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Summary

• Minimal information exists on whole-plant dynamics of mineral flow through *Arabidopsis thaliana* or on the source tissues responsible for mineral export to developing seeds. Understanding these phenomena in a model plant could help in the development of nutritionally enhanced crop cultivars.

• A whole-plant partitioning study, using sequential harvests, was conducted to characterize growth and mineral concentrations and contents of rosettes, cauline leaves, stems, immature fruit, mature fruit hulls, and seeds of three WT lines (Col-0, L*er*, and Cvi) and one mutant line (Col-0::*ysl1ysl3*).

• Shoot mineral content increased throughout the life cycle for all minerals, although tissue-specific mineral partitioning differed between genotypes. In particular, iron (Fe), zinc (Zn), and copper (Cu) were aberrantly distributed in *ysl1ysl3*. Remobilization was observed for several minerals from various tissues, including cauline leaves and silique hulls, but the amounts were generally far below the total mineral accretion observed in seeds.

• When YSL1 and YSL3 are nonfunctional, Cu, Fe, and Zn are not effectively remobilized from, or do not effectively pass through, leaf and maternal fruit tissues. With respect to seed mineral accretion in *Arabidopsis*, continued uptake and translocation of minerals to source tissues during seed fill are as important, if not more important, than remobilization of previously stored minerals.

Key words: biofortification, copper (Cu), iron (Fe), mineral partitioning, seed mineral content, YSL family, zinc (Zn).

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Introduction

On a worldwide basis, plants are the primary source of nutrients for human nutrition. Staple seed crops such as rice, maize, wheat, and bean supply the majority of daily dietary nutrients for billions of people. However, these foods have a low density of mineral nutrients, and for those whose diets are high in staple foods, micronutrient malnutrition is widespread (Grusak & DellaPenna, 1999). Iron and zinc deficiency each affects an estimated three billion people. Traditional interventions, such as fortification of foods and use of supplements, can alleviate malnutrition, but for a variety of reasons are difficult to implement successfully and permanently. In recent years, efforts have been made to use plant breeding and/or transgenic approaches to increase the mineral concentration of edible portions of staple crops (Poletti *et al.*, 2004; White & Broadley, 2005). This strategy, termed biofortification, has the potential to be more sustainable than traditional interventions, because a continued supply of supplements or fortified food would not be required. In addition, affected populations would not be required to change preferred dietary habits.

One of the barriers to biofortification of seeds is the lack of knowledge of how minerals are loaded into seeds, resulting in uncertainty about the best genes or pathways to target for modification. In recent years, much has been learned about the processes for uptake from the rhizosphere for several minerals, such as potassium (K) (Very & Sentenac, 2003), phosphorus (P) (Leggewie *et al.*, 1997; Raghothama & Karthikeyan, 2005), sulfur (S) (Smith *et al.*, 1997; Takahashi *et al.*, 2000), iron (Fe) (Curie & Briat, 2003), and zinc (Zn) (Ghandilyan *et al.*, 2006). However, little is known about the downstream steps that move minerals into or out of vascular tissues, translocation to vegetative tissues, or loading of minerals into seeds (Colangelo & Guerinot, 2006; Kramer *et al.*, 2007), although recent studies have indicated that FRD3 is important for movement of Fe from roots to shoots (Durrett *et al.*, 2007), as are HMA2 and HMA4 for root-to-shoot Zn translocation (Hussain *et al.*, 2004; Verret *et al.*, 2004). Some transgenic biofortification strategies have been attempted in rice. Initial seed biofortification efforts for Fe and Zn in rice have focused on increasing the iron storage protein ferritin (Goto *et al.*, 1999; Vasconcelos *et al.*, 2003) or root ferric reductase activity (Vasconcelos *et al.*, 2004). Grain Zn and Fe concentrations were increased in barley (*Hordeum vulgare*) expressing the Zn transporter *ZIP1* from *Arabidopsis thaliana* (Ramesh *et al.*, 2004), and were decreased in wheat (*Triticum aestivum*) expressing RNAi constructs that lowered *NAM* family gene expression (Uauy *et al.*, 2006). Another strategy was to constitutively express the root ferric reductase *FRO2* from *A. thaliana* in soybean (Vasconcelos *et al.*, 2006). In certain hydroponic growth conditions, the transgenic plants showed a threefold increase in leaf Fe concentration, but only a 10% increase in seed Fe. These results suggest that additional transport processes and regulatory mechanisms must be manipulated to move more Fe from leaves into seeds. Similar observations were seen with the *brz* mutant of *Pisum sativum*, which overaccumulates Fe in leaves but has normal seed Fe concentrations (Grusak, 1994), again suggesting that in addition to increasing net mineral uptake, the processes that control movement of minerals from vegetative tissues must also be targeted to accomplish large increases in seed mineral concentration. Thus, the most successful breeding or transgenic approaches will likely target multiple genes simultaneously.

The source tissues and processes responsible for the remobilization and supply of nitrogen to seeds has received considerable attention (Ta & Weiland, 1992; Schjoerring *et al.*, 1995; Hortensteiner & Feller, 2002; Schiltz *et al.*, 2005), and these studies have provided insight into source-sink partitioning. Minerals other than nitrogen may be remobilized from vegetative sources (Hocking & Pate, 1977; Drossopoulos *et al.*, 1996; Himelblau & Amasino, 2001), although a major portion of minerals in seeds are likely supplied through continuous uptake and translocation to developing seeds (Pate & Hocking, 1978). A few studies have addressed the sources of minerals other than N in a quantitative manner (Hocking & Pate, 1977; Hocking, 1994; Miller *et al.*, 1994; Garnett & Graham, 2005), but none of these studies compared different germplasm. In this paper, we use diverse germplasm to assess growth dynamics of above-ground organs (rosettes, cauline leaves, stems, immature fuits, mature silique hulls, and mature seeds) over the life cycle of the model plant *Arabidopsis thaliana.* We also describe the concentrations and contents of nine mineral nutrients (Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn) in these tissues over time. To address the question of whether there is genetic diversity for mineral partitioning and movement of minerals to seeds, we compare these parameters in three wild-type lines, Columbia (Col-0), Landberg *erecta* (L*er*-1), and Cape Verde Islands (Cvi). These ecotypes comprise the parents of two commonly studied RIL (recombinant inbred line) populations, Col X L*er* (Lister & Dean, 1993) and Cvi X L*er* (Alonso-Blanco *et al.*, 1998). Additionally, we studied the dynamics of mineral partitioning in the *ysl1ysl3* mutant, which is known to have low seed Cu, Fe, and Zn concentrations (Waters *et al.*, 2006).

Materials and Methods

Plant material and growth conditions

Seeds of *Arabidopsis thaliana* (L.) Heynh. were imbibed in 0.1% agarose for 3–5 d at 4°C and planted onto commercial potting mix (MetroMix 360) at a density of three to six plants per square 3.5 inch pot, and later thinned to a maximum density of three plants per pot. Accessions used were Columbia (Col-0), Landsberg *erecta* (L*er*-1), and Cape Verde Islands (Cvi), and a mutant line, *ysl1ysl3*. This mutant contains two T-DNA insertions, one in *YSL1* (At4g24120) and one in *YSL3* (At5g53550), in the Col-0 background (Alonso *et al.*, 2003). Plants were grown in an air-conditioned glasshouse under shadecloth, with supplemental fluorescent lighting of a 16 h photoperiod. These conditions provided light at approx. 100 μmol m^{-2} s^{-1} with brief periods of up to 300 μmol m^{-2} s^{-1}. Plants were watered as needed (usually twice a wk) by subirrigation of a nutrient solution of the following composition: 1.2 mM KNO_3, 0.8 mM $Ca(NO_3)_2$, 0.8 mM NH_4PO_4, 0.3 mM KH_2PO_4, 0.2 mM $MgSO_4$, 25 μM $CaCl_2$, 25 μM H_3BO_3, 2 μM $MnSO_4$, 2 μM $ZnSO_4$, 0.5 μM $CuSO_4$, 0.5 μM H_2MoO_4, 0.1 μM $NiSO_4$, 10 μM Fe-EDDHA as Sprint 138 (Becker-Underwood, Ames, IA, USA).

Tissue analysis

Plant parts were separated into rosettes, cauline leaves, stems (including flowers), immature fruits (of all developmental stages except mature fruits), mature silique hulls (valves), and mature seeds. Silique hulls and seeds were collected by fitting plants with seed collectors as described (http://www.arabidopsis.org/comguide/chap_1_plants/4_arab_seed_harvester.html). Plant tissues were dried in a 60°C oven and dry weight was determined. Tissues were digested to dryness at 220°C with

nitric : perchloric acid (4 : 1) and residues were dissolved in 15 ml 2% nitric acid. All acids were trace metal grade (Fisher Scientific, Pittsburgh, PA, USA) and water was filtered through a MilliQ system to at least 18 MΩ resistivity. Concentrations of Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn were determined by ICP-OES (CIROS ICP Model FCE12; Spectro, Kleve, Germany). Standards digested by this procedure confirmed that S was not volatilized during the digestion process.

Mineral content was determined by multiplying each sample's concentration by dry weight. Rosettes were not collected on the final or last two time points because of degradation of leaf tissue (63 d for L*er*-1, 70 d for Col-0, 84 and 90 d for Cvi, and 69 and 77 d for *ysl1ysl3*). To estimate total DW and mineral content at these time points (for partition quotient calculation, see later discussion), the missing rosette DWs were estimated to be equal to the average DW at time points following cessation of rosette growth, and rosette content was estimated to be equal to the average mineral content at time points following cessation of rosette growth. In a second experiment, rosette leaves were collected at all time points. For estimation of net mineral content loss from leaves, a linear curve was fitted from the point with the highest mineral content to the end of the experiment in order to reduce the influence of outlying data points. The equation of this line was used to estimate maximum and minimum mineral content. Net mineral content change was estimated by subtracting the final mineral content from the maximal mineral content. For minerals that had a decrease in content, the net loss was compared with final total seed mineral content to determine the contribution of remobilized minerals to seed mineral content.

For dissections of immature and mature siliques, full-length but still green (immature) fruits were collected, oven-dried, and dissected into seeds and remaining maternal tissues. Mature siliques that had not shattered were collected and opened, and mature silique hulls were separated from mature seeds. The mineral concentrations and contents (on a per-fruit basis) of the immature and mature seeds and silique hulls were determined by ICP-OES, and whole-fruit contents were calculated from these data.

Partition quotient calculation

To evaluate the partitioning of Cu and Zn within a plant during its life cycle, changes in each tissue's content were normalized to changes in each tissue's weight, relative to the whole plant. The DW of each organ was calculated as a percentage of total plant weight at each time point, and mineral content of each organ was calculated as a percentage of total plant mineral content at each time point. Using these values, the normalized partitioning of that mineral within the plant was calculated by dividing each organ's percentage mineral content by its percentage DW, and multiplying by 100, which we refer to as the partition quotient (PQ).

Results

Growth dynamics

Whole-plant and organ-specific growth dynamics throughout the life cycle for Col-0, L*er*-1, Cvi, and the mutant line *ysl1ysl3* are presented in Fig. 1. Above-ground plant size was similar for Col-0, L*er*-1, and *ysl1ysl3*, whereas Cvi was much larger and continued to grow through the final harvest of this study (Fig. 1a). Plant DW was separated into its component organs in Fig. 1(b)–(e). In all lines, new cauline leaves were produced each week during the period of cauline leaf growth, even as older leaves senesced and died. Also, new inflorescence bolts continued to emerge during the later time points, and new fruits were continuously produced, although not as prolifically as at earlier time points. Thus, although the experiment was ended when most fruits on the primary inflorescence stems were mature, a substantial amount of immature fruit tissue was still present in Col-0 and Cvi. At the later time points, stems were the largest tissue, with immature fruits as the second largest tissue during mid-to-late time course, except in Cvi where immature fruit mass barely exceeded rosette mass. L*er*-1 immature fruit mass was similar to stem mass at time points 42 and 49 d, and then nearly all siliques matured in the following 2 wk. Seed was the second largest proportion of total shoot weight in all lines at the final time point. Rosettes reached maximal DW at 39, 35, 70, and 42 d for Col-0, L*er*-1, Cvi, and *ysl1ysl3*, respectively. Cauline leaf DW reached near maximum DW approximately mid-experiment, but slightly increased throughout the remainder of the time course. In all lines except L*er*-1, cauline leaf was the tissue with the lowest mass at the end of the time course.

