Supporting Information:

A different sequence of events than previously reported leads to arsenic-induced damage in *Ceratophyllum demersum* L.

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TABLE S1: Composition of the nutrient solution optimised for growth of submerged macrophytes (SMNS, personal communication from HK, recipe of preparation and tests of performance will be published separately).

Substance	Concentration in the medium [µM]
Ca	40
Mg	150
K	119
Na	60
Cl	87
N	30
C	100
S	150
P	1 or 10
B	0.16
Co	0.01
Cr	0.02
Cu	0.01
Fe	0.4
Fe ligand: EDDHA	0.2
I	0.05
Mn	0.4
Mo	0.01
Ni	0.01
Zn	0.05
Hepes (as buffer)	500
pH (with KOH)	7.8

TABLE S2. Explanations of fluorescence kinetic parameters used in this work and references to earlier works that defined, extensively used and/or reviewed them.

Term	Explanation	References
F_0	Minimal fluorescence yield of a dark adapted sample in non-actinic measuring light (PQ-pool max. oxidised).	review: Maxwell and Johnson (1) ; used also in all other works cited in this table
F_m	Maximum fluorescence yield of a dark-adapted sample after supersaturating irradiation pulse (PQ-Pool max. reduced).	
F_m'	Maximum fluorescence yield of a light-adapted sample after supersaturating irradiation pulse (PQ-Pool max. reduced).	
F_v/F_m	$(F_m-F_0)/F_m$ = maximal dark-adapted quantum yield of photochemistry of the PSII reaction centre.	
F_p	Fluorescence yield at the P level of the induction curve after the onset of actinic light exposure (PQ-Pool partially reduced).	
Indices i1, r6, r5	These indices in the Φ_{PSII} and NPQ parameters refer to the sequence of measuring them. The timing of these measurements if as follows. i1: after 10 s of actinic light; i6: after 200 s of actinic light; r5: after 200s relaxation in the dark after the actinic light period.	Küpper *et al* (2), Andresen *et al* (3)
Light saturation	Measured by the increased amplitude of F_p relative to F_m after subtraction of F_0. $(F_p-F_0)/(F_m-F_0)$ is mostly dependent on the ratio of functional antenna molecules to functional reaction centres and electron transport chains. Under constant actinic irradiance for measuring F_p, a large antenna capturing photons and delivering them to its reaction centre will cause more of the "electron traffic jam" that leads to F_p than a small antenna.	Küpper *et al* (2), Mijovilovich *et al* (4)
Φ_{PSII}	$\Phi e = (F_m'-F_t')/F_m'$ = the light-acclimated efficiency of PS II. In the current manuscript the use of this parameter is extended to the relaxation period after the end of actinic light to analyse the return of the system to its dark-acclimated state as measured by F_v/F_m.	Genty *et al* (5), review: Maxwell and Johnson (1)
NPQ	Non-photochemical quenching, in this manuscript used as an acronym for the name of this phenomenon. In this manuscript, we measure non-photochemical quenching as $q_{CN} = (F_m - F_m')/ F_m$ = "complete non-photochemical quenching of Chl fluorescence", i.e. with normalisation to F_m.	Maxwell and Johnson (1) Küpper *et al* (2)

Literature Cited

1. Maxwell, K.; Johnson, G. N. Chlorophyll fluorescence: a practical guide. *J. Exp. Bot.* **2000**, 51**,** 659–668.
2. Küpper, H.; Parameswaran, A.; Leitenmaier, B.; Trtilek, M.; Setlik. I. Cadmium induced inhibition of photosynthesis and long-term acclimation to cadmium stress in the hyperaccumulator *Thlaspi caerulescens*. *New Phytol.* **2007** ,175, 655–674.
3. Andresen, E.; Lohscheider, J.; Setlikova, E.; Adamska, I.; Simek, M.; Küpper, H. Acclimation of *Trichodesmium erythraeum* ISM101 to high and low irradiance analysed on the physiological, biophysical and biochemical level. *New Phytol.* **2010**,185, 173–188.
4. Mijovilovich, A.; Leitenmaier, B.; Meyer-Klaucke, W.; Kroneck, P .M. H.; Götz, B.; Küpper, H. Complexation and toxicity of copper in higher plants. II. Different mechanisms for copper versus cadmium detoxification in the copper-sensitive cadmium/zinc hyperaccumulator *Thlaspi caerulescencs* (Ganges ecotype). *Plant Physiol.* **2009**,151, 715-731.
5. Genty, B.; Briantais, J.; Baker, N. R. The relationship between the quantum yield of photosynthetic electron-transport and quenching of chlorophyll fluorescence. *Biochem. Biophys. Acta.* **1989,** 990, 87-92.

