Supplemental Figures:

Supplemental Figure 1: S-content is not affected during growth

Total cellular S content was quantitated by ICP-MS/MS during logarithmic and stationary growth phase, normalized to cell numbers measured by a hemocytometer. Cells were grown in replete Fe (20 μM, black) or excess Fe condition (200 μM, red), collected at the indicated hours post-inoculation. Averages are shown with error bars indicating standard deviation of three independent cultures.
Supplemental Figure 2: Transcript changes in stationary cells do not show increased import capacities for trace metals
Transcript abundance changes (log₂ FC) of transporters involved in Fe, Cu, Zn, P, Mn and Ca transport in between four-day stationary and logarithmically growing cultures (2×10⁶ cells/ml, dark red) (Lv et al., 2013), late log (8×10⁶ cells/ml) and logarithmically growing alga (2×10⁶ cells/ml, light red) (Kropat et al., 2015), Fe-limited and Fe-replete cells (blue) (Urzica et al., 2012), Cu-deficient and Cu-replete conditions (green) (Castruita et al., 2011), Zn-limited and Zn-replete settings (orange) (Malasarn et al., 2013) and in P-limited and P-replete growth medium (purple) (Schmollinger et al., 2014). Transcripts changing more than 4 fold are highlighted in bold colors with black outlines.
**Supplemental Figure 3: Transient S accumulation in alkaline conditions correlates with biomass**

(A) Total cellular S content of cells inoculated into TAP media containing either 20 (black) or 200 (red) μM Fe, and starting at either pH 7.0 (filled circles) or pH 8.5 (open circles), collected at the indicated hours post-inoculation. S content was measured by ICP-MS/MS and cells in each culture were counted using a hemocytometer. (B) Carbon content, as a proxy of biomass, was measured as non-purgeable organic carbon content in cells by NPOC analysis. Averages between three independent cultures are shown with error bars indicating standard deviation. (C) Correlation of the S content and biomass data in (A) and (B) using all individual data points. R² value was determined via linear regression fitting.

**Supplemental Figure 4: Fe precipitates in cell-free TAP medium at pH 9.5 and higher**

TAP medium containing 200 μM Fe was titrated from pH 7.0 to pH 8.5, 9.0, 9.5, 10.0, 10.5, and 11.0 with KOH (A), or reversely from the higher pHs to pH 7.0 with acetic acid (B). The samples were left at room temperature for 30 mins at each titration point. An aliquot of each sample was filtered through a 0.2 μm filter to remove aggregates. Fe content in both the filtered (white) and unfiltered (grey) samples was quantified by ICP-MS/MS. Asterisks indicate significant differences (t-test, p < 0.05) between the filtered and unfiltered samples. Averages are shown with error bars indicating standard deviation of three independent samples.
Supplemental Figure 5: Fe in alkaline conditions accumulates within cells in foci

Fe and chlorophyll distribution in cells grown in pH 8.5 TAP medium with 20 or 200 μM Fe. From left to right, brightfield image of cell (BF), chlorophyll autofluorescence (Chl, green), IP1 to determine Fe(II) distribution (IP1, violet), overlay of IP1 and chlorophyll (Chl+IP1), overlay of brightfield, chlorophyll and IP1 (BF+Chl+IP1). The confocal images were collected on a Zeiss LSM 880 microscope. Scale bar = 5 μm. Rows with grey background were used in the main paper.
Supplemental Figure 6: LysoSensor identifies acidic compartments in alkaline cells

LysoSensor DND-189 detection of acidic pH compartments (purple) and overlay with chlorophyll autofluorescence in cells grown in pH 7.0 or 8.5 TAP medium with 200 μM Fe. The confocal images were collected on a Zeiss LSCM 880 microscope using Airyscan in channel mode. Scale bar = 5 μm.
Supplemental Figure 7: XFM shows Fe accumulate with Ca and P in alkaline conditions

From left to right, cell image, S, P, Ca, Fe distribution and overlay (P = blue; Ca = red; Fe = green) in cells grown in pH 8.5 or pH 7 TAP medium with 200 μM Fe, determined by X-ray fluorescence microscopy. The elemental distributions are depicted between the minimal (black) and maximal (white) elemental concentrations in μg/cm². Scale bar = 2 μm.
Supplemental Figure 8: NanoSIMS indicates Fe accumulate with Ca and P in alkaline conditions

Correlated EM (top left) and C, Fe, P and Ca distribution in cell sections of cultures grown in pH 8.5 (A) and pH 7.0 (B) TAP medium with 200 μM Fe, as well as the overlay (P = blue; Ca = red; Fe = green). The red box identifies the cell analyzed with higher resolution (Figure 4 C). B). White lines outline the cell regions used for the correlative quantification (Figure 4 D). C). Sections of fixed cells were imaged in positive secondary ion mode. Scale bars indicate 5 μm.
Supplemental Figure 9: Subtle reduction of S, trace metal, Ca and P at elevated temperatures

Sulfur (A) and carbon content (B) of cultures grown at 18, 21, 24, 27, and 30°C, sampled at mid-log (2-4 x 10^6 cells/ml) and stationary (five days post-inoculation, ~1 x 10^7 cells/ml) cell densities in Fe-replete (20 μM) and Fe-excess (200 μM) conditions. S content was measured by ICP-MS/MS, C content was measured as non-purgeable organic carbon by NPOC analysis. (C) Correlation of the total cellular S and C content. Cell-associated Fe (D), Cu (E), Zn (F), Mn (G), Ca (H), and P (I) abundance, as measured by ICP-MS/MS, collected during mid-log growth (2-4 x 10^6 cells/ml). Averages are shown with error bars indicating standard deviation of three independent cultures. No significant difference (t-test, p < 0.013, multiple Bonferroni-corrected) to cells grown at 24°C was noted.
Supplemental Figure 10: Vessel size does not affect growth or trace element quota

Doubling time (A) and number of generations between inoculation and stationary (B) of cells grown in different-sized vessels between 125- and 2800-ml capacity under replete or excess Fe condition (20 vs. 200 μM Fe). Cell-associated Fe (C), Cu (D), Zn (E), Mn (F), Ca (G) and P (H) content under replete (20 μM) and excess (200 μM) Fe conditions, as measured by ICP-MS/MS during mid-log growth (2-4 x 10⁶ cells/ml). Averages are shown with error bars indicating standard deviation of three independent cultures. Asterisk indicates significant differences (t-test, p < 0.013, multiple Bonferroni-corrected) to cells grown at 250-ml Erlenmeyer with 200 μM Fe. No significant difference was noted within the 20 μM Fe dataset.
Supplemental Figure 111: PCA

Principal component analyses of the individual experiments presented in the paper.
The light spectrum of the incubator used in all experiments collected during cell cultivation was measured using a SpectraPen SP 110. The probe was placed in the center of the incubator where cultures were positioned.
Supplemental References