Mineral concentration dynamics

Mineral concentrations (μg g^{-1}) of certain organs changed substantially during the course of the life cycle (Supplementary material, Tables S1–S6). Ca increased in concentration in both rosettes (Table S1) and cauline leaves (Table S2) over time in both Col-0 and L*er*-1, whereas Ca in leaves of Cvi and *ysl1ysl3* increased during the early growth period and then maintained steady concentrations, a pattern observed for Mg in both rosette and cauline leaves for Col-0, L*er*-1, and Cvi. Leaf Mg concentrations were similar at all time points for *ysl1ysl3*. Leaf Fe and Mn concentrations in all genotypes fluctuated throughout the time course, as did leaf P concentration in Col-0 and L*er*-1. Cvi rosette P concentration fluctuated, while cauline P concentration reached a maximum at 63d and thereafter dropped greatly. Leaf P concentration of *ysl1ysl3* peaked at 42 d and declined for the remainder of the experiment. All genotypes had similar patterns for rosette and cauline leaf K concentration, which was high early in the experiment, and then decreased substantially and maintained lower but similar concentrations

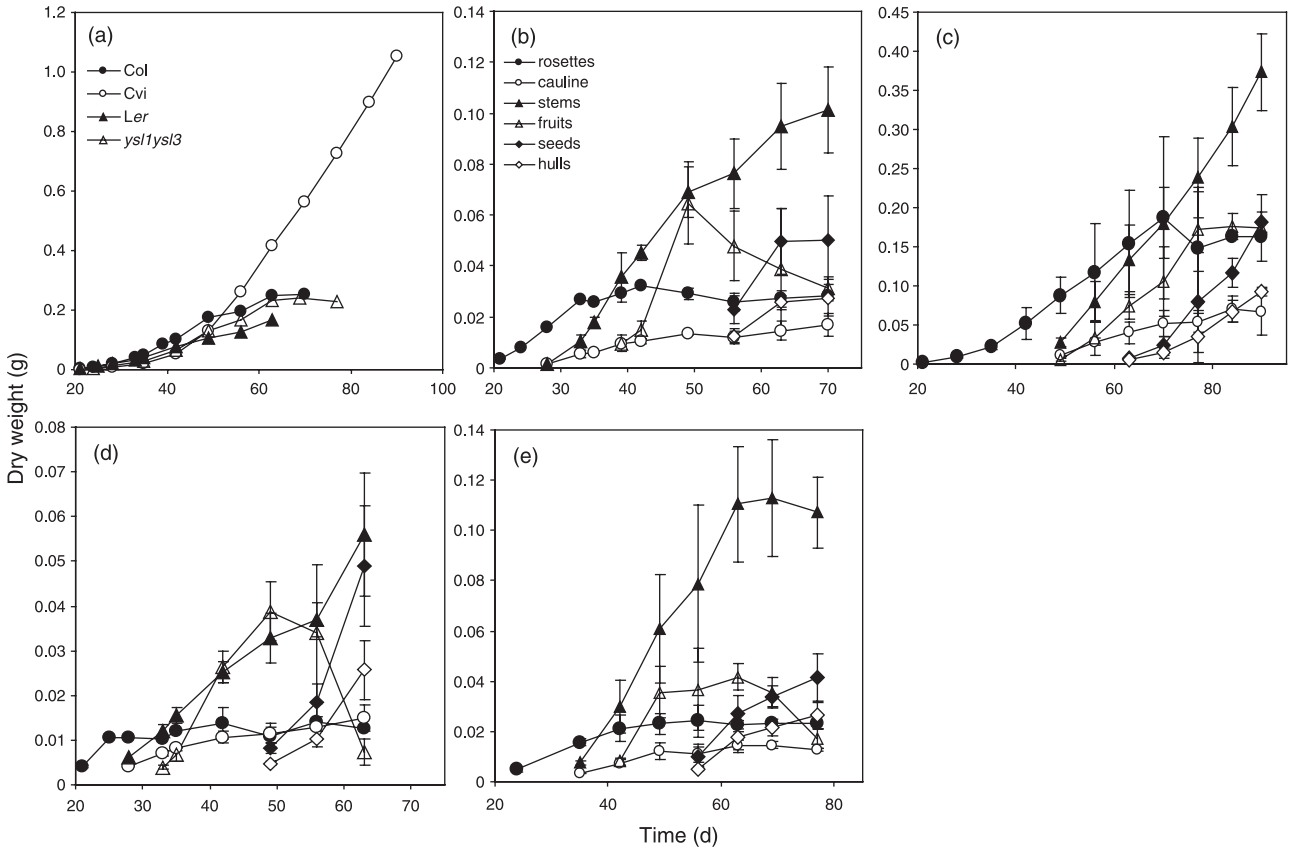

Fig. 1 Dry weights of shoot tissue over time. (a) Total shoot dry weight (sum of averages of all shoot tissues); (b–e) dry weights of shoot tissues of Arabidopsis lines. Tissues collected are rosettes (intact rosettes), cauline (all cauline leaves), stems (inflorescence stems minus fruits and cauline leaves, includes flowers), fruits (all immature fruits), seeds (all mature seeds, including fallen seeds and those that fell by gentle agitation), and hulls (valves from mature fruits, including fallen hulls and those that fell by gentle agitation). (b) Col-0 tissue dry weights; (c) Cvi tissue dry weights; (d) L*er*-1 tissue dry weights; (e) *ysl1ysl3* tissue dry weights. Symbols are mean DW ± SD. Some error bars do not extend beyond symbols.

late in the time course. The exception to this was Cvi cauline leaves, which had lower K concentration at early time points. Col-0, Cvi and *ysl1ysl3* rosettes exhibited increasing S concentration in early time points, which then decreased in later time points, whereas L*er*-1 rosette S concentration did not decrease. A similar pattern was observed for Cvi and *ysl1ysl3* cauline leaves, while cauline leaves of Col-0 and L*er*-1 had decreasing S concentration throughout the experiment. Cu concentration in leaves of all three wild-types decreased throughout the experiment, but Cu concentration in *ysl1ysl3* leaves was substantially higher and remained high with little or no decrease. In general, rosettes of all four genotypes exhibited Zn concentrations that peaked during early growth and then declined; this was also true of cauline leaves, although the extent of decrease was greater. Values of Cu and Zn concentration at comparable time points were usually higher in *ysl1ysl3* than in Col-0. Copper concentrations at comparable time points were usually two- to threefold higher in *ysl1ysl3* leaf tissues. Zinc rosette and cauline leaf concentrations were approx. 30% higher in *ysl1ysl3* than in Col-0 at 42, 49, and 56d. Iron concentrations at comparable time points were generally 25% lower in *ysl1ysl3* rosettes than in Col-0 rosettes and approx. 40% lower in *ysl1ysl3* cauline leaves.

Stem mineral concentrations of Ca, Fe, K, Mg, Mn, and Zn were relatively constant during the growth of the plants (Table S3), except for Zn concentration of L*er*-1 stems, which decreased during the life cycle. For the averaged values of all time points, Fe and Zn were 38 and 31% lower, respectively, in *ysl1ysl3* stems than in the parental line Col-0. As seen in leaves, Cu concentration decreased over time in stems in all three wild-type lines, but not in *ysl1ysl3*, which, conversely to leaf concentrations, was 52% lower in *ysl1ysl3* than in Col-0. P and S concentrations decreased in stems of Col-0, Cvi, and *ysl1ysl3*.

In general, the dynamics of mineral concentrations of immature fruits were similar to those of leaves; that is, higher at early time points and lower at later time points (Table S4). It should be pointed out that during early time points, younger fruits made up proportionally more of the immature fruit pool, and at later time points, fruits in the later stages of development were of greater abundance, especially by weight. There were no clear patterns of changing mineral concentrations

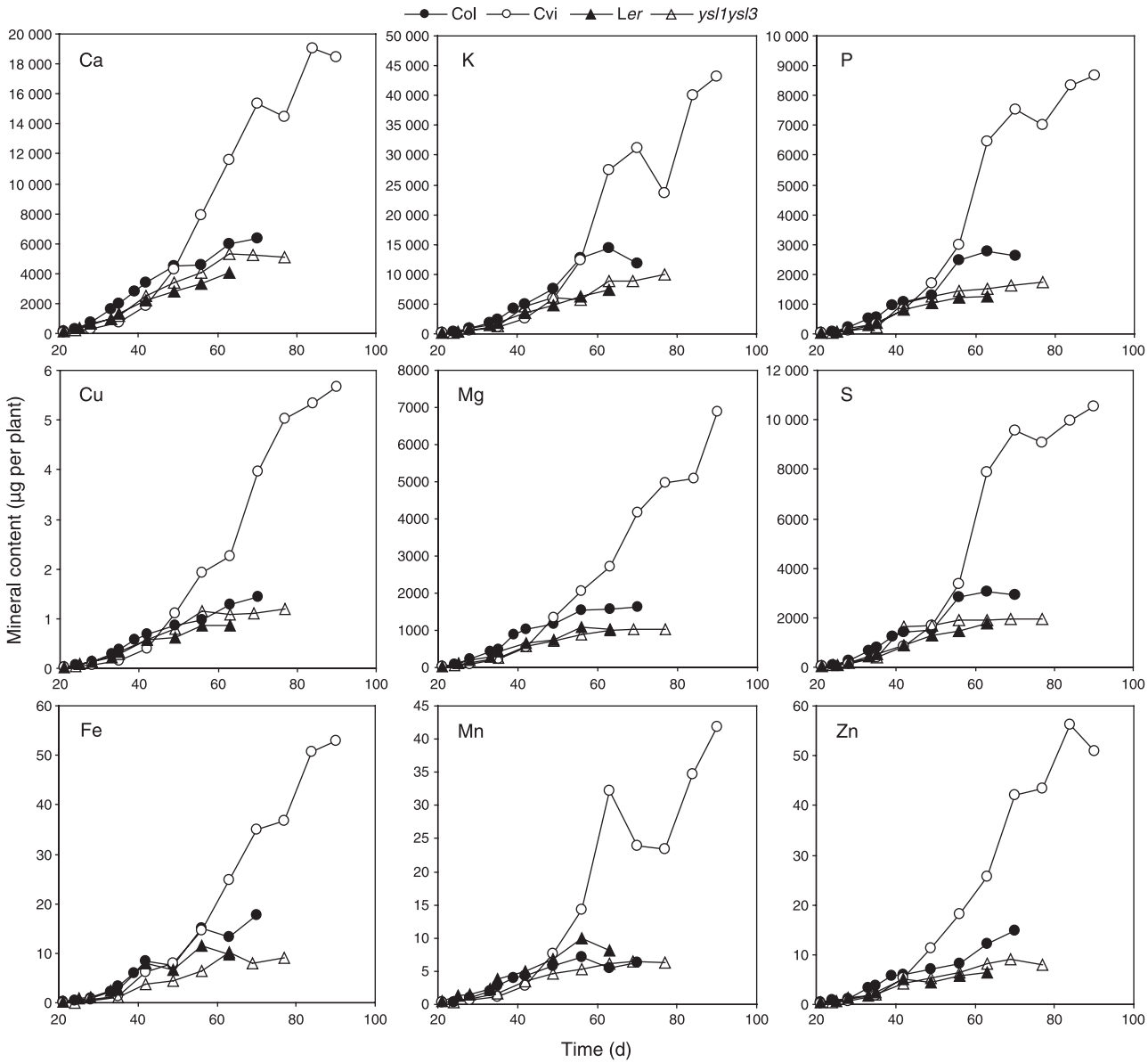

Fig. 2 Total mineral contents of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn) in Arabidopsis shoots over time.

in mature hulls (Table S5) or seeds (Table S6), although at all time points, S concentration in Cvi hulls was much higher than in the other lines (on average, sixfold higher than L*er*-1, 4.6-fold higher than *ysl1ysl3*, and 2.5-fold higher than Col-0).

At the final time point, Cu, P, S, and Zn seed mineral concentrations (Table S6) were usually higher than concentrations of other tissues. By contrast, seed Ca, Mg, and Mn concentrations were lower than those of any other tissue. Col-0 had highest seed mineral concentrations for K, Mg, P, S, and Fe. Cvi had the highest Cu concentration at 8.2 µg g^{-1} DW. There were differences between several seed mineral concentrations in *ysl1ysl3* and the wild types, most notably Cu, Fe, and Zn, which were 82, 63, and 45% lower, respectively, than the parental Col-0 wild type.

