

The serine–glycine–one-carbon metabolic network orchestrates changes in nitrogen and sulfur metabolism and shapes plant development

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Abstract

L-serine (Ser) and L-glycine (Gly) are critically important for the overall functioning of primary metabolism. We investigated the interaction of the phosphorylated pathway of Ser biosynthesis (PPSB) with the photorespiration-associated glycolate pathway of Ser biosynthesis (GPSB) using *Arabidopsis thaliana* PPSB-deficient lines, GPSB-deficient mutants, and crosses of PPSB with GPSB mutants. PPSB-deficient lines mainly showed retarded primary root growth. Mutation of the photorespiratory enzyme Ser-hydroxymethyltransferase 1 (SHMT1) in a PPSB-deficient background resumed primary root growth and induced a change in the plant metabolic pattern between roots and shoots. Grafting experiments demonstrated that metabolic changes in shoots were responsible for the changes in double mutant development. PPSB disruption led to a reduction in nitrogen (N) and sulfur (S) contents in shoots and a general transcriptional response to nutrient deficiency. Disruption of SHMT1 boosted the Gly flux out of the photorespiratory cycle, which increased the levels of the one-carbon (1C) metabolite 5,10-methylene-tetrahydrofolate and S-adenosylmethionine. Furthermore, disrupting SHMT1 reverted the transcriptional response to N and S deprivation and increased N and S contents in shoots of PPSB-deficient lines. Our work provides genetic evidence of the biological relevance of the Ser–Gly–1C metabolic network in N and S metabolism and in interorgan metabolic homeostasis.

IN A NUTSHELL

Background: Photorespiration, in which Rubisco adds oxygen to RuBP instead of adding carbon dioxide, thus lowering photosynthetic output, is a key metabolic pathway that will be affected by rising temperatures and increasing atmospheric CO₂ levels. For a long time, this pathway has been considered a wasteful process that consumes energy in the form of adenosine triphosphate (ATP) and reducing power. It also leads to the futile loss of carbon units as CO₂, consequently constraining plant growth and yield. However, recent studies suggest that future climate conditions, which may reduce photorespiratory activity, may also enhance plant biomass albeit at the cost of decreased crop nutritional value.

Question: Should we consider photorespiration only as a loss-inducing process?

Findings: Our research reveals the crucial role of glycine flux regulation by photorespiration in maintaining nitrogen, carbon, and sulfur balance in plants. Redirecting glycine flux out of the photorespiratory pathway has a positive effect on plant nitrogen and sulfur levels. Our findings shed light on the molecular mechanisms through which reduced photorespiration adversely affects crop nutritional value. In mammals, the serine–glycine–one-carbon metabolic network functions as a central integrator of nutrient status. We propose a similar role for this network in plants.

Next steps: Our findings open new avenues for enhancing crop nutritional quality under future climate change conditions, particularly with regard to nitrogen content, an essential component of proteins and nucleic acids. Validating the feasibility of this biotechnological approach will require additional experiments conducted in natural environments across different species and under varying conditions of temperature, light, and CO₂.

Introduction

The L-serine (Ser)–L-glycine (Gly)–one carbon (1C) metabolic network is required for the biosynthesis of nucleotides, proteins, and lipids. This network also drives the methyltransferase reactions governing epigenetic regulation and the supply of almost all methylated metabolites (Fig. 1). In mammals, the Ser–Gly–1C network supports the uninhibited proliferation of cancer cells and is associated with tumor progression (Locasale et al. 2011; Possemato et al. 2011; Pacold et al. 2016; Ducker and Rabinowitz 2017; Reina-Campos et al. 2019; Geeraerts et al. 2021). As a major 1C donor, Ser plays a crucial role in the regulation and function of Ser–Gly–1C metabolism in mammals (Locasale 2013; Yang and Vousden 2016) where it is mainly synthesized by the so-called phosphorylated pathway of Ser biosynthesis (PPSB) located in the cytosol. Ser biosynthesis in plants is much more complex and consists of 3 pathways. The first pathway, the glycolate pathway of Ser biosynthesis (GPSB), is associated with photorespiration and operates in mitochondria. In addition, there are 2 alternative nonrelated photorespiratory pathways: the PPSB (located in plastids) and the glycerate pathway (in peroxisomes).

In plants, the PPSB was long considered to be a minor pathway. However, functional characterization of PPSB genes demonstrated that this pathway is essential for male gametophyte and embryo development and for root growth (Benstein et al. 2013; Cascales-Miñana et al. 2013; Toujani et al. 2013; Wulfert and Krueger 2018). Cell proliferation and elongation analysis revealed that PPSB is indispensable for normal meristem development (Zimmermann et al. 2021). The plant PPSB defines a branch point for the 3-phosphoglycerate (3-PGA) produced in plastidial glycolysis and comprises 3 sequential reactions catalyzed by 3-PGA

dehydrogenase (PGDH), 3-phosphoSer aminotransferase (PSAT), and 3-phosphoSer phosphatase (PSP; Fig. 1) (Ho and Saito 2001). In *Arabidopsis thaliana* (Arabidopsis), 3 genes encode PGDHs (PGDH1, PGDH2, and PGDH3), 2 encode PSAT (PSAT1 and PSAT2), and one encodes PSP (PSP1) (Ros et al. 2014).

The GPSB has been regarded as the plant's most important Ser biosynthesis pathway due to the high flux borne by photorespiration (Tolbert 1997; Douce et al. 2001). The GPSB is the result of the oxygenase activity of Rubisco, which depends on the CO₂/O₂ ratio in the atmosphere (Ogren and Bowes 1971; Ogren 2003). This CO₂/O₂ ratio determines whether Rubisco acts as a carboxylase, producing 2 molecules of 3-PGA in the Calvin–Benson cycle, or as an oxygenase, producing one molecule of 3-PGA and one of the potent enzyme inhibitor 2-phosphoglycolate (2-PG). This 2-PG is recycled to 3-PGA through a series of enzymatic reactions of the photorespiratory cycle that takes place in the chloroplasts, peroxisomes, and mitochondria (Fig. 1). In this cycle, 2 molecules of 2-PG (2C each) are needed to recover one molecule of 3-PGA (3C), and one of the 4C atoms is released as CO₂. In addition, the photorespiratory cycle loses reduced nitrogen (N) as NH₃, which has to be reassimilated in a high energy-consuming pathway called the photorespiratory nitrogen cycle (Fig. 1). For this reason, photorespiration was long considered to be a futile cycle with the largest energy losses in plants (Bauwe et al. 2010; Peterhansel et al. 2010).

However, photorespiration integrates and participates in other pathways (Shi and Bloom 2021). First, photorespiration contributes to the pools of Gly and Ser. The photorespiratory Gly-decarboxylase complex (GDC) and Ser-hydroxymethyltransferase 1 (SHMT1) in the mitochondria of photosynthetic cells convert 2 Gly molecules into one Ser molecule (Fig. 1).

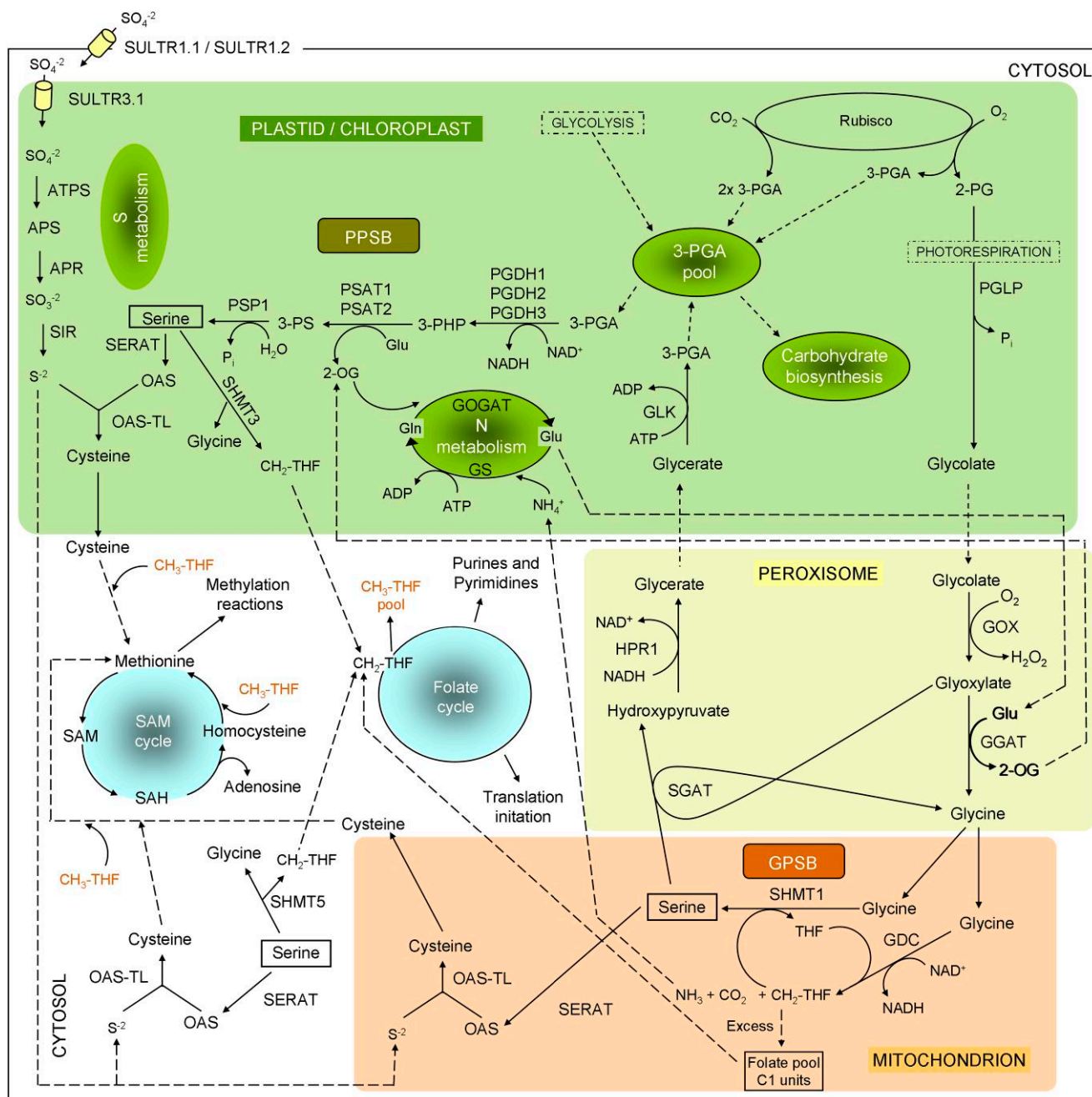


Figure 1. Schematic representation of the phosphorylated pathway (PSPB) and glycolate pathway (GPSB) of serine biosynthesis and putative interactions with other primary metabolic pathways. Enzymes and transporters: APR, APS reductase; ATPS, ATP sulfurylase; GDC, glycine-decarboxylase complex; GGAT, glutamate-glyoxylate aminotransferase; GLK, glycerate kinase; GOGAT, glutamine-oxoglutarate aminotransferase; GOX, glycolate oxidase; GS, glutamine-synthetase; HPR1, hydroxypyruvate reductase; OAS-TL, O-acetylserine-(thiol)-lyase; PGDH, 3-phosphoglycerate dehydrogenase; PGLP, 2-phosphoglycolate phosphatase; PSAT, 3-phosphoserine aminotransferase; PSP1, 3-phosphoserine phosphatase; SERAT, serine-acetyltransferase; SHMT, serine-hydroxymethyltransferase; SIR, sulfite reductase. Metabolites: 3-PHP, 3-phosphohydroxypyruvate; 3-PS, 3-phosphoserine; CH₃-THF, 5-methyl-THF; APS, adenosine 5'-phosphosulfate; C1, one-carbon metabolite; CH₂-THF, 5,10-methylene-THF; Gln, glutamine; Glu, glutamate; OAS, O-acetylserine; SAH, S-adenosylhomocysteine.

One Gly molecule is cleaved by GDC releasing CO₂ and NH₃. The remaining methylene C of Gly is transferred to tetrahydrofolate (THF) to form 5,10-methylene-THF (CH₂-THF) and NADH, which react with a second Gly to form Ser in a reaction catalyzed by SHMT (Fig. 1). The GDC complex activity

exceeds that of SHMT1 in vitro (Rebeille et al. 1994; Douce et al. 2001), which led to the assumption that CH₂-THF accumulates in the mitochondria relative to THF. Ser produced by SHMT in mitochondria and that synthesized by PSPB in plastids can be transported to the cytosol, where they can also be

used to produce 1C-units by the reverse reaction catalyzed by the cytosolic SHMTs (Mouillon et al. 1999; Engel et al. 2007). Cytosolic 1C units are employed for methionine (Met) biosynthesis, which is then incorporated into proteins or used for the production of the universal methyl donor S-AdenosylMet (SAM) (Fig. 1). Moreover, 1C units are also utilized to synthesize purines and pyrimidines (Rébeillé et al. 2006; Hanson and Gregory 2011; Gorelova et al. 2017; Mohanta et al. 2019). Although the SHMT1/GDC activity has been considered to be the major source of 1C units for plants (Bauwe and Kolukisaoglu 2003), genetic and metabolic evidence about the contribution of these photorespiratory reactions to the folate and SAM cycles is missing. Moreover, the contribution of the PPSB-derived Ser to 1C-folate metabolism is unknown.

