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Biosensor-Mediated In Situ Imaging Defines the Availability Period of Assimilatory Glutamine in Maize Seedling Leaves Following Nitrogen Fertilization

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Received: 22 June 2017; Accepted: 18 July 2017; Published: 19 July 2017

Abstract: The amino acid glutamine (Gln) is an important assimilatory intermediate between root-derived inorganic nitrogen (N) (i.e., ammonium) and downstream macromolecules, and is a central regulator in plant N physiology. The timing of Gln accumulation after N uptake by roots has been well characterized. However, the duration of availability of accumulated Gln at a sink tissue has not been well defined. Measuring Gln availability would require temporal measurements of both Gln accumulation and its reciprocal depletion. Furthermore, as Gln varies spatially within a tissue, whole-organ in situ visualization would be valuable. Here, the accumulation and subsequent disappearance of Gln in maize seedling leaves (*Zea mays* L.) was imaged in situ throughout the 48 h after N application to roots of N-deprived plants. Free Gln was imaged by placing leaves onto agar embedded with bacterial biosensor cells (*GlnLux*) that emit luminescence in the presence of leaf-derived Gln. Seedling leaves 1, 2, and 3 were imaged simultaneously to measure Gln availability across tissues that potentially vary in N sink strength. The results show that following root N fertilization, free Gln accumulates and then disappears with an availability period of up to 24 h following peak accumulation. The availability period of Gln was similar in all seedling leaves, but the amount of accumulation was leaf specific. As Gln is not only a metabolic intermediate, but also a signaling molecule, the potential importance of regulating its temporal availability within plant tissues is discussed.

Keywords: glutamine; *Zea mays*; assimilation; nitrogen use efficiency; utilization; biosensor; regulation

1. Introduction

Nitrogen (N) is a limiting plant macronutrient, especially in non-leguminous crops such as maize (*Zea mays* L.). N is required for the synthesis of macromolecules including nucleic acids, amino acids and chlorophyll [1]. Plant roots uptake soil N, primarily nitrate (NO_3^-) and ammonium (NH_4^+), and then assimilate this inorganic N into the amino acid glutamine (Gln) beginning with conversion of nitrate to ammonium. Glutamate (Glu) is then used as a substrate by the glutamine synthetase/glutamine oxoglutarate aminotransferase (GS/GOGAT) cycle to assimilate ammonium, which can become the amide group of Gln [2,3]; this is considered a major route by which inorganic N is incorporated into organic N [4–6]. Although the synthesis of other amino acids primarily uses Glu or aspartate (Asp) as the direct substrate for aminotransferases, Gln is also used as a N donor for amino acid synthesis by enzymes such as asparagine synthetase, releasing Glu [2,3,7]. As a primary assimilatory product, Gln displays extremely sensitive and rapid responses to N application in maize tissues; however, because it is a donor of N for downstream metabolism and hence has a transitory

role, free Gln forms only a fraction of the steady-state amino acid pool, reported to comprise 4–6% of the total amino acid content in leaves [8,9]. Therefore, the appearance and subsequent disappearance of Gln from plant tissue may serve as a useful biomarker for tracking the progression and rate of N uptake, assimilation and utilization. Although the rate and timing of appearance of assimilatory Gln following N fertilization have been well characterized in many plants grown in laboratory conditions, the rate of consumption and hence availability of free Gln in sink tissues is less defined despite its implications for N metabolism and regulation.

Previously [10], an *Escherichia coli* biosensor auxotrophic for Gln (named *GlnLux*) was engineered with a constitutive *lux* operon to emit luminescence upon exposure to external Gln. Advantages of *GlnLux* include a low cost and tissue requirement, and simplified tissue preparation steps compared to most biochemical approaches including high-performance liquid chromatography (HPLC). The biosensor cells could be embedded into agar (*GlnLux* agar), upon which leaves were placed, freeze-thawed to cause Gln leakage, and then incubated at 37 °C to activate the biosensor cells. Following root N fertilization, spatial luminescence-output from maize seedling leaves could then be imaged using a photon-capture camera, corresponding to free Gln tissue-localization [11]. However, in our previous study, there were few timepoints, resulting in low temporal resolution, and furthermore N was applied continuously for 24 h following N deprivation, corresponding to the entire duration of the experiment. As a result, while rapid assimilation to free Gln was observable following N fertilization, the rate of subsequent conversion of free Gln to downstream products could not be discerned.

Here, an in situ leaf imaging experiment using *GlnLux* agar was designed to characterize the timing of Gln availability in maize leaves in the 48 h following a pulse of root N fertilizer. All seedling leaves were imaged simultaneously to measure Gln availability across organs that potentially vary in N sink strength. Nine distinct timepoints were imaged allowing for high temporal resolution.