Mineral content dynamics

One objective of this study was to characterize the accumulation of minerals within the plant, and to compare partitioning between different lines. As mentioned previously, some mineral concentrations (in µg g^{-1}) changed over the course of this experiment, as did weight (in g) as the plants grew. Thus, mineral content (concentration multiplied by DW, in µg per organ) was also dynamic. Total mineral content is presented in Fig. 2. Mineral content was closely associated with plant DW

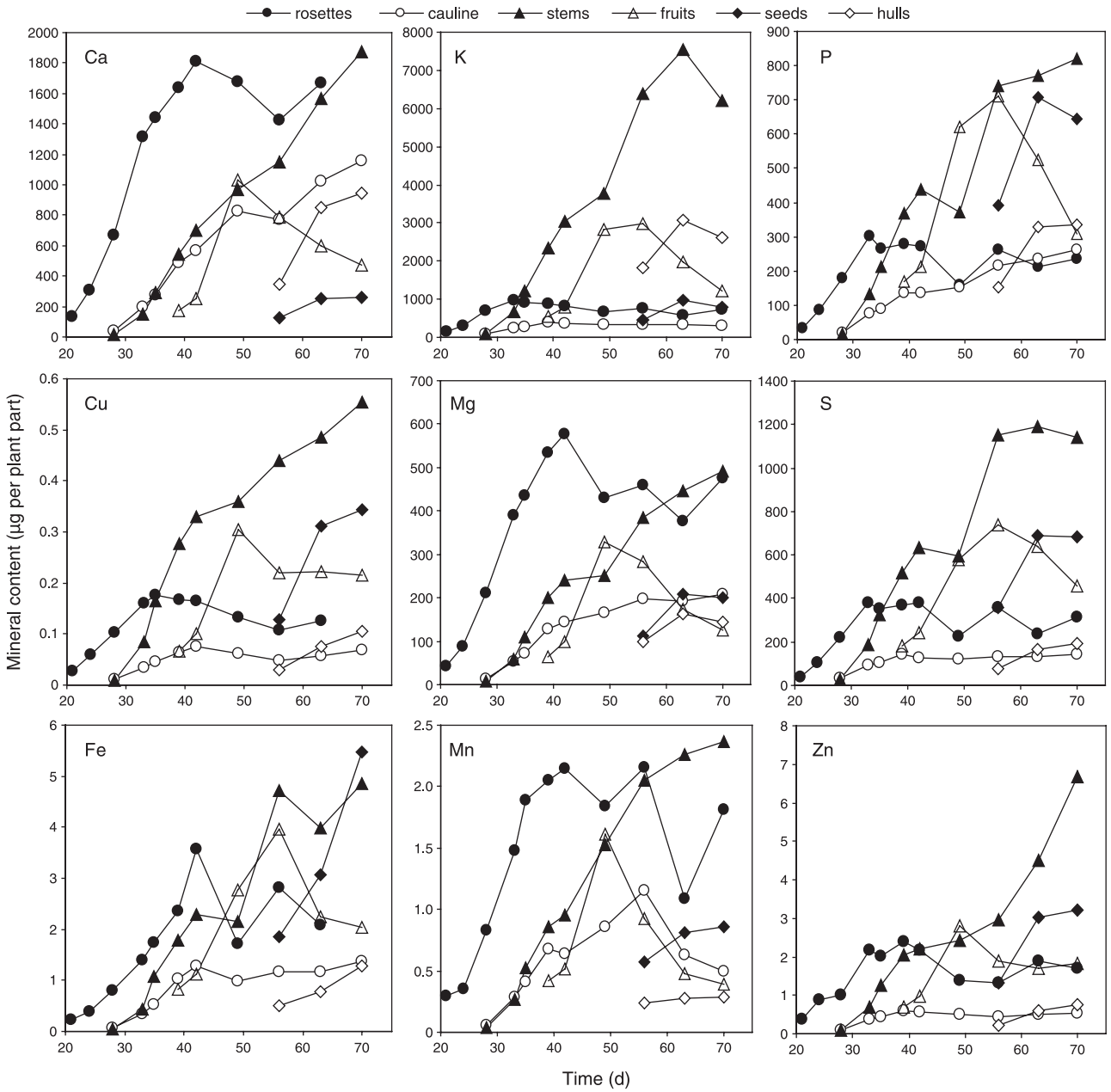

Fig. 3 Mineral contents of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn) in Arabidopsis Col-0 shoot tissues over time. Rosettes, whole rosettes; cauline, all cauline leaves; stems, inflorescence stems minus fruits and cauline leaves, including flowers; fruits, all immature fruits; seeds, mature seeds; hulls, mature silique hulls.

and accumulated throughout the experiment. For the most part, mineral content was higher in larger than in smaller plants, with the exception of Fe, where the smaller L*er*-1 accumulated more Fe than *ysl1ysl3*, and Mn, where L*er*-1 accumulated more Mn than both Col-0 and *ysl1ysl3*. Mineral contents in the different organs are presented in Figs 3–6. Contents of Ca and Mn generally increased in rosettes, stems, and cauline leaves over the time course. Rosette Mg content decreased in Col-0, Cvi, and *ysl1ysl3*.

To gauge the potential of vegetative tissues as a source of stored minerals to be remobilized to seeds, we estimated the net loss of mineral content of Cu, Fe, K, P, S, and Zn from cauline leaves by subtracting final estimated content from maximal estimated content (see Materials and Methods section for details). Net mineral content loss from rosette leaves was not calculated because of missing final data points. However, a second time course was conducted in which Col-0 and L*er*-1 cauline leaves and rosettes were collected at all time points. The

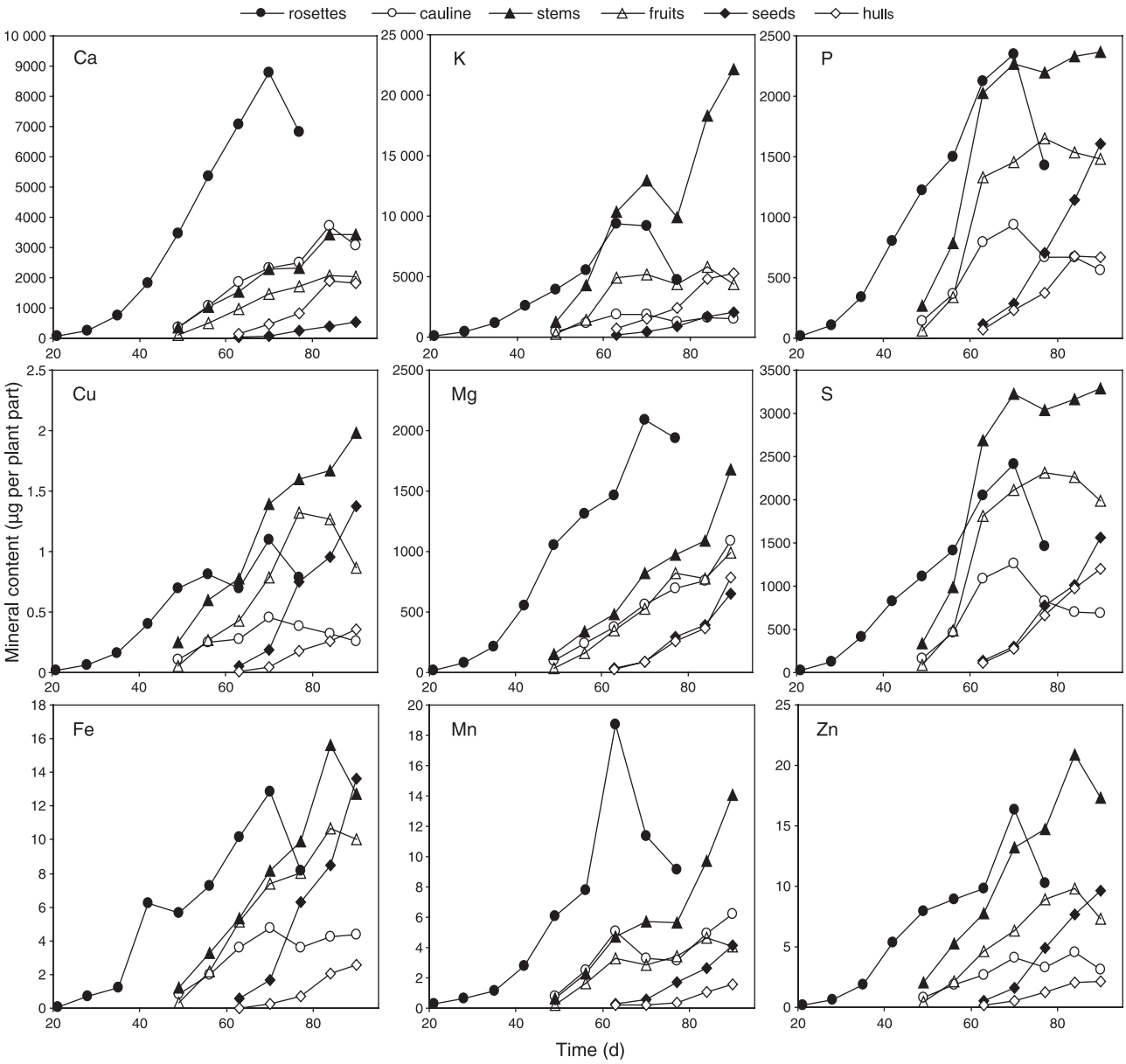

Fig. 4 Mineral contents of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn) in Arabidopsis Cvi shoot tissues over time. Rosettes, whole rosettes; cauline, all cauline leaves; stems, inflorescence stems minus fruits and cauline leaves, including flowers; fruits, all immature fruits; seeds, mature seeds; hulls, mature silique hulls.

mineral contents of these tissues (normalized to maximum content) are presented in Fig. S1. A net loss of mineral content of Cu, K, P, S, and Zn could be discerned in both rosettes and cauline leaves of both lines, and Fe content decreased in cauline leaves of both lines. The net loss of mineral content as a percentage of estimated maximum mineral content is presented in Fig. 7(a) and (c), and the maximum possible contribution to seed mineral content, assuming that the total net loss of each mineral was translocated to seeds before the final collection point, is presented in Fig. 7(b) and (d). Despite net losses of up to 70% of total leaf content for some minerals, this amount could have contributed at most only 40% of total seed mineral content for Cu, Fe, K, P, S, and Zn, and potentially contributed less than 10% in many cases.

Although some mineral concentrations decreased in stems, no loss of mineral content was observed in stems, with the exception of S in *ysl1ysl3* stems (Fig. 6). In Col-0 (Fig. 3) and Cvi (Fig. 4), S content did not increase over the last three to four time points, although stem DW continued to increase (Fig. 1), indicating that stems may be a source of remobilized S. Similarly, Cvi stem P was constant over the last four time points. Although content of several minerals in *ysl1ysl3* stems

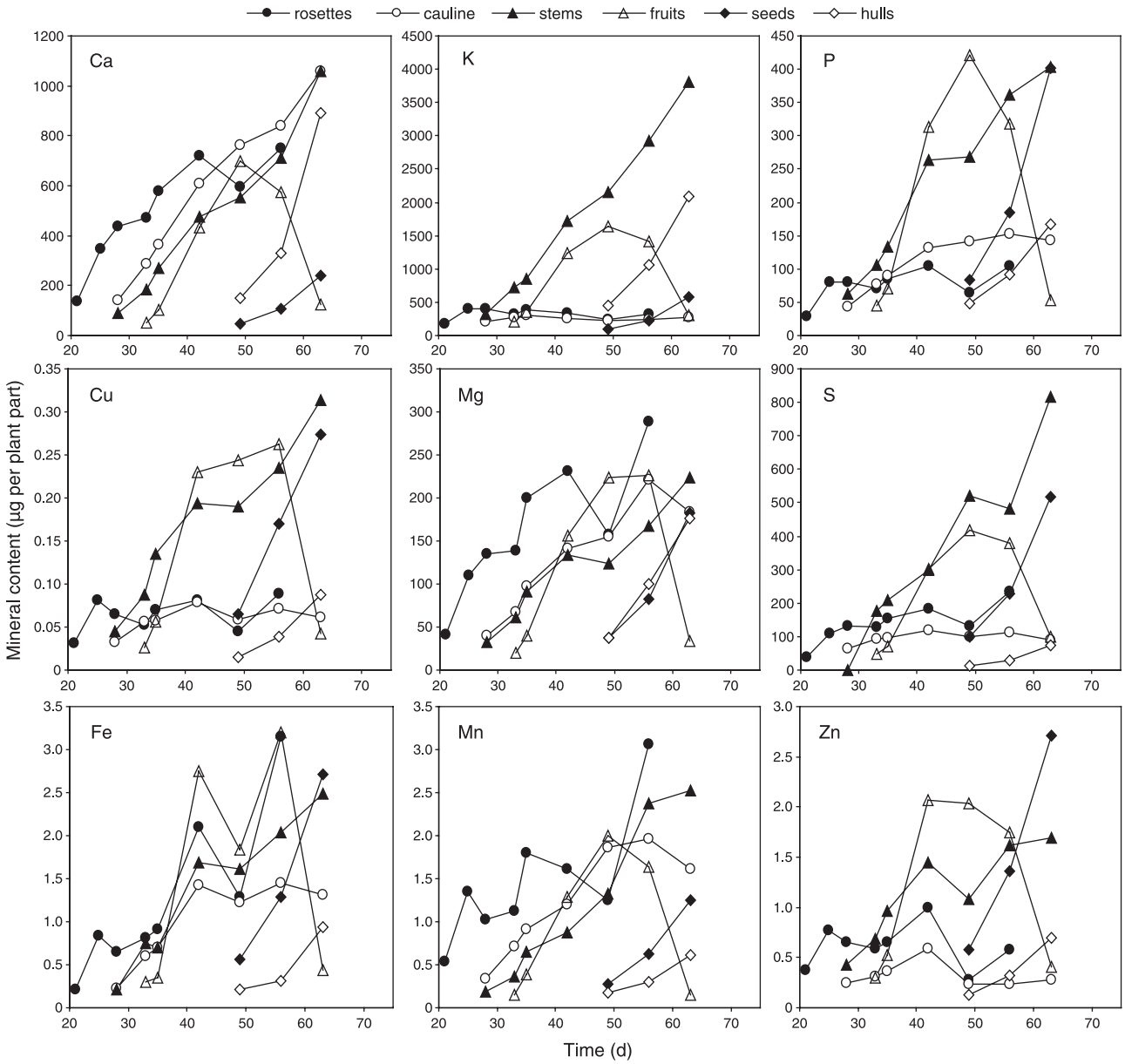

Fig. 5 Mineral contents of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn) in Arabidopsis L*er*-1 shoot tissues over time. Rosettes, whole rosettes; cauline, all cauline leaves; stems, inflorescence stems minus fruits and cauline leaves, including flowers; fruits, all immature fruits; seeds, mature seeds; hulls, mature silique hulls.

were constant over the last three time points, DW was also constant at these times.

