*Journal of Plant Nutrition and Soil Science*

WILEY-VCH

**RESEARCH ARTICLE** OPEN ACCESS

# Lentils can Absorb Amino Acids as a Nitrogen Source Supporting Early Growth

Alex A. Kröper[1,2] | Sabine Zikeli[2] | Monika A. Wimmer[1] | Christian Zörb[1]

[1]Institute of Crop Science, Quality of Plant Products and Viticulture (340e), University of Hohenheim, Stuttgart, Germany | [2]Center for Organic Farming (309), University of Hohenheim, Stuttgart, Germany

**Correspondence:** Christian Zörb (Christian.zoerb@uni-hohenheim.de)

**Received:** 12 November 2024 | **Revised:** 28 February 2025 | **Accepted:** 6 March 2025

**Academic Editor**: Kurt Möller

**Funding:** The project "Selektion geeigneter Sortentypen von Linsen (Lens culinaris) für nachhaltige Anbausysteme" was financed by the German Federal Ministry of Food and Agriculture, funding code 2815EPS050.

**Keywords:** amino acid pattern | ammonium | nitrate | organic agriculture | urea

## ABSTRACT

**Background:** Lentils (*Lens culinaris* Medik.) are a valuable crop due to their high nutritional content, low environmental impact, and nitrogen-fixing ability via rhizobacteria. Early in development, before this symbiosis is established, lentils require external nitrogen, typically supplied through fertilizers or already present in soils.

**Aim:** This study explores whether lentils can utilize amino acids as a nitrogen source and how amino acid supplementation affects growth and nitrate uptake.

**Results:** The findings show that lentils can absorb amino acids from soil, with no adverse effects on growth compared to mineral N fertilizers. The amino acid patterns show only slight changes in individual amino acids. NPF/NRT1, NRT2, AMT2, and DUR3 were expressed in all treatments in root tissue. LHT1 plays a minor role in the distribution of N in the shoots of lentil plants.

**Conclusion:** Although amino acid uptake is less efficient than that of nitrate, it may still benefit young plants in organic farming until rhizobacterial symbiosis is established.

## 1 | Introduction

Organic agriculture is a sustainable method of food production that emphasizes the use of organic fertilizers, crop rotation, and the cultivation of legumes, which are known to improve soil fertility through nitrogen fixation. In addition to ecosystem benefits, integrating legumes into crop rotations can also be economically positive for farmers (Reckling et al. 2016). Among legumes, the lentil (*Lens culinaris* Medik.) is a valuable edible crop for human diets in many countries because of its high protein content, dietary fiber, and high mineral content (Heseker and Heseker 2013; Vasconcelos et al. 2020). As legumes, lentils can fix $N_2$ through root colonization with rhizobacteria and formation of nodules, where nitrogen gas is converted into ammonium ($2\ NH_3 + 2\ H_2$ or $NH_4^+$) that the plant can utilize (Becana and Sprent 1987). This comes at the expense of photoassimilates, which are delivered in exchange to the rhizobia. Thus, the plant must grow to a certain developmental stage, which enables it to deliver additional photoassimilates to "feed" the bacteria (Silsbury 1989), which is seen as a symbiosis but also has an aspect of competition (Sachs et al. 2018). Once nodules are formed, no further N fertilization is needed. However, before this symbiosis is established, lentil growth and productivity depend on the availability of nitrogen, and N limitation can lead to reduced or

---

https://doi.org/10.1002/jpln.202400504

stunted growth, delayed plant development, yellowing of leaves, and reduced yield. If legumes were fertilized with sufficient nitrogen throughout their entire growing season, they would not develop functional nodules (Harper and Gibson 1984). To use N fixation efficiently, the right balance between N supply for the young plants and promoting the formation of nodules must be found.

Since lentils in the northern hemisphere are often grown in mixed cultivation with supporting crops such as barley or oats, there is competition for N with the supporting crop but also with other lentil plants during their juvenile development (R. S. Jones et al. 1981). Therefore, depending on soil $N_{min}$ status, it might make sense to apply a low-to-moderate amount of nitrogen fertilizer at sowing (Epie et al. 2023).

Lentil plants in a late stage of development especially when they switch from the vegetative to generative phase have a high demand for nitrogen, especially since their seeds have a high protein content. Nitrate uptake involves several transporters and enzymes. Key transporters involved in N uptake in plants such as nitrate transporter from the NPF family (NRT1.1, NRT2) and ammonium transporter (AMT2) as well as amino acid transporter (DUR3, LHT1) genes may change their abundance according to the N source and amount available (Nacry et al. 2013). Amino acid transporters such as LHT1 are primarily involved in the uptake and transport of amino acids, especially small neutral and acidic amino acids, such as glutamine. It has high affinity for a broad range of amino acids. Moreover, all these transporters play a crucial role in the uptake of nitrogen from the soil solution and its subsequent transport from the root to the shoot tissues for assimilation into amino acids and other nitrogen-containing compounds. While much is known about N acquisition in lentils by rhizobia, little is known about uptake of different N forms from soil solution.

We provide data on sugar concentrations in shoots and roots because the interplay between energy metabolism and nitrogen acquisition is critical for meeting the high energy demands associated with amino acid uptake and assimilation pathways, particularly under conditions of limited mineral nitrogen. Changes in the expression patterns of the genes involved are important to evaluate whether lentils can effectively take up amino acids.

The objective of this article is therefore to discuss the uptake systems for N sources such as amino acids in comparison to nitrate or ammonium, especially in the early development phase before symbiosis is active. Specifically, we hypothesize that (1) lentil plants can take up amino acids as an N-source and (2) application of amino acids as a single N-source influences the amino acid pattern in lentil roots and shoots.