As the oxygenase activity of Rubisco depends on the CO₂/O₂ ratio, the supply of Ser–Gly–1C by GPSB to plants will be influenced by increases in the atmospheric CO₂ concentration resulting from anthropogenic activity. Furthermore, as Ser is recycled by photorespiration to 3-PGA (Fig. 1), the absolute contribution of GPSB to the supply of Ser for plant metabolism and development remains controversial. Modeling and isotopic labeling studies estimated that under ambient CO₂ (aCO₂) concentrations, a substantial fraction of the photorespiratory Gly and Ser is not recycled back to 3-PGA, but it is used in other metabolic pathways as precursors of molecules required for growth, such as nucleotides or proteins (Abadie et al. 2016; Busch et al. 2018; Fu et al. 2023). However, under elevated CO₂ (eCO₂) growth conditions, when GPSB activity is restricted, increase in Arabidopsis biomass provided that PPSB remains functional, indicating that the PPSB-derived Ser contributes more to plant growth than the GPSB-derived Ser (Zimmermann et al. 2021). These findings highlight the complexity of the interaction of both pathways and indicate that their contribution to other metabolic networks is not completely understood. In this regard, it has been described that photorespiration and PPSB may contribute to N and sulfur (S) assimilation (Rachmilevitch et al. 2004; Bloom et al. 2010; Abadie et al. 2016; Busch et al. 2018; Samuilov et al. 2018a, 2018b; Abadie and Tcherkez 2019; Anoman et al. 2019; Zimmermann et al. 2021). The role of PPSB and GPSB in these metabolic networks may be especially important for crops in a scenario in which the continuous increase in atmospheric CO₂ concentrations may reduce photorespiration and thus limit crop nutritional quality (Bloom et al. 2010; Bloom et al. 2012, 2014; Jauregui et al. 2015, 2016; Walker et al. 2016). Nonetheless, the connections between PPSB and GPSB with N and S metabolism and especially their biological relevance in terms of their contribution to overall plant nutrient status and development remain unclear.

In this study, we aimed to clarify the relative contributions of both PPSB and GPSB to Ser–Gly–1C biosynthesis and to gain insight into how they interact with N, S, and C metabolism. Previous attempts to block both PPSB and photorespiration have been unsuccessful. Therefore, studies were

conducted using PPSB-deficient lines under conditions that favored or disfavored photorespiration (Zimmermann et al. 2021). Here, we used a combination of PPSB-deficient and SHMT1-deficient lines. In this way, we could bypass the reaction producing Ser in the GPSB without short-circuiting the photorespiratory cycle. We show that the regulation of Ser/Gly flux by PPSB and GPSB affects N, C, and S homeostasis. Our work provides genetic evidence of the biological significance of the Ser–Gly–1C metabolic network in N and S metabolism and in organ developmental patterns in Arabidopsis. Our study unravels essential steps of photorespiration, which might be used to develop new crops with higher nutritional value.

Results

Interactions of PPSB and GPSB modify the developmental pattern of aerial parts/roots of plants

Figure 2 shows that both *PGDH1*- and *PSP1*-deficient Arabidopsis lines (*c-psp1* and *c-pgdh1*), with low background levels of *PGDH1* and *PSP1*, respectively (Cascales-Miñana et al. 2013; Casatejada-Anchel et al. 2021), displayed more dramatic growth phenotypes under eCO₂, where GPSB activity is reduced, than under aCO₂ (Fig. 2, A and B), which corroborates the notion that PPSB and GPSB cooperate in supplying Ser for plant growth (Zimmermann et al. 2021). Accordingly, *PGDH1* and *PGDH2*, the 2 main *PGDH* family genes (Casatejada-Anchel et al. 2021), were induced in shoots of wild-type (WT) plants under eCO₂ growth conditions (Fig. 2C). Under these conditions, *PGDH1* and especially *PGDH2* were also induced in *c-psp1* lines, as well as *PGDH2* in *c-pgdh1* lines, suggesting the general upregulation of the PPSB in response to *PSP1* or *PGDH1* inactivation (Fig. 2D). However, *SHMT1* was repressed in PPSB-deficient lines. Besides, neither *PGDH1* nor *PGDH2* was induced in the roots of a mutant of *SHMT1* (*shmt1.2*, henceforth *shmt1*) in which *PGDH2* was even repressed. These results indicate that the interactions between the 2 Ser biosynthetic pathways are complex.

In the first attempt to complement the growth phenotype of PPSB-deficient lines, we blocked photorespiration at the level of serine-glyoxylate aminotransferase (SGAT), which is the peroxisomal enzyme that converts Ser into hydroxypyruvate in the photorespiratory cycle (Fig. 1). SGAT could be a key point in the control of the Ser pool since it was shown that *sgat* mutants have increased Ser levels and that SGAT overexpression reduces Ser levels (Somerville and Ogren 1980; Modde et al. 2017). In our experimental conditions, the *sgat1* mutants accumulated more Ser than WT when grown under aCO₂ and eCO₂ conditions (Supplemental Fig. S1). However, the *sgat1* mutation in the *PGDH*-silenced lines was lethal.

The finding that *SHMT1* was repressed in PPSB-deficient lines led us to introduce the *shmt1* mutation into the PPSB-deficient mutant background (*shmt1 c-psp1* and

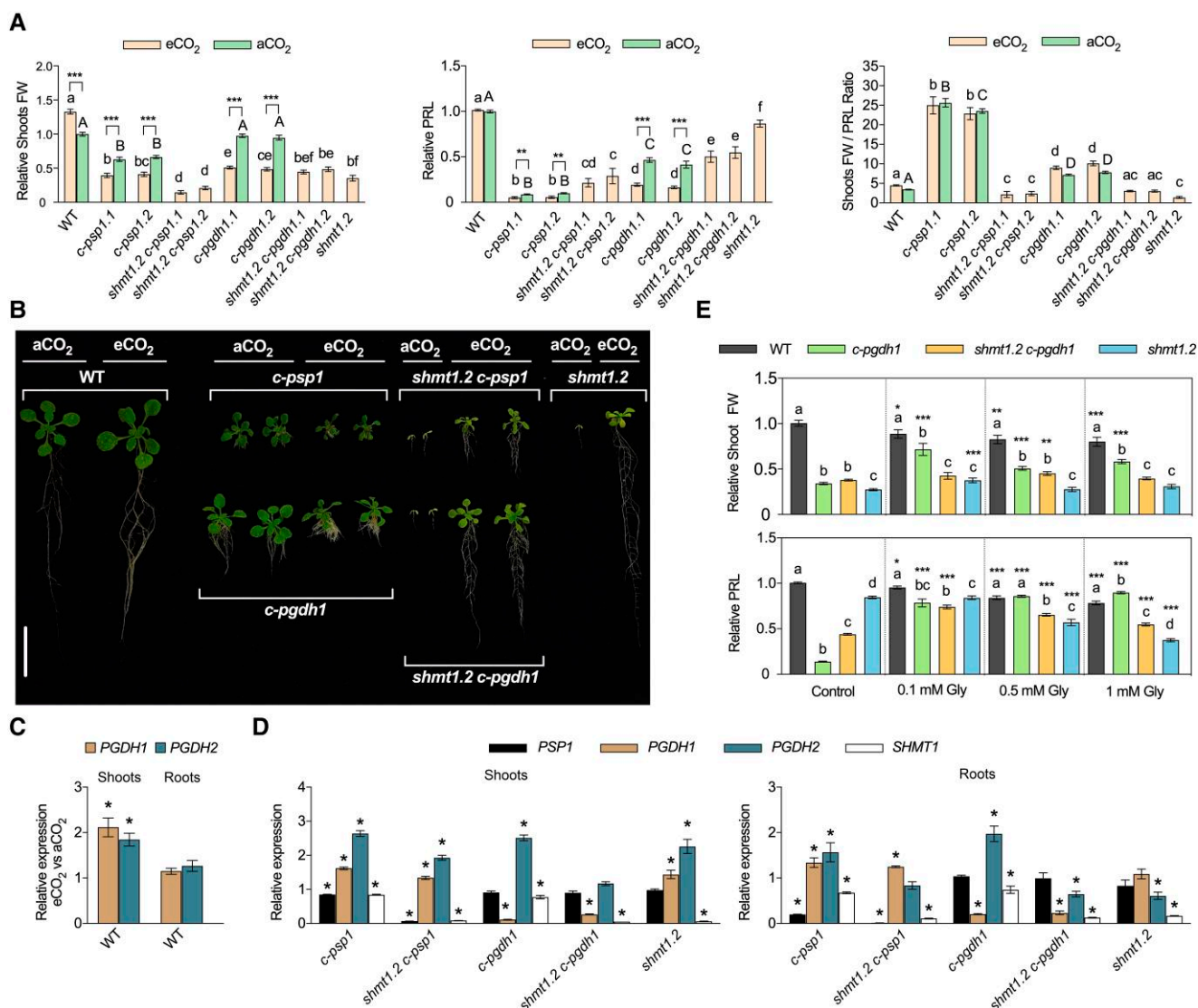


Figure 2. Characterization of PPSB-deficient (*c-psp1* and *c-pgdh1*) and SHMT1-deficient (*shmt1.2*, *shmt1.2 c-psp1*, and *shmt1.2 c-pgdh1*) lines. **A)** Relative shoot fresh weight (FW), primary root length (PRL), and shoot FW/PRL ratio of different lines grown under aCO₂ or eCO₂ conditions compared to WT plants. **B)** Photograph of representative individuals of each line grown under aCO₂ or eCO₂ conditions. **C)** PGDH1 and PGDH2 expression in shoots and roots of WT lines under eCO₂ compared with aCO₂ conditions. **D)** PSP1, PGDH1, PGDH2, and SHMT1 expression in different lines grown under eCO₂ compared with WT. **E)** Relative shoot FW and PRL of different lines grown under eCO₂ supplemented with Gly. In **A)** and **E)** (mean $\pm$ SE, $n \geq 7$; data represent the mean of at least 10 plants), values are normalized to the mean calculated for the WT under aCO₂ **A)** or eCO₂ conditions **E)**; different letters indicate significant differences between lines ($P < 0.05$) under the same growth conditions; significant differences between growth conditions, as determined by Student's *t*-test, are denoted by * ($P < 0.05$), ** ($P < 0.01$), or *** ($P < 0.001$). **C** and **D)** Values (mean $\pm$ SE; $n \geq 4$ independent biological replicates of pools of 40 plants) are normalized to the gene expression under aCO₂ conditions **C)** or to the WT background **D)**; significant differences between aCO₂ and eCO₂ gene expression **C)** or between mutants and WT **D)**, as determined by Student's *t*-test, are denoted by * ($P < 0.05$). Scale bar = 2 cm **B)**.

shmt1 c-pgdh1). In contrast to PPSB-deficient lines, PGDH2 expression was no longer induced in *shmt1 c-pgdh1* shoots and *shmt1 c-psp1* roots, while it was even repressed in the roots of *shmt1 c-pgdh1* lines, indicating a general downregulation of the PPSB pathway in these mutant backgrounds. We characterized single and double mutants under eCO₂ and aCO₂ whenever possible. In single mutants, the PPSB deficiency (*c-psp1* and *c-pgdh1*) mainly affected primary root growth, while the SHMT1 mutation mostly affected shoot

growth (Fig. 2, A and B). For instance, under aCO₂, the shoot fresh weight of the *c-pgdh1* lines was unchanged, while the primary root length was reduced by more than 50% compared with WT. Under eCO₂, the same growth trend, albeit more exacerbated, was observed for both *c-psp1* and *c-pgdh1*. Under these conditions, *shmt1* showed a 65% reduction in shoot biomass, while the primary root length was only reduced by 13%. The *shmt1* mutation in PPSB-deficient lines maintained (*shmt1 c-pgdh1*) or reduced (*shmt1 c-psp1*) shoot

growth versus their respective PPSB-deficient lines (*c-pgdh1* or *c-psp1*). Interestingly, the primary root length recovered in the double mutants between 2.6-fold and 5.2-fold longer than in the respective PPSB-deficient lines. These results indicate a change in the shoot/root developmental pattern in the double mutants, as quantified by the shoot fresh weight/primary root length ratio (Fig. 2A). This ratio was always higher in *c-pgdh1* than in the *shmt1 c-pgdh1* lines, while the lowest shoot fresh weight/primary root length ratio was found in the *shmt1* line (Fig. 2A). Altogether, phenotypic characterization and expression data suggest that Ser homeostasis might be modified in the double-mutant lines.