Mineral partitioning

A PQ value, representing the proportional mineral content in a tissue relative to the proportional DW of that tissue, was calculated for Cu and Zn to allow comparison of the dynamics of partitioning of minerals between different lines regardless of differences in plant size. PQ curves for Cu and Zn in *ysl1ysl3* rosettes and cauline leaves (Fig. 8) were substantially different from the WT lines. Over time, the *ysl1ysl3* Cu and Zn rosette PQ curves rise sharply to values *c*. 250 for Cu and 170 for Zn, whereas the WT lines' PQ values are close to 100 for both minerals. A similar curve is seen for cauline leaves, but partitioning of Cu into this tissue is much higher, at values of over 300 by the later time points, while the PQ curves for Cu for all three WT lines sloped slightly downward. Zinc PQ curves for Col-0 and Cvi rosettes fluctuated *c*. 100, while the curve for Zn for L*er*-1 was downward-sloping. Both Col-0 and L*er*-1 Zn PQ curves sloped downward for cauline leaves, but the curve for Cvi was flat. For the *ysl1ysl3*

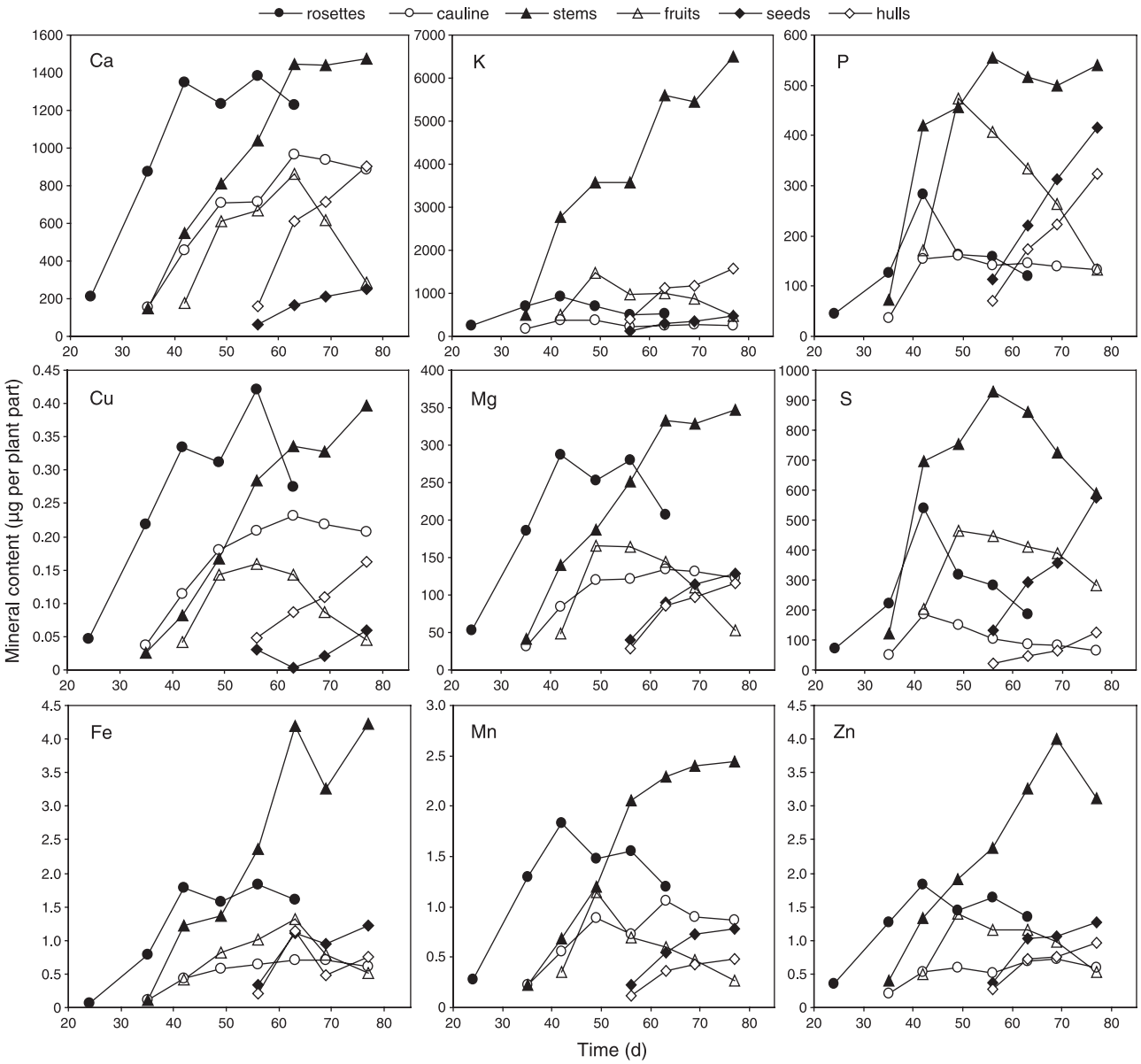

Fig. 6 Mineral contents of calcium (Ca), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), phosphorus (P), sulfur (S), and zinc (Zn) in Arabidopsis *ysl1ysl3* shoot tissues over time. Rosettes, whole rosettes; cauline, all cauline leaves; stems, inflorescence stems minus fruits and cauline leaves, including flowers; fruits, all immature fruits; seeds, mature seeds; hulls, mature silique hulls.

mutant, Zn PQ curves for both leaf tissues were upward-sloping, similar to Cu curves. PQ for *ysl1ysl3* stem Zn was generally lower than all WT lines, and Cu PQ values were much lower in the mutant. As a result of fluctuating Fe contents, Fe PQ curves were quite 'noisy' and difficult to interpret (not shown). Fluctuating content measurements were observed for some other minerals as well, and likely resulted from variation in both mineral concentrations and DW among individual plants.

To compare partitioning of Cu, Fe, and Zn in tissues with whole-plant concentrations of these minerals, total shoot Cu, Fe and Zn content was divided by total DW to give weight-normalized whole-plant Cu, Fe, and Zn accumulation (Fig. S2). Relative Zn accumulation was similar for *ysl1ysl3* and Col-0, whereas *ysl1ysl3* had higher Cu and lower Fe relative accumulation, indicating that relative Zn uptake (i.e. weight-normalized) is similar, relative Fe uptake is lower, and relative Cu uptake is higher in *ysl1ysl3* than in Col-0.

To determine whether maternal silique tissues are a source of seed minerals, full-size but still green fruits (immature) of Col-0 and L*er*-1 and dry but nonshattered fruits (mature) were collected, oven-dried, and dissected in order to separate hulls from seeds and to quantify mineral concentrations and contents on a per-fruit basis. The mineral contents and DW are presented

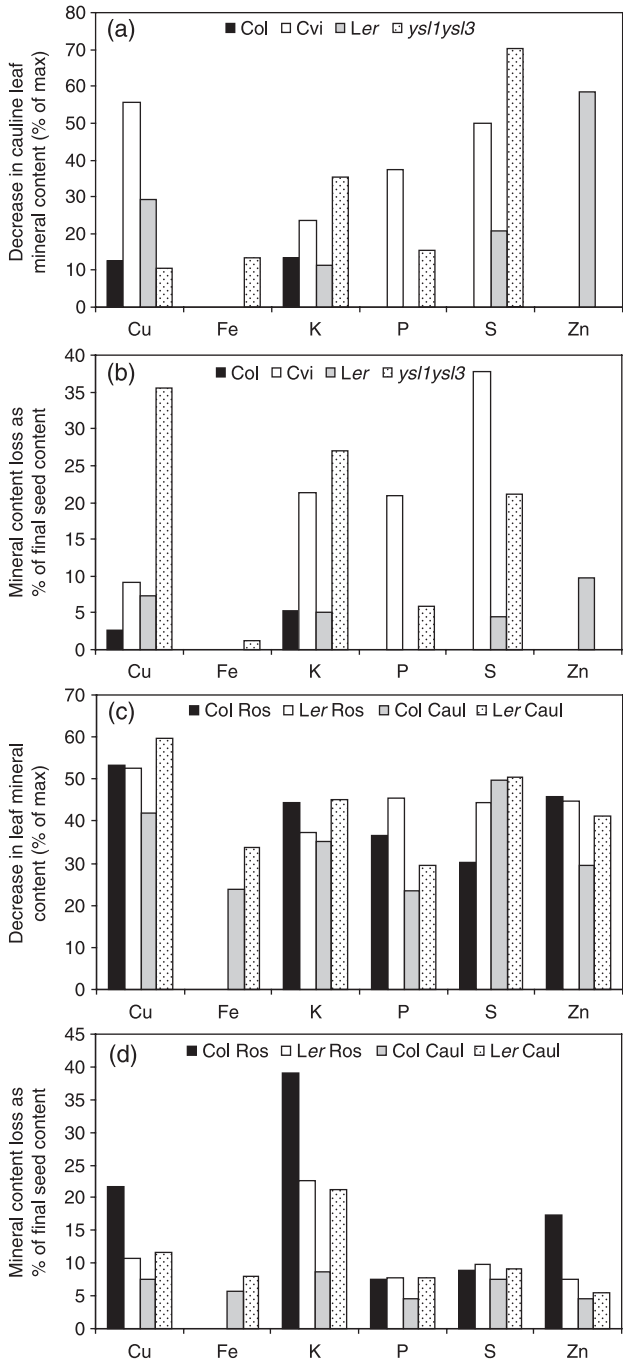

Fig. 7 Decrease in mineral content of copper (Cu), iron (Fe), potassium (K), phosphorus (P), sulfur (S), and zinc (Zn) from selected tissues of Arabidopsis, and potential contribution of remobilized minerals to seed mineral content. (a) Percentage decrease in cauline leaf mineral content for Col-0, L*er*-1, Cvi, and *ysl1ysl3* in primary study. (b) Mineral content lost as a percentage of total seed mineral content at final time point in primary study. (c) Percentage decrease in rosettes and cauline leaf mineral content for Col-0 and L*er*-1 in study presented in Fig. S1 (Supplementary material). (d) Mineral content lost as a percentage of total seed mineral content at the final time point in study presented in Fig. S1 (Supplementary material).

in Fig. 9. As the fruits matured, hulls of both Col-0 and L*er*-1 gained Ca and K (Fig. 9a). Although hull tissues gained a small amount of DW (Fig. 9e), both lines lost some P, S, Cu, Fe, and Zn content (Fig. 9a,c), suggesting that there was remobilization of these minerals from hulls to seeds during this period. Seeds gained content for all minerals except K when comparing immature and mature dissected fruits (Fig. 9b,d). Both Ca and K had lower contents in mature seeds than in mature hulls, while Cu, Fe, P, S, and Zn had higher contents in mature seeds.

To compare the distribution of minerals within mature fruit of the four lines, the percentage of total mature fruit mineral content contained in seeds at the final time point was calculated for each mineral (Fig. 9f). For mature fruit of the WT lines, more Cu, Fe, Mn, P, S, and Zn were partitioned to the seed fraction than in hulls (i.e. > 50% of mature fruit content). Mg was nearly equal in each fraction, and more Ca and K were partitioned to hulls. Across all lines (both WT and mutant), the distribution patterns for Ca, K, Mg, and Mn were similar, whereas the *ysl1ysl3* mutant had lower partitioning to seeds for Cu (61% lower than Col-0), Fe (20% lower than Col-0), P (11% lower than Col-0), and Zn (27% lower than Col-0). Seed distribution of S differed for Cvi, which had 56% of fruit S in seeds, compared with 77, 88, and 82% for Col-0, L*er*-1, and *ysl1ysl3*, respectively.