## 2 | Material and Methods

### 2.1 | Plant Material and Growth Conditions

Lentil seeds cv. Anicia (green marbled, thousand kernel weight 36 g) were sterilized by 95% ethanol and 3% sodium hypochlorite and germinated in sand in a greenhouse. After 11 days, at the two leaf stage, seedlings were transferred into black plastic pots containing 4.5 L hydroponic solution (1 mM $KH_2PO_4$, 1 mM $K_2SO_4$, 1 mM $MgSO_4$, 100 µM Fe(III)-EDTA, 100 µM KCl, 20 µM $H_3BO_3$, 3 µM $MnSO_4$, 3 µM $ZnSO_4$, 0.4 µM $CuSO_4$, 0.4 µM $Na_2MoO_4$, 0.15 µM $CoCl_2$, and 0.1 µM $NiSO_4$). Additionally, the nutrient solutions contained one of five different N forms, namely 1 mM $Ca(NO_3)_2$, 1 mM $(NH_4)_2SO_4$, 1 mM $CH_4N_2O$, 0.5 mM $C_6H_{14}N_4O_2$ (arginine), or 1 mM $C_5H_{10}N_2O_3$ (glutamine). All treatments, other than the one treated with $Ca(NO_3)_2$, additionally received 1 mM $CaSO_4$ to adjust the calcium concentrations. The pH of the nutrient solution was set to 6.8 by adding sulfuric acid or potassium hydroxide. To avoid osmotic shock in the seedlings, the nutrient concentration was increased by 25% increments every 2 days until the final full concentration was reached. At the plant transplantation stage, it was confirmed that nodules were not yet present. The nutrient solution was renewed twice a week and every second day in the last week before harvest. Lentil plants were cultivated for 32 days in a greenhouse at ambient temperature. A completely randomized design with four replications of three plants at each pot ($n = 4$) was chosen. The three plants of one replicate were harvested together and pooled to one sample.

At harvest, roots were separated from the shoots, rinsed for 10 s with deionized $H_2O$ and gently blotted dry with tissue paper. Approximately 2 cm of the root tips and the first fully developed leaf with 5 mm of the stem were separated to obtain reliable samples of the above- and below-ground parts of the plant for RNA extraction, shock frosted in liquid nitrogen, and stored at −80°C until further processing. The fresh weight of the three plants of each replicate was determined immediately at harvest, and the dry weight after drying the plant material at 60°C for 3 days in a ventilated oven until a constant weight was reached.

Statistical analyses of the data were done by one-way analysis of variance (ANOVA) followed by post hoc Tukey honestly significant difference (HSD), accepting a type 1 error rate of 0.05, performed with SAS (Statistical Analysis Systems ver. 9.4, SAS Institute Inc., Cary, NC, USA). To ensure homogeneity of variance and normal distribution of the data for the calculation of the statistics, transformations were performed using either the natural logarithm, square root, or inverse.

### 2.2 | Nitrate

Nitrate concentrations were determined as described by Zhang et al. (2021), based on the colorimetric method by nitration of salicylic acid described by Cataldo et al. (1975). Absorbance was detected using a plate reader (Infinite M Nano, Tecan Trading AG, Switzerland) at a wavelength of 410 nm. Each sample was technically repeated three times.

### 2.3 | Soluble Sugars

Sucrose and glucose were extracted according to Wedeking et al. (2016) and determined enzymatically as described by Gomez et al. (2007) except that the assay volume was scaled-up in order to use a spectrophotometer (SPECORD 50 PLUS, Analytik Jena, Germany) instead of a microplate reader for measurement. Before and after cleavage of sucrose, the reaction product NADH catalyzed by D-glucose-6-phosphate-dehydrogenase phosphorylated by hexokinase from D-glucose was measured at 340 nm.

### 2.4 | Amino Acids

Analysis of free amino acids was done according to commission Regulation (EC) No. 152/2009 III F: CELEX No 02009R0152-20130212 (Commission of the European Communities 2009). Samples were hydrolyzed with hydrochloric acid and then separated by ion exchange chromatography (oxidized feedstuff column, type cation exchanger resin, 20 × 4.6 mm column) and determined by photometric detection at 570 nm (primary amino acids) and 440 nm (secondary amino acids) after post-column reaction with ninhydrin using an Amino Acid Analyzer (Biochrom 30, Biochrom Ltd., UK).

### 2.5 | RNA Isolation, Preparation, and Transcript Expression

An aliquot of 150 mg of homogenized frozen plant material was incubated on ice with lysis buffer (100 mM Tris-HCl, pH 8.0; 2 % lauroyl sarcosyl; 25 mM EDTA, pH 8.0; 25 mM EGTA, pH 8.0; 100 mM ß-mercaptoethanol; dissolved in 1% SDS, 5 mM DTT). Phenol and chloroform (each 400 µL) were added, mixed, and centrifuged at 13,000 *g*, 4°C, 10 min. This procedure was repeated using the supernatant and only using 400 µL chloroform. After centrifugation, 60 % v/v isopropanol and 10 % v/v 3 M Na acetate (pH 5.2) were added to the supernatant and precipitated at −20°C overnight. The RNA precipitation was centrifuged 12,000 *g*, 15 min, 4°C and subsequently washed with ice cold ethanol (70%). After completely removing the ethanol, the total RNA was dissolved in 20–50 µL and stored at −80°C.

The cDNA was reverse transcribed using the SuperScript IV Vilo Master Mix (Invitrogen, USA) according to the manufacturer's instructions. The cDNA served as the template for qRT-PCR measurements with iTaq Universal SYBR Green Supermix (Bio-Rad, USA) following the manufacturer's instructions. The conditions for qRT-PCR amplifications were as follows: 95°C for 4:00 min, 40 cycles of 95°C for 20 s, 60°C for 30 s, 72°C for 50 s followed by 95°C for 1 min 65°C for 5 s increment (1°C steps) to 95°C for measuring the melting point. For every transporter, three technical replicates were measured of any of the three biological replicate ($n = 4$) (C1000 Touch Thermal Cycler CFX96 Real-Time System, Bio-Rad, USA).

Two reference genes were chosen according to Sinha et al. (2019): elongation factor 1*α* (EF1*α*) and tubulin (Tub). The following transcripts were analyzed: NPF/NRT1, important role in $NO_3^-$uptake (Morère-Le Paven et al. 2011); NRT2, high affinity $NO_3^-$ uptake system (Pellizzaro et al. 2015); AMT2, uptake and transport of $NH_4^+$ (Straub et al. 2014); DUR3, uptake and transport of urea (Liu et al. 2003); LHT1, transporter for root amino acids uptake (Chen and Bush 1997; Gratz et al. 2021; Hirner et al. 2006; Svennerstam et al. 2007). Primers (10 pmol µL$^{-1}$) are given in Table S1 and were purchased from Thermo Fischer, USA, and cross checked by BLASTN against nucleotide collections of closely related legume species at https://www.ncbi.nlm.nih.gov/nucleotide/. The size of amplified DNA was checked by agarose gels, and amplicons were ligated into a plasmid vector (TOPO TA Cloning, Invitrogen, USA) according to the manufacturer's instructions and sequenced (Macrogen Europe, Netherlands). Sequence data were compared with corresponding gene bank sequences. The target amplicons were 80–123 bp in length (Table S1).

