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Variation in the capacity for organic nitrogen acquisition along the root length of rice and wheat

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Abstract: Oligopeptides constitute an important yet understudied component of soil's dissolved organic nitrogen (DON) pool, representing a primary breakdown product of proteins. However, the mechanisms of oligopeptide uptake and utilisation by crop roots remain poorly understood in a plant nutrition context. We investigated the rate and spatial uptake pattern of ^{14}C-labelled alanine and di- to pentapeptides of alanine in wheat and rice under sterile hydroponic conditions. Both species demonstrated the capacity to absorb N through amino acids and oligopeptides, with rice roots showing higher peptide uptake than wheat. Specifically, alanine absorption exceeded peptide uptake by 3–7-fold in rice and 6–9-fold in wheat. Using phosphor imaging, we demonstrated that alanine and oligopeptide uptake occurred throughout the root system, with the highest accumulation in the root tip and root hair regions. Further, spatial analysis revealed that peptide absorption rates in rice were 2–5 times higher in the 0–1 cm root section and 1.5–4 times higher in the 1–2 cm section compared to corresponding wheat root segments. We conclude that plants can directly take up amino acids and oligopeptides to acquire exogenous N, with marked differences occurring among species in both uptake efficiency and spatial uptake patterns.

Keywords: amino acid uptake; crop nutrition; dissolved organic carbon; peptide transport

Nitrogen (N) is the most critical and largest input in agroecosystems, serving as the primary nutrient driving plant development and agricultural productivity (Mokhele et al. 2012, Mao et al. 2025). Traditionally, ammonium (NH_4^+) and nitrate (NO_3^-) are considered to be the primary inorganic N sources for plants (Hachiya and Sakakibara 2017, Ye et al. 2022); however, extensive N fertiliser application, particularly in China, has resulted in very low N utilisation efficiency (ca. 30–50%; Moran-Zuloaga et al. 2015, Mahboob et al. 2023). These intensive agricultural practices have led to significant N losses, causing soil quality degradation and environmental contamination through eutrophication of freshwater ecosystems, alongside substantial

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ammonia (NH_3) and nitrous oxide (N_2O) emissions (Shen et al. 2014, Sainju et al. 2019).

In N-limited natural ecosystems, dissolved organic nitrogen (DON) often dominates the soluble N pool, primarily from proteins released by dead plant residues and soil microorganisms (Farrell et al. 2013). Generally, these proteins are degraded by soil microorganisms into shorter-chain proteins, oligopeptides, which are then further cleaved into their constituent amino acids and ultimately mineralised to NH_4^+ for plant uptake (Farrell et al. 2013). Research has demonstrated that plants can utilise a wide range of low molecular weight DON compounds (e.g., urea, amino acids, oligopeptides) not solely through microbial mineralisation, but *via* direct uptake using a range of active transporters (Tegeder and Rentsch 2010, Xiu et al. 2019, Tang et al. 2023, 2024), such as maize (*Zea mays* L.) (Biernath et al. 2008), tundra sedges (Schimel and Chapin 1996). These transporters may also be involved in the recapture of DON lost into the soil *via* passive root exudation or for the exchange of peptide signals with symbionts (Wu et al. 2007, Jones et al. 2009, Drechsler et al. 2018). This direct uptake represents a metabolically efficient "short-circuit" in the N cycle, potentially reducing energy expenditure and minimising N losses through processes like nitrification-denitrification and leaching (Näsholm et al. 2009, Moran-Zuloaga et al. 2015, Farzadfar et al. 2021, Ma et al. 2021, 2025). In agricultural systems, inorganic N often dominates due to fertiliser addition; however, numerous studies have also documented the importance of DON in soil N cycling (Ros et al. 2009, Liang et al. 2021), especially amino acid uptake in plant nutrition (Näsholm et al. 2009, Hill et al. 2011a, Moreau et al. 2019). Murphy et al. (2000) reported that the soil soluble organic N pool can be as abundant as the inorganic N pool, and each accounts for approximately 50% of the total soluble N pool. These values can be influenced by various factors such as soil type, cropping system, and fertilisation practices (Hill et al. 2011a, Farrell et al. 2013). For example, soil organic N content was significantly higher under long-term fertilisation than under conventional mineral fertilisation (Liu et al. 2023). However, the contribution of this N pool to crop plant nutrition remains controversial (Näsholm et al. 2001, Reeve et al. 2009, Kuzyakov and Xu 2013).

