Title

Comparison of corn and lupin in respect to As mobilisation, uptake and release in an arsenic contaminated floodplain soil.

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Introduction

Phosphate (P^V) and arsenate (As^V) are adsorbed by the same surface binding sites, in particular Fe(hydr)oxides. For corn, mobilisation of P^V was primarily due to release of citrate in an artificial quartz substrate with different amounts of goethite and spiked with arsenate (Vetterlein et al. 2007).

In this experiment simultaneous mobilisation of arsenate was not observed. Modelling of the soil solution composition showed that competition between As^V, P^V and organic acid anions for binding sites on goethite depends on the proportion of total binding sites to empty binding sites (surface coverage) and the relative abundance of the three anions (Szegedi et al., submitted).

Lupin (*Lupinus albus*) is known to release higher quantities of organic anions compared to corn (Dinkelaker et al., 1989; Jones & Darrah 1995) and is thus potentially more efficient in mobilizing P^V and probably also As^V. At the same time lupin is much more susceptible to As^V toxicity. Corn growth is not reduced in the artificial quartz substrate with 1 g kg^{-1} of goethite and 5 mg kg^{-1} As^V (corresponding to soil solution concentration of 30 μM), whereas for lupin out of 12 seeds only 1 germinated and even this one died off after two weeks. Lupin and corn also differ in Si requirement (Marschner 1995), which is linked to arsenic metabolism as aquaglyceroporins identified as Si transporters have been shown to mediate As^{III} influx and efflux (Zhao et al. 2009).

Recent hydroponic studies with tomato and rice (Xu et al. 2007) have shown strong efflux of As^{III} from plants, which is in line with the identification of aquaglyceroporins as channels for bidirectional As^{III} transport (Bienert et al. 2008; Ma et al. 2008). The amount of As^{III} released was related to plant species and P nutrition (Xu et al. 2007).

The aim of the present study was to compare plant As uptake and release of lupin and corn and elucidate the changes in soil solution P and As concentration in the rhizosphere. The experiment was conducted with (P 100) and without (P 0) addition of P to establish differences in relative abundance of P and As anions in soil solution and differences in plant P status.

Material and methods

Zea mays and *Lupinus albus* were grown under controlled conditions (23 °C, 75 % rel. humidity and a 12-h photo-period) in boxes in which the root compartment was separated from the bulk soil compartment by a nylon mesh (Vetterlein and Jahn, 2004). Micro-tensiometers and micro suction cups were aligned horizontally with a spatial resolution of 45 mm and 6 mm, respectively.

The experiments were set up with three replications in a randomised block design. Each compartment system was equipped with 2 microtensiometers and 15 micro suction cups and placed on a balance for determination of water consumption.

An As contaminated floodplain soil from the Mulde floodplain in Saxony (detailed description in Ackermann et al. submitted) in which As was predominantly associated with the Fe(hydr)oxide fraction was used as substrate (Ackermann et al. submitted). While total As concentration (342 mg kg^{-1}) was much higher compared to the artificial quartz substrate used in previous experiments, soil solution As concentration (0.4µM) was two orders of magnitude lower due to the high concentrations of Fe(hydr)oxides.

For each plant species two treatments were established without (P 0) and with P fertilization (P 100) at a rate of 100 mg kg^{-1}. Soil solution was sampled simultaneously from all micro suction cups 0, 7, 14, 21, 28 and 35 days after planting. Soil solution was collected with a suction of 30 kPa for 6 hours on the respective day. Sample volume ranged from 100 to 200 µl per suction

cup. Soil solution samples were bulked for each suction cup position for the measurement of As-Species (HPLC-ICP-MS), P (EPOS-Photometer) and pH (ISFET-electrode). At harvest (35 DAP) plants were divided in roots, old leaves, and young leaves. Only half of the samples were used for dry weight determination (65°C for 48 h) and digestion with HNO_3 in a pressure unit (Seiff). P was determined by ICP-OES (Jobin Yvon). The other half of the sample was frozen in liquid nitrogen and stored at -80°C for As-species analysis. Plant samples stored in liquid nitrogen were pulverized in liquid nitrogen using a micro-dismembrator and stored at -80°C until extraction. 1-2 g plant sample were homogenized with 10 ml deionised water and centrifuged for 10 min (22000 g, 4°C). The supernatant was analysed immediately by IC-ICP-MS (Mattusch et al., 2000). The method allowed the detection of As^{III}, As^{V}, methylarsonicacid (MMA), dimethylarsinic acid (DMA), arsenobetain, and arsenocholin.