Supplement 3

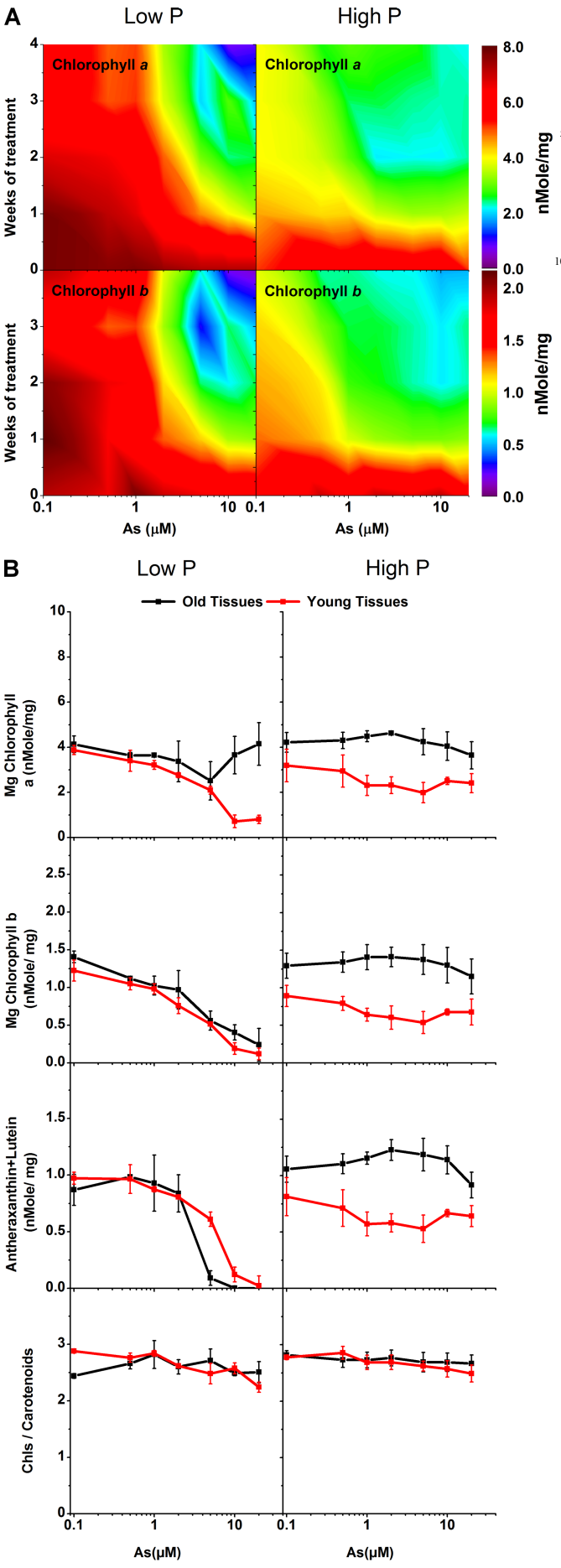
Additional Methodology

Oxygen Exchange

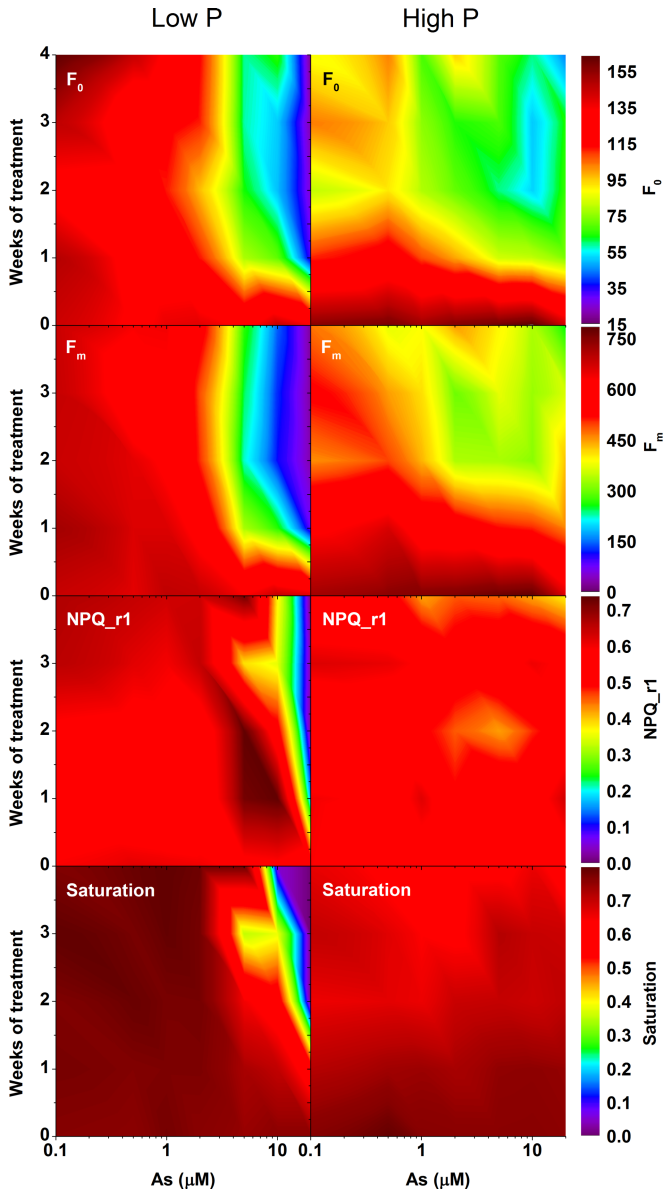
The whole plant was placed into a lab-made 200 mL measuring chamber, maintained at 25°C, and oxygen exchange was measured by a WTW CellOx 325 oxygen electrode connected to an inoLab Oxi 740 terminal (Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany). Oxygen uptake by dark respiration was measured by applying darkness for 20 min before measuring net photosynthetic oxygen release, for which the plant was exposed to increasing irradiance. At the end, respiratory oxygen uptake in darkness was measured again. Data were recorded using the OxyCorder device with the software Oxywin 2.71 (Photon Systems Instruments, Brno, Czech Republic), and further data analysis was done in Microcal Origin Professional 8.1 (Originlab, Northampton, USA).