2. Materials and Methods

2.1. Maize Growth Conditions

Zea mays L. hybrid CG60 X CG102 seed [12] was used. Two independent trials were conducted in a greenhouse set to 28 °C/20 °C day/night (16 h/8 h), with 1000 W high pressure sodium and 1000 W metal halide lamps supplemented with GroLux bulbs (Osram Sylvania Inc., Wilmington, MA, USA). Average light intensity was 768–923 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Trial 1) and 749–896 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (Trial 2) (canopy level at noon). Seeds were surface sterilized by soaking for 4 min in 70% ethanol, 2 min in 4% NaClO, and then washed five times in sterile double distilled (dd)H_2O. Seeds were transferred to 18-cell (1 seed per cell, 8.5 × 8.5 × 9 cm) growth flats of Turface® (Profile Products, Buffalo Grove, IL, USA), a clay gravel containing only trace plant-available nitrogen (N) [13]. The flats were placed into sub-irrigation trays containing ddH_2O with no nutrients. Stiff plastic webbing (L1020 injection molded web tray, Landmark Plastic, Akron, OH, USA) was placed in between the flats and sub-irrigation trays for easy transfer of the flats. Flats were watered as needed, with daily randomization.

Seedlings were grown for seven days, after which the remaining ddH_2O was emptied. Flats were moved to different sub-irrigation trays containing modified N-free Hoagland's nutrient solution (No. 2 Basal Salt Mixture HOP03-1LT, Caisson Labs, Smithfield, UT, USA) in which all other macro and micronutrients were provided as 2.86 $mg \cdot L^{-1}$ H_3BO_3, 554.90 $mg \cdot L^{-1}$ $CaCl_2$, 0.045 $mg \cdot L^{-1}$ $CuCl_2$, 33.00 $mg \cdot L^{-1}$ $C_{10}H_{12}N_2NaFeO_8 \cdot 3H_2O$, 240.33 $mg \cdot L^{-1}$ $MgSO_4$, 1.81 $mg \cdot L^{-1}$ $MnCl_2 \cdot 4H_2O$, 0.025 $mg \cdot L^{-1}$ $Na_2MoO_4 \cdot 2H_2O$, 372.70 $mg \cdot L^{-1}$ KCl, 136.025 $mg \cdot L^{-1}$ KH_2PO_4, and 0.1 $mg \cdot L^{-1}$ $ZnCl_2$. Seedlings remained in the solution for 24 h before sampling to prevent nutrient imbalance during N application.

2.2. Two Hour Nitrogen Pulse and Leaf Tissue Sampling

Growth flats served as the experimental units for repeated measurements, arranged as a randomized complete block design. The first leaf sampling was conducted just before applying +/−N treatments to the seedlings (time 0; the starting point). Leaves were harvested and placed in liquid N_2. Leaf 1 (the first leaf to emerge) was entirely removed at the ligule. Leaves 2 and 3 had not fully developed, and only the completely unfurled segment was harvested. Four replicates of each leaf, from each N treatment, from individual plants grown in separate growth flats were stored at −80 °C until imaging. After the starting timepoint, +/−N treatment was performed by moving all flats to new sub-irrigation trays containing either the same N-free Hoagland's solution described above (−N) or supplemented with 20 mM N (+N) provided as ammonium nitrate (NH_4NO_3).

Plant N uptake was permitted for 2 h. Maize leaves were sampled again, as described above, after 1 and 2 h of N uptake. Flats were then removed from the sub-irrigation trays, and a water hose was applied directly over the top of each flat for 5 min, followed by the bottom of each flat for 1 min to flush N present in the growth media. Previous experimental steps were staggered to account for the flushing period. Flats were placed into new sub-irrigation trays of N-free Hoagland's solution for the remainder of the experiment. Leaf sampling was performed again at 4 h, 6 h, 12 h, 24 h, 36 h and 48 h after the initial transfer to the N-uptake treatments, again by sampling the three different leaves of four replicate plants from each N treatment harvested from separate growth flats. Two additional harvest timepoints were included in Trial 2 at 18 h and 30 h to increase the temporal resolution.

2.3. Generating Leaf In Situ Images of Free Glutamine

Direct in situ imaging was performed with *GlnLux* biosensor cells (Figure 1) as previously described [11]. Briefly, biosensor cells were incorporated into solid *GlnLux* agar plates (150 × 15 mm Petri dishes). Images of free glutamine (Gln) were generated by placing freeze-thawed leaves on the surface of the *GlnLux* agar. Plates were incubated at 37 °C for 2.5 h, and imaged with a photon capture camera (7383-0007, Princeton Instruments, Trenton, NJ, USA) pre-cooled to −100 °C with an exposure time of 1000 s. The methodology was validated in a previous publication to ensure that image variability between plates was not significant [11]. Raw image output was converted to false-colour heat maps with WinView software (version 2.5.16.5, Princeton Instruments).