Disrupting SHMT1 profoundly alters N and C metabolism in PPSB-deficient lines

To investigate the effect of disrupting *SHMT1* on central metabolism, we measured the major C and N metabolites in plants grown under eCO₂ conditions under which SHMT1-deficient lines are viable (Fig. 3). Glycolate measurements in shoots, a key metabolite at the beginning of the photorespiratory pathway and the product of the metabolization of toxic 2-PG, showed that its content in *shmt1 c-pgdh1* and *shmt1* was not higher than in WT or *c-pgdh1* lines (Supplemental Fig. S2). This result suggests that metabolites upstream of glycolate in the photorespiratory pathway, such as 2-PG, did not accumulate more in mutants with a *shmt1* background than in other lines under the low photorespiratory growth conditions used in this work (eCO₂ and 100 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light intensity). However, principal component analysis (PCA) of metabolites revealed clear differences between lines (Fig. 3, A and D). The metabolite whose levels differed most strongly between lines in shoots and roots was the amino acid Gly. Like the *shmt1* parental lines, double *shmt1* PPSB-deficient lines showed a dramatic increase in Gly levels in shoots and roots compared with the other lines. These lines also displayed higher Ser levels than their respective single PPSB-deficient lines, although the changes were not as dramatic as for Gly. In fact, the common pattern of all SHMT1-deficient lines (*shmt1*, *shmt1 c-psp1*, and *shmt1 c-pgdh1*) had a much higher shoot Gly/Ser ratio than WT or PPSB-deficient lines, suggesting that Gly metabolism was more strongly altered than the Ser metabolism in these lines (Fig. 3B). Therefore, diverting the photorespiratory flux before Ser biosynthesis by SHMT1 changes the Gly/Ser ratio in shoots. Gly-feeding experiments confirmed that this amino acid can complement the root growth phenotypes of PPSB-deficient mutants (Fig. 2E). However, externally supplied Gly differently affected the root growth response, depending on the mutant background and the concentration. Thus, Gly concentrations of 0.5 mM or higher clearly inhibited primary root growth compared with controls without Gly supplementation in WT and *shmt1* plants, especially the latter. However, these Gly concentrations still had a positive effect on the primary root growth of *c-pgdh1* lines. These results indicate that greater levels of external Gly are required in PPSB-deficient lines.

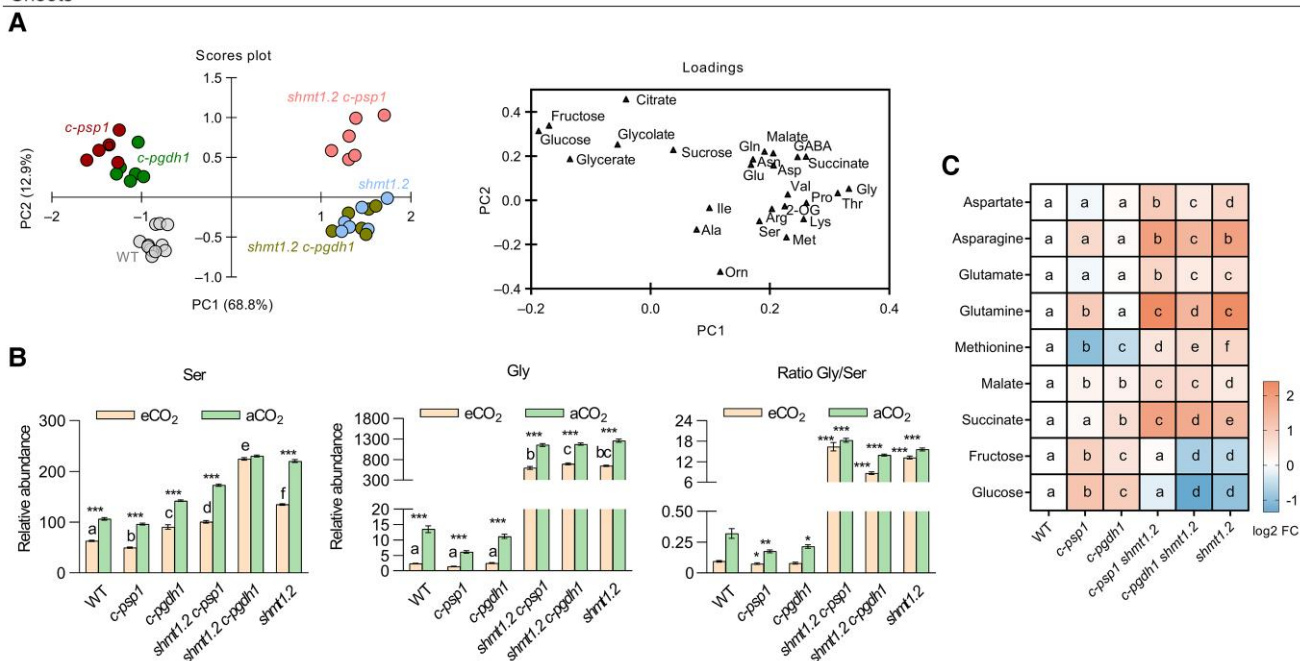
Other major metabolite changes between lines were the accumulation of transport amino acids, such as glutamate (Glu), glutamine (Gln), and aspartate (Asp), and Asp-derived amino acids, such as asparagine (Asn) and Met in shoots of SHMT1-deficient lines compared with WT and PPSB-deficient lines (Fig. 3, A and C). By contrast, the levels of soluble sugars such as glucose and fructose were reduced in these lines: in roots, Glu, Gln, Asp, and Asn no longer accumulated in SHMT1-deficient versus PPSB-deficient lines (Fig. 3F). Conversely, some of these amino acids (Gln and Asn) and others like alanine overaccumulated in PPSB-deficient lines. As in shoots, glucose and fructose levels decreased in the roots of SHMT1-deficient lines, with a clear opposite trend to that in the PPSB-deficient lines. These differences in the amino acids and sugar profiles between shoots and roots of PPSB- and SHMT1-deficient lines point to changes in N and C homeostasis between organs in different mutant backgrounds.

To more precisely identify how Ser–Gly homeostasis in shoots and roots was affected by the lack of SHMT1 activity in the PPSB-deficient background, we shifted plants grown under eCO₂ to aCO₂ conditions, where photorespiration is much more active (Fig. 3B; Supplemental Fig. S3). In WT shoots, as expected, the Ser and Gly levels dropped when plants were grown under eCO₂ conditions compared with aCO₂ (Fig. 3B; Supplemental Fig. S3). The Gly/Ser ratio indicated that the drop of Gly levels in shoots under eCO₂ was much greater than that of Ser in both PPSB-deficient and WT lines (Fig. 3B). Other relevant changes found in the shoots of SHMT1-deficient lines shifted from eCO₂ to aCO₂ conditions were strong increases in Glu and Asp levels and decreases in 2-oxoglutarate (2-OG) levels (Supplemental Fig. S3). In roots, Gly and Ser levels also decreased in all lines transferred from eCO₂ to aCO₂ conditions, and these changes were more drastic in SHMT1-deficient lines (Fig. 3E; Supplemental Fig. S3). The shift from eCO₂ to aCO₂ primarily affected amino acid homeostasis in roots (Supplemental Fig. S3). In addition to the above-mentioned Gly, the transport amino acids Glu, Gln, Asp, and Asn were more drastically affected in the SHMT1-deficient mutants than in other lines, confirming the influence of photorespiration on amino acid status in roots (Supplemental Fig. S3). Overall, these results indicate that SHMT1 activity has clear consequences for amino acid metabolism and distribution between roots and shoots.

Root phenotypes in PPSB-deficient lines are dependent on SHMT1/GDC activity in shoots

SHMT1 is mainly expressed in shoots. We performed grafting experiments to elucidate the role of shoots in the changes in root developmental patterns in the mutants. Using the *shmt1 c-psp1* shoot as the scion, the root growth of the *c-psp1* stock was rescued (Fig. 4; Supplemental Fig. S4), confirming that the changes in developmental patterns in the *shmt1* PPSB-deficient lines are shoot-dependent.

Shoots



Roots

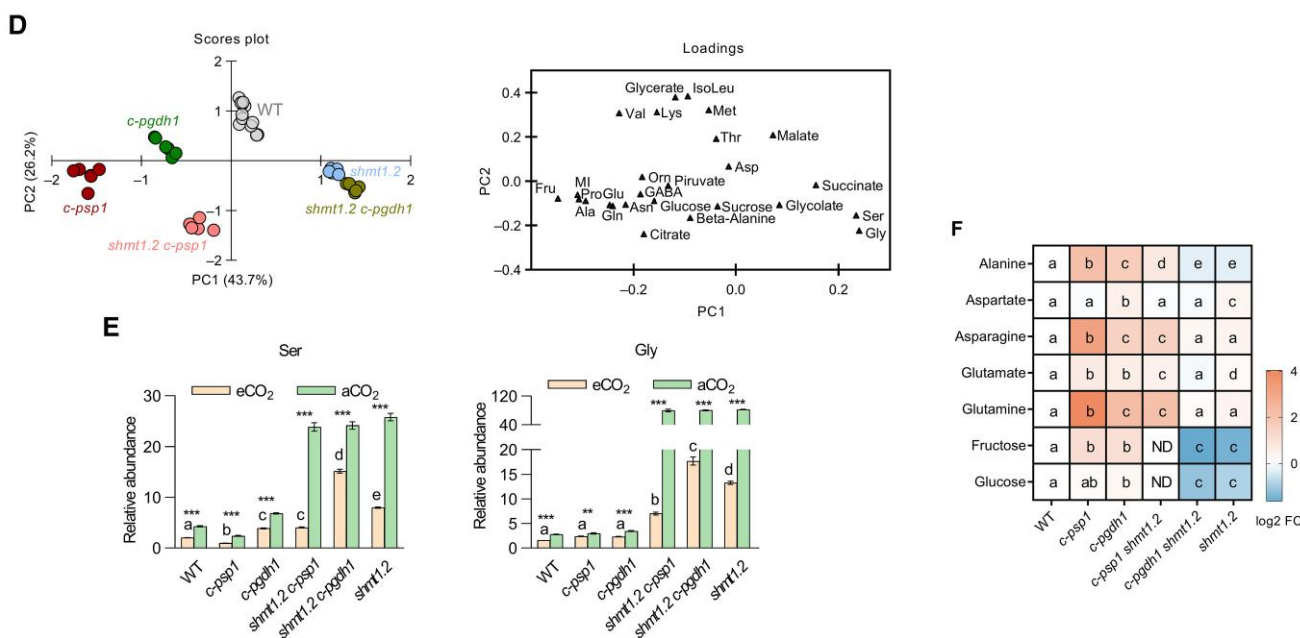


Figure 3. Metabolite profiles of WT, PPSB-deficient (*c-psp1* and *c-pgdh1*), and SHMT1-deficient (*shmt1.2*, *shmt1.2 c-psp1*, and *shmt1.2 c-pgdh1*) lines grown under eCO₂ conditions. **A** and **D**) PCA and loading plots of metabolites in shoots **A**) and roots **D**). Data from GC-MS analysis were evaluated using PCA with the 2 first components accounting for at least 70% of total metabolic variance. Values in parenthesis give the relative contribution of each component to the total variance observed in the dataset. **B** and **E**) Relative Gly and Ser content in shoots **B**) and roots **E**) of different mutant backgrounds under eCO₂ conditions or after a 24 h shift to aCO₂ conditions compared with WT. **C** and **F**) Heat map showing most relevant changes in the metabolite contents of shoots **C**) and roots **F**) under eCO₂ conditions. Values represent the mean $\pm$ SE, $n \geq 6$ of pools of 40 plants from 2 different lines for each genotype; different letters indicate significant differences between lines ($P < 0.05$) under eCO₂ conditions; significant differences between the same line under aCO₂ and eCO₂ conditions, as determined by Student's *t*-test, are denoted by * ($P < 0.05$), ** ($P < 0.01$), and *** ($P < 0.001$). In the Gly/Ser ratio, lines are compared with the WT under the same growth conditions.

To investigate the specific role of SHMT1 activity in the observed developmental patterns, we introduced the *SHMT2* mutation (*shmt2.2*, henceforth *shmt2*) into the PPSB-

deficient lines. SHMT2 is the second mitochondrial SHMT isoform in Arabidopsis. Its activity represents only a very small fraction of total SHMT activity in shoots, which is

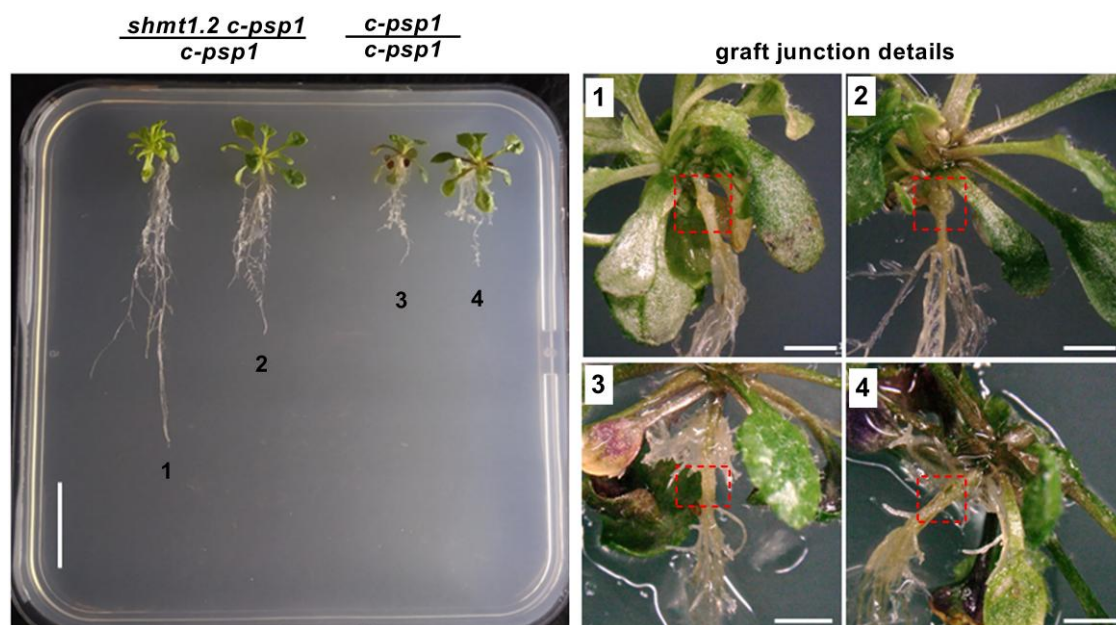


Figure 4. Grafting of scion *shmt1.2 c-psp1* shoots onto *c-psp1* roots under eCO_2 . Squares mark the junction between shoots and roots. Scale bars = 2 cm (left) and 2 mm (right).

confined to vascular tissues (Engel et al. 2011) (<http://www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi>). *shmt2 c-pgdh1* lines were viable under aCO_2 and eCO_2 conditions, but the *SHMT2* mutation did not rescue the inhibited primary root length of *c-pgdh1* and even enhanced it under aCO_2 (Supplemental Fig. S5). The most important difference between the *shmt1 c-pgdh1* and *shmt2 c-pgdh1* mutants was the increase in Ser and especially Gly contents in the shoots of *shmt1 c-pgdh1* (Fig. 3B; Supplemental Fig. S5). Overall, these data demonstrate that the change in the developmental patterns observed in *shmt1* PPSB-deficient lines is related to the lack of photorespiration-associated *SHMT1* activity in shoots.

