Biology and Fertility of Soils (2023) 59:1007–1011
https://doi.org/10.1007/s00374-023-01754-0

SHORT COMMUNICATION

Amino mapping: possibility to visualize amino-N compounds in the rhizosphere of *Zea Mays* L.

Sajedeh Khosrozadeh[1,2] · Andrey Guber[3,4] · Farshid Nourbakhsh[2] · Banafshe Khalili[2] · Evgenia Blagodatskaya[1]

Received: 15 December 2022 / Revised: 16 May 2023 / Accepted: 27 June 2023 / Published online: 22 July 2023

Abstract

Understanding N uptake by plants, the N cycle, and their relationship to soil heterogeneity has generated a great deal of interest in the distribution of amino-N compounds in soil. Visualization of the spatial distribution of amino-N in soil can provide insights into the role of labile N in plant-microbial mechanisms of N acquisition and plant N uptake, but until now, it has remained technically challenging. Here, we describe a novel technique to visualize the amino-N distribution at the root-soil interface. The technique is based on time-lapse amino mapping (TLAM) using membranes saturated with the fluorogenic OPAME reagent (*O*-phthalaldehyde and β-mercaptoethanol). OPAME in the membrane reacts with organic compounds containing a NH_2 functional group at the membrane-soil interface, generating a fluorescent product visible under UV light and detectable by a digital camera. The TLAM amino-mapping technique was applied to visualize and quantify the concentration of amino-N compounds in the rhizosphere of maize (*Zea Mays* L.). A ten times greater amino-N concentration was detected in the rhizosphere compared to non-rhizosphere soil. The high content of amino-N was mainly associated with the root tips and was 3 times larger than the average amino-N content at seminal roots. The amino-N rhizosphere was 2 times broader around the root tips than around other parts of the roots. We concluded that TLAM is a promising approach for monitoring the fate of labile N in soils. However, the technique needs to be standardized for different soil types, plant species, and climate conditions to allow wider application.

Keywords Time-lapse amino mapping (TLAM) · OPAME reagent · Amino-N compound distribution · Rhizosphere

Introduction

Although organic compounds containing NH_2 functional groups (e.g., amino acids, amino sugars) account for only a minor proportion of dissolved organic N in soils (Roberts et al. 2007; Warren 2017), they have attracted considerable interest as contributors of labile N in natural ecosystems due to their rapid turnover and intact uptake by plants and microorganisms (Jones et al. 2005a; Nannipieri and Paul 2009). Living and dead roots, microbial exudation, litter inputs (Jones et al. 2005a; Moe 2013; Holz et al. 2017), and microbial debris (Roberts et al. 2007) are key sources of amino acids and amino sugars in soil. Rhizodeposition accelerates microbial activity in the rhizosphere, resulting in stiff competition for amino acids as the most available N source in the root zone (Moe 2013; Holz et al. 2017; Blagodatskaya et al. 2021). Plants, in turn, are able to recapture large amount of exogenous amino acids from soil (Jones et al. 2005b). Therefore, plant roots might be both a major source and sink of amino acids.

Numerous methods have been developed to assay total and specific amino acid/sugar concentrations in soil. For example, a chromogenic dye assay using ninhydrin (Moore and Stein 1954) and a fluorometric assay using OPAME (*O*-phthalaldehyde and β-mercaptoethanol) have been adapted for the measurement of free amino acids (Jones

✉ Sajedeh Khosrozadeh
s.khosrozadeh@ag.iut.ac.ir

✉ Evgenia Blagodatskaya
evgenia.blagodatskaya@ufz.de

[1] Department of Soil Ecology, Helmholtz-Centre for Environmental Research – UFZ, 06120 Halle (Saale), Germany

[2] Department of Soil Science, College of Agriculture, Isfahan University of Technology, Isfahan 84156-83111, Iran

[3] Department of Soil, Plant and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA