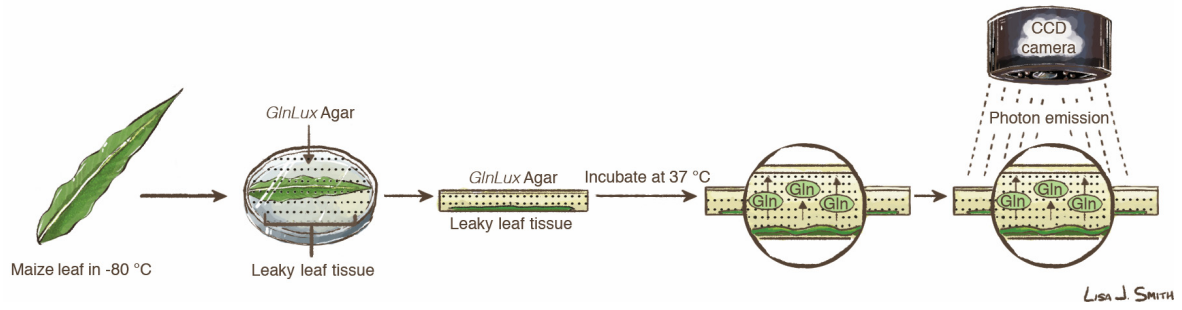

Figure 1. Schematic image of the *GlnLux* in situ imaging assay, courtesy of Lisa Smith (University of Guelph). The dots shown in the Petri dish represent *GlnLux* biosensor cells embedded in the agar. The figure may be re-used under the Creative Commons BY license. CCD, charge-coupled device.

3. Results and Discussion

3.1. GlnLux Imaging Demonstrates the Availability Period of Assimilatory Gln

Roots of seedlings grown without N for 8 days were provided with a transitory 2 h pulse of N fertilizer (Figures 2A and 3A). Immediately before the N fertilizer application, no differences in leaf *GlnLux* luminescence output were observed between the +/−N treated plants (0 h: Figures 2B and 3B). In Trial 1, *GlnLux* output from plants treated with the +N solution increased until 12–24 h after

the onset of the N pulse (Figure 2B). In Trial 2, additional 18 h and 30 h timepoints were examined (Figure 3B), but peak accumulation occurred in the same 12–24 h period. Pronounced differences between the N treatments persisted until 30 h (Figure 3B). Following accumulation, Gln depletion was of a similar rate, evident by 36 h and complete by 48 h in both Trials 1 and 2 (Figures 2B and 3B). At the latter timepoint, no differences between the +/−N treatments were visible. The results suggest that the availability period of free Gln in maize seedling leaves is 24 h following peak accumulation.