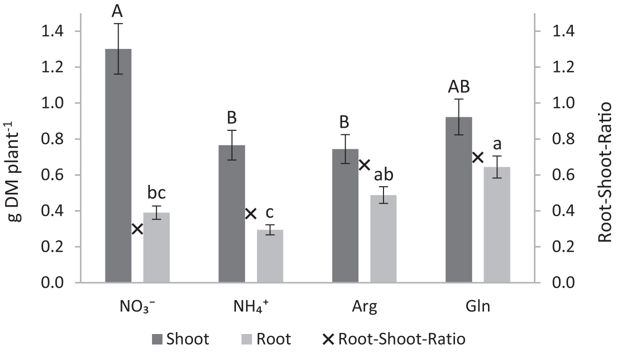

**FIGURE 1** | Plant dry matter (g plant$^{-1}$) of shoot (black) and roots (gray) and root-shoot-ratio (X) after application of: nitrate ($NO_3^-$), ammonium ($NH_4^+$), arginine (Arg), and glutamine (Gln) as N source. Bars represent log back transformed standard errors of the mean ($n = 4$). Similar letters above bars indicate no significant difference between the treatments for shoots (capital letters) or roots (lower case letters) ($p \leq 0.05$).

## 3 | Results

### 3.1 | Plant Biomass

Shoot dry matter was the highest under $NO_3^-$ treatment, while root dry matter was low. Shoot dry matter showed no significant differences in the other treatments, indicating similar effects of ammonium and amino acid supply. Even shoot dry matter under Gln treatment was similar to the $NO_3^-$ treatment. In roots, dry matter was the highest with amino acid supply. The $NH_4^+$ treatment had the lowest root biomass. Accordingly, the root–shoot ratios (x in Figure 1) increased from $NO_3^-$ (0.30) < $NH_4^+$ (0.38) < Arg (0.66) < Gln (0.70).

### 3.2 | Nitrate Concentration

The nitrate concentration in root and shoot tissue was significantly highest at $NO_3^-$ treatment (Figure 2). However, at amino acid nutrition or ammonium nutrition, the nitrate concentrations in roots and shoots were ca. 30 times lower. No significant differences were observed in nitrate concentrations among the $NH_4^+$, Arg, and Gln treatments in either roots or shoots.

### 3.3 | Sugar Concentration

Total sugar concentration in roots was lower at amino acid nutrition. The $NO_3^-$ nutrition had significantly highest sugar concentrations in roots (Figure 3a). The share of glucose was between 4% and 7% of the total sugar concentration in roots. In shoots, the total sugar concentration was 3.5 times higher

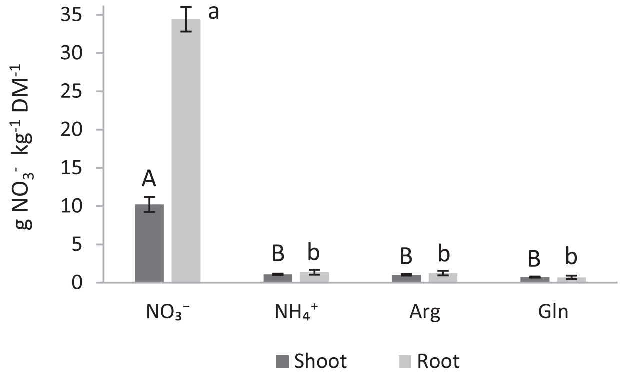

**FIGURE 2** | Nitrate concentration (mg g$^{-1}$ dry matter) of roots (gray) and shoots (black) after application of ($NO_3^-$), ammonium ($NH_4^+$), arginine (Arg), and glutamine (Gln) as N source. Bars represent log back transformed standard errors of the mean ($n$ = 4). Similar letters above bars indicate no significant difference between the treatments for shoots (capital letters) or roots (lower case letters) ($p \leq 0.05$).

than in roots. However, no significant differences of all nutrition treatments were detected (Figure 3b) indicating comparable conditions for photosynthesis and assimilation. The share of glucose in the total sugar concentration in the shoot was between 33% and 56%, which is much higher than in the root.

### 3.4 | Amino Acid Concentrations

Amino acids are found in the rhizosphere as a result of degradation by soil proteases or cellular efflux from plant roots and microbes (D. L. Jones and Hodge 1999). According to Moe (2013), amino acids are appreciated as a key intermediary in the soil nitrogen cycle, and their measured abundance and diversity in soil can be considered a snapshot of the processes resulting in their appearance (organic matter degradation and release from cells) and their disappearance (mineralization and assimilation). Abiotic factors can be considered to account for amino acid variability in soil such as temperature and soil nutrient status (Ivarson et al. 1970). These abiotic processes are intricately linked with biotic processes. Additional work indicates that the composition of the soil amino acid pool changes with plant diversity levels, suggesting differences in organic nitrogen input as well as differences in rates of organic nitrogen mineralization according to the aboveground flora (Sauheitl et al. 2010). Several methods have been used to determine total free amino acid content in soil (Ivarson and Sowden 1969; D. L. Jones et al. 2002), consistently finding low micromolar concentrations (6.7–22.4 μmol L$^{-1}$) across ecosystems and reporting that free amino acids make up 10%–40% of total soluble nitrogen.

In addition to relevant transporter studies of mostly *Arabidopsis* plants (e.g., Forsum et al. 2008), there is not much literature about direct evidence for the uptake of amino acids in crop plants. There are reports in Australian plant communities (Schmidt and Stewart 1999) as well as in tropical sugarcane (Vinall et al. 2012).