[4] DOE Great Lakes Bioenergy Research Center, Michigan State University, East Lansing, MI 48824, USA

et al. 2002; Darrouzet-Nardi et al. 2013) and amino sugars (Roberts et al. 2007) in soil. The balance between amino acid production and consumption determines the total amino acid concentration measured in a homogenized soil sample (Hill et al. 2019). Due to high soil heterogeneity, greater microbial activity and faster nutrient turnover rates occur in soil hotspots enriched with easily degradable organic matter (Kuzyakov and Blagodatskaya 2015; Ghaderi et al. 2022; Hao et al. 2022), where dynamic inputs of amino acids (either plant or microbial origin) are expected (Inselsbacher et al. 2011; Hill et al. 2019; Blagodatskaya et al. 2021). Indeed, compared to non-rhizosphere soil, larger concentrations of amino acids have been detected in the rhizosphere (Inselsbacher et al. 2011), biopores, and detritusphere hotspots by a microdialysis approach (Hill et al. 2019). Despite the successful determination of amino acids in soil microhabitats, the latter method only determines amino acid fluxes and cannot quantify the spatial distribution of NH_2-containing compounds in the soil microenvironments.

The aim of the present study was to visualize and quantify the spatial distribution of amino–N compounds at the root-soil interface of *Zea Mays* L. We employed a new technique, termed time-lapse amino mapping (TLAM), that combined the principle of membrane soil zymography (Spohn and Kuzyakov 2013; Sanaullah et al. 2016) with a time-lapse approach (Guber et al. 2021). The technique involved applying an OPAME reagent (*O*-phthalaldehyde and β-mercaptoethanol) saturated membrane on the soil and at the soil-root interface. The OPAME reagent reacted with NH_2 functional groups in compounds at the membrane-soil interface to generate a fluorescent product visible under UV light. Development of the fluorescence signal in the membrane over time was recorded using a DSLR camera (time-lapse approach). We hypothesized that amino-N compounds are mainly produced by roots and root-associated microorganisms, i.e., the rhizo-microbiome. Therefore, their concentrations were expected to be greater in the rhizosphere than in non-rhizospheric soil.

Materials and methods

The soil was collected from a depth of 0–50 cm of a loamy Haplic Phaeozem in an agricultural area planted with oilseed rape (Vetterlein et al. 2021) near Schladebach, Saxony-Anhalt, Germany (51.3087° N, 12.1045°E). The soil had a loam texture (sand 33%, silt 48%, and clay 19%) with 8.6 g kg^{-1} total organic carbon, 0.84 g kg^{-1} total nitrogen, and pH of 6.4 ($CaCl_2$).

Soil and seeds were prepared as described by Vetterlein et al. (2021) as part of the DFG Priority Program 2089 "Rhizosphere spatiotemporal organization—a key to rhizosphere function." The soil was air-dried, passed through a 2 mm sieve, and kept at room temperature. The soil was treated with 50 mg N (NH_4NO_3), 50 mg K (K_2SO_4), 25 mg Mg ($MgCl_2$ $6H_2O$), and 40 mg P ($CaHPO_4$) per kg dry mass, and a 1 mm sieve was used to uniformly distribute the fertilizer. To visualize the spatial distribution of amino-N at the root–soil interface, a rhizobox setup was established to grow maize plants. Sieved and fertilized soil was packed in transparent rhizoboxes (3 × 8.8 × 17.8 cm, H × W × L; Clickbox® Germany) to a final bulk density of 1.26 g cm^{-3}. Sterilized maize (*Zea mays* L.) seeds were sown 1 cm below the soil surface in three replicate rhizoboxes. Soil water content was maintained at ~ 22% (v/v) in the rhizoboxes during the growth period (21 days), and distilled water was added regularly to the soil to compensate for evapotranspiration losses. During the experiment, the rhizoboxes were inclined at 50° to the horizontal to direct root growth along the front rhizobox walls, as suggested by Spohn and Kuzyakov (2013) and Sanaullah et al. (2016).

For amino mapping, the OPAME reagent was prepared according to Jones et al. (2002). Briefly, 50 mg of *O*-phthalaldehyde was dissolved in 5 ml of methanol, then 100 µl of mercaptoethanol was added, and the mixture was vortexed in a fume hood (OPAME concentrate). To prepare the working solution, 200 ml of borate buffer (0.02 M, pH 9.5) was added to the OPAME concentrate. Various membrane types (cellulose, regenerated cellulose, polyamide, polycarbonate, and cellulose nitrate) were tested to determine the best membrane for amino mapping (see the supplement for details). Consequently, 140 µm thick cellulose nitrate membranes with 0.2 µm pores size were chosen as the most appropriate for amino mapping due to their hydrophilicity, non-auto fluorescent properties, and high signal-to-noise ratio.