¹⁵N labeling experiments confirm that Gly is prioritized over Ser in the double mutants, affecting the Ser–Gly–1c

The ^{15}N -labeling experiments (Fig. 5) showed that the de novo incorporation of ^{15}N into Gly was much greater in *SHMT1*-deficient lines than in WT and *c-pgdh1* (Fig. 5, A and B). However, the de novo incorporation of ^{15}N into Ser was higher in *c-pgdh1* than in *SHMT1*-deficient lines, especially in roots. The enrichment of both Gly and Ser, a useful measure of the turnover rate, was lower in the *SHMT1*-deficient lines than in WT or *c-pgdh1*. However, the enrichment of Ser in *SHMT1*-deficient lines was much lower than that of Gly (Fig. 5, A and B). Thus, much more Gly is incorporated and is metabolized more quickly than Ser in *SHMT1*-deficient lines. These data, plus the higher Ser steady-state values, suggest lesser Ser flux into other metabolites in the *SHMT1*-deficient mutants.

Considering that the differences between the lines with and without *SHMT1*-deficiency were mostly in the Gly incorporation rate rather than in enrichment, our results suggest that a considerable amount of Gly not being metabolized by *SHMT1* is diverted to other metabolic reactions in the mutants. These reactions could include other *SHMT* isoforms, such as the plastidial *SHMT3*, whose gene was upregulated in *shmt1 c-pgdh1* shoots, or enhanced GDC activity, as deduced by the induction of some genes encoding isoforms of the GDC complex (*GLDP1* and *GLDP2*) in *shmt1 c-pgdh1* (Fig. 6). In contrast to *shmt1 c-pgdh1*, both GDC (*GLDP1* and *GLDP2*) and *SHMT1* gene expression was repressed in *c-pgdh1* (Figs. 2D and 6C). These results suggest that Gly could also be taken out of the photorespiratory cycle in mitochondria of this mutant at a higher rate than in WT and might be converted to Ser in other compartments or organs to compensate for the Ser deficiency. This notion was substantiated by the greater Gly enrichment found in *c-pgdh1* shoots, which indicates a higher Gly metabolization rate (Fig. 5A).

The ^{15}N flux measurements also indicated that Glu did not display a higher de novo biosynthesis rate in *SHMT1*-deficient versus PPSB-deficient shoots (Fig. 5A), suggesting that Glu accumulation observed at steady state might be due to lower turnover caused by inhibited photorespiratory flux. However, the de novo ^{15}N label incorporation into Asp and Asp-derived amino acids such as Met was greater in *SHMT1*-deficient than in PPSB-deficient shoots. Unlike shoots, ^{15}N flux analysis in roots indicated greater Asp and Glu biosynthesis in *c-pgdh1* than in *shmt1 c-pgdh1* (Fig. 5B), which could explain the amino acid accumulation in the roots of PPSB-deficient lines.

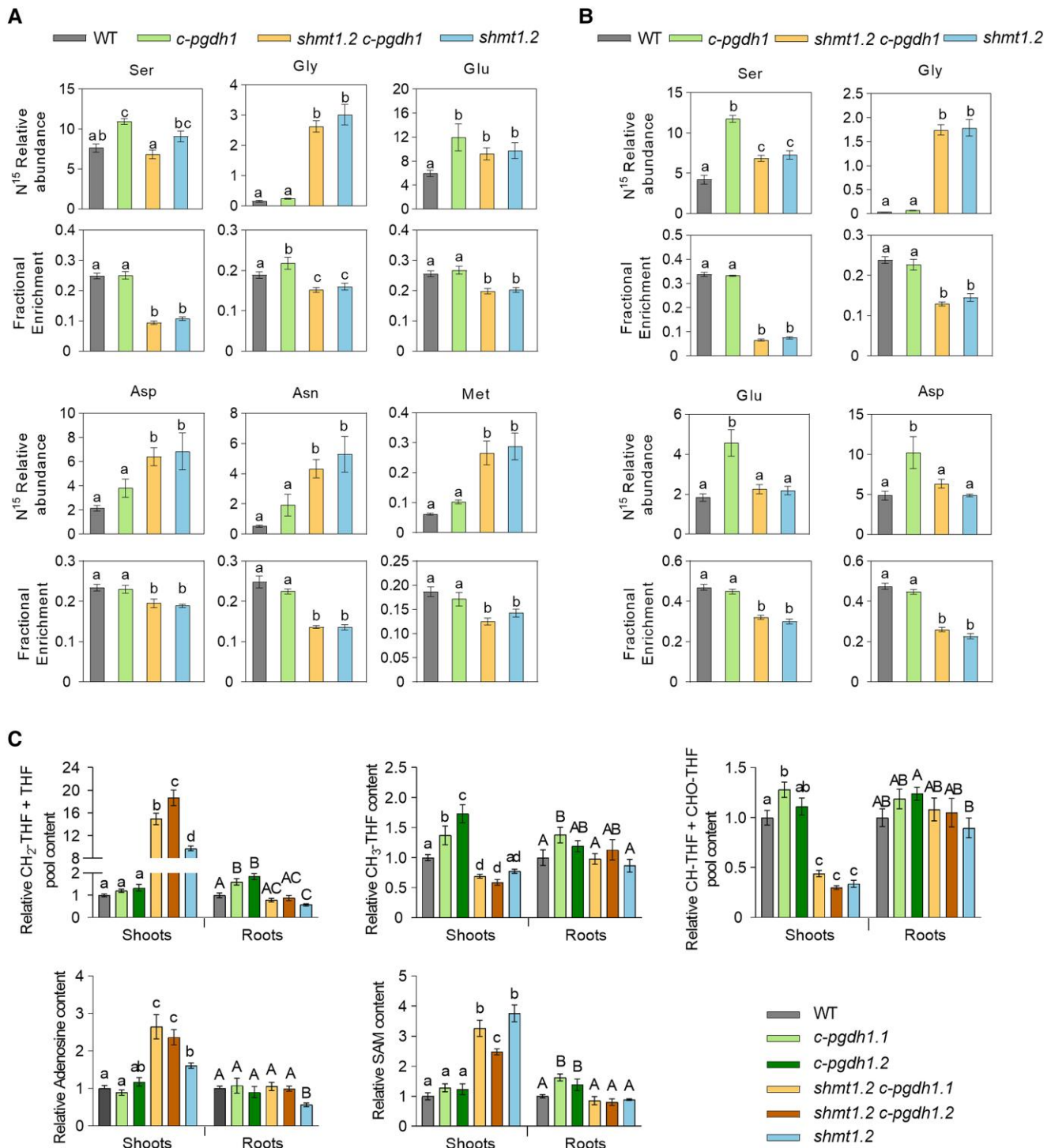


Figure 5. SHMT1-deficient lines (*shmt1.2* and *shmt1.2 c-pgdh1*) incorporate more Gly and Asp-family amino acids and synthesize more folates and SAM intermediates than PPSB-deficient (*c-pgdh1*) lines. **A** and **B**, Quantification of ^{15}N -labeled major amino acids in PPSB- and SHMT1-deficient lines grown under eCO₂ conditions; values of shoots **A**) and roots **B**) are shown as relative abundance (upper panel) and fractional enrichment (lower panel). **C**) Relative contents of folate and SAM cycle intermediates in the shoots and roots of different mutant backgrounds grown under eCO₂ conditions compared with WT. In **A**) and **B**), values represent the mean $\pm$ SE, $4 \leq n \leq 10$ pools; data represent the mean of at least 45 plants from 2 different lines for each genotype. In **C**), values represent the mean $\pm$ SE, $n \geq 6$ pools data represent the mean of at least 40 plants. Different letters indicate significant differences between lines ($P < 0.05$).

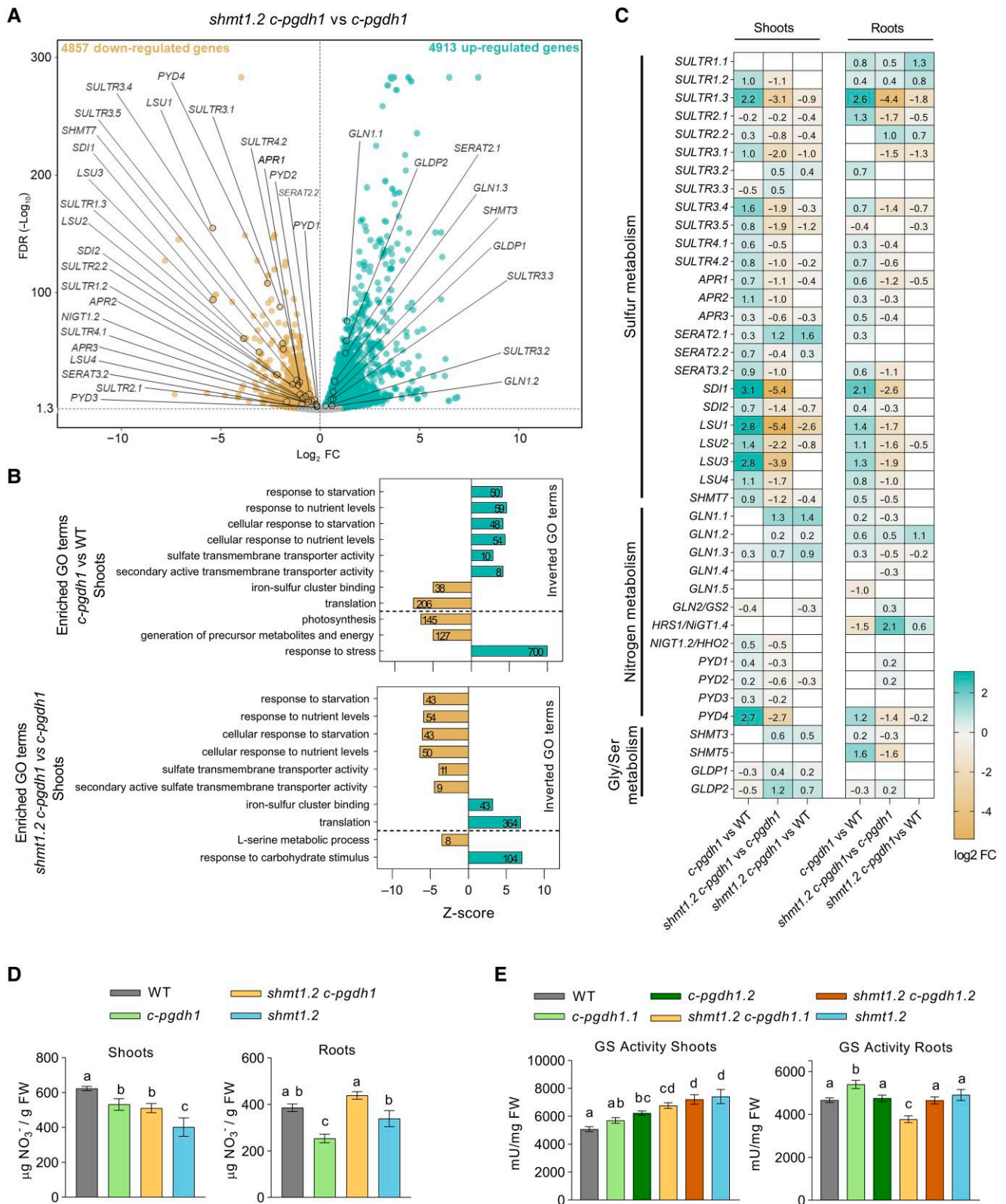


Figure 6. Transcriptomics profiles of PPSB-deficient (*c-pgdh1*) and SHMT1-deficient (*shmt1.2 c-pgdh1*) lines under eCO₂. **A**) Volcano plots for differentially expressed genes between *shmt1.2 c-pgdh1* and *c-pgdh1* in shoots. Brown and green dots represent upregulated and downregulated genes, respectively (FDR < 0.05). **B**) Functional category analysis for transcriptional responses in shoots. In each bar, the number of genes differently upregulated or downregulated is shown. **C**) Heat map showing the most relevant changes in the expression of genes related to Gly/Ser, sulfur, and nitrogen metabolism in shoots and roots. Numbers inside boxes stand for log-fold change. **D**) Nitrate (NO₃⁻) content in shoots and roots of different lines. **E**) Glutamine synthetase (GS) activity in shoots and roots of different lines. Values represent the mean ± SE, 4 ≤ n ≤ 8 pools of 40 plants from 2 different lines for each genotype; different letters indicate significant differences between lines (P < 0.05).