Results

Mean soil solution P concentration was doubled by P fertilizer application (2.08 and 5.51 µM in P 0 and P 100 respectively). Concomitantly, As^{V} concentration increased from 0.41µM to 0.72 µM due to competition between As^{V} and P^{V} for sorption sites. With time there was a decrease in mean soil solution P concentration at both levels of P supply. At P 0 no difference between corn and lupin was observed, while at P 100 soil solution P concentration was lower in lupin with between plant species differences increasing with time (Fig. 1).

Corn dry weight and plant P uptake increased 3.5 fold with P application. Lupin showed a smaller growth response to P application (1.3 fold) and a much better growth and higher P uptake at P 0 compared to corn (Fig. 2). As a consequence P concentrations in the shoots were similar for both P treatments in corn (0.71 and 0.75 g kg^{-1}) while they increased slightly for lupin with increasing P supply (0.87 and 1.1 g kg^{-1}, respectively). The shoot:root dm ratio was similar in lupin and corn.

As uptake was increased in both plant species by P application. Total As uptake was higher in lupin at P 0 but similar for both plant species at high P supply (Fig. 2).

In general, translocation of As from roots to shoots was lower in lupin (shoot:root ratio 0.14 – 0.16) compared to corn (shoot:root ratio 0.33).

The lower translocation in lupin resulted in lower mean shoot As concentrations but higher root As concentrations in lupin compared to corn (Fig. 3).
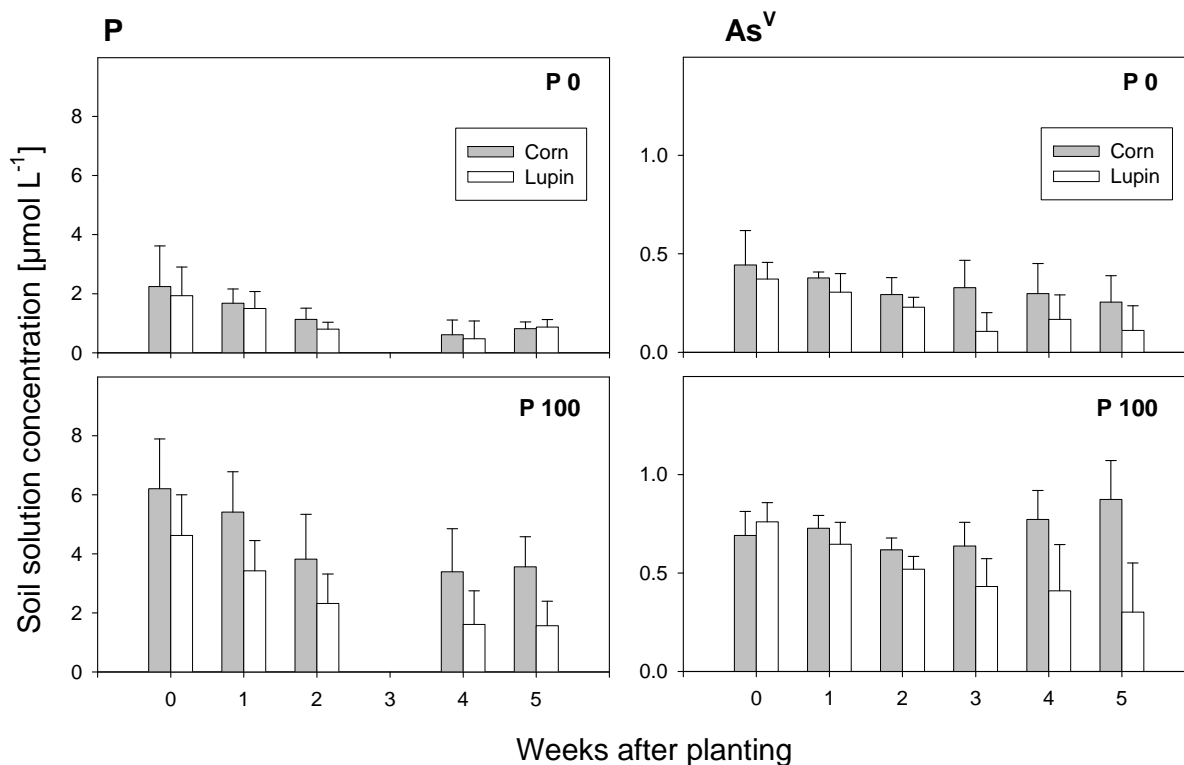
Fig. 1: Changes in P and As^V concentration in soil solution with time for lupin and corn plants grown in As contaminated floodplain soil at two levels of P supply.
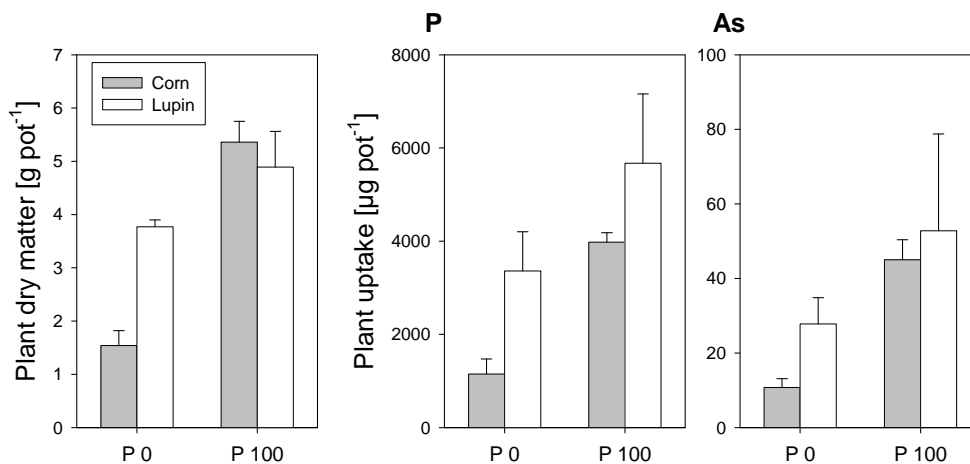