Determination of Starch Accumulation

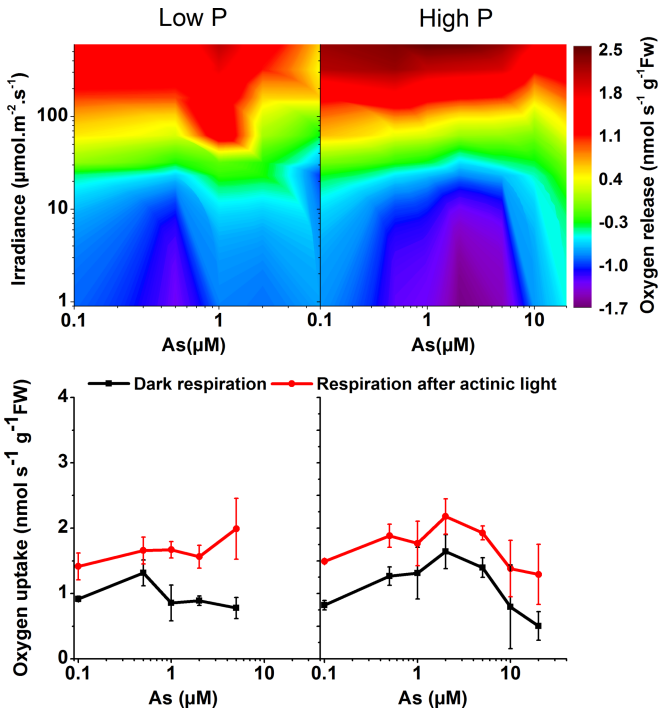
Starch in the harvested plant samples was measured after four weeks of As treatment. A total starch assay kit (improved AOAC Method 996.11 and AACC Method 76.13; Megazyme, Ireland) was used but with modifications for our 20x smaller sample amounts (5 mg of lyophilized tissue): amounts and volumes were scaled down, 1.5 ml microcentrifuge tubes were used, and centrifugation was done at 16,000× *g*. The measurement was done in 1000 µL semi-micro cuvettes using the spectrophotometer Lambda750 (Perkin-Elmer, Waltham, MA, USA).



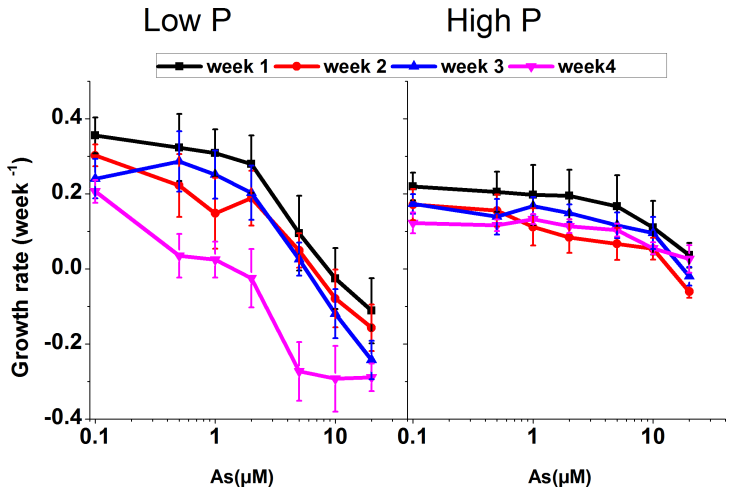
Supplementary Fig. S1. Effect of As on photosynthetic pigments of *Ceratophyllum demersum*. Plants were exposed to As under low and high P conditions. A, Effect on Chl a and Chl b content during four weeks of As exposure. Data are mean of three experiments (two-dimensional figure showing the time course on the vertical axis and the As concentration series on the horizontal axis). B, Effect on Chl a, Chl b, antheraxanthin+lutein and Chl/carotenoid ratio after four weeks of As exposure. Data are mean ±SE of three experiments.



