents of humic acids fractionated with respect to molecular weight. J. Soil Sci. 23, 50-57.

*Yuan, T. L.* (1980): Adsorption of phosphate and water-extractable soil organic material by synthetic aluminum silicates and acid soils. Soil Sci. Soc. Am. J. 44, 951-955.

*Ziechmann, W.* (1980): Huminstoffe. Verlag Chemie, Weinheim FRG.

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