Discussion

We and others are interested in increasing seed mineral concentrations of plants consumed by humans. Arabidopsis can serve as an excellent model for identification of genes or pathways that could be targeted in crops (Maloof, 2003; Borevitz & Ecker, 2004; Bevan & Walsh, 2005; Mitchell-Olds & Schmitt, 2006; Schmid *et al.*, 2006). In this study, we have characterized organ-specific changes in dry matter and mineral content to monitor the net flow of minerals into and through the plant over the life cycle. Any increase in mineral content in one organ must have resulted from uptake and translocation from the soil, or from remobilization from one organ to another. By harvesting all shoot tissues and tracking the mineral partitioning in these tissues over time, remobilization can be estimated for each mineral. In this work, remobilization is defined as the net loss of stored or recycled mineral content from one organ or tissue over time, with the mineral loss representing movement into another tissue or organ. One caveat is that this method is able to measure net mineral content changes only, and cannot trace the movement of minerals introduced to the plant at a specific point in time, as the use of stable or radioisotopes would allow. A net mineral content decrease for 2 wk or more was interpreted as remobilization. As one of the major goals of this study was to determine the sources of seed minerals (e.g. leaves and fruit hulls) and the flux of minerals through the plant en route to the seed, the potential for remobilization of previously stored minerals from

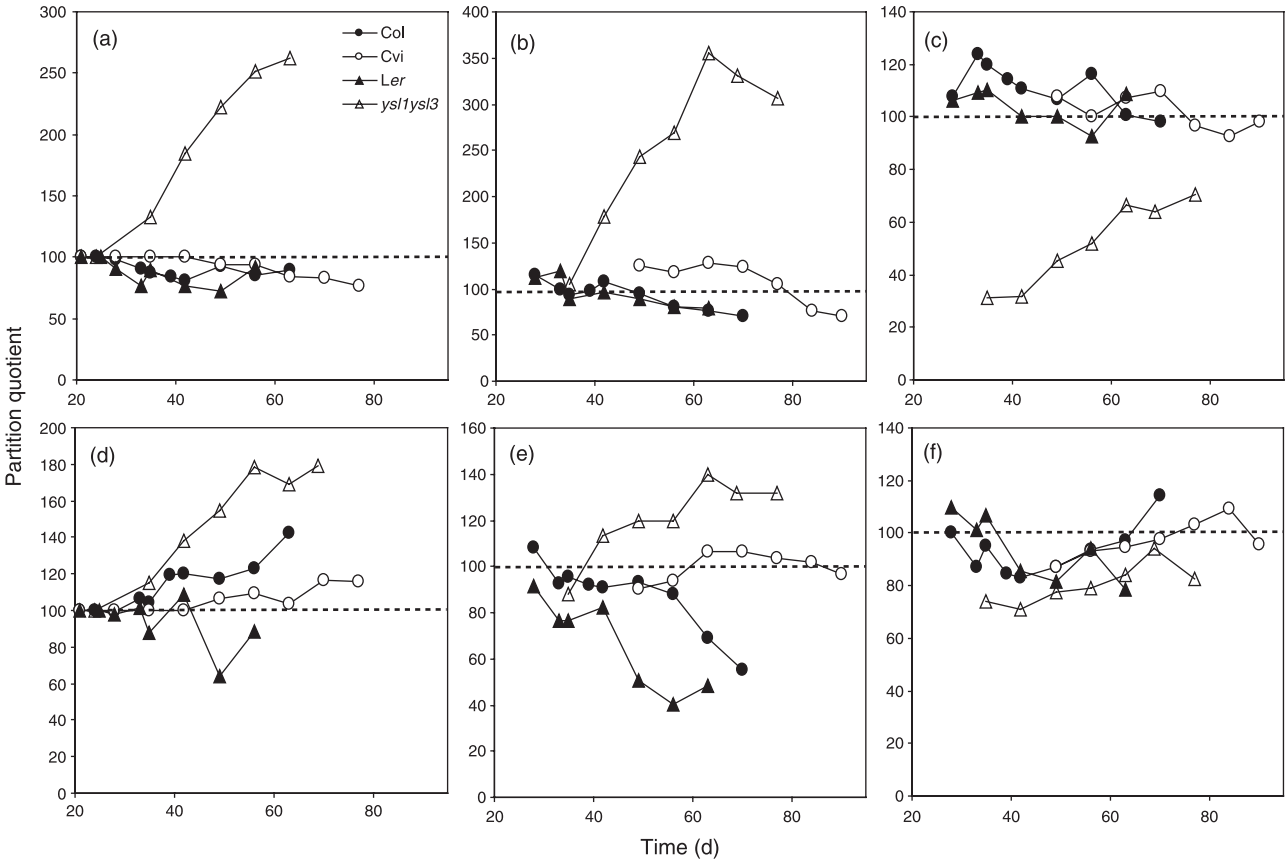

Fig. 8 Partition quotients (PQ) for copper (Cu) and zinc (Zn) in vegetative tissues over time. (a–c) Cu PQ for rosettes (a), cauline leaves (b), and stems (c). (d–f) Zn PQ for rosettes (d), cauline leaves (e), and stems (f). Dashed horizontal line represents PQ of 100.

vegetative tissues was evaluated against the need for continued mineral translocation from roots (to vegetative source tissues) during seed development and seed fill.

Growth and mineral dynamics

Mineral concentrations and contents of Cu, K, P, S, and Zn decreased in leaf tissues in the WT lines over time (Tables S1, S2; Figs 3–5). These dynamics are consistent with those observed in wheat (Hocking, 1994; Miller *et al.*, 1994), walnut (Drossopoulos *et al.*, 1996) and cotton (Zhao & Oosterhuis, 1999) leaves. Presumably, mineral content is lost from senescing leaves, although loss from nonsenescing leaves cannot be ruled out. When collecting rosettes or cauline leaves from midway through the growth period, some of the leaves were senescing, while some were young and still expanding. Thus, absolute mineral concentration or content differences could be declining in all leaves, or net loss in senescing leaves could be tempered by mineral gains in younger leaves.

L*er*-1 remobilized a higher percentage of Cu and Zn from cauline leaves than Col-0 in both time course experiments (Fig. 7), demonstrating genetically influenced differences in mineral dynamics. Sulfur remobilization from Cvi hulls was notably different from the other lines, and these differences were reflected in Fig. 9(f). Although seed S concentration was lowest in Cvi (Table S6), mature hull S concentrations were 2.5-fold higher than Col-0, the next highest line (Table S5). It would be interesting to profile contents of sulfur-containing molecules, such as storage proteins, metallothionein, phytochelatins, and glucosinolates, in Cvi and other accessions to determine which S-containing compounds are lower in Cvi. In a recent study of lines from an Arabidopsis RIL population, some lines exhibited drastic decreases in S concentration of the first six rosette leaves following the onset of senescence, while others did not; this was true for P as well, demonstrating that there is genetic control over mineral remobilization (Diaz *et al.*, 2005). To exploit genetic differences that control mineral export from leaves, such as for Cu, Zn, P, and S mentioned earlier, quantitative trait locus mapping and proteomic techniques could be used to identify genes and proteins that are involved with mineral fluxes through, or remobilization from, leaves, as has been recently done for N remobilization (Mickelson *et al.*, 2003; Schiltz *et al.*, 2004). Genes that influence net mineral loss from leaf tissues could be targeted in breeding programs for seed mineral biofortification.

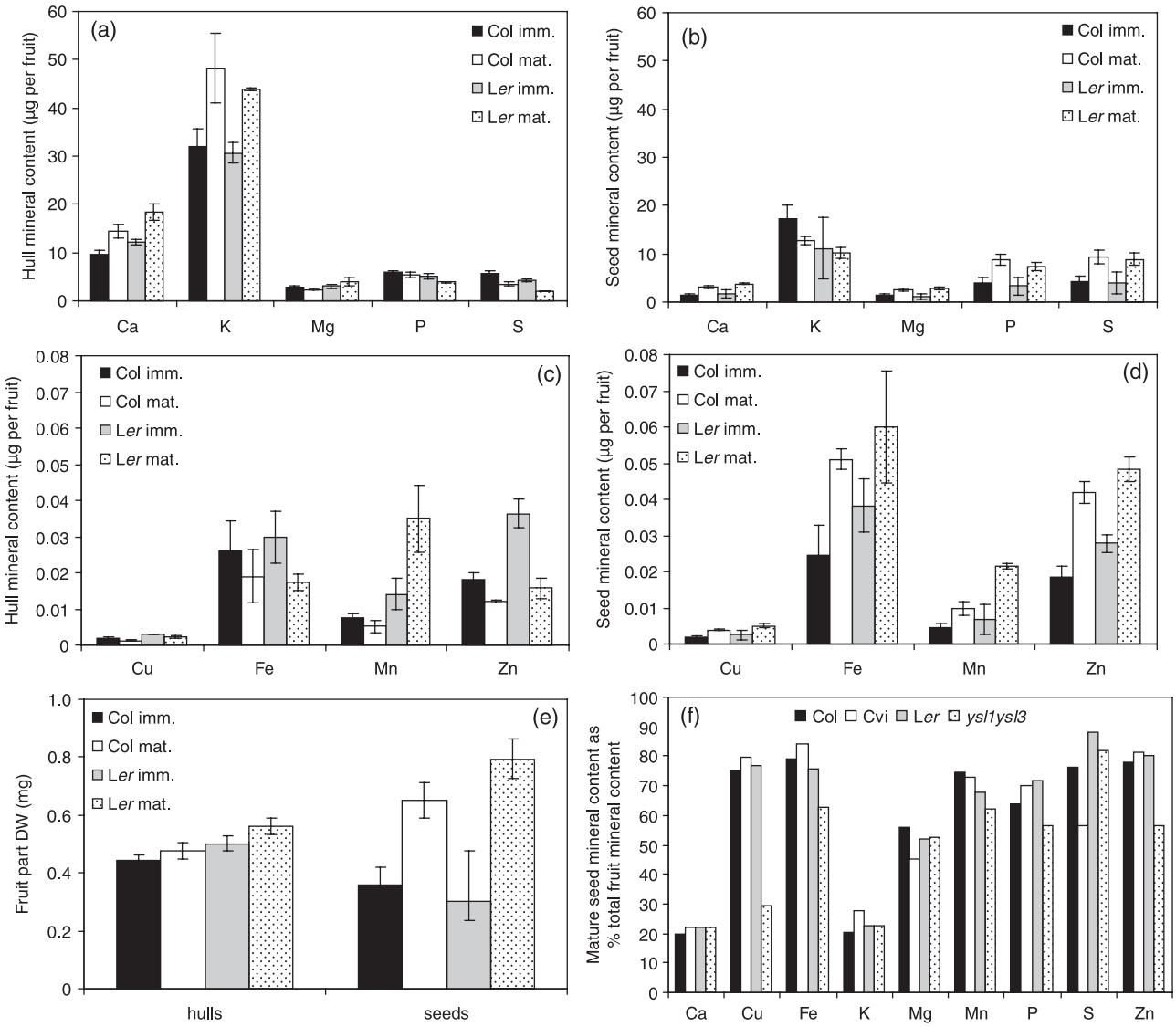

Fig. 9 Mineral partitioning in maternal fruit tissues and seeds of Arabidopsis. Macronutrient mineral contents in hulls (a) and seeds (b) of dissected immature and mature fruits of Col-0 and L*er*-1. Micronutrient mineral contents in hulls (c) and seeds (d) of dissected immature and mature fruits of Col-0 and L*er*-1. (e) Dry weights of dissected immature and mature hulls and seeds of Col-0 and L*er*-1. (f) Partitioning of mature fruit minerals in seeds (% of mature seed and mature hull total mineral content) of Col-0, L*er*-1, Cvi, and *ysl1ysl3* in time course study (final time points).

In all lines, K concentration in leaf tissues decreased before fruit maturation, and K concentration was much higher in mature hulls than in any other tissue, with stems as the second highest tissue (Tables S1–S6). A possible explanation for this is that Arabidopsis might use K as a major solute to provide phloem sap osmolality, thereby contributing to the driving force for bulk flow of sap from source tissues to fruits (Pate *et al.*, 1984). In Col-0 and L*er*-1 immature and mature fruit dissections, K was the only element that did not gain content between immature and mature seeds; in fact, K content decreased (Fig. 9b,d). During seed development, water is recirculated from the seed apoplasm to the maternal plant through the xylem (Zhang *et al.*, 2007), and it is possible that some K is lost from seeds in this manner.

What are the sources of seed minerals? Remobilization vs continued supply from roots

Remobilization of leaf mineral reserves to supply seeds with minerals has been emphasized in previous studies (Uauy *et al.*, 2006), but the absolute contribution of stored minerals to total seed mineral content is unclear. It is expected that a minimal amount of each mineral is incorporated into structural or protein molecules and thus unavailable for mobilization, and that source tissues would have to accumulate minerals in excess of this minimal amount to allow mobilization to growing tissues such as seeds. This was the case for S in soybean leaves (Sunarpi & Anderson,

1996), where a soluble S pool was available for remobilization and an insoluble S pool could not be mobilized. The size of the soluble pool was dependent on S nutrition. Leaves of soybean in one experiment remobilized P, while in a second experiment no remobilization occurred, yet seeds of both experiments had comparable seed P concentrations (Crafts-Brandner, 1992). In the present study, plants were watered with a complete nutrient solution in an attempt to provide all minerals in excess of minimal requirements. As such, vegetative tissues should have been able to store quantities above the structural minimum, which would have provided excess minerals for remobilization. Alternatively, with the abundant mineral supply at the root level, continued uptake during seed fill may have reduced or precluded the need for remobilization to serve as a source of minerals for seeds. In our study, the remobilization results were not exactly consistent between experiments (Fig. 7); differences were found in the minerals that were remobilized and the amounts remobilized. Thus, our results indicate that remobilization of minerals from Arabidopsis leaves is not absolutely required for seeds to acquire minerals.