Briefly, the most important difference between lines is the higher Gly flux in shoots and roots of SHMT1-deficient lines. The increased Gly flux in shoots of SHMT1-deficient lines is channeled into Asp family amino acids, especially Asn and Met, linking N with S and folate metabolism.

Folate and SAM cycles are affected by changes in Ser/Gly flux

Because Ser and Gly participate in 1C-folate metabolism via SHMT and GDC enzymes, we measured steady-state folate pools. The most striking difference between the lines was the 15-fold higher CH₂-THF + THF pool size in *shmt1 c-pgdh1* shoots compared with the WT (Fig. 5C). These values are consistent with higher GDC activity in *shmt1 c-pgdh1* lines. No method is currently available to distinguish between CH₂-THF and THF in plant tissues. However, the increase in the CH₂-THF + THF pool in *shmt1* and *shmt1 c-pgdh1* probably reflects a rise in the level of CH₂-THF, the product of GDC activity, because *shmt1* mutants are unable to convert the CH₂-THF produced by GDC into Ser and THF in the mitochondria. Other minor changes were found in the folate pool of *shmt1 c-pgdh1* shoots, i.e. a reduction in the pools of 5-methyl-THF (CH₃-THF) and 5,10-methenyl-THF (CH-THF) + 10-formyl-THF (HCO-THF), with these metabolite levels showing an opposite trend to that in *c-pgdh1* shoots. Differences in folate pools between lines were much smaller in roots than in shoots. The CH₂-THF + THF pool decreased in *shmt1* roots but increased in *c-pgdh1* roots (Fig. 5C). However, no major changes appeared in the folate levels of *shmt1 c-pgdh1* lines, probably due to the balancing of the opposite effects from the 2 single mutants.

The changes in the folate pool found between lines and organs could affect other pathways like the SAM cycle because the methyl moiety of SAM is derived from 1C-folates. The levels of SAM and adenosine, another product of the SAM cycle, increased 2-fold to 3-fold in shoots of SHMT1-deficient lines (Fig. 5C). Increased SAM cycle activity could also explain the depletion of CH₃-THF, which is the donor of 1C groups for SAM, in SHMT1-deficient lines. Briefly, changes in Ser/Gly flux strongly affected folate and SAM metabolism, modifying the metabolite equilibrium between roots and shoots and affecting S-metabolism.

Transcriptomic analysis confirms alterations of the N–C–S networks between shoots and roots of *c-pgdh1* and *shmt1 c-pgdh1* lines

In agreement with previous results, PCA of the transcriptomic data indicated that the main differences between lines appeared in shoots and not in roots (Supplemental Fig. S6). When comparing *c-pgdh1* with *shmt1 c-pgdh1* shoots, 4,857 genes were downregulated and 4,913 were upregulated (Fig. 6A). Compared with WT, in *c-pgdh1* shoots, several categories of genes related to response to nutrient level and starvation were upregulated, including marker genes for S and N deficiency (Fig. 6B). When comparing *shmt1 c-pgdh1*

with *c-pgdh1*, however, genes in the same categories that respond to nutrient levels were downregulated. In shoots of *c-pgdh1*, there was a general induction of genes encoding enzymes participating in pyrimidine catabolism, the so-called PYD genes, which respond to N deficiency (Fig. 6C; Supplemental Fig. S7). In particular, PYD4, the last gene of the pyrimidine catabolic pathway, was strongly upregulated in *c-pgdh1* lines and strongly repressed in *shmt1 c-pgdh1*. PYD genes are induced under N-limited conditions to use pyrimidines as an N source (Zrenner et al. 2009; Witte and Herde 2020). Accordingly, downregulation of genes encoding enzymes participating in the biosynthesis of pyrimidine intermediates occurred in *c-pgdh1* shoots compared with WT, while the opposite trend was noted when comparing *shmt1 c-pgdh1* lines versus *c-pgdh1* (Supplemental Fig. S7).

In roots, the *HSR1/NIGT1.4* was strongly downregulated in *c-pgdh1* but upregulated in *shmt1 c-pgdh1* compared with the WT (Fig. 6C). *HSR1/NIGT1.4* is a member of the NIGT clade that is specifically expressed in roots, encoding a transcriptional repressor of the NO₃[−] transporter gene *NTR2.1* (Maeda et al. 2018; Ueda et al. 2020). The downregulation of *HSR1/NIGT1.4* in *c-pgdh1* roots suggests that NO₃[−] uptake was activated as a response to N deficiency. Accordingly, the NO₃[−] content in *c-pgdh1* roots was approximately 35% lower than in WT (Fig. 6D), while the NO₃[−] content in *shmt1 c-pgdh1* roots was significantly higher than that of *c-pgdh1* and similar to that of WT (even a 14% increase was observed). Another NIGT clade member, *NIGT1.2*, was upregulated in *c-pgdh1* shoots and downregulated in *shmt1 c-pgdh1* shoots compared with *c-pgdh1*. These data show that NO₃[−] signaling between shoots and roots is differentially altered in PPSB-deficient and SHMT1-deficient lines, probably reflecting a change of NO₃[−] allocation between the organs. The gene expression pattern of glutamine synthetase (GS) isoforms was also altered in *c-pgdh1* versus *shmt1 c-pgdh1*. Major changes occurred in the cytosolic GS isoforms *GLN1.1*, *GLN1.2*, and *GLN1.3*, showing an opposite expression pattern between shoots and roots (Fig. 6C).

NH₄⁺ content showed a similar increase in shoots of both *c-pgdh1* and *shmt1 c-pgdh1* mutant lines compared with WT (Supplemental Fig. S8). To determine whether this higher NH₄⁺ content was related to changes in a biosynthetic process, we measured GS activity. Compared with the WT, GS activity was greater in *shmt1 c-pgdh1* shoots, but similar to or even lower than that in *c-pgdh1* roots (Fig. 6E). These results indicate that N signaling between shoots and roots is altered in *shmt1 c-pgdh1* compared with *c-pgdh1*, confirming that SHMT1 activity can profoundly affect N homeostasis.

Genes encoding sulfate transporters (SULTRs) and proteins involved in S metabolism in general were downregulated in *shmt1 c-pgdh1* shoots compared with *c-pgdh1* (Fig. 6B). Thus, genes described as S deficiency markers, such as *SHMT7*, *LSU1*, *LSU2*, *LSU3*, *LSU4*, *SDI1*, and *SDI2*, which were upregulated in *c-pgdh1* shoots and roots compared with WT, showed the opposite trend in *shmt1 c-pgdh1*. Some of these genes (*LSU1*, *LSU2*, *SHMT7*, and *SDI2*) showed

Table 1. Carbon (C), nitrogen (N), and sulfur (S) contents (mg/g dry weight) in shoots and roots of WT, PPSB-deficient (*c-pgdh1*), and SHMT1-deficient (*shmt1.2 c-pgdh1*, *shmt1.2*) lines growth under eCO₂ conditions

genotype	C	N	S	N/C ratio	S/C ratio
Shoots					
WT	400.1 ± 0.6 ^a	67.86 ± 0.27 ^a	9.12 ± 0.15 ^a	0.170 ± 5.49·10 ⁻⁴ a	0.023 ± 4.03·10 ⁻⁴ a
<i>c-pgdh1</i>	419.9 ± 0.7 ^b	62.80 ± 0.41 ^b	7.37 ± 0.12 ^b	0.150 ± 9.04·10 ⁻⁴ b	0.018 ± 3.08·10 ⁻⁴ b
<i>shmt1.2 c-pgdh1</i>	390.5 ± 1.0 ^c	70.69 ± 0.29 ^c	10.84 ± 0.10 ^c	0.181 ± 7.75·10 ⁻⁴ c	0.028 ± 3.26·10 ⁻⁴ c
<i>shmt1.2</i>	397.2 ± 3.0 ^a	69.90 ± 0.26 ^c	11.57 ± 0.26 ^d	0.176 ± 3.42·10 ⁻³ c	0.029 ± 8.17·10 ⁻⁴ c
Roots					
WT	418.8 ± 0.7 ^a	51.75 ± 0.34 ^a	7.30 ± 0.09 ^a	0.124 ± 7.68·10 ⁻⁴ a	0.017 ± 2.25·10 ⁻⁴ a
<i>c-pgdh1</i>	427.9 ± 0.8 ^b	54.92 ± 0.16 ^b	9.77 ± 0.07 ^b	0.128 ± 5.18·10 ⁻⁴ b	0.023 ± 1.36·10 ⁻⁴ b
<i>shmt1.2 c-pgdh1</i>	403.8 ± 0.1 ^c	57.45 ± 0.40 ^c	8.97 ± 0.10 ^c	0.142 ± 1.08·10 ⁻³ c	0.022 ± 2.35·10 ⁻⁴ b
<i>shmt1.2</i>	413.7 ± 1.2 ^d	57.12 ± 1.31 ^c	7.39 ± 0.22 ^a	0.138 ± 2.78·10 ⁻³ d	0.018 ± 4.85·10 ⁻⁴ c

Values represent the mean ± SE, $n \geq 6$ of pools of 40 plants from 2 different lines per genotype; different letters (^{a, b, c, d}) indicate significant differences between lines ($P < 0.05$).

even lower expression levels in *shmt1 c-pgdh1* shoots than in the WT, which agrees with the notion that S metabolism is activated in these mutant lines (Fig. 6C). SULTRs and adenosine phosphosulfate reductase (APR) enzymes play predominant roles in controlling sulfate assimilation in plastids (Ristova and Kopriva 2022). All 3 APR genes were upregulated in *c-pgdh1* compared with WT but downregulated in *shmt1 c-pgdh1* compared with *c-pgdh1* (Fig. 6C). SULTR genes showed different expression patterns depending on their functions in plants. *SULTR1.1* and *SULTR1.2*, whose major functions involve root uptake, were upregulated in roots of both *c-pgdh1* and *shmt1 c-pgdh1*, but with higher expression levels in the latter (Fig. 6C). However, an opposite expression trend appeared in most of the other 10 SULTR genes in both mutants, especially in shoots. Notably, *SULTR1.3* and *SULTR3.1* were highly upregulated in *c-pgdh1* and highly downregulated in *shmt1 c-pgdh1*. *SULTR1.3* is a high-affinity SULTR required for sulfate uptake and for maintaining S metabolism in the sieve element companion cell complex (Yoshimoto et al. 2007). Deletion of this high-affinity SULTR restricted transfer of ³⁵S from cotyledons to shoot meristems and roots in Arabidopsis (Yoshimoto et al. 2007). *SULTR3.1* participates in sulfate transport at chloroplast/plastid envelopes (Cao et al. 2013). Taken together, these transcriptional data indicate that the expression of genes responding to sulfate signaling and sensing was altered in *shmt1 c-pgdh1* compared with *c-pgdh1*, with the upregulation of major sulfate uptake transporter genes in roots but downregulation of APR genes, and most SULTR genes involved in intercellular and intracellular sulfate transport.

Unlike genes that respond to nutrient levels, genes related to carbohydrate metabolism were among the most highly upregulated in *shmt1 c-pgdh1* shoots (Fig. 6B). For example, among the 154 differentially regulated genes in the “response to carbohydrate stimulus” category in *shmt1 c-pgdh1* shoots, 104 were upregulated (Fig. 6B; Supplemental Data Set 1). This also indicates a shift in C metabolism between *c-pgdh1* and *shmt1 c-pgdh1*. Therefore, we measured total N, C, and S levels in roots and shoots (Table 1). In *c-pgdh1*, an alteration occurred in the distribution of S and N between shoots and roots versus WT, with a deficiency in shoots and an

excess in roots. However, the C content increased in both shoots and roots of *c-pgdh1* versus WT. In *shmt1 c-pgdh1*, however, the S and N contents increased in shoots to levels even higher than in the WT, but the C content decreased in both shoots and roots. The most important differences between *shmt1 c-pgdh1* and *c-pgdh1* were, once again, found in shoots, particularly in the S content. Compared with the WT, the S content of *c-pgdh1* shoots decreased by approximately 20% but increased by ~20% in *shmt1 c-pgdh1*, following a similar trend in *shmt1*. Due to the changes in C, S, and N contents, the N/C and S/C ratios in shoots decreased in PPSB-deficient lines and increased in SHMT-deficient lines. In roots, the most important differences between both lines were changes in the C content, which dropped in *shmt1 c-pgdh1* and rose in *c-pgdh1*. We also measured total N, S, and C contents under aCO₂ growth conditions in the *c-pgdh1* line, confirming that the reduction in N and S contents and the increase in C content in shoots of the PPSB-deficient lines occurred independently of photorespiratory activity (Supplemental Table S1).