In this study, the sum of concentrations of total amino acids (free plus hydrolyzed) was the highest in roots at the two amino acid nutrition treatments but did not differ under nitrate and ammonium treatment (Table 1). In shoot, the sum of amino acids was comparable to those of roots but with significantly highest concentration of Arg nutrition (Table 1). This may also give evidence that lentil roots are able to uptake amino acids in comparable amounts as under nitrate and ammonium nutrition. Generally, in roots and shoots, the amino acid concentration showed a similar pattern. In shoots, there was no significant effect of all four treatments. Shoots had the highest Asp concentrations followed by Leu, Gln, and Lys, and all others were lower. In shoots, all nutrition treatments had no significant effects on amino acid concentrations except for Asp (Figure 4b).

**TABLE 1** | Total amino acid concentration (mg g DM$^{-1}$) in root and shoot (free plus hydrolyzed amino acids).

| Treatment | Root | Shoot |
|---|---|---|
| $NO_3^-$ | 185.0 b | 199.6 b |
| $NH_4^+$ | 193.8 b | 205.8 b |
| Arg | 203.2 b | 216.9 a |
| Gln | 253.9 a | 204.8 b |

*Note:* Significances according to Tuckey test, different letters indicate significant differences for shoot or for root separately.

### 3.5 | Gene Expression

A series of transcripts most relevant transporter could be detected in root tissue via qPCR. The relative transcript abundance of NPF/NRT1, NRT2, AMT2, DUR3, and LHT1 for all nutrition treatments is shown as fold change compared to the expression of NPF/NRT1 under $NO_3^-$ nutrition (Figure 5). The direction of arrows shows the transcript tendency with upward pointing arrows indicating ≥ twofold higher expression and downward pointing arrows showing ≤ 0.5-fold lower expression compared to the expression of the reference. In roots, the NPF/NRT1 transcript abundance remains unchanged under $NH_4^+$ and both amino acid treatments (Figure 5a). The expressions of the transcripts NRT2, AMT2, DUR3, and LHT1 were higher compared to NPF/NRT1 under $NO_3^-$ treatment (Figure 5a). The transcripts of NRT2 increased 139-fold under $NO_3^-$ or 22-fold under Arg treatment. In roots, under $NH_4^+$ treatment, transcripts of NRT2 and LHT1 decreased.

In shoots, only NRT2 and AMT2 in the $NO_3^-$ treatment and AMT2 in the Arg treatment were higher (Figure 5b). Most of the other transcripts, mainly LHT1, decreased substantially. If Gln was the N form provided, all transcripts decreased.

## 4 | Discussion

The results clearly indicate that young lentil plants were able to take up amino acids from the nutrient solution (Figure 1). Despite nitrate nutrition had slightly higher biomass production, amino acid nutrition did produce comparable plants. Moreover, the effect of ammonium nutrition on biomass growth was comparable to amino acid nutrition (Figure 1). Roots had even more com-

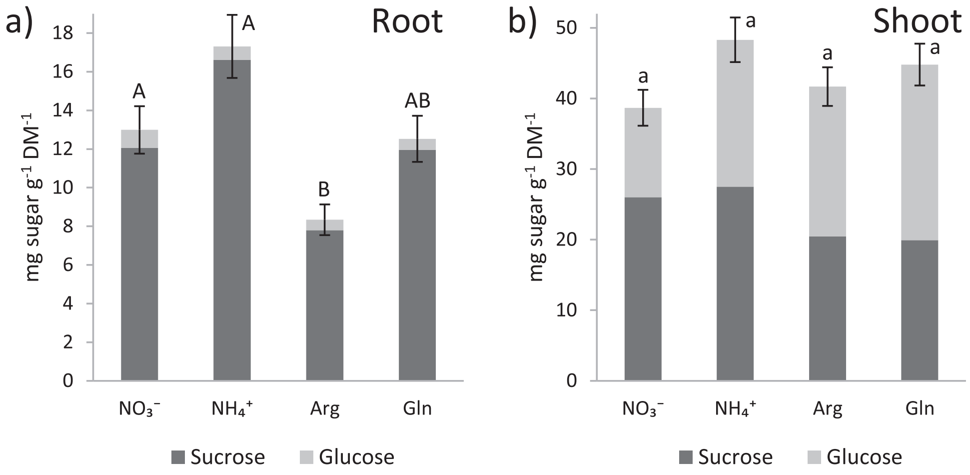

**FIGURE 3** | Sucrose concentrations (dark gray) and glucose concentration (gray) (mg g$^{-1}$ dry matter) of roots (a) and shoots (b) after application of nitrate ($NO_3^-$), ammonium ($NH_4^+$), arginine (Arg), and glutamine (Gln) as N source. Bars represent log back transformed standard errors of the mean ($n = 4$) of the sum of sucrose and glucose. Similar letters above bars indicate no significant difference between the treatments for shoots (capital letters) or roots (lower case letters) (sum of sucrose and glucose; $p \leq 0.05$).

parable biomass growth with high impact on Gln and Arg nutrition. Therefore, at early growth stages, when amino acids were available, those seedlings had no disadvantages. Despite nitrogen concentrations in root and shoot tissues were lower at amino acid nutrition, the biomass production was comparable (Figure 2). This indicates that nitrate concentration even below 10 mg g$^{-1}$ FW in tissues was adequate for early growth under such conditions. Moreover, the higher root–shoot ratio at nutrition with amino acids (Figure 1) may indicate that N availability was sufficient (Brouwer 1962). It remains unclear if with nitrate nutrition the pool of nitrate in vacuoles might be greater, but the amount of nitrogen needed for growth was also sufficient with amino acid nutrition. This would indicate that legumes such as lentils are able to take up amino acids through their roots. However, there may be certain differences in plant development when different N sources are applied; it has been reported that amino acid uptake has an impact on root architecture (Forde et al. 2013; Walch-Liu et al. 2006). Particularly biomass accumulation with amino acid treatment was less than with nitrate treatment but might be sufficient for lentil plants in the early stage (Figure 1). Moreover, this conclusion is supported by the fact that the total sugar concentration in both the shoot and root was similar at $NO_3^-$ and Gln nutrition. However, less sugar was measured in roots after N was provided in the form of Arg (Figure 3) but was sufficient in all treatments, indicating that there were enough photoassimilates for growth, or at least that photosynthetic assimilates were not restricted under amino acid nutrition. Therefore, we can support the first hypothesis that the roots of lentil plants have the ability to use amino acids such as Gln and Arg as a direct source of N. To our knowledge, this finding for lentils is new and has some relevance in organic agriculture which may differ in amino acid concentration (Scheller and Raupp 2005) because organic manure is used as a source of N. Organic residues, especially proteins, which release amino acids and fragments thereof after degradation, can also be taken up at a substantial rate. This may deliver sufficient nitrogen for the establishment of young plants until symbiosis is functional and therefore is of high relevance for organic agriculture.