Three cellulose nitrate membranes (circle, plain, 0.2 µm, 50 mm ∅, Whatman®, Cytiva 10401314) were soaked in OPAME reagent. The rhizoboxes were opened from the root side and placed in a dark chamber with 15 W blue-black ultraviolet lamps (Erolite® Germany) as sources of UV light. The OPAME-saturated membranes were placed directly on soil-root surfaces for amino mapping. A transparent glass sheet was placed over the membranes to keep them in contact with the soil and prevent evaporation of the substrate from the membranes. A Nikon D3500 DSLR camera equipped with an AF-P DX NIKKOR 18–55 mm f/3.5–5.6G VR lens (Nikon Inc., Melville, NY) was used to capture images. The object distance, lens aperture, and shutter speed were 210 mm, f/6.3, and 1/125 s, respectively. Membrane images were captured during a 10-min time series (every 10 s for the first minute and then every 30 s during the next 9 min).

Similarly to fluorometric microplate assay, L-leucine was used as a standard for amino mapping. L-leucine has been recommended as an affordable, representative standard, and the units of L-leucine equivalents have

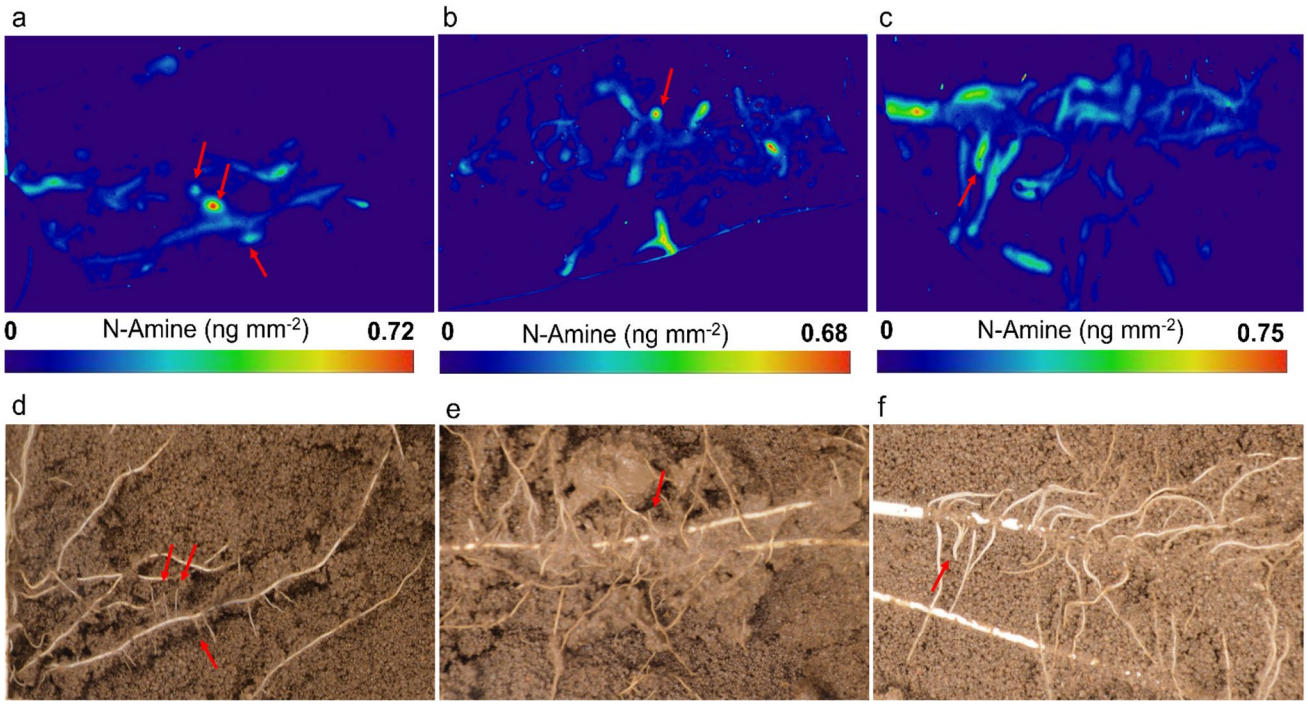

Fig. 1 Aminograms (**a**–**c**) of maize roots (*Zea Mays* L.). Daylight photographs of maize roots (**d**–**f**) taken before TLAM application. The red arrows show the root tips