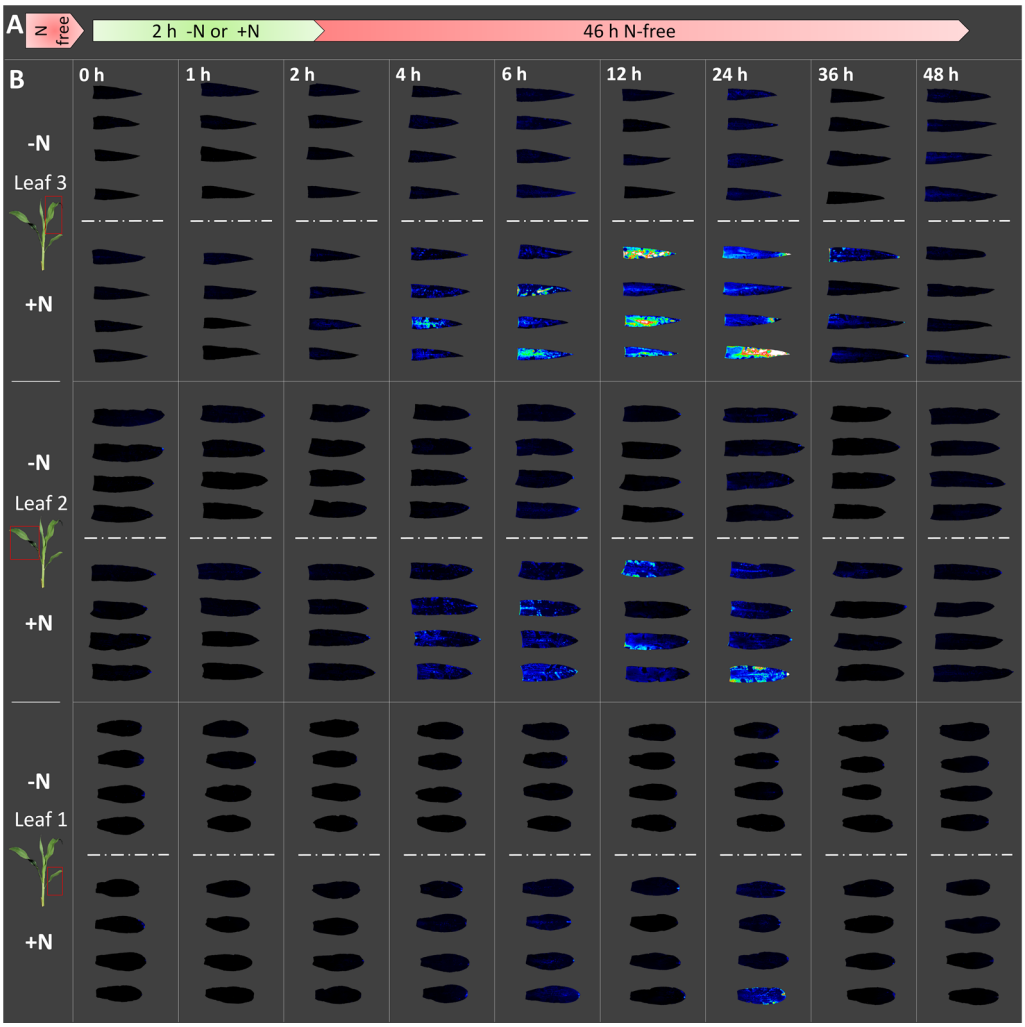

Figure 2. Luminescence imaging of free Gln from maize seedling leaves: Trial 1 (July 2016). Maize plants were grown in N-free media from germination for 8 d. Plants were then transferred for 2 h to a nutrient solution containing either 0 mM (−N) or 20 mM (+N) total nitrogen provided as ammonium nitrate (**A**). Three different seedling leaves were harvested and imaged using *GlnLux* agar (**B**). Harvesting was performed just prior to application of the −/+N treatments, and twice during the treatments after 1 h and 2 h of N uptake. Following the 2 h N-uptake period, all plants were transferred to N-free nutrient solution. Leaves were again harvested after 4 h, 6 h, 12 h, 24 h, 36 h, and 48 h. Displayed are *GlnLux* false-colour images of four replicates of each leaf from both the –N (above dashed lines) and +N (below dashed lines) treatments at equivalent harvest timepoints, roughly corresponding to panel **A**. For display purposes, the three leaves from each plant are arranged along a single column, and the four biological replicates are shown in the same order within each box. The intensity of *GlnLux* output, from greatest to least, is white, red, yellow, green, and blue, with black indicating absence of *GlnLux* output. The reader is encouraged to magnify the images.