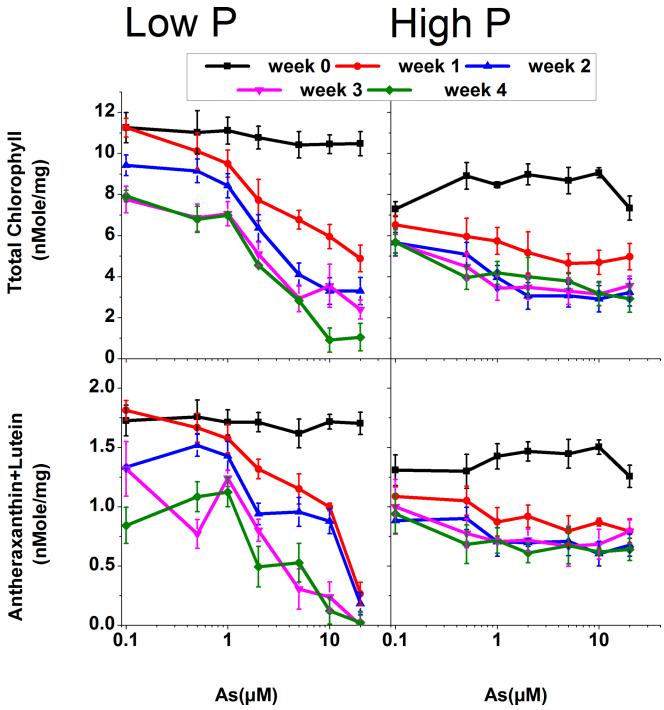
Supplementary Fig. S2. Effect of As on photosynthetic parameters (two-dimensional figure showing the time course on the vertical axis and the As concentration series on the horizontal axis). Plants were exposed to As for four weeks under low and high P conditions. Data are mean of three experiments. F_0; dark-adapted minimal fluorescence yield in absence of actinic light, F_m; Maximal dark-adapted fluorescence quantum yield in dark adapted samples subjected to a supersaturating light pulse, NPQ_r1; non-photochemical energy quenching after 10 s of dark phase, Saturation of PSII = $(F_p-F_0)/(F_m-F_0)$; semi-quantitatively displaying the ratio of functional antennae to functional electron transport chains (Küpper et al., 2007a).



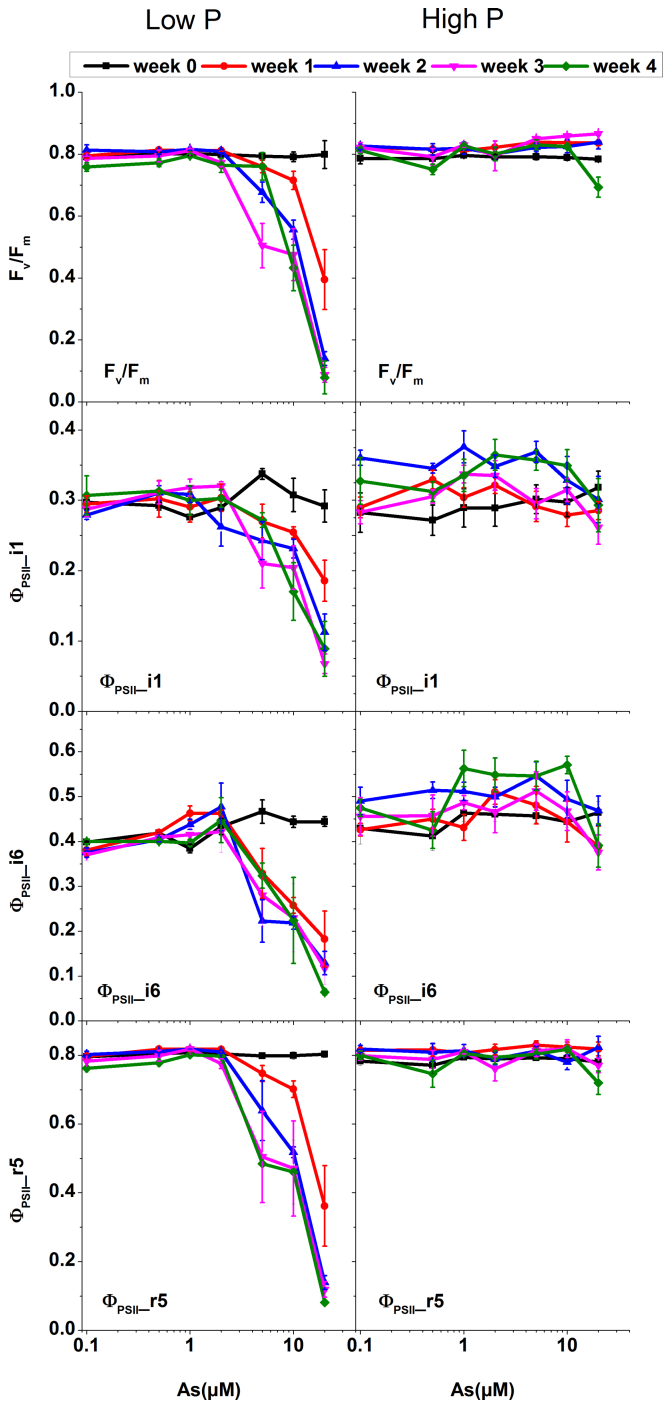
Supplementary Fig. S3. Effect of As on photosynthesis and respiration parameters. Plants were exposed to As under low and high P conditions, and measurements were done after 4 weeks. Top: Net photosynthetic oxygen release at different irradiances (two-dimensional figure showing the irradiance during measurement on the vertical axis and the As concentration series on the horizontal axis). Bottom: Oxygen uptake by dark respiration. Data are mean ±SE of three experiments.