Therefore, the deficiency of N and S that occurred in *c-pgdh1* shoots was corrected by bypassing the photorespiratory flux at the SHMT1 level. This implies that PGDH1 and SHMT1 activities play important roles in regulating C, N, and S distribution within and between plant cells and organs.

Protein and carbohydrate metabolism is differentially affected in PPSB- and SHMT-deficient lines

We performed protein biosynthesis experiments using a labeled ³⁵SCys/³⁵SMet cocktail in shoots. Neither *c-pgdh1* nor *shmt1 c-pgdh1* showed higher amino acid incorporation into proteins than WT (Fig. 7A). However, the enrichment of ³⁵SCys/³⁵SMet was lower in SHMT1-deficient lines than in WT or *c-pgdh1*, which suggests a lower metabolization rate. This could indicate a greater preference of N-containing metabolites as a C sink instead of carbohydrates and would agree with the increased N and S and the decreased soluble sugar contents in these lines. We checked the growth responses of *c-pgdh1* and *shmt1 c-pgdh1* in the presence of exogenous 2% sucrose. While the shoot fresh weight of *shmt1*

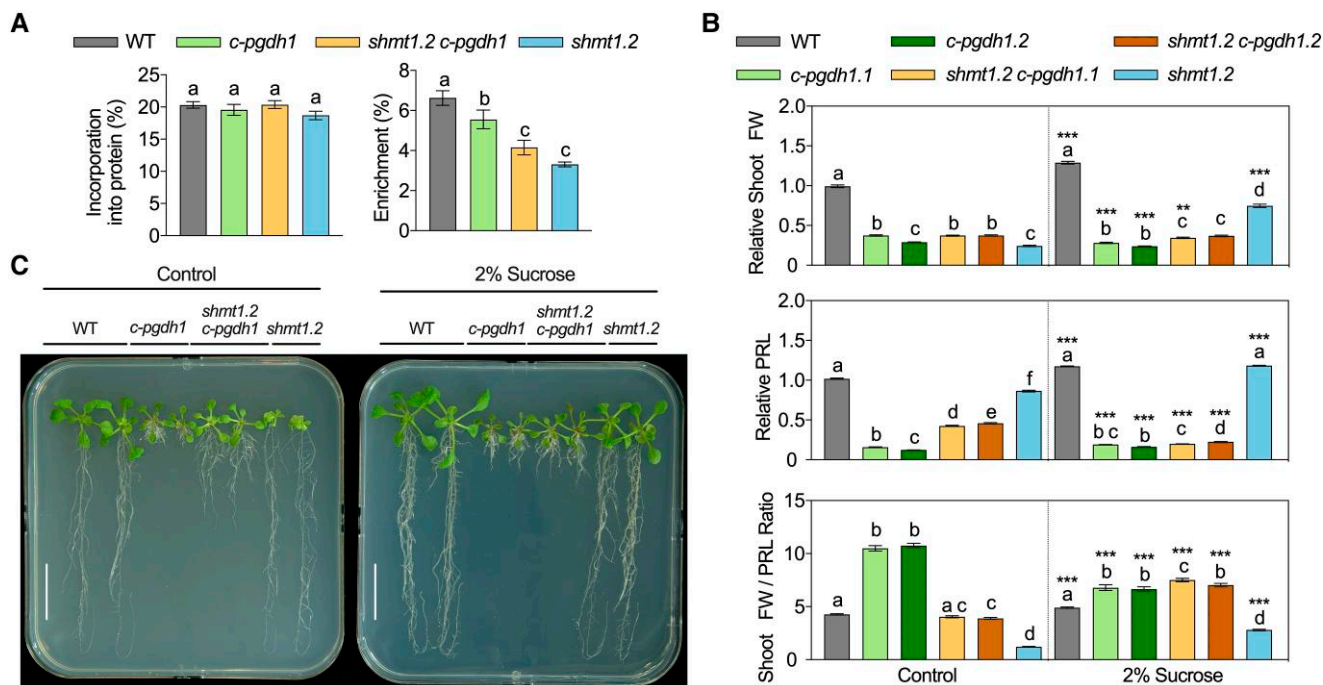


Figure 7. Protein and carbohydrate metabolism is differentially affected in PPSB-deficient (*c-pgdh1*) and SHMT1-deficient (*shmt1.2* and *shmt1.2 c-pgdh1*) lines under eCO₂ conditions. **A)** Protein synthesis in shoots measured by ³⁵S-Met/³⁵S-Cys labeling experiments. Values are shown as incorporation into proteins (left panel) and enrichment (right panel). **B)** Relative shoot fresh weight (FW), primary root length (PRL), and shoot FW/PRL ratio of different lines supplemented ±2% sucrose. **C)** Photograph of representative individuals of each line. In **A)**, values represent the mean ± SE, *n* ≥ 6 pools of 40 plants from 2 different lines for each genotype; different letters indicate significant differences between lines (*P* < 0.05). In **B)**, values (mean ± SE; *n* ≥ 40 plants from 2 different lines for each genotype) are normalized to the mean calculated for the WT. Different letters indicate significant differences between lines (*P* < 0.05); significant differences between the same line under control growth conditions, as determined by Student's *t*-test, are denoted by * (*P* < 0.05), ** (*P* < 0.01), or *** (*P* < 0.001). Scale bars = 2 cm (C).

increased almost 3-fold, the shoot growth of *c-pgdh1* was negatively affected by this treatment (Fig. 7, B and C). Interestingly, sucrose treatment drastically decreased the primary root length of *shmt1 c-pgdh1* but increased the primary root length of *c-pgdh1* (Fig. 7, B and C). Consequently, sucrose treatment altered the plant developmental pattern depending on the genetic background, as shown by the shoot fresh weight/primary root length ratio, which increased in the SHMT-deficient lines but decreased in *c-pgdh1* compared with control medium without sucrose (Fig. 7B). These results confirm the notion that SHMT1-deficient lines have impaired carbohydrate metabolism compared with the other lines and that developmental changes in PPSB- and SHMT-deficient mutants involve nutrient sensing and signaling mechanisms in different plant organs, as well as shoot–root communications processes.

Discussion

Evidence supports the involvement of photorespiratory Gly in the maintenance of Ser homeostasis at the whole plant level

Crosstalk has been proposed between photorespiration and PPSB during Ser biosynthesis. This assumption is based on

the observation that Ser levels drop to a lesser extent than Gly levels under low photorespiratory conditions in WT leaves (Kleczkowski and Givan 1988; Fig. 3B), which might be due to PPSB induction when photorespiration is compromised (Modde et al. 2017; Fig. 2, C and D). However, we have shown that steady-state levels of Ser also drop to a much lesser extent than Gly in PPSB-deficient lines under photorespiratory-reduced conditions (Fig. 3B). We have also shown that not only Gly but also steady-state Ser levels increase in double mutants devoid of PGDH and SHMT1 activities. ¹⁵N-flux measurements indicated that less Ser was incorporated into shoots and roots in the *shmt1 c-pgdh1* lines than in PPSB-deficient lines (Fig. 5, A and B), as expected after eliminating the major enzymes responsible for its synthesis. Therefore, we propose that some of the Gly leaving photorespiration is used for Ser synthesis in the cytosol and plastids. This conversion of Gly into Ser through cytosolic and plastidial SHMTs is not thermodynamically favored. However, the high Gly/Ser ratio in the SHMT1-deficient lines could shift the reaction equilibrium in favor of Ser formation in these compartments.

Gly and Ser levels in roots also decreased in all lines in response to the transition from aCO₂ to eCO₂ conditions, especially in SHMT1-deficient lines. This implies that fractions of the root Gly and Ser pools are also associated with

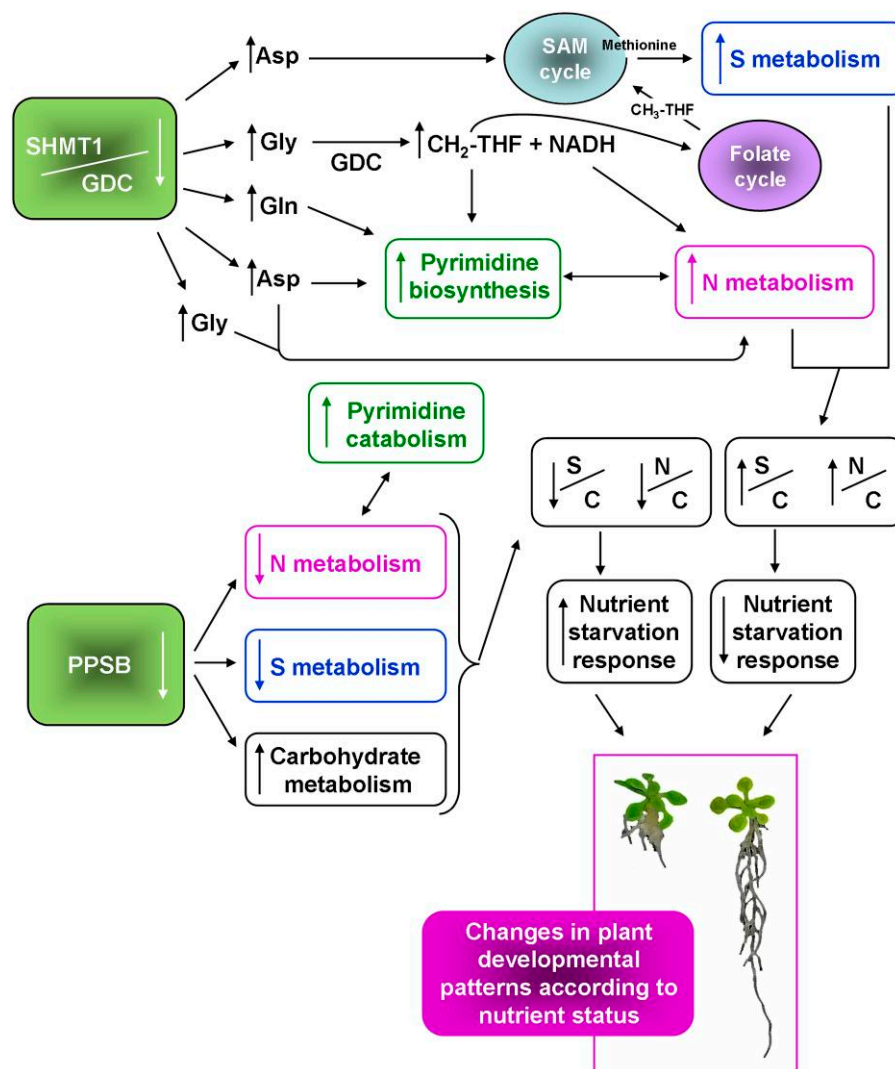


Figure 8. Proposed model for metabolic pathways interacting with the PPSB and GPSB. Reduced PPSB activity negatively affects nitrogen (N) and sulfur (S) metabolism in shoots and induces the nutrient starvation response. Lack of SHMT1 activity increases glycine, aspartate as well as CH₂-THF content, which has an impact on pyrimidine metabolism, activating biosynthetic and repressing catabolic processes. SAM is also activated. All these changes, along with possible increases in NADH content, positively affect N, S, and carbon (C) metabolism, repress the nutrient starvation response, and change the N/C and S/C ratios, which modify the plant developmental pattern. Enzymes: GDC, glycine-decarboxylase complex; SHMT1, serine-hydroxymethyl transferase 1. Metabolites: CH₃-THF, 5-methyl-THF, Asp, aspartate; CH₂-THF, 5,10-methylene-THF; Gln, glutamine.

photorespiratory activity, suggesting that both amino acids are transported from shoots to roots. These data collectively indicate the involvement of photorespiratory Gly in maintaining Ser homeostasis at the whole plant level. Thus, the regulation of SHMT/GDC activity could be a crucial component of the contribution of photorespiration to Ser–Gly–1C homeostasis in plants. The provision of more or less Gly from photorespiration could be a physiological strategy, as suggested by the downregulation of *SHMT1* and *GDC* genes in PPSB-deficient lines to provide Ser and folates for growth and metabolism when other pathways such as the PPSB are restricted. However, other questions remain. For instance, why do PPSB-deficient mutants have such strong phenotypes? Thus, the vast majority of the observed metabolic

changes in PPSB-deficient lines may not be related to Ser starvation directly, which was not always observed in PPSB-deficient lines, but are most likely caused by alterations in nutrient signaling and metabolism. As we have shown that changes in shoots are responsible for the phenotypes of roots (Fig. 4), our results suggest that metabolic changes in shoots led by Gly might modulate root development (Fig. 8).

Folates and Gly as activators of N metabolism

Photorespiration was long considered to be a process that reduces photosynthetic efficiency, especially in C₃ plants, and considerable efforts have been made to reduce its activity or bypass the metabolic pathway (the so-called photorespiratory bypasses) in order to enhance crop productivity

(Kebeish et al. 2007; Peterhansel et al. 2010; Betti et al. 2016; Cotton et al. 2018; Shen et al. 2019; South et al. 2019; Baslam et al. 2020; Fernie and Bauwe 2020). However, several studies have indicated that reduced photorespiratory rates due to the increasing atmospheric CO₂ concentrations will positively affect plant biomass (Ainsworth and Long 2020), most likely causing a decrease in their nutritional quality (Myers et al. 2014; Medek et al. 2017). Our study supports the notion that regulating photorespiratory activity might provide benefits for N, S, and folate status, which is important for the nutritional quality of crops.