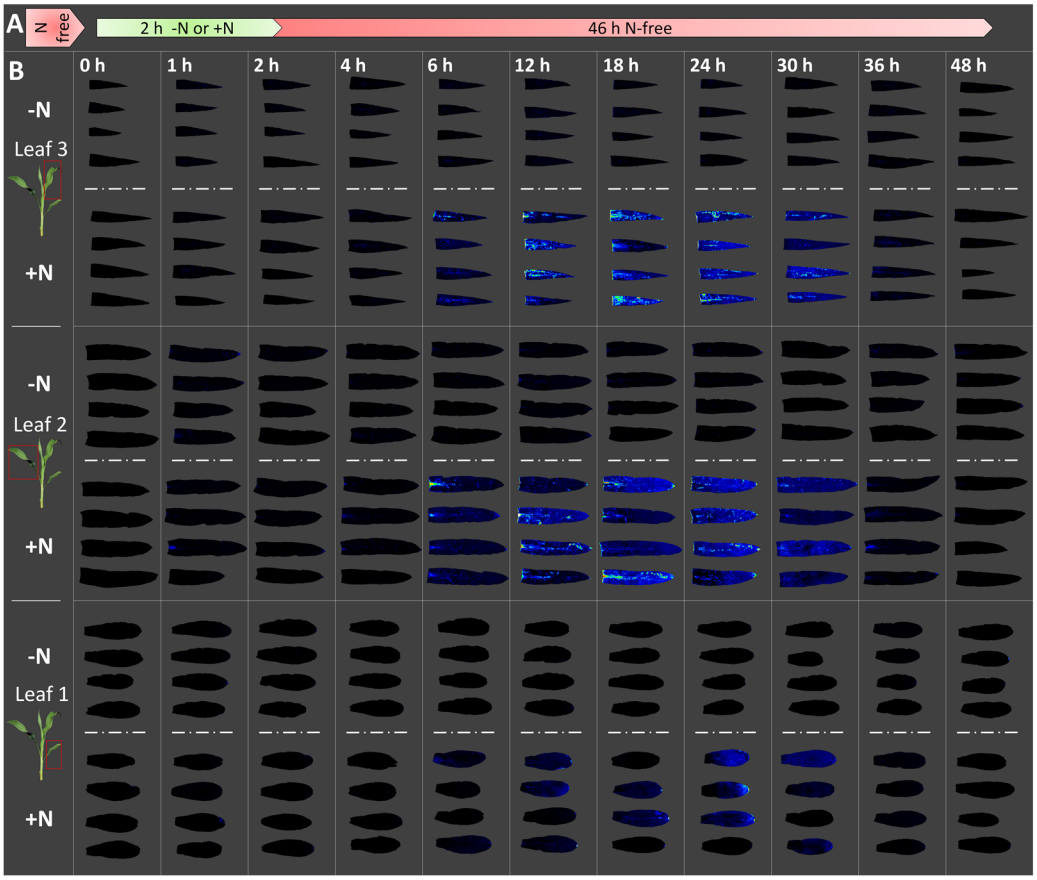

Figure 3. Luminescence imaging of free Gln from maize seedling leaves: Trial 2 (December 2016). Maize plants were grown in N-free media from germination for 8 d. Plants were then transferred for 2 h to a nutrient solution containing either 0 mM (−N) or 20 mM (+N) total nitrogen provided as ammonium nitrate (**A**). Three different seedling leaves were harvested and imaged using *GlnLux* agar (**B**). Harvesting was performed just prior to application of the −/+N treatments, and twice during the treatments after 1 h and 2 h of N uptake. Following the 2 h N-uptake period, all plants were transferred to N-free nutrient solution. Leaves were again harvested after 4 h, 6 h, 12 h, 18 h, 24 h, 30 h, 36 h, and 48 h. Displayed are *GlnLux* false-colour images of four replicates of each leaf from both the –N (above dashed lines) and +N (below dashed lines) treatments at equivalent harvest timepoints, roughly corresponding to panel **A**. For display purposes, the three leaves from each plant are arranged along a single column, and the four biological replicates are shown in the same order within each box. The intensity of *GlnLux* output, from greatest to least, is white, red, yellow, green, and blue, with black indicating absence of *GlnLux* output. The reader is encouraged to magnify the images.

The results infer the timing by which free Gln is consumed by downstream reactions (e.g., conversion to other amino acids). Future experiments might be conducted in which the molecular regulation of enzymes such as asparagine synthetase and their products are described in a similar timecourse. For example, glutamate, asparagine, and aspartate are important downstream products of assimilatory Gln [7], and their quantification may provide a layer of data with which to annotate *GlnLux* images. Additionally, genetic mutants or chemical inhibitors [14,15] that block Gln conversion, or N^{15} labeling experiments [9] would provide confirmation that reduction of *GlnLux* output over time is the result of Gln conversion to downstream molecules. The exact timing of Gln disappearance may also be characterized with analytical chemistry methods [9], or an alternative *GlnLux* methodology which analyzes plant extracts for more quantitative results [11].

3.2. The Timing of Gln Accumulation and Depletion Occurs Symmetrically along the Leaf-Age Sink Gradient

GlnLux imaging was performed simultaneously in all leaves to detect differences in Gln accumulation and depletion among leaves with potential high N sink strength (youngest growing leaf 3) and low sink strength (leaf 1) [16,17]. There was not a leaf-specific effect in terms of the timing of *GlnLux* accumulation (all peaked at 12–24 h) and depletion (common disappearance by 48 h) but intensity differences were observed (Figures 2B and 3B). Specifically, Gln accumulation was greatest in leaf 3 (youngest leaf), followed by leaf 2, then leaf 1 (oldest leaf) (Figures 2B and 3B), likely to fulfill the extra nutrient demand required to support growth of the younger leaves [16–19]. *GlnLux* output persisted slightly longer in leaf 3 in Trial 1 than Trial 2 (Figure 2B) for an unknown reason, either biological or technical.

One interpretation of these results is that Gln shuttling to N sinks is regulated to maintain a metabolic balance between supporting the N requirement for growth in young tissue, with maintaining the ongoing metabolic needs of older leaves (e.g., photosynthesis) which were non-senescent at the assayed stage. These results are consistent with those of a previous publication in terms of the onset of timing of peak Gln accumulation across maize seedling leaves [11], but also demonstrate that the timing of Gln depletion and hence Gln availability is similar across the leaf-age sink gradient.

3.3. The Timing of Gln Availability May Be Specific to the Conditions Used

In general, these timing results should be interpreted cautiously. The exact timing of Gln accumulation/disappearance and hence availability may be specific to the conditions of this study. The results might be different with other developmental stages, genotypes, or environmental conditions. Previous experiments have also demonstrated that the tissue location of N assimilation (i.e., root vs. shoot) is specific to the inorganic soil N concentration [20–22] and source (i.e., ammonium vs. nitrate) [8,23,24]. In this study, *GlnLux* output across N treatments and timepoints was generally greater in Trial 1 (Figure 2B) compared to Trial 2 (Figure 3B), which were conducted in July and December 2016, respectively. Despite the controlled greenhouse environment, plants in July (summer) received higher light intensities, day lengths, and temperatures, compared to December (winter). N uptake is in part regulated by the rate of carbon fixation and photosynthesis, which ensures the adequate supply of carbon skeletons for assimilatory products [25,26]. These physiological responses to environmental variation may have contributed to observed differences between the trials.

Despite the variable nature of N metabolism, various studies have consistently shown that GS and/or Gln rapidly accumulate within minutes to hours after application of N following a period of N starvation [21–23,27,28], consistent with the in situ visualization presented here. It is worth noting that free Gln in maize leaves does not represent all of N absorbed by roots—for example, nitrate can also be transported to foliage for storage [29–32].