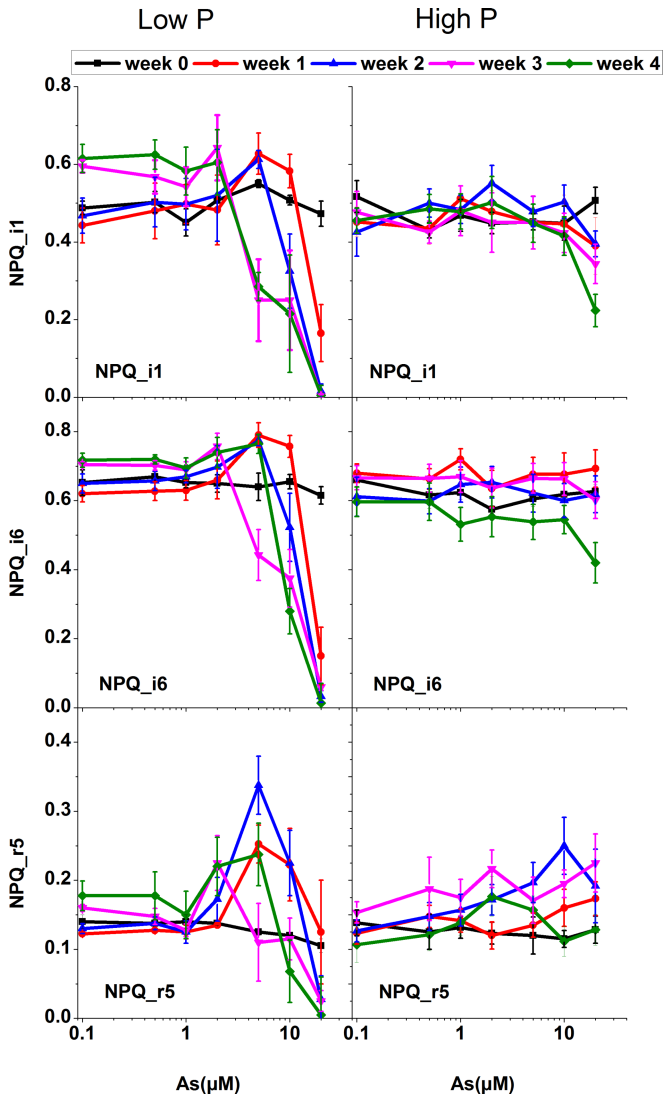
Supplementary Fig. S4. Growth response of *Ceratophyllum demersum* exposed to As under low and high P conditions. Growth rate of the plants per week calculated on the basis of fresh weight . Negative growth rates mean net loss of biomass i.e. more tissue dies off and decays from the lower part of the plant than what is growing new at the tip of the plant. The data are the mean ±SE of three experiments.