### 4.1 | Application of Amino Acids as N-Source Influence Amino Acid Pattern

The total (free + hydrolyzed) amino acids (Table 1) indicate no fundamental difference in N uptake between amino acid, ammonium, and nitrate nutrition. The amino acid pattern in lentil roots and also in leaves was not generally affected by amino acid nutrition (Figure 4), and only some amino acids such as Ala, Gln, Gly, and Thr in roots were significantly increased. According to the studies conducted by Moran-Zuloaga et al. (2015), microorganisms take up the majority of amino acids in soils, which are then made available to plants again in digested fragments through the rhizosphere soil solution.

### 4.2 | Amino Acid Uptake

The results of the gene expression analysis were related to the expression of NPF/NRT1, which serves as a reference. Nitrate is an important N source for plants in the soil solution (Crawford 1995) and NPF/NRT1 belongs to the family of nitrate transporters, which are responsible for the uptake of nitrate at higher concentrations (Orsel et al. 2006). Compared to NPF/NRT1 in $NO_3^-$ treatment, other N uptake systems tend to be upregulated in roots (Figure 5a). Therefore, it can be concluded that the lentil plants tend to access a broad spectrum of N sources via the root. The transcript of LHT1, which is known to play an important role in amino acid uptake and transport (Hirner et al. 2006), shows increased expression when N is provided as Arg compared to $NO_3^-$.

For the transport of N within the plant shoot, the AMT2 was upregulated under Arg nutrition (Figure 5b). All other transporter transcripts were unchanged or decreased. This might reflect the fact that for long distance transport, there seems no need for the upregulation of transporter to maintain N status of the plant, and these findings are in agreement with Tegeder and Masclaux-Daubresse (2018). The uptaken amino acids can be directly used

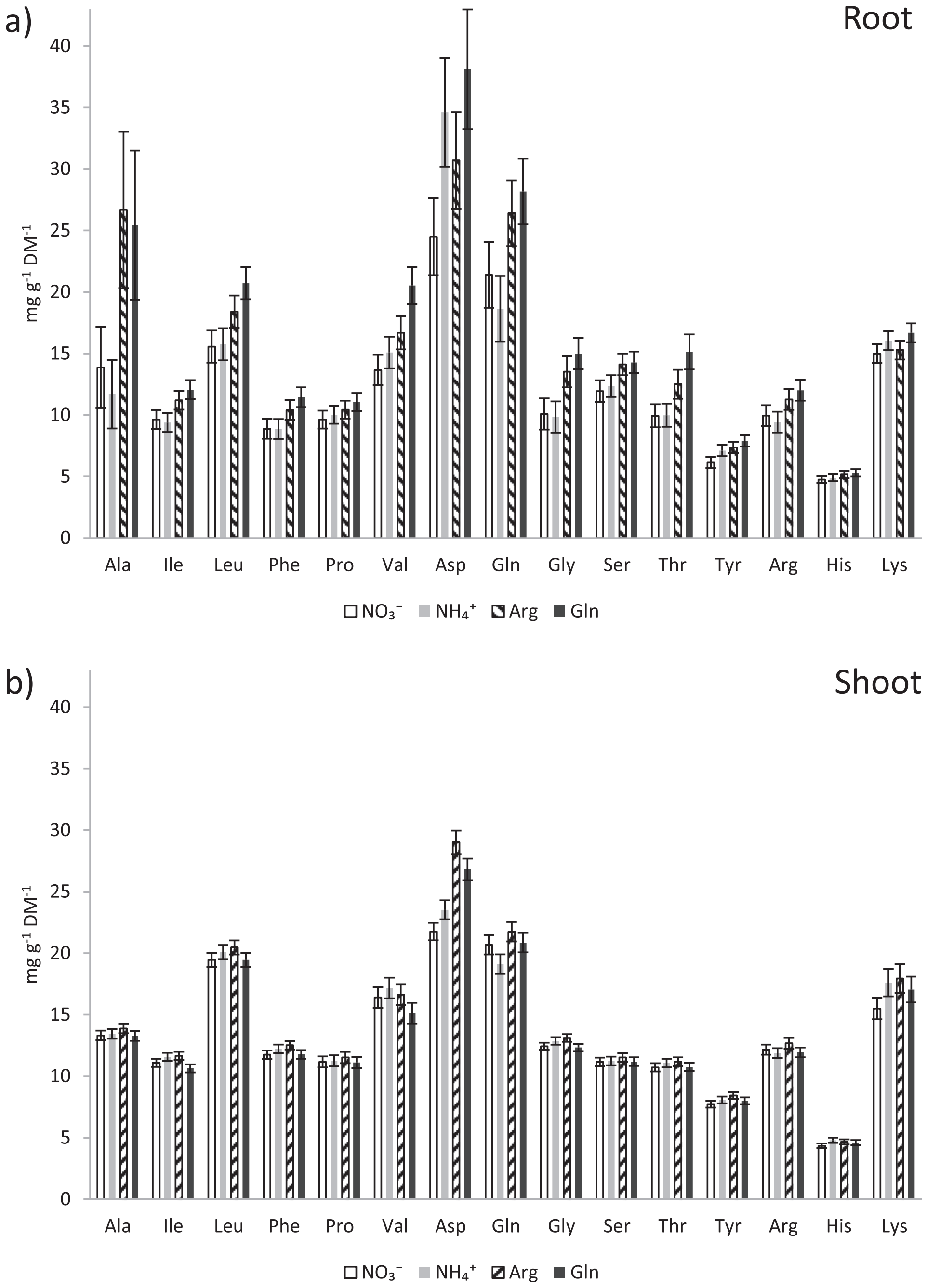

**FIGURE 4** | Concentration of total amino acids (mg $g^{-1}$ dry matter) of roots (a) and shoots (b) after application of nitrate ($NO_3^-$, white), ammonium ($NH_4^+$, light gray), arginine (Arg, hatched gray), and glutamine (Gln, black) as N source. Bars represent log back transformed standard errors of the mean $p \leq 0.05$ ($n = 4$).

in the root physiology contributing to growth and maintenance or might be transported to the shoot or moreover can be converted to other small N containing substances including small peptides such as glutathione and therefore may need other transporter transcripts. Moreover, ammonium transporters such as AMT2 play a role in the storage of amino acids in the vacuole (Howitt and Udvardi 2000; Martinoia et al. 2007).