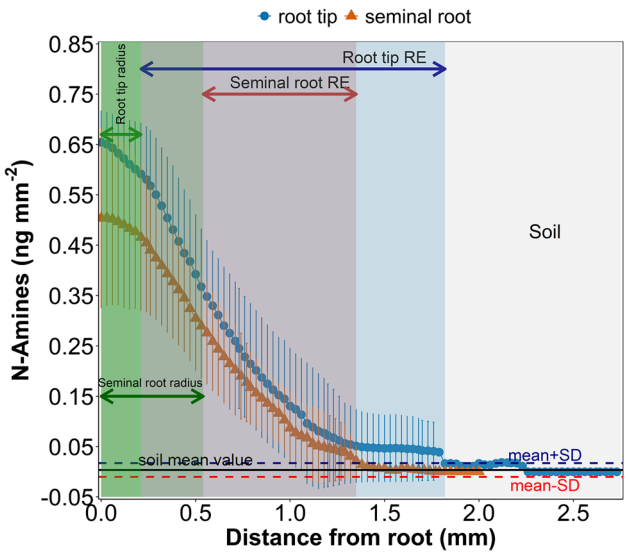

Fig. 2 Distribution of amino-N compounds measured from the center of a maize (*Zea Mays* L.) root tip or seminal root to non-rhizospheric soil: zero distance denotes the center of the maize root. RE indicates the extent of the rhizosphere

been shown to provide an approximation of the true total free primary amine concentration (Darrouzet-Nardi et al. 2013). For quantitative analysis of amino-mapping images (aminograms), 5 µl of an L-leucine standard solution of concentration 0, 0.1, 0.5, or 1 mM (corresponding to 0, 7, 35, and 70 ng of L-leucine-N, respectively) and 10 µl of OPAME were applied to the membranes, then they were covered with a transparent glass sheet to mimic the amino-mapping settings. Membranes saturated with the standard solutions were photographed under UV light with the same camera settings as used for TLAM.

The standard solution images were used for calibration and processed using ImageJ software (Schindelin et al. 2012) to determine the relationship between image brightness and equivalent N content in image pixels. The calibration images were split into red, blue, and green channels and converted to 8-bit format. The brightness in the image pixels across the calibration membranes was determined by subtracting average grayscale values (GSVs) of images with zero L-leucine content from the GSVs of the calibration membranes separately for each channel. Linear regression analysis between the applied N content and GSV sums in the pixels of the calibration images acquired 5 min after application of the L-leucine standards was used to calculate the calibration coefficient (a) from

$$M_i = a \sum_1^n G_i^j F_i^j \quad 1 < j < 255$$

where M_i is the N amount applied to the membrane (ng), G_i^j is the GSV in pixels, F_i^j is the number of pixels in each gray-scale group j, and a is the calibration coefficient (ng of N G-value^{-1} pixel^{-1}). The best calibration was obtained for the green channel with $a = 1.015 \times 10^{-5}$ ng G^{-1} pixel^{-1} (Fig. S5). As this type of calibration depends on the individual imaging process and camera settings, it should be re-evaluated for each experimental setup. To acquire aminograms, the time series amino mapping was processed using a modified time-lapse procedure proposed by Guber et al. (2021). Specifically, the N-amino content in the membranes was calculated based on the maximum G values detected in individual pixels of the green channel during 10 min of amino mapping. The rhizosphere extent of the N-amino compounds was analyzed using the ImageJ Plot Profile tool from cross-sections drawn across selected roots perpendicular to their axes by determining the distance between a region with high amino-N concentration in the soil (mean soil concentration + SD) and the root border. The average GSV of areas corresponding to non-rhizosphere soil was defined as the mean soil concentration of amino-N.