In wheat, mineral remobilization from leaves was observed in two studies, for Cu, K, Mg, P, S, and Zn (Hocking, 1994), and for Cu, K, P, S, and Zn (Miller *et al.*, 1994). In these two experiments, remobilization of these minerals could account for approx. 40–70% of the total seed mineral content if all mineral content lost from leaves went entirely to seeds. Iron was mobilized in a third wheat study (Garnett & Graham, 2005), but little mobilization of Zn was detected, and the authors concluded that all Zn that entered the seeds was taken up after anthesis. Therefore, a major proportion of mineral content in seeds probably comes from nonstorage sources, that is, continued root uptake and translocation during the seed fill period (although some remobilization from potential root mineral stores cannot be ruled out). This is consistent with our data of total mineral content of shoot tissues (Fig. 2), which continued to increase even as seeds were maturing. We did observe remobilization of Cu, K, P, S, Zn, and in some instances Fe from leaves (Figs 3–7, Fig. S1), and at least a portion of these minerals was likely incorporated into growing tissues, such as stems and fruits. In the unlikely event that 100% of the content of each mineral lost from leaves went to seeds, this could account for, at most, 48% of seed K for Col-0 (Fig. 7d). This source could account for 6–30% of seed Fe, P, S, Zn, and Cu. No remobilization of Ca, Mg, or Mn was reliably observed. Thus, enhancing the plants' ability to remobilize certain minerals could be a reasonable target for crop improvement, assuming the plant has excess minerals to be remobilized. However, targeting the capacity for continued import of minerals from roots and the multiple (and largely unknown) processes involved in mineral translocation during seed development/seed fill may be more practical targets for modification to improve seed mineral concentration.

The *ysl1ysl3* mutant gives insight into metal micronutrient partitioning

Previously (and in this work), it was shown that the *ysl1ysl3* mutant, which has disruption of two metal-chelate transporter genes, had low seed Cu, Fe, and Zn concentrations and impaired movement of Cu and Zn from senescing rosette leaves (Waters *et al.*, 2006). One anticipated outcome of the present work was that the source or upstream tissues would be revealed by differences in partitioning of Cu, Fe, and Zn between the mutant and the parental line Col-0. Mutant rosette and cauline leaf Fe concentrations were generally lower than WT, and Cu and Zn concentrations were higher. As can be discerned from the concentrations (Tables S1, S2) and PQ values (Fig. 8), Cu and Zn highly accumulate in leaf tissues of *ysl1ysl3*, and this accumulation began before the start of leaf concentration and content decreases (remobilization) in the WT lines. The cauline PQ curves for Zn in Col-0 and Cvi are relatively flat, while the L*er*-1 cauline curve has a distinct downward slope, reflecting remobilization or net loss from this tissue (Fig. 8). By contrast, *ysl1ysl3* stem PQ values for Zn and especially for Cu were quite low at early points, and stem Cu PQ never reached values comparable to any of the WT lines.

Seed concentrations for all minerals except Mn were altered between Col-0 and *ysl1ysl3* (Table S2), in that concentrations were lower, except for Ca, which was significantly higher. However, most of these differences are still within range of other wild types and are unlikely to be detrimental; for example, the *ysl1ysl3* seed concentrations of P and S were not different from L*er*-1 and Cvi, *ysl1ysl3* seed K was only 9% lower than L*er*-1, and *ysl1ysl3* seed Mg concentration was only 7% lower than Cvi. By contrast, *ysl1ysl3* seed Cu concentration was 82% lower, Fe was 63% lower, and Zn was 45% lower than the parental line Col-0 (*ysl1ysl3* Cu concentration was 87% lower than Cvi, and Zn was 53% lower than L*er*-1). The high rosette and cauline leaf Cu and Zn concentrations and low stem concentrations (Tables S1–S3) suggest that these minerals are not distributed properly within the *ysl1ysl3* plants.

In addition to vegetative tissues, maternal fruit tissues have been shown to be mineral sources for seeds. In soybean, S was mobilized from pods into seeds (Sunarpi & Anderson, 1997). In a study of three legume species, N, K, P, Mg, Ca, Fe, Mn, Cu, and Zn losses from pods accounted for a minimum of 5% of seed Fe (in *Pisum sativum*) to a maximum of 38.9% for Mg (in *Lupinus albus*) (Hocking & Pate, 1977). In wheat, Zn first accumulated in glumes, lemma, and palea, then Zn content of these tissues decreased as seed Zn content increased (Pearson & Rengel, 1994). Our results indicate that Arabidopsis silique hulls are a source of minerals for seeds (Fig. 9). There was a notable difference between the WT lines and *ysl1ysl3* when it came to Cu, Fe, and Zn concentrations in hulls, indicating that, as in leaves (Fig. 8), these metals are not effectively mobilized from or translocated through the fruit hulls.

All of these observations suggest that in *ysl1ysl3*, Cu and Zn are 'trapped' in source tissues (leaves and fruit hulls) to the exclusion of these minerals from stem and seed tissues. This Cu and Zn accumulation pattern in the mutant suggests that minerals destined for seeds in the phloem pathway do not go directly from roots to seeds, but must first flux or pass through leaves. Radiolabeled S was noted to move sequentially from mature to younger leaves before moving to seeds (Sunarpi & Anderson, 1996), and minerals were modeled to flux through leaves and/or pods before entering the phloem sap for translocation to seeds (Hocking & Pate, 1977). Clearly, minerals must pass through the stem to reach cauline leaves, which is likely by xylem transport. Since Cu and Zn apparently do not readily move from rosettes or cauline leaves of *ysl1ysl3*, our results indicate a physiological role for YSL1 and YSL3 in phloem transport of Cu, Zn, and Fe, probably by moving these metals to the proper compartments for phloem loading, consistent with roles proposed for YSL family members previously (DiDonato *et al.*, 2004; Koike *et al.*, 2004; Le Jean *et al.*, 2005; Waters *et al.*, 2006).

Studies have provided functional evidence that YSL proteins transport Fe(II)-NA (nicotianamine) (DiDonato *et al.*, 2004; Koike *et al.*, 2004), Cu(II)-NA (DiDonato *et al.*, 2004), and Mn(II)-NA (Koike *et al.*, 2004), and seeds of a *ysl1* T-DNA knockout had lower seed Fe and NA concentrations (Le Jean *et al.*, 2005). Transport of Zn(II)-NA by YSLs has not been demonstrated, but the low seed Zn and high leaf Zn concentrations in *ysl1ysl3* (Table S2) suggest that YSL1 and/or YSL3 may transport Zn(II)-NA (or some other Zn complex). If metal-NA transport and involvement in phloem loading is indeed the molecular role of the YSL family, then NA may be accumulating in the *ysl1ysl3* leaves and altering metal homeostasis and feedback of shoot mineral status to roots. NA has long been proposed to be essential for sensing cellular Fe status (Stephan & Grun, 1989; Pich & Scholz, 1991; Pich *et al.*, 2001). Mutant plants that lack NA usually have a leaf metal concentration phenotype that is nearly opposite to the *ysl1ysl3* phenotype; that is, these leaves have lower Cu (Pich & Scholz, 1996; Pich *et al.*, 2001; Takahashi *et al.*, 2003), higher Fe (Pich & Scholz, 1996; Pich *et al.*, 2001), and lower Zn (Pich *et al.*, 2001; Takahashi *et al.*, 2003). Tobacco plants that overexpressed NA synthase (NAS) had higher seed Zn, Mn (Kim *et al.*, 2005), Cu, and Fe (Takahashi *et al.*, 2003; Kim *et al.*, 2005) than control plants.

Localization of *YSL* (DiDonato *et al.*, 2004; Koike *et al.*, 2004; Waters *et al.*, 2006) and *NAS* (Inoue *et al.*, 2003) expression to vascular tissue further support the idea that YSLs are involved in loading metal-NA complexes into phloem tissue. *YSL1* and *YSL3* expression was also observed in minor veins during leaf senescence (Waters *et al.*, 2006), and movement of Cu and Zn from senescing *ysl1ysl3* leaves was impaired. To assess which genes might be redundant to, or might work with, *YSL1* and/or *YSL3*, we used these genes as bait to probe publicly available microarray data for genes with similar expression patterns using the Botany Array Resource Expression Angler (Toufighi *et al.*, 2005) with a cutoff value of 0.5 (Table S7). In addition to five uncharacterized putative transporters, there was one ferric reductase, *FRO8*, which is primarily expressed in vascular tissue (Wu *et al.*, 2005), and two chelator synthesis genes: phytochelatin synthase (Ha *et al.*, 1999) and *NAS3*. The coexpression of *NAS3* and *YSL1* are particularly interesting, as NAS3 would provide the substrate for YSL1 to transport metal-NA complexes. In recent comparative genomic studies of *A. thaliana* and the Zn and Cd hyperaccumulator species *Arabidopsis halleri*, three *NAS* genes were more highly expressed in roots and one *NAS* gene more highly expressed in shoots of *A. halleri* (Becher *et al.*, 2004; Weber *et al.*, 2004; Talke *et al.*, 2006), as was *AhYSL6* in *A. halleri* shoots (Talke *et al.*, 2006). Similarly, three *YSL* genes were more highly expressed in the Zn, Cd, and Ni hyperaccumulator *Thlaspi caerulescens* than in *A. thaliana* (Gendre *et al.*, 2007). A recent microarray study categorized 240 putative senescence-associated transporters (Van der Graaff *et al.*, 2006), and it is interesting to note that *YSL1*, *YSL3*, *YSL7*, and *YSL8* were all among the identified transporters, as well as putative transporters for Cu, Fe, K, P, S, Zn, and oligopeptides (some of which may be metal chelators). These results, in combination with our current results, suggest that overexpression of *NAS* and *YSL* genes in tandem, or selection of lines with naturally higher expression of these genes, may improve uptake and translocation of mineral micronutrients such as Cu, Fe, and Zn into seeds.

In summary, this study suggests that with respect to the transport of minerals to seeds, continued uptake and translocation of minerals to source tissues during seed fill are as important as, if not more important than, remobilization of previously stored shoot minerals. Our results also suggest that minerals move into and through leaves and silique hulls before their translocation to seeds. Thus, in addition to targeting source tissues for increased mineral remobilization, researchers should also target root uptake and leaf efflux transporters to increase mineral accumulation in seeds. The *ysl1ysl3* mutant, with its reduced movement of Cu, Zn, and Fe out of leaves, could be an excellent tool to help identify other molecular components involved in source–sink mineral partitioning.

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Supplementary Material

The following supplementary material is available for this article online:

Fig. S1 Mineral contents (% of maximum content) of Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn of Col-0 and L*er*-1 rosettes and cauline leaves over time.

Fig. S2 Whole shoot relative accumulation of Cu, Fe, and Zn ($\mu g\ g^{-1}$). A, weight-normalized Cu accumulation for Col-0 and ysl1ysl3; B, dry weight-normalized Fe accumulation for Col-0 and *ysl1ysl3*; C, weight-normalized Zn accumulation for Col-0 and *ysl1ysl3*.

Table S1 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in rosettes over time

Table S2 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in cauline leaves over time

Table S3 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in stems (inflorescence stems, including flowers, minus fruits and cauline leaves) over time

Table S4 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in immature fruits (of all developmental stages before maturity) over time

Table S5 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in hulls (valves from mature fruits, includes fallen hulls and those removed by gentle agitation) of mature fruits over time

Table S6 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in seeds (seeds from mature fruits, including fallen seeds and those removed by gentle agitation) over time

Table S7 Genes showing coexpression with *YLS1* or *YSL3* in microarray experiments, as determined by the Botany Array Resource Expression Angler

This material is available as part of the online article from:
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Supplementary Material

Table S1 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in rosettes over time (± SD). Values without SD are single measurements.

Macronutrients (mg g-1)

	Ca (mg g-1)				K (mg g-1)				Mg (mg g-1)				P (mg g-1)				S (mg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
21	37±0	28±1	34±2		45±1	53±0	43±3		12±0	9±0	10±0		9±0	10±0	7±0		11±1	12±0	9±1	
24	38		33	42±0	39		39	47±1	11		10	10±0	11		8	9±0	13		10	14±1
28	42±2	30±0	41±11		44±2	55±3	38±1		13±1	9±1	13±0		11±0	12±0	8±0		14±1	14±0	12±1	
33	49±2		46±1		36±1		31±1		15±1		13±1		11±1		7±0		14±1		13±0	
35	56±3	34±2	50±2	56±3	35±1	54±3	29±1	44±4	17±1	10±1	17±1	12±1	10±1	16±1	7±0	8±1	14±1	18±2	12±1	14±2
42	58±3	36±3	52±5	64±2	26±2	50±1	24±3	44±2	18±1	11±2	17±2	14±1	9±1	16±2	8±1	13±1	12±1	16±0	13±1	25±2
49	58±3	40±3	56±4	53±2	23±1	45±5	22±2	30±3	15±1	12±3	15±3	11±1	5±0	14±1	6±0	7±1	8±1	13±2	12±2	14±2
56	55±4	46±2	54±6	57±3	29±1	48±4	23±5	20±2	18±4	11±2	21±1	12±1	10±1	13±1	7±1	7±1	14±1	12±2	17±2	12±2
63	61±1	46±5		54±1	22±0	61±14		23±1	14±1	10±1		9±0	8±1	14±4		5±0	9±1	13±4		8±1
70		47±4				49±9				11±2				13±3				13±3		
77		46±4				32±5				13±3				10±3				9±2		

Micronutrients (µg g-1)

	Cu (µg g-1)				Fe (µg g-1)				Mn (µg g-1)				Zn (µg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
21	7.2±0.4	8±0.2	7.6±0.6		64.2±7	45.9±7	50.9±7		80.7±3	145.3±7	130.7±25		99.7±3	84±2	91.2±19	
24	7.3		7.8	9.1±0.4	48.1		80.3	12.5±3	43.7		129.7	54.1±26	107.4		73.9	70±1
28	6.5±0.4	7.2±0.7	6.2±0.2		50.4±6	81.9±33	61.8±5		52.5±6	77.3±25	97.3±17		63.3±18	67.5±12	61.8±10	
33	6.0±0.5		5.1±0.1		52.2±7		78.7±5		55.2±12		108.8±12		81±9		57.2±7	
35	6.8±0.7	7.3±0.5	7.1±0.4	13.9±1.1	67.2±11	56.7±6		49.9±18	73.2±17	50.9±6	155.2±21	82.5±15	78.5±19	82.9±12	51.2±8	81.2±5
42	5.3±0.5	7.8±1.5	5.9±0.6	15.8±0.9	114.4±13	121.3±20	152.1±44	84.3±19	68.5±9	54.3±48	116.7±62	86.3±18	69.8±9	104.3±23	72.6±10	86.8±5
49	4.5±0.6	7.9±1.5	4.2±0.7	13.5±0.9	59.1±10	64.5±9	120.3±54	68.5±13	63.4±20	69.5±40	117.3±34	64.2±5	47.3±12	91.2±23	26±9	62.7±3
56	4.2±0.8	6.9±2.2	6.3±1.6	17.5±3.3	109.3±31	62±6	225.1±23	76.4±9	83.8±33	66.5±38	218.2±29	64.4±7	51±16	76.5±28	41±11	68.2±12
63	4.6±0.3	4.6±0.8		12.1±1.1	76.6±4	66.3±19		70.9±4	40±19	121.8±23		52.6±1	69.3±13	63.7±7		59.4±5
70		5.8±1.2				68.7±14				60.5±44				87.3±35		
77		5.3±1.2				55.1±19				61.4±36				69.1±32		

Table S2 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in cauline leaves over time (± SD). Values without SD are single measurements.