However, based on our findings, the effectiveness of amino acids as a nitrogen source is lower than that of $NO_3^-$, but they might be important under conditions without mineral nitrogen fertilization, such as in organic agriculture. The use of amino acids in such systems, as highlighted by Gonzalez Perez et al. (2015), underlines the significance of this nitrogen pool in agricultural systems. In general, it seems relevant in the growth phase before the symbiosis of young plants is established.

## 5 | Conclusion

Lentil plants are able to take up amino acids from the rhizosphere and use them as a nitrogen source. This finding is particularly relevant for organic agriculture, where only organic fertilizers are used. Despite the differential expression of investigated

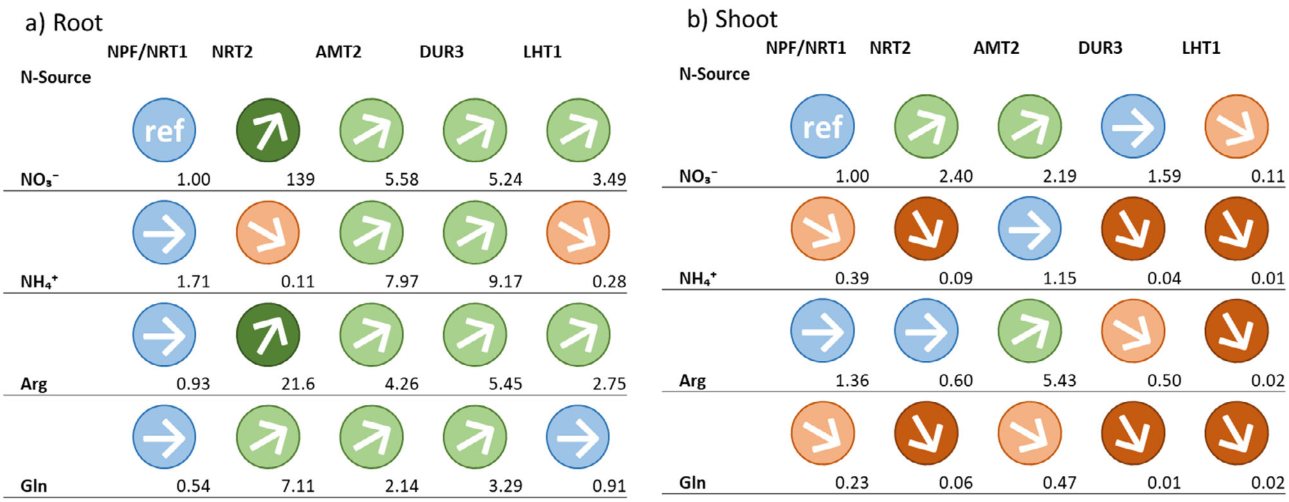

**FIGURE 5** | Relative gene expression of the genes NPF/NRT1, NRT2, AMT2, DUR3, and LHT1 in (a) root and (b) shoot tissue after cultivation with the nitrogen sources $NO_3^-$, $NH_4^+$, arginine, and glutamine as fold change compared to the expression of NPF/NRT1 cultivated under $NO_3^-$ application. Rising arrows indicate greater than twofold, light green ≥ 10-fold, dark green increase and falling arrows < 0.5-fold, light red ≤ 0.1-fold, dark red decrease of gene expression. Numbers show the measured increase and decrease in fold change as a factor compared to the reference ($n = 4$).

uptake and transport systems, the amino acid pattern in roots and shoots does only marginally change with different nitrogen sources. Amino acids can significantly support the nitrogen supply in lentil plants, particularly during the juvenile stage before functional nodules for nitrogen fixation are fully formed.

### Author Contributions

A.A.K., S.Z, M.A.W., and C.Z. contributed to writing and concept. A.A.K. analyzed data and prepared the figures. All authors have read and agreed to the published the manuscript.

### Acknowledgments

This article is dedicated to Dr. Sabine Gruber. We acknowledge the technical assistance of *Ms. Dagmar Repper* and *Christiane Beierle* and also acknowledge the role of *Dr. Sabine Gruber* for her role as PI. The project "*Selektion geeigneter Sortentypen von Linsen* (*Lens culinaris*) *für nachhaltige Anbausysteme*" was financed by the *German Federal Ministry of Food and Agriculture, funding code 2815EPS050*.

Open access funding enabled and organized by Projekt DEAL.

### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

### References

Becana, M., and J. I. Sprent. 1987. "Nitrogen Fixation and Nitrate Reduction in the Root Nodules of Legumes." *Physiologia Plantarum* 70, no. 4: 757–765. https://doi.org/10.1111/j.1399-3054.1987.tb04335.x.

Brouwer, R. 1962. "Nutritive Influences on the Distribution of Dry Matter in the Plant." *Netherlands Journal of Agricultural Science* 10, no. 5: 399–408. https://doi.org/10.18174/njas.v10i5.17581.

Cataldo, D. A., M. Maroon, L. E. Schrader, and V. L. Youngs. 1975. "Rapid Colorimetric Determination of Nitrate in Plant Tissue by Nitration of Salicylic Acid." *Communications in Soil Science and Plant Analysis* 6, no. 1: 71–80. https://doi.org/10.1080/00103627509366547.

Chen, L., and D. R. Bush. 1997. "LHT1, a Lysine- and Histidine-Specific Amino Acid Transporter in *Arabidopsis*." *Plant Physiology* 115, no. 3: 1127–1134. https://doi.org/10.1104/pp.115.3.1127.