Results and discussion

Application of OPAME-saturated membranes enabled visualization of the spatial distribution of amino-N, which was mainly associated with the maize roots and rhizosphere (Fig. 1). A higher concentration of amino-N compounds was detected in the rhizosphere than in non-rhizospheric soil (Fig. 1 a–c), most likely due to root exudation and high microbial activity in the rhizosphere. Amino acids are known to predominate in root exudates, which are released in vast quantities from roots to soil (Jones et al. 2005a; Moe 2013).

The concentration of amino-N compounds decreased with increasing distance from the root surface (Fig. 2) and extended up to a 2 mm zone around the center of roots (Fig. 2). The amino-N concentration was 10 times higher in the vicinity of roots than in the non-rhizosphere soil, supporting our initial hypothesis. Despite the approximately two times smaller radius of the root tips than the average size of seminal roots, the area of elevated amino-N concentration was almost doubled around the former. Thus, the relative rhizosphere extent (as a ratio of the root radius) was about 5 times smaller at seminal roots (1.5) than at their tips (7.6). The higher concentration of amino-N at the root tips (Fig. 2) provided visual evidence supporting the common assumption that amino acids are mainly released from the root tips of *Zea Mays* L. (Jones and Darrah 1994). Previous observations of high amino acid concentrations at a fine scale in biopores and the detritusphere (Hill et al. 2019) suggest that high amino-N content may indicate soil hotspots.

Conclusions

An amino-mapping approach was successfully developed and tested to visualize the two-dimensional distribution of amino-N compounds at an undisturbed root-soil interface. However, further work is required to standardize the method and test it for different soil types, plant species, vegetation stages, and physiological situations, such as drought stress or nutrient deficiency.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-023-01754-0.

Funding Open Access funding enabled and organized by Projekt DEAL. This work was done at Helmholtz-Centre for Environmental Research—UFZ and was financially supported by this research center. This work was conducted within the framework of the priority program 2089 "Rhizosphere spatiotemporal organization – a key to rhizosphere functions," funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)—Project number: 403664478. Seeds of the maize were provided by Caroline Marcon and Frank Hochholdinger (University of Bonn). Support for this research was provided in part by the NSF DEB Program (Award # 1904267); by the NSF LTER Program (DEB 1027253) at the KBS; by the GLBRC, U.S. DOE under Award Number DE-SC0018409 and by USDA-NIFA Program (Award# 2022–67019-36104). The first author is grateful for her scholarship to the Isfahan University of Technology, Isfahan, Iran.

Data availability Data are available from the corresponding authors upon request.

Declarations

Competing interests The authors declare no competing interests.

References

Blagodatskaya E, Tarkka M, Knief C, Koller R, Peth S, Schmidt V, Spielvogel S, Uteau D, Weber M, Razavi BS (2021) Bridging microbial functional traits with localized process rates at soil interfaces. Front Microbiol 12:625697. https://doi.org/10.3389/fmicb.2021.625697

Darrouzet-Nardi A, Ladd MP, Weintraub MN (2013) Fluorescent microplate analysis of amino acids and other primary amines in soils. Soil Biol Biochem 57:78–82. https://doi.org/10.1016/j.soilbio.2012.07.017

Ghaderi N, Schmidt H, Schlüter S, Banfield C, Blagodatskaya E (2022) Development of micro-zymography: visualization of enzymatic

activity at the microscopic scale for aggregates collected from the rhizosphere. Plant Soil 478:253–271. https://doi.org/10.1007/s11104-022-05573-4

Guber A, Blagodatskaya E, Juyal A, Razavi BS, Kuzyakov Y, Kravchenko A (2021) Time-lapse approach to correct deficiencies of 2D soil zymography. Soil Biol Biochem 157:108225. https://doi.org/10.1016/j.soilbio.2021.108225

Hao C, Dungait JAJ, Wei X, Ge T, Kuzyakov Y, Cui Z, Tian J, Zhang F (2022) Maize root exudate composition alters rhizosphere bacterial community to control hotspots of hydrolase activity in response to nitrogen supply. Soil Biol Biochem 170:108717. https://doi.org/10.1016/j.soilbio.2022.108717