One of the main problems associated with increasing atmospheric CO₂ levels is the reduction in N and protein contents in plants, which alters the N/C ratio (Wieser et al. 2008; Bloom et al. 2010; Myers et al. 2014; Medek et al. 2017; Bloom et al. 2020). Under eCO₂ conditions, N content is constrained in leaves of C₃ plants but not in roots (Jauregui et al. 2016). PPSB-deficient lines also showed altered N and S allocation between roots and shoots (lower in shoots and higher in roots than in WT; Table 1). In *shmt1 c-pgdh1* shoots, however, the N and S content increased, and marker genes for S and N deficiency were downregulated (Table 1 and Figs. 6, B and C). Thus, our data demonstrate that a shift in photorespiratory flux affects the N status in shoots of PPSB-deficient plants at the transcriptional and metabolic levels, corroborating the important impact of photorespiration on N metabolism.

How the N/C ratio is altered by eCO₂ has been the subject of intense debate in recent years (Bloom et al. 2010, 2012, 2014; Bloom 2015; Jauregui et al. 2015, 2016; Walker et al. 2016; Eisenhut et al. 2019; Andrews et al. 2020). Diminished photorespiratory flux has been suggested as one of the possible causes of reduced NO₃[−] assimilation (and uptake) in shoots, although the mechanism is not fully understood (Rachmilevitch et al. 2004; Bloom 2015; Krämer et al. 2022). One plausible hypothesis to explain the relationship between photorespiration and NO₃[−] assimilation points to the high demand for electrons required to power NO₃[−] reduction by nitrate reductase. Bloom and Lancaster (2018) postulated an alternative photorespiratory pathway that increases photorespiratory energy efficiency by generating malate in the chloroplast. Low photorespiratory activity under eCO₂ could impair the malate:2-OG shuttle in the chloroplast, decreasing the reducing power in the form of NADH in the cytosol for nitrate reductase activity (Bloom and Lancaster 2018; Shi and Bloom 2021). In *shmt1 c-pgdh1* lines, we did not find any differences in the levels of glycolate (Supplemental Fig. S2), one of the products of the postulated alternative photorespiratory reaction, which could explain changes in the N status between lines.

Alternatively, other sources of higher NADH content in *shmt1 c-pgdh1* could increase the reducing power for nitrate reductase activity in these lines. In *shmt1 c-pgdh1* lines, photorespiration is arrested before the hydroxypyruvate reductase reaction; this enzyme consumes NADH in the peroxisome (Fig. 1). Besides, *shmt1 c-pgdh1* lines showed increased GDC gene expression in shoots (Fig. 6C), which

could provide extra NADH in mitochondria. Therefore, excess NADH levels in the mitochondria and peroxisomes of *shmt1 c-pgdh1* could also help provide the NADH required for nitrate reductase activity in the cytosol through the malate shuttles. Supporting this idea, *shmt1 c-pgdh1* displayed higher malate levels compared with the other lines.

Increases in the levels of other metabolites associated with SHMT1/GDC activity in SHMT1-deficient shoots, such as Gly itself and those of the CH₂-THF + THF pool (Figs. 3B and 5C), could also have an impact on N and S metabolism. The over-accumulation of Gly in SHMT1-deficient lines may play a key role in stabilizing the C/N balance by consuming photosynthetic products and providing amino groups for N metabolism. It has been suggested that, under photorespiratory conditions, plants divert a considerable amount of C into amino acids such as Gly and Ser, which in turn stimulate amino acid biosynthesis and N assimilation (Busch et al. 2018). As described by Abadie et al. (2016), the Gly/Ser stoichiometry of photorespiration is close to 2 but increases at high photorespiratory rates. Thus, subtle changes in SHMT1/GDC catalytic activity would build up Gly, sequestering N, which would need to be compensated for by increased N assimilation (Abadie et al. 2016). Photorespiratory flux is up to 100-fold greater than NO₃[−] reduction (Bloom et al. 2010), so even a slight imbalance in photorespiratory recycling may affect the plant N budget. By increasing the availability of Gly, we modified N/C and S/C ratios in plants, confirming earlier predictions.

It has been hypothesized that the deposition of already fixed N as Gly is important for effective de novo N assimilation, because Gly (and Ser) are N sinks with low C content, leaving C skeletons (2-OG) available for de novo N assimilation (Krämer et al. 2022). SHMT1-deficient lines divert a large amount of Gly from the photorespiratory cycle compared with other lines, which affects N accumulation in shoots (Fig. 5A and Table 1). Besides, Gly is metabolized more quickly in PPSB-deficient mutants than in other lines, likely to obtain Ser, which may account for their lower N content in shoots under both photorespiratory and nonphotorespiratory conditions. Since high photorespiratory rates stimulate NO₃[−] assimilation and uptake in plants (Rachmilevitch et al. 2004; Bloom 2015), high Gly levels (or metabolites derived from Gly) in shoots may positively affect root NO₃[−] uptake, as suggested by the increased NO₃[−] content in roots of *shmt1 c-pgdh1* (Fig. 6D). If this were so, the drop in Gly content observed when WT plants grow under eCO₂ could be one of the signals to reduce N assimilation. As Gly and Ser contents in plant cell are lower than the contents of other major amino acids, Gly/Ser status may not serve as an N reservoir but may instead function as the metabolic signal to modulate NO₃[−] uptake and assimilation.

The high Gly contents in the SHMT1-deficient lines led to a change in the folate content, especially the CH₂-THF + THF pool (Fig. 5C), most likely indicating an increase in the CH₂-THF content. Thus, photorespiration might also be related to N metabolism via its impact on folate metabolism.

Folates were reported to play important roles in signaling cascades (Stokes et al. 2013), as well as in N and C metabolism (Jiang et al. 2013; Meng et al. 2014; Li et al. 2021), but the action mechanisms remain largely unknown. Folates are substrates for the synthesis of purines and pyrimidines; these major N-containing molecules are required for DNA and RNA biosynthesis. Transcriptomic data indicate that the pyrimidine catabolism pathway was strongly upregulated in *c-pgdh1* lines (Supplemental Fig. S7), likely as a response to organic N depletion, while pyrimidine biosynthesis was downregulated. The large supply of CH₂-THF, Gln, and Asp in the SHMT1-deficient lines, which are all required for pyrimidine biosynthesis, could fuel the pyrimidine biosynthetic pathway and restore the N balance in cells. These data allow us to hypothesize that Gly, along with the 1C-folate pathway, acts on nutrient signaling networks leading to the regulation of N metabolism and represents links between photorespiration and N metabolism (Fig. 8).

Overall, our results support the idea that the regulation of SHMT1/GDC may play a key role in the crosstalk between photorespiration and N-metabolism. Much speculation has been made as to why low photorespiration rates reduce N and protein contents. Our results provide genetic evidence for the biological relevance of regulating SHMT1/GDC activity on N metabolism (Fig. 8). We also provide clues about the underlying molecular mechanisms.

SHMT1/GDC activity links S with folate and SAM metabolism

The molecular mechanisms controlling S sensing and signaling in plants are not fully elucidated. We show that changes in the Gly/Ser flux alter the metabolic and transcriptional responses to S (Figs. 5C, 6B, and 6C). At the transcriptional level (Fig. 6, B and C), sulfate signaling and sensing were altered in *shmt1 c-pgdh1* in an opposite manner to that in *c-pgdh1*, pointing to the importance of the PPSB–GPSB interaction for balancing S homeostasis between heterotrophic and autotrophic tissues and also between cellular compartments. 1C units are required to synthesize the S-containing amino acid Met (Fig. 1) and could thus be involved in the activation of S-metabolism in shoots of SHMT1-deficient lines. SHMT1-deficient lines showed high levels of CH₂-THF + THF but reduced levels of other THF forms such as CH₃-THF or CH-THF (Fig. 5C). This finding suggests that there is not equilibrium between different THF pools among plant cell compartments. Alternatively, changes in folate homeostasis could be related to the activation of other metabolic pathways in SHMT1-deficient lines, such as the SAM cycle in the cytosol (Ravanel et al. 2004). The levels of SAM cycle components adenosine, SAM, and Met increased in SHMT1-deficient lines compared with the WT (Fig. 5C). SAM not only serves as a methyl donor in methylation reactions, but it is also an important S-containing metabolite and a form of reduced S that undergoes long-distance transport (Rennenberg et al. 1979; Bonas et al. 1982; Lappartient et al.

1999; Davidian and Kopriva 2010; Tan et al. 2010; Watanabe et al. 2021). Thus, the S-containing metabolites of the SAM cycle could be the link between SHMT/GDC activity and S metabolism.

Our results suggest that Asp and its derived amino acids may participate in bottleneck reactions in the shoots of *shmt1* PPSB-deficient lines. Under eCO₂ conditions, Asp levels increased in all lines, as expected, since photorespiration drains amino groups out of the Asp pools (Novitskaya et al. 2002). However, under these eCO₂ conditions at which SHMT1-deficient lines are viable, we found higher de novo incorporation of Asp as well as much higher Asp pools in shoots of SHMT1-deficient lines than in other lines (Figs. 3C and 5A). This greater incorporation could be directly related to the increased accumulation of Glu in these lines, from which Asp is formed. Increased levels of SAM cycle metabolites might also be involved in the activation of the Asp pathway (or vice versa) in SHMT1-deficient lines. Indeed, the Asp pathway is finely regulated by SAM and Cys feedback loops (Sauter et al. 2013; Galili et al. 2016). Asp is a precursor of the essential amino acids Met, lysine, threonine, and isoleucine and of nucleotides and NAD⁺, which serve as key metabolites for cell proliferation. This coregulation is consistent with the changes in folate levels in shoots and roots of SHMT1-deficient lines and supports the connection between folates and the S metabolism. Taken together, we can reasonably infer that the change in Asp concentration is one of the key reasons for the metabolic changes observed in SHMT1-deficient lines. To date, the mechanisms controlling Asp homeostasis are not fully elucidated. Thus, our results may reveal an important link between Asp and the regulation of SHMT1/GDC activity. We propose that this connection links photorespiration to S metabolism through the folate and SAM cycles (Fig. 8).

Crosstalk of C, S, and N metabolism shapes root development

In contrast to amino acids, glucose and fructose levels were drastically reduced in the mutants with a SHMT1-deficient background (Fig. 3C). The low hexose levels in SHMT1-deficient lines could not only be explained by slow recycling of photorespiration-derived 3-PGA (Eisenhut et al. 2017; Flügel et al. 2017; Timm et al. 2021) but also by their greater use in the synthesis of Asp family amino acids, as shown in mammals (Ritterhoff et al. 2020). The lower turnover of proteins in *shmt1 c-pgdh1* could mean that these lines prioritize protein accumulation as sink to the detriment of other metabolites such as sugars in order to channel the excess amino acids. In the end, the upregulation of genes responding to carbohydrate stimulus and the reduction in total C content in *shmt1 c-pgdh1* are consistent with a C deficiency in SHMT1-deficient lines. In *c-pgdh1*, however, there is an excess of C (glucose, fructose, and total C; Fig. 3C and Table 1), likely due to the lack of 3-PGA utilization for Ser synthesis by PGDHs.

These lines also showed a deficiency of S and N in shoots. We propose that the imbalanced S/C and N/C ratios might

affect root growth in PPBS mutants (Fig. 8). The strong reduction in primary root growth in the *shmt1 c-pgdh1* mutant in the presence of sucrose corroborated this hypothesis. Since one of the inhibitory effects of sucrose on photosynthesis is the inhibition of Rubisco activity itself (Quentin et al. 2013; Lobo et al. 2015), we assume that, under these growth conditions, photorespiration may be further inhibited, while plants maintain high levels of C provided by sucrose. Changes in the developmental pattern in the *shmt1 c-pgdh1* mutant could therefore be explained by the following: (i) supplied sucrose affects the C/N and C/S ratios directly in *shmt1 c-pgdh1* by increasing the C content, (ii) the further reduction in Rubisco oxygenase activity in *shmt1 c-pgdh1* affects the N and S status, or (iii) a combination of both factors. In line with this hypothesis, the combination of PPBS and SHMT1 mutant backgrounds compensated for the imbalance in C, N, and S levels and modified the shoot/root developmental pattern by stimulating root versus shoot growth (Fig. 8).

The interaction between metabolism and development in plants is poorly understood. Our work provides important clues about how Gly/Ser fluxes modulate N/C and S/C ratios and shape plant developmental patterns in response to nutrient status. We show that PPBS is necessary for correct N and S partitioning between shoots and roots. Disrupting PPBS led to an imbalance in this partitioning, leading to an N and S deficit in shoots and a general response to nutrient deficiency. Forcing the exit of Gly out of the photorespiratory cycle by inhibiting SHMT1 led to changes in folate, SAM, and Asp metabolism that reversed the changes in N and S deficiency in shoots of the PPBS-deficient lines. Therefore, the regulation of the Ser–Gly–1C network is crucial for N, C, and S homeostasis. Several hypotheses have been put forward to explain the role of photorespiration in N and S metabolism. Our results provide genetic and biochemical evidence that the regulation of the SHMT1/GDC activity ratio is a key target linking photorespiration to N and S metabolism at the metabolic and transcriptional levels. Therefore, both the photorespiratory and PPBS pathways modulate N, S, and C fluxes through the Ser–Gly–1C network. In mammals, this network is considered to be a central integrator of nutrient status (Locasale 2013). We propose a similar role for the Ser–Gly–1C network for plants. Genetic engineering of SHMT/GDC and PGDH activity is expected to be a useful target to improve the N and S contents of crops under forthcoming climate change conditions. To validate the viability of this biotechnological approach, additional experiments will need to be conducted in natural environments under varying temperature, light, and CO₂ conditions.