Commission of the European Communities. 2009. *Commission Regulation (EC) No 152/2009*. European Union.

Crawford, N. M. 1995. "Nitrate: Nutrient and Signal for Plant Growth." *The Plant Cell* 7, no. 7: 859. https://doi.org/10.1105/tpc.7.7.859.

Epie, K. E., P. J. Bauer, K. C. Stone, and A. M. Locke. 2023. "Nitrogen Fertilizer Effects on Soybean Physiology, Yield Components, Seed Yield and Protein Content in the Southeastern United States." *Journal of Plant Nutrition* 46, no. 3: 462–472. https://doi.org/10.1080/01904167.2022.2084106.

Forde, B. G., S. R. Cutler, N. Zaman, and P. J. Krysan. 2013. "Glutamate Signalling via a MEKK 1 Kinase-Dependent Pathway Induces Changes in *Arabidopsis* Root Architecture." *The Plant Journal* 75, no. 1: 1–10. https://doi.org/10.1111/tpj.12201.

Forsum, O., H. Svennerstam, U. Ganeteg, and T. Näsholm. 2008. "Capacities and Constraints of Amino Acid Utilization in Arabidopsis." *New Phytologist* 179, no. 4: 1058–1069. https://doi.org/10.1111/j.1469-8137.2008.02546.x.

Gomez, L., D. Bancel, E. Rubio, and G. Vercambre. 2007. "The Microplate Reader: An Efficient Tool for the Separate Enzymatic Analysis of Sugars in Plant Tissues—Validation of a Micro-Method." *Journal of the Science of Food and Agriculture* 87, no. 10: 1893–1905. https://doi.org/10.1002/jsfa.2924.

Gonzalez Perez, P., R. Zhang, X. Wang, J. Ye, and D. Huang. 2015. "Characterization of the Amino Acid Composition of Soils Under Organic and Conventional Management After Addition of Different Fertilizers." *Journal of Soils and Sediments* 15: 890–901. https://doi.org/10.1007/s11368-014-1049-3.

Gratz, R., I. Ahmad, H. Svennerstam, et al. 2021. "Organic Nitrogen Nutrition: LHT1. 2 Protein From Hybrid Aspen (*Populus tremula* L. x tremuloides Michx) Is a Functional Amino Acid Transporter and a Homolog of Arabidopsis LHT1." *Tree Physiology* 41, no. 8: 1479–1496. https://doi.org/10.1093/treephys/tpab029.

Harper, J. E., and A. H. Gibson. 1984. "Differential Nodulation Tolerance to Nitrate Among Legume Species." *Crop Science* 24, no. 4: 797–801. https://doi.org/10.2135/cropsci1984.0011183X002400040040x.

Heseker, B., and H. Heseker. 2013. *Nährstoffe in Lebensmitteln: die Große Energie- und Nährwerttabelle, 4. Ed. UZV-Fachbuch*. Umschau Zeitschriften Verlag.

Hirner, A., F. Ladwig, H. Stransky, et al. 2006. "Arabidopsis LHT1 Is a High-Affinity Transporter for Cellular Amino Acid Uptake in Both Root Epidermis and Leaf Mesophyll." *The Plant Cell* 18, no. 8: 1931–1946. https://doi.org/10.1105/tpc.106.041012.

Howitt, S. M., and M. K. Udvardi. 2000. "Structure, Function and Regulation of Ammonium Transporters in Plants." *Biochimica et Biophysica Acta (BBA)-Biomembranes* 1465, no. 1–2: 152–170. https://doi.org/10.1016/S0005-2736(00)00136-X.

Ivarson, K. C., and F. J. Sowden. 1969. "Free Amino Acid Composition of the Plant Root Environment Under Field Conditions." *Canadian Journal of Soil Science* 49, no. 1: 121–127. https://doi.org/10.4141/cjss69-014.

Ivarson, K. C., F. J. Sowden, and A. R. Mack. 1970. "Amino-Acid Composition of Rhizosphere as Affected by Soil Temperature, Fertility and Growth Stage." *Canadian Journal of Soil Science* 50, no. 2: 183–189. https://doi.org/10.4141/cjss70-026.

Jones, D. L., and A. Hodge. 1999. "Biodegradation Kinetics and Sorption Reactions of Three Differently Charged Amino Acids in Soil and Their Effects on Plant Organic Nitrogen Availability." *Soil Biology and Biochemistry* 31, no. 9: 1331–1342.

Jones, D. L., A. G. Owen, and J. F. Farrar. 2002. "Simple Method to Enable the High Resolution Determination of Total Free Amino Acids in Soil Solutions and Soil Extracts." *Soil Biology and Biochemistry* 34, no. 12: 1893–1902. https://doi.org/10.1016/S0038-0717(02)00203-1.

Jones, R. S., R. P. Patterson, and C. D. Raper. 1981. "The Influence of Temperature and Nitrate on Vegetative Growth and Nitrogen Accumulation by Nodulated Soybeans." *Plant and Soil* 63: 333–344. https://doi.org/10.1007/BF02370034.

Liu, L. H., U. Ludewig, W. B. Frommer, and N. von Wirén. 2003. "*AtDUR3* Encodes a New Type of High-Affinity Urea/$H^+$ Symporter in *Arabidopsis*." *The Plant Cell* 15, no. 3: 790–800. https://doi.org/10.1105/tpc.007120.

Martinoia, E., M. Maeshima, and H. E. Neuhaus. 2007. "Vacuolar Transporters and Their Essential Role in Plant Metabolism." *Journal of Experimental Botany* 58, no. 1: 83–102. https://doi.org/10.1093/jxb/erl183.

Moe, L. A. 2013. "Amino Acids in the Rhizosphere: From Plants to Microbes." *American Journal of Botany* 100, no. 9: 1692–1705. https://doi.org/10.3732/ajb.1300033.

Moran-Zuloaga, D., M. Dippold, B. Glaser, and Y. Kuzyakov. 2015. "Organic Nitrogen Uptake by Plants: Reevaluation by Position-Specific Labeling of Amino Acids: Reevaluation of Organic N Uptake by Plants by Position-Specific Labeling." *Biogeochemistry* 125: 359–374. https://doi.org/10.1007/s10533-015-0130-3.