Hill EJ, Jones DL, Paterson E, Hill PW (2019) Hotspots and hot moments of amino acid N in soil: real-time insights using continuous microdialysis sampling. Soil Biol Biochem 131:40–43. https://doi.org/10.1016/j.soilbio.2018.12.026

Holz M, Zarebanadkouki M, Kuzyakov Y, Pausch J, Carminati A (2017) Root hairs increase rhizosphere extension and carbon input to soil. Ann Bot 121:61–69. https://doi.org/10.1093/aob/mcx127

Inselsbacher E, Öhlund J, Jämtgård S, Huss-Danell K, Näsholm T (2011) The potential of microdialysis to monitor organic and inorganic nitrogen compounds in soil. Soil Biol Biochem 43:1321–1332. https://doi.org/10.1016/j.soilbio.2011.03.003

Jones DL, Darrah PR (1994) Amino-acid influx at the soil-root interface of Zea mays L. and its implications in the rhizosphere. Plant Soil 163:1–12. https://doi.org/10.1007/BF00033935

Jones DL, Owen AG, Farrar JF (2002) Simple method to enable the high resolution determination of total free amino acids in soil solutions and soil extracts. Soil Biol Biochem 34:1893–1902. https://doi.org/10.1016/S0038-0717(02)00203-1

Jones DL, Healey JR, Willett VB, Farrar JF, Hodge A (2005a) Dissolved organic nitrogen uptake by plants—an important N uptake pathway? Soil Biol. Biochem 37:413–423. https://doi.org/10.1016/j.soilbio.2004.08.008

Jones DL, Shannon D, Junvee-Fortune T, Farrar JF (2005b) Plant capture of free amino acids is maximized under high soil amino acid concentrations. Soil Biol Biochem 37:179–181. https://doi.org/10.1016/j.soilbio.2004.07.021

Kuzyakov Y, Blagodatskaya E (2015) Microbial hotspots and hot moments in soil: concept & review. Soil Biol Biochem 83:184–199. https://doi.org/10.1016/j.soilbio.2015.01.025

Moe LA (2013) Amino acids in the rhizosphere: from plants to microbes. Am J Bot 100:1692–1705. https://doi.org/10.3732/ajb.1300033

Moore S, Stein WH (1954) A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J Biol Chem 211:907–913. https://doi.org/10.1016/S0021-9258(18)71178-2

Nannipieri P, Paul EA (2009) The chemical and functional characterization of soil N and its biotic components. Soil Biol Biochem 41:2357–2369. https://doi.org/10.1016/j.soilbio.2009.07.013

Roberts P, Bol R, Jones DL (2007) Free amino sugar reactions in soil in relation to soil carbon and nitrogen cycling. Soil Biol Biochem 39:3081–3092. https://doi.org/10.1016/j.soilbio.2007.07.001

Sanaullah M, Razavi BS, Blagodatskaya E, Kuzyakov Y (2016) Spatial distribution and catalytic mechanisms of β-glucosidase activity at the root-soil interface. Biol Fertil Soils 52:505–514. https://doi.org/10.1007/s00374-016-1094-8

Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, Preibisch S, Rueden C, Saalfeld S, Schmid B, Tinevez J-Y, White DJ, Hartenstein V, Eliceiri K, Tomancak P, Cardona A (2012) Fiji: an open-source platform for biological-image analysis. Nat Methods 9:676–682. https://doi.org/10.1038/nmeth.2019

Spohn M, Kuzyakov Y (2013) Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – coupling soil zymography with 14C imaging. Soil Biol Biochem 67:106–113. https://doi.org/10.1016/j.soilbio.2013.08.015

Vetterlein D, Lippold E, Schreiter S, Phalempin M, Fahrenkampf T, Hochholdinger F, Marcon C, Tarkka M, Oburger E, Ahmed M, Javaux M, Schlüter S (2021) Experimental platforms for the investigation of spatiotemporal patterns in the rhizosphere—laboratory and field scale. J Plant Nutr Soil Sci 184:35–50. https://doi.org/10.1002/jpln.202000079

Warren CR (2017) Changes in small organic N during early stages of soil development. Soil Biol Biochem 110:44–55. https://doi.org/10.1016/j.soilbio.2017.03.004