3.4. Implications of a Limited Gln-Availability Period in Maize Leaf Tissue

GlnLux imaging suggests that after inorganic N from soil is assimilated and transported in the shoot, a portion accumulates as free Gln in leaves. Following peak accumulation, free Gln remains up to 24 h before downstream utilization. Assimilatory processes can be influenced by different growing conditions, tissue ages, and genetic characteristics, as recently demonstrated and reviewed [11]. However, the literature has indicated 24 h as an important timepoint for a variety of plant species grown under different conditions. In an older study [8], leaf levels of nitrate and especially ammonia similarly began to plateau after 24 h following N fertilization in maize grown from seed for two weeks under N deprivation. The same study also showed that nitrate reductase activity peaked at the 24 h timepoint and then decreased [8]. The 24 h time-frame of Gln (and nitrate, ammonia) availability at a N sink may have implications for our understanding of N metabolism. It is well known that N uptake and assimilation genes, enzymes and metabolites such as Gln are under circadian control [25,26,33]. However, it may be that plants regulate the availability period of free Gln not because

it is an intermediate to build other N-containing molecules—in this role, it may be advantageous for free Gln to persist locally. Rather, plants may regulate the persistence of Gln because it acts as a critical signaling molecule of the internal N status which can, for example, negatively regulate root N transporters [34,35]. An inverse correlation has been observed between the Gln concentration and nitrate transporter proteins in the roots of multiple species [28,36–38]. When Gln was administered to maize seedlings via tip-cut leaves, root N uptake was shown to decrease [37]. In addition to transporter regulation, increased Gln was shown to control at least 35 general stress response genes in rice [39,40]. Increased Gln has also been implicated in coordinating sulphate and N nutrition in barley [41], and to exert control over the C4 photosynthetic pathway in maize [42]. Therefore, the ~24 h period of Gln availability may be an important interval during which free Gln (or lack thereof) can affect a range of plant metabolic processes. In the future, *GlnLux* may be used to help probe the relationship between free Gln and these other metabolic processes, allowing metabolic/transcriptomic heatmaps to be overlaid with both temporal and spatial resolution.

4. Conclusions

Following root nitrogen fertilization, based on in situ imaging, the availability period of assimilatory Gln in maize seedling leaves was shown to be 24 h after peak accumulation. The temporal availability period of glutamine was similar in all seedling leaves, however the amount of Gln accumulation was leaf specific. As Gln is not only a metabolic intermediate, but also a signaling molecule, this temporal availability period may also have importance in the regulation of other plant processes.

Acknowledgments: We thank Dietmar Scholz and Sue Couling (University of Guelph) for assistance in maintaining the greenhouse experiments. We thank Elizabeth A. Lee (University of Guelph) for the gift of maize seed. T.L.G. was supported in part by scholarships from the University of Guelph, and an Alexander Graham Bell Canada Graduate Scholarship award from the Natural Sciences and Engineering Research Council of Canada (NSERC). Grants were awarded to M.N.R. from the Ontario Ministry of Agriculture, Food and Rural Affairs (OMAFRA), Grain Farmers of Ontario and the NSERC CRD Program.

Author Contributions: T.L.G. and M.N.R. conceived and designed the experiments; T.L.G. performed the experiments, analyzed the data, and wrote the paper. M.N.R. edited the paper.

Conflicts of Interest: A US patent has been issued on the *GlnLux* biosensor technology (US 61/499286) and a conflict of interest may be present, however the technology is not currently commercialized.

References

1. Masclaux-Daubresse, C.; Daniel-Vedele, F.; Dechorgnat, J.; Chardon, F.; Gaufichon, L.; Suzuki, A. Nitrogen uptake, assimilation and remobilization in plants: Challenges for sustainable and productive agriculture. *Ann. Bot.* **2010**, *105*, 1141–1157. [CrossRef] [PubMed]
2. Bernard, S.M.; Habash, D. The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. *New Phytol.* **2009**, *182*, 608–620. [CrossRef] [PubMed]
3. Xu, G.; Fan, X.; Miller, A.J. Plant nitrogen assimilation and use efficiency. *Annu. Rev. Plant Biol.* **2012**, *63*, 153–182. [CrossRef] [PubMed]
4. Miflin, B.J.; Lea, P.J. The pathway of nitrogen assimilation in plants. *Phytochemistry* **1976**, *15*, 873–885. [CrossRef]
5. Joy, K.W. Ammonia, glutamine, and asparagine: A carbon-nitrogen interface. *Can. J. Bot.* **1988**, *66*, 2103–2109. [CrossRef]
6. Cren, M.; Hirel, B. Glutamine synthetase in higher plants: Regulation of gene and protein expression from the organ to the cell. *Plant Cell Physiol.* **1999**, *40*, 1187–1193. [CrossRef]
7. Lam, H.M.; Coschigano, K.T.; Oliveira, I.C.; Melo-Oliveira, R.; Coruzzi, G.M. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1996**, *47*, 569–593. [CrossRef] [PubMed]