Morère-Le Paven, M.-C., L. Viau, A. Hamon, et al. 2011. "Characterization of a Dual-Affinity Nitrate Transporter MtNRT1. 3 in the Model Legume *Medicago truncatula*." *Journal of Experimental Botany* 62, no. 15: 5595–5605. https://doi.org/10.1093/jxb/err243.

Nacry, P., E. Bouguyon, and A. Gojon. 2013. "Nitrogen Acquisition by Roots: Physiological and Developmental Mechanisms Ensuring Plant Adaptation to a Fluctuating Resource." *Plant and Soil* 370: 1–29. https://doi.org/10.1007/s11104-013-1645-9.

Orsel, M., F. Chopin, O. Leleu, et al. 2006. "Characterization of a Two-Component High-Affinity Nitrate Uptake System in *Arabidopsis*. Physiology and Protein-Protein Interaction." *Plant Physiology* 142, no. 3: 1304–1317. https://doi.org/10.1104/pp.106.085209.

Pellizzaro, A., T. Clochard, E. Planchet, A. M. Limami, and M. C. Morère-Le Paven. 2015. "Identification and Molecular Characterization of *Medicago Truncatula* NRT2 and NAR2 Families." *Physiologia Plantarum* 154, no. 2: 256–269. https://doi.org/10.1111/ppl.12314.

Reckling, M., G. Bergkvist, C. A. Watson, et al. 2016. "Trade-offs Between Economic and Environmental Impacts of Introducing Legumes Into Cropping Systems." *Frontiers in Plant Science* 7: 669. https://doi.org/10.3389/fpls.2016.00669.

Sachs, J. L., K. W. Quides, and C. E. Wendlandt. 2018. "Legumes versus Rhizobia: A Model for Ongoing Conflict in Symbiosis." *New Phytologist* 219, no. 4: 1199–1206. https://doi.org/10.1111/nph.15222.

Sauheitl, L., B. Glaser, M. Dippold, K. Leiber, and A. Weigelt. 2010. "Amino Acid Fingerprint of a Grassland Soil Reflects Changes in Plant Species Richness." *Plant and Soil* 334: 353–363. https://doi.org/10.1007/s11104-010-0387-1.

Scheller, E., and J. Raupp. 2005. "Amino Acid and Soil Organic Matter Content of Topsoil in a Long Term Trial With Farmyard Manure and Mineral Fertilizers." *Biological Agriculture & Horticulture* 22, no. 4: 379–397. https://doi.org/10.1080/01448765.2005.9755299.

Schmidt, S., and G. R. Stewart. 1999. "Glycine Metabolism by Plant Roots and Its Occurrence in Australian Plant Communities." *Functional Plant Biology* 26, no. 3: 253–264. https://doi.org/10.1071/PP98116.

Silsbury, J. H. 1989. "Nodulation and Nitrogen Fixation (Acetylene Reduction) of Four Cultivars of Chickpea." *Australian Journal of Experimental Agriculture* 29, no. 5: 663–669. https://doi.org/10.1071/ea9890663.

Sinha, R., T. R. Sharma, and A. K. Singh. 2019. "Validation of Reference Genes for qRT-PCR Data Normalisation in Lentil (*Lens culinaris*) Under Leaf Developmental Stages and Abiotic Stresses." *Physiology and Molecular Biology of Plants* 25, no. 1: 123–134. https://doi.org/10.1007/s12298-018-0609-1.

Straub, D., U. Ludewig, and B. Neuhäuser. 2014. "A Nitrogen-Dependent Switch in the High Affinity Ammonium Transport in *Medicago truncatula*." *Plant Molecular Biology* 86: 485–494. https://doi.org/10.1007/s11103-014-0243-4.

Svennerstam, H., U. Ganeteg, C. Bellini, and T. Nasholm. 2007. "Comprehensive Screening of *Arabidopsis* Mutants Suggests the Lysine Histidine Transporter 1 to Be Involved in Plant Uptake of Amino Acids." *Plant Physiology* 143, no. 4: 1853–1860. https://doi.org/10.1104/pp.106.092205.

Tegeder, M., and C. Masclaux-Daubresse. 2018. "Source and Sink Mechanisms of Nitrogen Transport and Use." *New Phytologist* 217, no. 1: 35–53. https://doi.org/10.1111/nph.14876.

Vasconcelos, M. W., M. A. Grusak, E. Pinto, et al. 2020. "The Biology of Legumes and Their Agronomic, Economic, and Social impact." In *The Plant Family Fabaceae: Biology and Physiological Responses to Environmental Stresses*, edited by M. Hasanuzzaman, S. Araújo, and S. Singh Gill, 3–25. Springer. https://doi.org/10.1007/978-981-15-4752-2_1.

Vinall, K., S. Schmidt, R. Brackin, P. Lakshmanan, and N. Robinson. 2012. "Amino Acids Are a Nitrogen Source for Sugarcane." *Functional Plant Biology* 39, no. 6: 503–511. https://doi.org/10.1071/FP12042.

Walch-Liu, P., L. H. Liu, T. Remans, M. Tester, and B. G. Forde. 2006. "Evidence That L-Glutamate Can Act as an Exogenous Signal to Modulate Root Growth and Branching in *Arabidopsis thaliana*." *Plant and Cell Physiology* 47, no. 8: 1045–1057. https://doi.org/10.1093/pcp/pcj075.

Wedeking, R., A. K. Mahlein, U. Steiner, E. C. Oerke, H. E. Goldbach, and M. A. Wimmer. 2016. "Osmotic Adjustment of Young Sugar Beets (*Beta vulgaris*) Under Progressive Drought Stress and Subsequent Rewatering Assessed by Metabolite Analysis and Infrared Thermography." *Functional Plant Biology* 44, no. 1: 119–133. https://doi.org/10.1071/FP16112.

Zhang, X., B. L. Franzisky, L. Eigner, C. M. Geilfus, and C. Zörb. 2021. "Antagonism of Chloride and Nitrate Inhibits Nitrate Reductase Activity in Chloride-STRESSED Maize." *Plant Growth Regulation* 93: 279–289. https://doi.org/10.1007/s10725-020-00685-2.

## Supporting Information

Additional supporting information can be found online in the Supporting Information section.