Macronutrients (mg g-1)

K (mg g-1)					Mg (mg g-1)				P (mg g-1)				S (mg g-1)			
ysl1ysl3	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
	52		48±2		9		9±1		14		10±0		21		15±1	
	46±1		38±1		10±0		10±0		14±1		11±0		18±1		13±1	
46±0	45±2		38±2	49±2	12±0		12±1	9±0	14±2		11±0	11±1	17±2		12±1	15±1
62±3	34±4		24±4	51±3	14±2		13±2	11±0	13±2		12±2	21±1	12±2		11±2	25±2
58±1	24±1	39±0	19±2	30±4	12±2	9±1	13±2	10±0	11±2	14±0	12±1	13±0	9±2	15±1	9±1	12±1
64±2	28±2	41±4	19±5	20±3	16±4	8±1	17±3	11±1	18±2	13±3	12±2	13±0	11±1	16±5	9±1	9±2
68±1	23±1	47±4	18±2	18±0	13±2	9±1	12±2	10±0	16±2	20±4	10±1	10±1	9±1	27±4	6±1	6±0
64±1	18±1	37±5		19±1	12±1	11±2		9±0	16±1	18±4		9±0	8±1	24±1		6±0
69±9		23±2		20±1		13±3		10±1		13±5		10±1		16±4		5±0
		23±3				11±2				10±1				10±2		
		23±2				16±3				8±1				10±1		

Micronutrients (µg g-1)

Cu (µg g-1)			Fe (µg g-1)				Mn (µg g-1)				Zn (µg g-1)			
Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
	7.7±0.4		48.3		54.2±2		37.3		80.8±16		69.1		58.2±19	
	7.9±1.7		64.9±3		84.5		52.8±12		100.5±40		70.4±5		43.3	
	7.0±0.9	11.0±0.3	86.7±13		85.6±25	33.8±9	65.8±3		111.9±39	66.1±12	71.9±3		44.6±10	62.3±4
	7.4±1.3	15.3±0.4	122.6±46		133.6±14	58.7±7	60.9±11		112±46	75±14	53±9		55±3	71.4±3
10.5±0.5	5.1±0.8	14.7±0.8	73.2±19	77.1±5	106.7±30	46.9±4	64.8±16	75.9±10	160.9±33	72.4±5	37.5±10	77.7±7	20.5±5	48.5±2
8.7±3.0	4.6±4.7	18.7±1.7	96.8±10	68.9±15	112.7±34	57.9±6	95.5±37	88±40	152.5±24	64.8±7	36.6±12	65.8±19	18.6±5	45.7±5
6.9±1.6	4.1±0.6	16.3±0.4	79.5±19	88.4±17	87.2±9	50.3±3	43±25	125.6±32	107.7±6	74.8±4	33.5±19	65.6±13	18.8±0	49.1±2
8.7±2.7		15.0±0.7	81.3±15	91.4±26		48.2±4	29.6±15	63.2±25		61.7±8	31.9±15	79.4±19		49.6±4
7.3±1.8		16.2±1.7		67.5±11		48.4±3		58.9±35		68±12		61.9±32		46.4±2
4.6±1.3				60.5±15				69.8±43				63.9±27		
3.8±0.5				64.7±12				92±21				46.9±20		

Table S3 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in stems (inflorescence stems including flowers, minus fruits and cauline leaves) over time (± SD). Values without SD are single measurements.

Macronutrients (mg g-1)

	Ca (mg g-1)				K (mg g-1)				Mg (mg g-1)				P (mg g-1)				S (mg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
28	11		15±0		63		54±2		5		5±0		12		10±0		18		15±0	
33	14±1		15±1		64±4		60±8		6±0		5±0		13±0		9±1		18±1		14±2	
35	17±2		18±1	19±0	69±7		55±8	65±4	6±1		6±0	5±0	12±1		9±1	9±1	18±2		12±2	16±1
42	15±2		19±0	18±1	67±5		68±7	93±4	5±1		5±1	5±0	10±1		10±1	14±1	14±2		16±2	23±2
49	14±2	13±1	17±1	13±1	55±2	45±2	65±3	59±8	4±1	6±0	4±1	3±0	5±1	10±1	8±1	7±1	9±1	12±1	13±1	12±1
56	15±1	13±2	19±2	13±2	84±7	53±5	79±6	46±6	5±2	4±1	5±0	3±0	10±1	10±2	10±1	7±1	15±1	12±3	15±2	12±2
63	17±1	11±1	19±1	13±0	79±6	78±11	68±5	51±3	5±1	4±0	4±1	3±0	8±1	15±2	7±1	5±0	13±1	20±3	10±2	8±0
70	19±2	13±2		13±1	61±13	72±5		48±5	5±0	5±1		3±0	8±2	13±2		4±0	11±1	18±3		6±1
77		10±1		14±1		41±2		61±2		4±1		3±0		9±1		5±0		13±2		6±0
84		11±1				60±8				4±1				8±1				10±1		
90		9±2				59±4				4±1				6±1				9±1		

Micronutrients (µg g-1)

	Cu (µg g-1)				Fe (µg g-1)				Mn (µg g-1)				Zn (µg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
28	7.1		7.3±0.5		31.2		35±3		24.1		30.3±5		63.8		69.5±9	
33	8.2±0.7		7.3±0.4		41.6±18		62.1±25		25.6±2		30.3±7		66.2±4		57±7	
35	9.3±1.1		8.7±0.5	3.3±0.3	60.8±8		44.8±4	14.4±8	29.5±4		41.7±10	28.9±4	71.5±10		62.2±9	52.3±5
42	7.3±0.6		7.7±1.3	2.7±0.2	50.7±7		66.7±17	40.6±9	21±3		34.9±13	22.8±2	48.4±6		57.5±3	44.6±2
49	5.2±0.4	9.1±0.9	5.8±0.4	2.7±0.3	31.1±3	43.2±9	49±24	22.4±4	22.1±7	22.9±4	40.5±13	19.6±3	35.2±9	74.7±11	33±6	31.4±3
56	5.8±0.7	7.4±2.2	6.4±0.3	3.6±0.5	61.9±15	40.6±13	55.3±7	30±4	26.9±11	28.4±5	64.4±17	26.1±4	38.8±5	65.1±19	43.8±2	30.2±1
63	5.1±0.8	5.8±1	5.6±1.1	3±0.2	41.9±10	39.9±6	44.6±13	38±12	23.8±10	35.3±7	45.2±14	20.8±9	47.4±26	58.3±15	30.3±9	29.4±3
70	5.5±0.7	7.7±2.9		2.9±0.4	48±3	45.2±17		28.8±10	23.4±13	31.7±9		21.3±4	66.1±25	73.1±21		35.4±9
77		6.7±1.6		3.7±0.5		41.6±25		39.4±21		23.6±7		22.8±6		61.8±23		29.1±5
84		5.5±1				51.4±30				32±5				68.7±24		
90		5.3±1.1				34.2±6				37.7±3				46.4±27		

Table S4 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in immature fruits (of all developmental stages prior to maturity) over time (± SD). Mean values are averages (± SD) of individual samples from all time points.

Macronutrients (mg g-1)

	Ca (mg g-1)				K (mg g-1)				Mg (mg g-1)				P (mg g-1)				S (mg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
33			13±0				54±3				5±0				11±0				13±2	
35			16±1				52±3				6±0				10±1				11±2	
42	17±2		16±0	22±2	52±4		47±4	62±4	7±1		6±1	6±0	14±2		12±1	21±2	16±2		11±1	25±2
49	16±1	17±1	18±1	17±1	44±3	48±1	42±2	42±4	5±0	7±0	6±1	5±0	10±1	11±0	11±1	13±2	9±1	15±1	11±1	13±2
56	16±1	14±3	17±2	18±1	62±10	42±9	42±4	27±7	6±2	5±1	7±1	4±0	15±2	10±2	9±0	11±1	16±2	14±5	11±1	12±1
63	15±0	13±1	17±0	21±1	51±7	66±9	41±1	24±1	5±1	5±0	5±0	3±0	13±1	18±2	7±2	8±0	16±1	24±1	13±4	10±1
70	15±1	14±4		17±1	40±6	49±5		25±2	4±0	5±1		3±0	10±1	14±2		7±1	15±1	20±2		11±1
77		10±3		16±3		25±5		28±3		5±1		3±0		10±2		8±0		13±2		16±2
84		12±2				33±1				4±1				9±1				13±1		
90		12±1				25±2				6±1				9±1				11±0		
mean	16±2	13±3	17±2	18±2	50±10	42±16	46±5	35±13	5±1	5±1	6±1	4±1	13±3	12±4	10±2	11±5	14±3	17±5	11±2	14±5

Micronutrients (µg g-1)

	Cu (µg g-1)				Fe (µg g-1)				Mn (µg g-1)				Zn (µg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
33			6.5±0.3				74.6±20				38.2±7				75.9±9	
35			8.4±0.7				51.3±9				56.8±19				77.8±9	
42	6.8±0.4		8.7±1.2	5.1±0.4	74.6±27		104.1±13	51.3±10	34.7±5		48.5±13	44±8	66.1±5		78±3	61±1
49	4.7±0.6	9.3±0.6	6.3±1.2	4±0.2	42.9±9	59.5±0	47.4±19	23.2±5	25±7	34.5±11	51.5±8	32.1±3	43.3±8	72.9±5	52.5±6	39±2
56	4.6±0.8	7.9±2.4	7.8±0.8	4.3±0.4	82.9±21	65.9±18	94.4±0	27.5±4	19.4±5	49.5±13	48.4±17	19±3	39.8±7	63.2±14	51.5±2	31.7±3
63	5.7±0.8	5.7±1.2	5.9±0.8	3.4±0.2	57.6±2	68.4±13	58.8±14	31.6±14	12.4±3	44.1±9	20.6±1	14.3±1	43.8±8	61.8±7	54.8±4	27.6±1
70	6.9±1.6	7.4±2.8		2.4±0.4	65.8±4	69.7±31		22.5±2	12.5±1	27.2±7		13.4±1	58.4±15	59.8±9		27.8±3
77		7.7±2		2.6±0.5		46.4±14		30.3±10		20.1±4		15.3±1		51.7±8		31±6
84		7.3±2.1				61±28				26.3±5				56.2±17		
90		5±0.6				57.9±9				23.4±4				42.4±10		
mean	5.7±1.3	7.0±2.2	7.3±1.4	3.7±0.9	65.9±22	61.5±20	68.7±26	29.1±12	21.6±9	31±12	47.3±16	23.6±11	50.5±13	57.2±12	63.2±14	35.8±11

Table 5 Concentrations of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in hulls (valves from mature fruits, includes fallen hulls and those removed by gentle agitation) of mature fruits over time (± SD). Values without SD are single measurements. Mean values are averages (± SD) of individual samples from all time points.