8. Sugiharto, B.; Sugiyama, T. Effects of nitrate and ammonium on gene expression of phosphoenolpyruvate carboxylase and nitrogen metabolism in maize leaf tissue during recovery from nitrogen stress. *Plant Physiol.* **1992**, *98*, 1403–1408. [CrossRef] [PubMed]
9. Cañas, R.A.; Yesbergenova-Cuny, Z.; Simons, M.; Chardon, F.; Armengaud, P.; Quilleré, I.; Cukier, C.; Gibon, Y.; Limami, A.M.; Nicolas, S.; et al. Exploiting the genetic diversity of maize using a combined metabolomic, enzyme activity profiling, and metabolic modeling approach to link leaf physiology to kernel yield. *Plant Cell* **2017**, *29*, 919–943. [CrossRef] [PubMed]
10. Tessaro, M.J.; Soliman, S.S.M.; Raizada, M.N. Bacterial whole-cell biosensor for glutamine with applications for quantifying and visualizing glutamine in plants. *Appl. Environ. Microbiol.* **2012**, *78*, 604–606. [CrossRef] [PubMed]
11. Goron, T.L.; Raizada, M.N. Biosensor-based spatial and developmental mapping of maize leaf glutamine at vein-level resolution in response to different nitrogen rates and uptake/assimilation durations. *BMC Plant Biol.* **2016**, *16*, 230. [CrossRef] [PubMed]
12. Khanal, R.; Earl, H.; Lee, E.A.; Lukens, L. The genetic architecture of flowering time and related traits in two early flowering maize lines. *Crop Sci.* **2011**, *51*, 146–156. [CrossRef]
13. Goron, T.L.; Bhosekar, V.K.; Shearer, C.R.; Watts, S.; Raizada, M.N. Whole plant acclimation responses by finger millet to low nitrogen stress. *Front. Plant Sci.* **2015**, *6*, 652. [CrossRef] [PubMed]
14. Monselise, E.B.I.; Kost, D. 15N NMR spectroscopic study of ammonium ion assimilation by *Spirodela oligorrhiza*—Lemnaceae—As affected by light and carbon supply in green and extoliated plants. *Israel J. Plant Sci.* **1998**, *46*, 255–264. [CrossRef]
15. Oaks, A.; Sivasankar, S.; Goodfellow, V.J. The specificity of methionine sulfoximine and azaserine inhibition in plant tissues. *Phytochemistry* **1998**, *49*, 355–357. [CrossRef]
16. Meiri, A.; Silk, W.K.; Lauchli, A. Growth and deposition of inorganic nutrient elements in developing leaves of *Zea mays* L. *Plant Physiol.* **1992**, *99*, 972–978. [CrossRef] [PubMed]
17. Poethig, R.S. Phase change and the regulation of developmental timing in plants. *Science* **2003**, *301*, 334–336. [CrossRef] [PubMed]
18. Neves-Piestun, B.G.; Bernstein, N. Salinity-induced changes in the nutritional status of expanding cells may impact leaf growth inhibition in maize. *Funct. Plant Biol.* **2005**, *32*, 141–152. [CrossRef]
19. Hu, Y.; Burucs, Z.; von Tucher, S.; Schmidhalter, U. Short-term effects of drought and salinity on mineral nutrient distribution along growing leaves of maize seedlings. *Environ. Exp. Bot.* **2007**, *60*, 268–275. [CrossRef]
20. Andrews, M. The partitioning of nitrate assimilation between root and shoot of higher plants. *Plant Cell Environ.* **1986**, *9*, 511–519.
21. Sakakibara, H.; Kawabata, S.; Takahashi, H.; Hase, T.; Sugiyama, T. Molecular cloning of the family of glutamine synthetase genes from maize: Expression of genes for glutamine synthetase and ferredoxin-dependent glutamate synthase in photosynthetic and non-photosynthetic tissues. *Plant Cell Physiol.* **1992**, *33*, 49–58.
22. Redinbaugh, M.G.; Campbell, W.H. Glutamine synthetase and ferredoxin-dependant glutamate synthase expression in the maize (*Zea mays*) root primary response to nitrate. *Plant Physiol.* **1993**, *101*, 1249–1255. [CrossRef] [PubMed]
23. Sukanya, R.; Li, M.; Snustad, D.P. Root- and shoot-specific responses of individual glutamine synthetase genes of maize to nitrate and ammonium. *Plant Mol. Biol.* **1994**, *26*, 1935–1946. [CrossRef] [PubMed]
24. Prinsi, B.; Espen, L. Mineral nitrogen sources differently affect root glutamine synthetase isoforms and amino acid balance among organs in maize. *BMC Plant Biol.* **2015**, *15*, 96. [CrossRef] [PubMed]
25. Lillo, C. Signalling cascades integrating light-enhanced nitrate metabolism. *Biochem. J.* **2008**, *415*, 11–19. [CrossRef] [PubMed]
26. Wang, K.; Okumoto, S.; Zhang, X.; Ervin, E. Circadian patterns of the major nitrogen metabolism-related enzymes and metabolites in creeping bentgrass and the influence of cytokinin and nitrate. *Crop Sci.* **2011**, *51*, 2145–2154. [CrossRef]
27. Magalhães, J.R.; Ju, G.C.; Rich, P.J.; Rhodes, D. Kinetics of 15NH4+ Assimilation in *Zea mays*. *Plant Physiol.* **1990**, *94*, 647–656. [CrossRef] [PubMed]