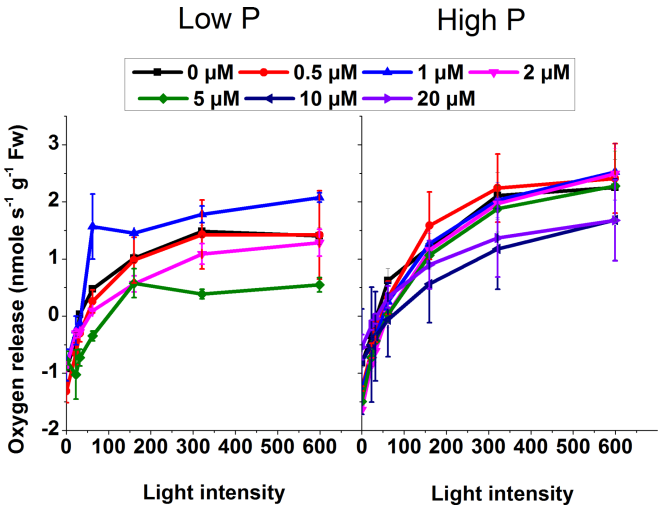
Supplementary Fig. S5. Effect of As on photosynthetic pigments under low and high P conditions. Effect on total chlorophyll and antheraxanthin+lutein during 4 weeks of As exposure. Data are mean ±SE of three experiments. Antheraxanthin and lutein could not be distinguished from each other due to almost identical UV absorption spectra. Thus, these were quantified together and termed 'antheraxanthin+lutein'.



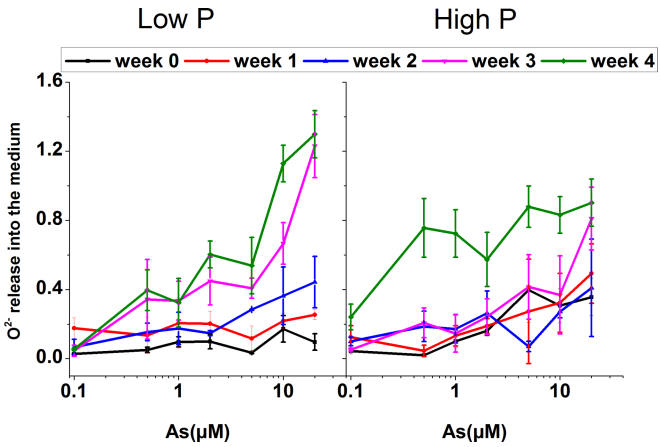
Supplementary Fig. S6. Effect of As on photosynthesis biophysics measured by *in vivo* chlorophyll fluorescence kinetic measurements. Plants were exposed to As for 4 weeks under low and high P conditions. Effect on photochemical parameters measured as $F_v/F_m = (F_m-F_0)/F_m$; maximal dark-adapted photochemical quantum yield of the photosystem II reaction centre, $\Phi_{PSII} = (F_m'-F_t')/F_m'$; Light-acclimated linear electron flow, Φ_{PSII}_i1 after 10 s of actinic light, Φ_{PSII}_i6 after 200 s of actinic light, Φ_{PSII}_r5 = Capacity of electron flow after 200 s of relaxation in the dark. Data are mean ±SE of three experiments.



Supplementary Fig. S7. Effect of As on photosynthesis biophysics measured by *in vivo* chlorophyll fluorescence kinetic measurements. Plants were exposed to As for 4 weeks under low and high P conditions. Effect on non-photochemical quenching of energy measured as NPQ = $(F_m\text{-}F_m')/F_m$, NPQ_i1; after 10 s of actinic light, NPQ_i6; after 200 s of actinic light, NPQ_r5; non-photochemical energy quenching after 200 s of dark phase. NPQ_relax = (NPQ_i6-NPQ_r5)/NPQ_i6; relaxation of non photochemical quenching within 200 s after end of actinic light. Data are mean ±SE of three experiments.



Supplementary Fig. S8. Effect of As on photosynthesis. Plants were exposed to As under low and high P conditions, and measurements were done after 4 weeks. Net photosynthetic oxygen release at different irradiances Data are mean ±SE of three experiments.



Supplementary Fig. S9. Effect of As on superoxide generation by leaves. Plants were exposed to As under low and high P conditions. Data are mean ±SE of three experiments.

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