Fig. 2: Dry matter and P and As uptake for lupin and corn plants grown in As contaminated floodplain soil at two levels of P supply for 35 days.

There was no difference in As species distribution in tissues of corn and lupin. As^{III} was the dominant As species in all tissues (Fig. 3).

Initially As^{III} could hardly be detected in the soil solution, but with time soil solution As^{III} concentrations increased up to 0.6 µM (Fig. 4). Soil solution As^{III} concentrations were higher for lupin compared to corn at both P levels. P fertilization had no significant effect on soil solution As^{III} concentrations, due to the large variability of As^{III} concentrations at P 100.
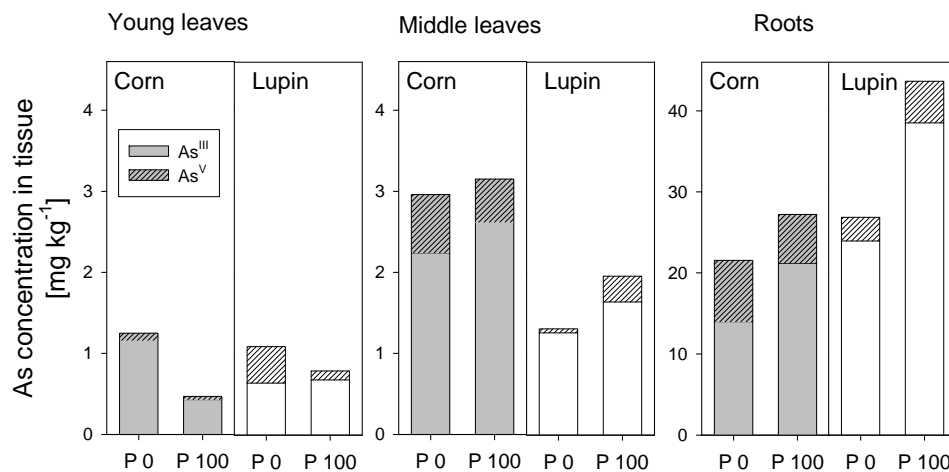
Fig. 3: Concentration of As^V and As^{III} in young, middle leaves and roots of lupin and corn plants grown in As contaminated floodplain soil at two levels of P supply for 35 days.
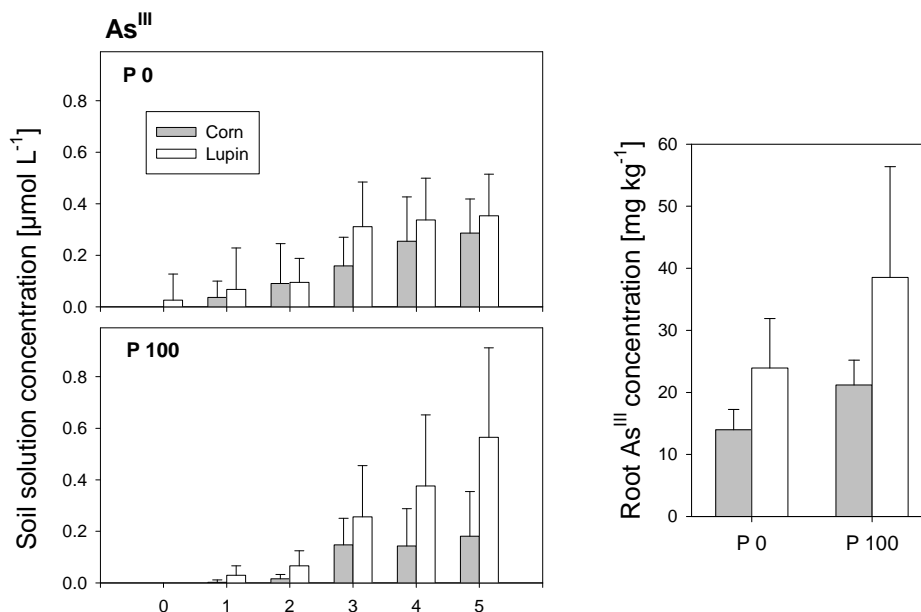