28. Vidmar, J.J.; Zhuo, D.; Siddiqi, M.Y.; Schjoerring, J.K.; Touraine, B.; Glass, A.D.M. Regulation of high-affinity nitrate transporter genes and high-affinity nitrate influx by nitrogen poolis in roots of barley. *Plant Physiol.* **2000**, *123*, 307–318. [CrossRef] [PubMed]
29. Ivanko, S.; Ingversen, J. Investigation on the assimilation of nitrogen by maize roots and the transport of some major nitrogen compounds by xylem sap. III. Transport of nitrogen compounds by xylem sap. *Physiol. Plant* **1971**, *24*, 355–363. [CrossRef]
30. Millard, P. The accumulation and storage of nitrogen by herbaceous plants. *Plant Cell Environ.* **1988**, *11*, 1–8. [CrossRef]
31. Miller, A.J.; Fan, X.; Orsel, M.; Smith, S.J.; Wells, D.M. Nitrate transport and signalling. *J. Exp. Bot.* **2007**, *58*, 2297–2306. [CrossRef] [PubMed]
32. Brien, A.O.; Vega, A.; Bouguyon, E.; Krouk, G.; Gojon, A.; Coruzzi, G.; Gutie, R.A. Nitrate transport, sensing, and responses in plants. *Mol. Plant* **2016**, *9*, 837–856. [CrossRef] [PubMed]
33. Li, H.; Liang, Z.; Ding, G.; Shi, L.; Xu, F.; Cai, H. A natural light/dark cycle regulation of carbon-nitrogen metabolism and gene expression in rice shoots. *Front. Plant Sci.* **2016**, *7*, 1318. [CrossRef] [PubMed]
34. Coruzzi, G.M.; Zhou, L. Carbon and nitrogen sensing and signaling in plants: Emerging "matrix effects". *Curr. Opin. Plant Biol.* **2001**, *4*, 247–253. [CrossRef]
35. Touraine, B.; Daniel-Vedele, F.; Forde, B.G. Nitrate uptake and its regulation. In *Plant Nitrogen*; Lea, P.J., Morot-Gaudry, J.F., Eds.; Springer: Berlin/Heidelberg, Germany, 2001; pp. 1–36.
36. Zhuo, D.; Okamoto, M.; Vidmar, J.J.; Glass, A.D.M. Regulation of a putative high-affinity nitrate transporter (Nrt2;1At) in roots of *Arabidopsis thaliana*. *Plant J.* **1999**, *17*, 563–568. [CrossRef] [PubMed]
37. Pal'ove-Balang, P.; Mistrik, I. Control of nitrate uptake by phloem-translocated glutamine in *Zea mays* L. seedlings. *Plant Biol.* **2002**, *4*, 440–445. [CrossRef]
38. Nazoa, P.; Vidmar, J.J.; Tranbarger, T.J.; Mouline, K.; Damiani, I.; Tillard, P.; Zhuo, D.; Glass, A.D.M.; Touraine, B. Regulation of the nitrate transporter gene *AtNRT2.1* in *Arabidopsis thaliana*: Responses to nitrate, amino acids and developmental stage. *Plant Mol. Biol.* **2003**, *52*, 689–703. [CrossRef] [PubMed]
39. Kan, C.; Chung, T.; Juo, Y.; Hsieh, M. Glutamine rapidly induces the expression of key transcription factor genes involved in nitrogen and stress responses in rice roots. *BMC Genom.* **2015**, *16*, 731. [CrossRef] [PubMed]
40. Kan, C.; Chung, T.; Hsieh, M. Gene expression profiling of rice seedlings in response to glutamine treatment. *Genom. Data* **2015**, *6*, 123–124. [CrossRef] [PubMed]
41. Karmoker, J.L.; Clarkson, D.T.; Saker, L.R.; Rooney, J.M.; Purves, J.V. Sulphate deprivation depresses the transport of nitrogen to the xylem and the hydraulic conductivity of barley (*Hordeum vulgare* L.) roots. *Planta* **1991**, *185*, 269–278. [CrossRef] [PubMed]
42. Sugiharto, B.; Suzuki, I.; Burnell, J.N.; Sugiyama, T. Glutamine induces the N-dependent accumulation of mRNAs encoding phosphoenolpyruvate carboxylase and carbonic anhydrase in detached maize leaf tissue. *Plant Physiol.* **1992**, *100*, 2066–2070. [CrossRef] [PubMed]