Macronutrients (mg g-1)

	Ca (mg g-1)				K (mg g-1)				Mg (mg g-1)				P (mg g-1)				S (mg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
49			31±2				95±11				8±2				10±1				2±1	
56	28±2		32±2	31±1	148±10		103±12	81±6	8±2		10±2	6±1	12±2		9±1	14±2	7±1		3±0	4±1
63	33±1	32	35±2	35±1	120±3	151	81±8	63±11	6±1	6	7±3	5±0	13±1	15	6±1	10±1	6±1	24	3±1	3±0
70	34±2	32±1		33±2	95±16	105±33		54±10	5±1	6±2		4±0	12±1	16±0		10±0	7±1	19±1		3±0
77		24±4		34±1		71±11		59±6		7±3		4±0		11±5		12±1		19±4		5±1
84		28±1				72±2				5±1				10±0				14±2		
90		20±6				57±7				8±2				7±2				13±1		
mean	32±3	25±6	33±2	34±2	119±26	78±29	91±14	61±11	6±2	7±2	8±3	5±1	12±1	11±4	8±2	11±2	7±1	17±4	3±0	4±1

Micronutrients (µg g-1)

	Cu (µg g-1)				Fe (µg g-1)				Mn (µg g-1)				Zn (µg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
49			3.1±0.4				42.8±19				35.1±3				25.9±3	
56	2.5±1.3		3.8±0.4	9.6±2.7	41.7±4		30.3±11	40.4±19	19.4±6		28.6±7	24.1±3	19±4		31.6±10	53.3±6
63	2.9±0.4	2.4	3.4±0.8	4.9±0.9	30.4±6	80.9	36.5±11	26.4±5	10.8±5	39.2	23.6±5	20.8±2	23±8	33.2	27.1±4	40.8±16
70	3.9±0.6	3.1±0.3		5.0±0.5	46.5±12	16.9±3		22.1±6	10.5±5	14.9±5		19.7±5	28±10	34.6±14		35.1±5
77		5.2±0.1		6.1±1.4		20.3±6		28.7±11		10±1		18±2		36.4±18		35.9±5
84		3.9±1.1				31.0±10				16.1±5				30.1±13		
90		3.8±0.4				27.4±7				16.7±2				23.4±4		
mean	3.2±1	3.9±1	3.4±0.6	5.8±1.8	40.3±10	29.1±17	35.9±12	27.2±10	13.5±6	16.5±8	27.8±7	20±3	23.8±8	30.4±11	28.2±6	38.9±11

Table S6 Concentrations (µg/g) of macronutrients (Ca, K, Mg, P, and S) and micronutrients (Cu, Fe, Mn, and Zn) in seeds (seeds from mature fruits, includes fallen seeds and those removed by gentle agitation) over time (± SD). Values without SD are single measurements. Mean values are averages (± SD) of individual samples from all time points. Mean values followed by different letters are significantly different (P<0.005) as determined by 2-tailed t-test.

Macronutrients (mg g-1)

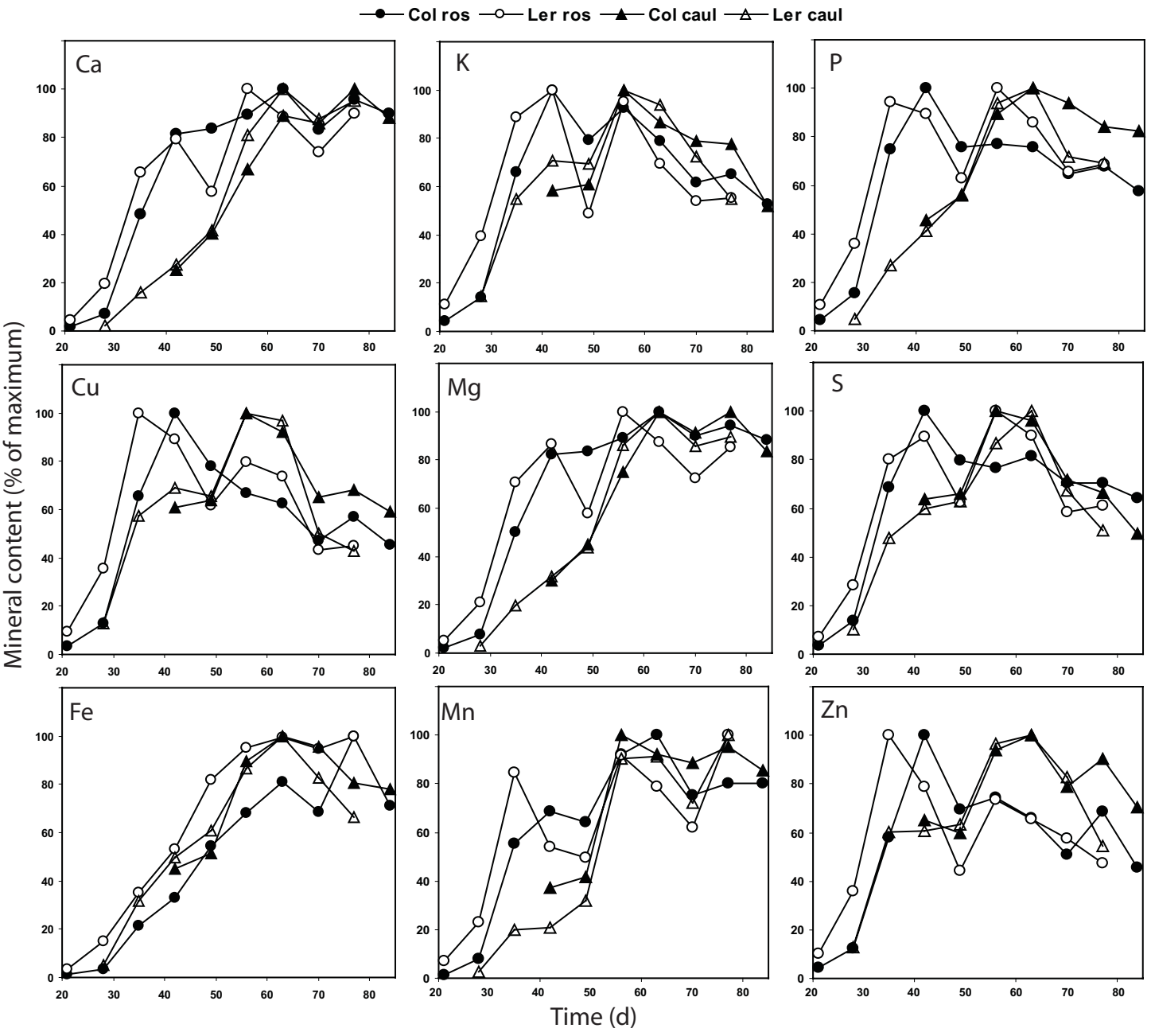
	Ca (mg g-1)				K (mg g-1)				Mg (mg g-1)				P (mg g-1)				S (mg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
49			6±0				12±1				5±0				10±1				12±0	
56	6±0		6±0	6±1	20±1		12±1	11±1	5±0		5±0	4±0	17±2		10±1	11±3	16±2		12±1	13±0
63	5±0	4	5±0	6±0	20±2	21	12±1	11±1	4±0	4	4±0	3±0	14±1	16	8±1	8±0	14±2	18	11±1	11±0
70	5±0	3±0		6±0	16±2	19±3		11±1	4±0	4±0		3±0	13±1	12±1		9±0	14±2	13±3		10±0
77		3±0		6±0		12±2		11±1		4±0		3±0		9±1		10±0		10±1		14±2
84		3±0				15±2				3±0				10±1				9±1		
90		3±0				11±1				4±0				9±0				9±1		
mean	5±0 *a*	3±0 *b*	5±0 *a*	6±0 *c*	18±3 *a*	14±3 *b*	12±1 *b*	11±1 *b*	4±0 *a*	4±0 *b*	4±0 *a*	3±0 *c*	14±2 *a*	10±2 *b*	9±1 *b*	9±1 *b*	14±2 *a*	10±3 *b*	12±1 *b*	12±2 *b*

Micronutrients (µg g-1)

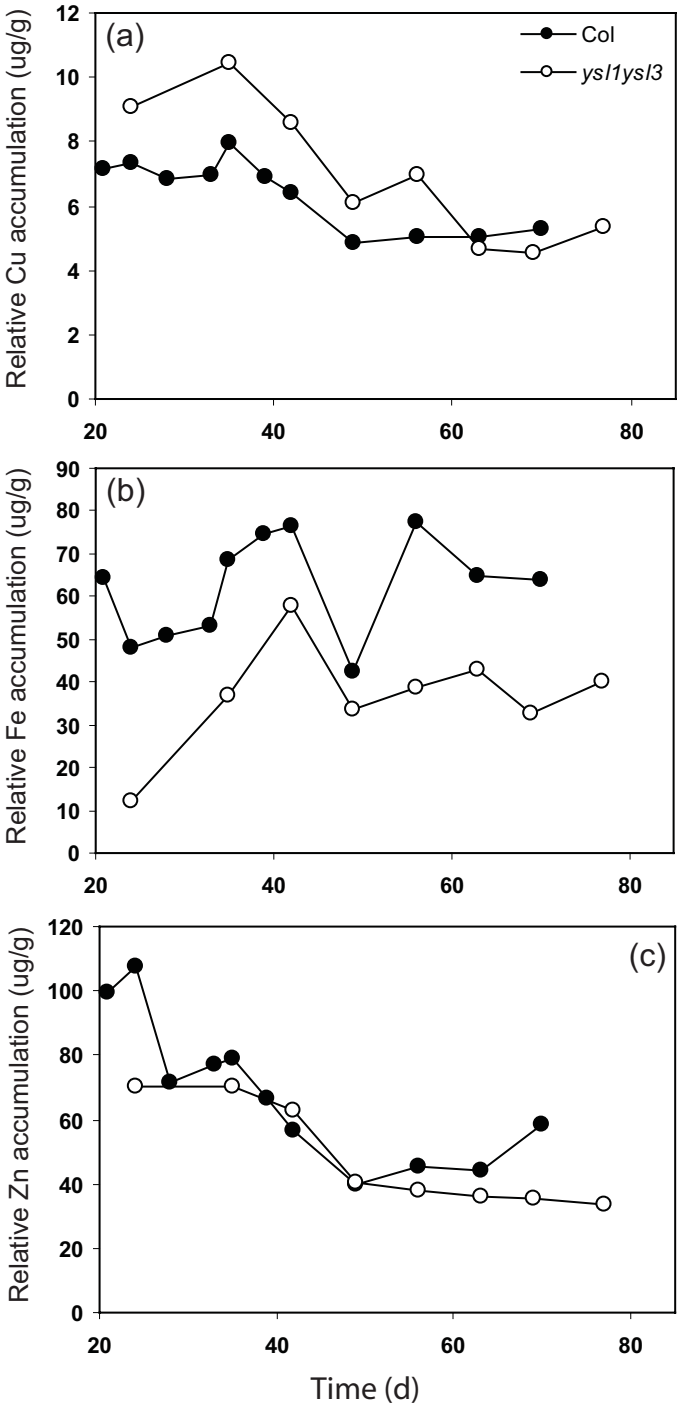
	Cu (µg g-1)				Fe (µg g-1)				Mn (µg g-1)				Zn (µg g-1)			
Time (d)	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*	Col	Cvi	Ler	*ysl1ysl3*
49			7.9±2.1				68.8±20				33.8±3				70.1±9	
56	5.7±0.3		9.3±2	3±1.1	81.9±13		112.1±56	33.6±23	25.0±2		34.2±6	21.8±3	58.1±5		74±10	36±9
63	6.3±1.2	6.6	5.6±0.4	0.1±0.4	61.8±18	79.9	55.5±9	40.8±33	16.3±3	32.9	25.5±1	19.9±1	61.1±27	76.1	55.3±3	38.1±20
70	6.8±1.1	8.2±2.4		0.6±0.4	109.2±20	73.5±8		27.8±12	17.1±3	24.5±5		21.4±1	64.3±8	69.5±10		31.2±1
77		9.5±2.2		1.4±1.4		79.5±10		29.7±7		21.4±2		18.9±1		62.6±11		30.6±2
84		8.2±2.3				72.7±12				23±2				66.2±9		
90		7.6±2				74.9±4				22.9±2				52.9±10		
mean	6.4±1.1 *a*	8.2±2.1 *b*	7.4±2.2 *ab*	1.1±1.2 *c*	78.6±19 *a*	75.4±8 *a*	64.5±16 *a*	29.0±10 *b*	18.8±5 *a*	23.3±3 *c*	30.5±6 *b*	20.3±2 *a*	57.7±9 *a*	61.9±11 *a*	66.6±11 *a*	31.5±4 *b*

Table S7 Genes showing co-expression with *YSL1* or *YSL3* in microarray experiments, as determined by BAR Expression Anger.

Locus	Gene
	YSL1 co-expressed
At1g09240	NAS2 (nicotianamine synthase)
At1g68570	proton-dependent oligopeptide transport (POT) family protein
At5g06530	similar to ABC transporter family
At1g69870	proton-dependent oligopeptide transport (POT) family protein
At5g50160	FRO8 (ferric reductase family)
At3g60160	similar to ABC transporter family
	YSL3 co-expressed
At5g44070	PCS1 (phytochelatin synthase)
At5g64410	OPT4 (oligopeptide transporter family)



Supplemental Figure 1. Mineral contents (% of maximum content) of Ca, Cu, Fe, K, Mg, Mn, P, S, and Zn of Col-0 and Ler-1 rosettes and cauline leaves over time.



Supplemental Figure 2. Whole shoot relative accumulation of Cu, Fe, and Zn (µg/g). A, weight normalized Cu accumulation for Col-0 and ysl1ysl3. B, Dry weight normalized Fe accumulation for Col-0 and ysl1ysl3. C, weight normalized Zn accumulation for Col-0 and ysl1ysl3.