Materials and methods

Plant material and growth conditions

Original Arabidopsis (*A. thaliana*) seed stocks (ecotype Columbia-0 or Landsberg erecta) were supplied by the

European Arabidopsis Stock Center (Scholl et al. 2000). Conditional mutants with reduced expression of PSP1 (*c-psp1.1* and *c-psp1.2*) and PGDH1 (*c-pgdh1.1* and *c-pgdh1.2*) were obtained as previously described (Cascales-Miñana et al. 2013; Casatejada-Anchel et al. 2021). The *shmt1.2* mutant (Voll et al. 2006) was kindly supplied by Prof Hermann Bauwe (Rostok, Germany). The mutant alleles of At5g26780 (*shmt2.2*; SALK 096265) and At2g13360 (*sgat1*; GT_5_6208) were selected in the SIGnAL Collection database at the Salk Institute (Alonso et al. 2003) and were identified by PCR genotyping with the primers listed in Supplemental Table S2. PPBS-deficient double mutants with *shmt1.2* and *shmt2.2* (*shmt1.2 c-psp1.1*, *shmt1.2 c-psp1.2*, *shmt1.2 c-pgdh1.1*, *shmt1.2 c-pgdh1.2*, *shmt2.2 c-psp1.1*, *shmt2.2 c-psp1.2*, *shmt2.2 c-pgdh1.1*, and *shmt2.2 c-pgdh1.2*) were generated by crossing single *shmt1.2* and *shmt2.2* mutants with PPBS-deficient mutants and identified by PCR genotyping with the primers listed in Supplemental Table S2.

Unless otherwise stated, seeds were sterilized and sown on 0.8% agar plates containing 1/5 strength MS medium with Gamborg vitamins buffered with 0.9 g L⁻¹ MES, adjusted to pH 5.7 with Tris (1/5 MS). After 2 to 4 d of stratification at 4 °C, the plates were vertically placed at 22 °C under a 16-h day/8-h night photoperiod at 100 μmol m⁻² s⁻¹ light intensity (Lumilux fluorescent cool white, OSRAM EAN 4050300517797) and under aCO₂ or eCO₂ (2000 ppm) conditions for 14 to 16 d before sampling the seedlings. When indicated, 2% sucrose was added to the growth medium.

Grafting experiments

To graft the double mutant *shmt1.2 c-psp1* shoot (scion) with the PPBS-deficient *c-psp1* root (root-stock) and for self-grafting of PPBS-deficient *c-psp1*, the protocol described by Thieme et al. (2015) was followed with some modifications. In short, plants were grown vertically on solid 1/5 MS medium containing 1.5% (w/v) agar at 22 °C and 2,000 ppm of CO₂ under a 16-h day/8-h night photoperiod and 100 μmol m⁻² s⁻¹ light. One day before grafting, plates containing the seedlings were covered with aluminum foil to favor the elongation of the mutant's hypocotyls. Seven to eight days after germination, evenly elongated hypocotyls were cut transversely in the upper half of the hypocotyl with a sterile razor blade, and the scions were combined with the root stock. Graft junctions were supported using a silicon tube with a 0.51 mm internal diameter, and grafted plants were transferred onto new sterile plates with 1/2 MS containing 1.5% (w/v) agar and grown under the same conditions as mentioned above. Every 3 d, the formation of adventitious roots originating from the scion was checked and whenever possible carefully removed.

Metabolite determination

Shoots and roots of 15-d-old plants grown on vertical plates were used to determine metabolite content in derivatized methanol extracts by gas chromatography-MS (GC-MS) as described by Lisec et al. (2006). Plants were sampled after

10-h of growth in the light. Foliates were analyzed according to Wu et al. (2018). SAM analysis was performed as previously described (Hung et al. 2013). For adenosine measurements, 100 mg (fresh weight) of tissue was resuspended in 100 μ L of HClO₄ 1 M. After a 10-min centrifugation at 11,500 rpm at 4 °C, the supernatant was analyzed by LC-MS-MS as described by Fung et al. (2001).

¹⁵N enrichment of the metabolome

Seeds were surface sterilized and sown on 1/5 MS plates containing 0.8% agar and kept in the dark at 4 °C for 4 d to synchronize germination. The plates were then incubated at 22 °C under a 16-h day/8-h night photoperiod at a light level of 100 μ mol m⁻² s⁻¹ and eCO₂ in a phytotron chamber. After 5 d of germination, the seedlings were transferred to hydroponic cultures as previously described (Erban et al. 2020). Briefly, seedlings were grown for 12 d in sterile glass containers with a metal net for support and liquid 1/5 MS medium. After reaching the morphological stage of 1.05 (Boyes et al. 2001), plants were transferred to new hydroponic medium containing 0.8 mM ammonium nitrate as the sole nitrogen source labeled or unlabeled with the stable isotope ¹⁵N, ¹⁵NH₄⁺¹⁵NO₃⁻ (Sigma-Aldrich, Ref. 366528), or ¹⁴NH₄⁺¹⁴NO₃⁻ (Sigma-Aldrich, Ref. 221244). After 3 d, shoots and roots were harvested separately and immediately frozen. The roots from the labeled media were washed with unlabeled 1/5 MS medium to detach the labeled ions. Extraction of metabolites was performed as described by Erban et al. (2020) and analyzed by GC-MS.

The raw chromatograms were baseline corrected and deconvoluted in ChromaTOF. Subsequently, the files were exported as .netCDF files. The .netCDF files were imported into TagFinder (Luedemann et al. 2008), setting the intensity threshold for upload into TagFinder to one, so as to include low abundance peaks such as multiply ¹⁵N-labeled isotopologs. Internal retention time standards of n-alkanes C₁₀ to C₃₆ (decane, dodecane, pentadecane, octadecane, nonadecane, docosane, octacosane, dotriacontane, hexatriacontane) were used to align the chromatograms. Peak intensities were scaled to the maximum of all peaks with the same m/z ratio and retention index window set to 0.05. Compounds were identified using the Golm Metabolome Database (Hummel et al. 2007). Due to isotopic envelope shifts, N-containing metabolites were manually annotated. ¹³C₆-Sorbitol was used as the internal standard of the polar phase. The normalization factor applied to each sample was the product of the fresh weight per sample multiplied by the intensity of the internal standard. Annotated mass features from the Golm Metabolome Database were used as targets for ¹⁵N isotopic tracing analysis of each N-containing metabolite. In particular, mass features that contained one or more N atoms in their molecular formulas were used. The natural isotopic abundance (NIA) of mass features was corrected for across all detected isotopologs. The sum of the corrected ¹⁵N-labeled isotopolog intensities divided by the total ¹⁴N- and ¹⁵N-pool was considered to be the mean fractional

enrichment. All calculations were performed as previously described (Huege et al. 2014; Heinrich et al. 2018; Millard et al. 2019). The ¹⁵N relative abundance reflects the relative amount of the metabolite pool that was newly synthesized since it incorporated the ¹⁵N during our experiment. The nominal values were considered to be the sum of all detected isotopologs per mass feature after correcting NIA. In cases where one isotopolog was used for correction, the ¹⁵N relative abundance is the NIA-corrected abundance of the first isotopolog.

Elemental analyses, nitrate, and ammonium quantification

NH₄⁺ analysis was performed using the Berthelot method as described by Weatherburn (1967). NO₃⁻ was determined as described by Zhao and Wang (2017). Elemental analysis was performed using the Pregl–Dumas method with a CHNS elemental analyzer (ThermoFisher SmartFlash model).

Gene expression analyses and RNA-seq

Reverse transcription quantitative PCR analysis was performed as described recently (Casatejada-Anchel et al. 2021). The primers used are listed in Supplemental Table S2. For RNA-seq, shoots and roots of 14-d-old plants vertically grown on 1/5 MS plates under eCO₂ conditions were used. Three independent biological replicates of each sample were harvested after the 10-h light period for analysis. Total RNA extraction, RNA-seq library preparation, and sequencing were performed as previously described (Anoman et al. 2019). Quality check, trimming, alignment, reads counting, and filtering of genes expressed at low levels were conducted as previously described (Sun et al. 2018). PCA was performed on the TMM-normalized gene matrix after removing genes that are not expressed using the R function prcomp. Filtered genes were used to perform differential gene expression analysis with EdgeR. Genes were considered to be significantly differentially expressed if the corrected *P*-value (false discovery rate, FDR) was <0.05. Gene Ontology Enrichment Analysis was performed in agriGO v2.0 (Tian et al. 2017) based on a hypergeometric test.

Analysis of glutamine synthetase activity

GS activity was determined from crude extracts using the biosynthetic enzyme assay as previously described by Márquez et al. (2005). Crude extracts were obtained as described by García-Calderón et al. (2012). Shoots and roots were harvested from 14-d-old plants grown under eCO₂ conditions on 1/5 MS plates.

Analysis of protein biosynthesis using ³⁵S-labeled Met/Cys and analysis of total protein content

The analysis of tracer incorporation into proteins using a ³⁵S-labeled L-Met/L-Cys cocktail (NEG77200MC) was performed as described previously (Zimmermann et al. 2021). Plant material was incubated in hydroponic culture

containing 1/5 MS medium supplemented with 0.1 mM Met traced by 6.3 μCi ^{35}S -labeled L-Met/L-Cys cocktail per well for 6-h before harvesting. Total protein content was quantified with Bradford reagent (Bio-Rad) using bovine serum albumin as a standard.

Statistical analysis

Experimental values represent mean values and SE ; n represents the number of independent samples. Significant differences compared with either the WT or control treatment were analyzed by Student's t -test algorithms (2-tailed) embedded in Microsoft Excel. Significant differences between groups were analyzed by 1-way ANOVA followed by Fisher's LSD test using IBM SPSS Statistics software. Bar plots were generated in GraphPad 8 software. The details of statistical analysis results are listed in [Supplemental Data Sets 1 and 2](#).

Accession numbers

The original RNA-seq data were submitted to SRA under BioProject accession number PRJNA911249 (released 2023-10-11). The Arabidopsis locus identifiers for genes used in this study are as follows: *PSP1* (At1g18640), *PGDH1* (At4g34200), *SGAT* (At2g13360), *SHMT1* (At4g37930), and *SHMT2* (At5g26780).

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Author contributions

S.R.-T., A.A.-E., R.C.-A., J.M.-B., and S.K. participated in investigation and methodology. S.R.-T. and A.A.-E. participated in data analysis. TM and S.R.-T. participated in RNA-seq analysis and data curation. J.M.-B. and P.A. participated in protein biosynthesis experiments. S.S. and C.L.B. participated in folate analysis under the supervision of S.R. D.B.-M., S.R.-T., A.E., and F.M.-S. supported GC-MS data processing, i.e. TagFinder processing, metabolite and mass feature annotation, natural isotopic abundance correction, and statistical analyses under

the supervision of J.K. and A.R.F. S.R.-T., R.R., and A.R.F. conceived and designed the research. R.R., S.R., and S.R.-T. participated in conceptualization of the work. R.R. wrote the manuscript. A.R.F., S.R., S.K., J.K., P.A., J.M.-B., and S.R.-T. revised the manuscript. R.R. and A.R.F. supervised the work and provided funding.

Supplemental data

The following materials are available in the online version of this article.

Supplemental Figure S1. Amino acid levels in *sgat* mutant and WT shoots under aCO_2 and eCO_2 conditions.

Supplemental Figure S2. Glycolate levels in shoots of PPSB-deficient and PPSB-SHMT1-deficient lines.

Supplemental Figure S3. Heat map showing variation in metabolite levels when plants were shifted from eCO_2 to aCO_2 growth conditions.

Supplemental Figure S4. Grafting of scion *shmt1.2 c-psp1* shoots into *c-psp1* roots.

Supplemental Figure S5. Phenotypic characterization of *shmt2* mutants.

Supplemental Figure S6. PCA of RNA-seq data.

Supplemental Figure S7. Differential gene expression in the pyrimidine metabolic pathway in PPSB-deficient lines versus WT and SHMT-deficient lines versus *c-pgdh1* lines.

Supplemental Figure S8. NH_4^+ content in shoots and roots of different lines grown under eCO_2 conditions.

Supplemental Table S1. Carbon (C), nitrogen (N), and sulfur (S) content (mg/g dry weight) in shoots of WT and PPSB-deficient (*c-pgdh1*) lines growth under aCO_2 conditions.

Supplemental Table S2. List of primers used in this work.

Supplemental Data Set 1. Statistical analysis of results in Tables and Figures.

Supplemental Data Set 2. Statistical analysis of results in Supplemental Tables and Supplemental Figures.

Conflict of interest statement. None declared.

Data availability

The materials generated in this work are available on reasonable request to the authors.

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