Fig. 4: Changes of As^{III} concentration with time in soil solution of As contaminated floodplain soil planted with lupin or corn at two levels of P supply for 35 days.

Soil solution pH was slightly but consistently higher with corn compared to lupin (data not shown). The pH in the rhizosphere was similar to that of the bulk soil, probably due to the high buffer capacity of the floodplain soil compared to the artificial quartz substrate used in previous experiments.

Discussion

The stronger decrease of soil solution P concentration, in particular in P 100 with lupin compared to corn and the higher P uptake of lupin in both P fertilization treatments is a clear

indicator for the higher P requirement and uptake efficiency of lupin compared to corn. This is in agreement with I_{max} values in the literature for a range of P concentrations. For lupin root segments Keerthisinghe et al. (1998) reported values ranging from 0.02 to 0.15 nM min^{-1} cm^{-1} including proteoid roots while Bhadoria et al. (2004) found a range of 0.01 to 0.02 nM min^{-1} cm^{-1} for corn. Higher I_{max} values probably allow lupins to compensate for their lower specific root length (1800 cm g^{-1} d.wt) compared to corn (2800 cm g^{-1} d.wt.). Lupins not only have a high uptake capacity for P but also have a lower difference in affinity of the uptake system for phosphate and arsenate than is usually observed for other plants (Esteban et al. 2003). In addition the down-regulation of the high affinity phosphate/arsenate uptake system by phosphate is slower compared to other plants (Esteban et al. 2003).

An increase of P concentration in the rhizosphere due to release of organic acid anions as it has been observed in substrates with low sorption capacity was not observed in the present experiment. Whether this is a result of a large number of empty sorption sites due to a low surface coverage of the sorbent or due to low organic acid exudation cannot be decided at present. A further analysis of organic acid anions in the soil solution is required. However, even if no organic acid anions in the soil solution are detected, exudation cannot be ruled out as the sorption of organic acid anions is very fast (Schulz & Vetterlein 2007; Grafe et al. 2002).

Regardless whether organic acid anion release was involved or not, higher P uptake of lupin was associated with stronger As acquisition of lupin compared to corn and this was not just a function of improved plant growth (Fig. 2). This is in agreement with the assumption that the major difference between lupin and corn is the uptake capacity per unit root length (see above) and thus associated with P transporters which provide the uptake pathway for As^V (Esteban et al. 2003).

As^{III} concentrations in soil solution increased with time and were related to root As^{III} concentrations, i.e. were higher for lupin than for corn. This is in agreement with the hypothesis of a diffusion driven As^{III} efflux via aquaglyceroporines previously described as Si transporters (Bienert et al. 2008; Ma et al. 2008). A negative effect of P supply on soil solution As^{III} concentration as shown by Xu et al. (2007) in hydroponics was not found in the present study, which is probably due to the fact that in soil As uptake is promoted by P fertilisation, due to the competition for sorption sites in soil. To our knowledge, there is currently no information available on Si transporters in lupin in contrast to corn (Mitani et al. 2009) and rice (Ma et al. 2006), but due to the general lower Si requirement of lupin compared to corn one might expect a lower Si and thus As^{III} transport capacity in lupin and thus less efflux at similar internal As^{III} concentrations unless transport capacity does not limit reaching equilibrium between cytoplasm and external solution. Our current results can be best explained by assuming equilibrium between inside and outside concentrations. However, more information is required on Si or As^{III} transporters in lupin and the sites of As^{III} release. The latter could be visualized by the application of As bioreporters as it has been recently demonstrated for corn by Kuppardt et al. (submitted).

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