Oligopeptides, as key components of the DON pool, represent a more labile and bioavailable fraction compared to proteins or complex organic N. Mengel et al. (1999) highlighted that ca. 60% of the soluble N extracted by 0.01 mol/L $CaCl_2$ from agricultural soil was in the form of amino acids and peptides. In agricultural soils of the UK, the content of soluble N present as small peptides (with a molecular weight < 1 kDa) is comparable to that present as free amino acids (Farrell et al. 2011, Hill et al. 2011b). While peptide transporters have been extensively characterised in model plants such as *Arabidopsis* (Tegeder and Rentsch 2010, Tegeder et al. 2018, Perchlik and Tegeder 2017), their specific localisation and functionality in crop species (e.g., rice, wheat, maize) remain poorly understood. Further, previous measurements of root peptide uptake have two major methodological constraints: (1) studies typically use unrealistically high concentrations (1–10 mmol/L; Soper et al. 2011, Matsumiya et al. 2012) compared to actual soil solution levels (1–100 µmol/L; Wilkinson et al. 2014); and (2) transporter expression studies often neglect to validate functional roles in N uptake under environmentally relevant conditions.

This study aimed to characterise the spatial distribution and kinetics of oligopeptide uptake across root zones in rice and wheat at relevant concentrations. We hypothesised that rice and wheat would demonstrate species-specific mechanisms of DON acquisition, reflecting their distinct evolutionary adaptations to different ecological environments.

MATERIAL AND METHODS

Preparation of experimental plants. Plant amino acid and oligopeptide uptake was measured under sterile hydroponic conditions to avoid microbial uptake of the substrates and interference by sorption to soil particles (Jones et al. 2005, Oburger and Jones 2018). Seeds of rice (*Oryza sativa* L. bilinear hybrid rice cv. Xinliangyou 223) and wheat (*Triticum aestivum* L. cv. Granary) were surface sterilised in 15% NaClO and 80% ethanol, and grown aseptically according to Hill et al. (2011b). Briefly, surface-sterilised seeds were germinated on 50% Murashige and Skoog (MS) agar for two weeks. Subsequently, the seedlings were transferred to sterile Phytatrays (Sigma Aldrich, Gillingham, UK) containing 50% MS basal medium in sterile perlite. All plants were grown in a climate-controlled cabinet with a temperature of 20 °C, 16 h photoperiod and light intensity of 500 µmol/m^2/s, until the three-leaf stage was reached.

Uptake and spatial mapping of amino acid and oligopeptide uptake in intact roots. Alanine was

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chosen as it is one of the most abundant amino acids in soil organic matter and root exudates. At the 3rd leaf stage, all plants were visually inspected and measured to ensure minimal variation within species. Root systems of individual plants (n = 3) were placed in 0.2 µm-filtered solutions containing either 100 µmol/L of ^{14}C-labelled alanine (Ala), di-alanine (Di-Ala), tri-alanine (Tri-Ala), tetra-alanine (Tetra-Ala), or penta-alanine (Penta-Ala) (5 kBq/mL; American Radiolabeled Chemicals, St Louis, USA). After a short incubation time (10 min), the roots were removed, then washed thoroughly in water and 0.1 mol/L $CaCl_2$ to remove ^{14}C-label retained in the apoplast (Farrar 1985) before oven-drying (80 °C, 48 h). Their ^{14}C distribution was then visualised using a Cyclone Plus phosphor-imager (PerkinElmer, Waltham, USA) to map ^{14}C-amino acid/oligopeptide uptake patterns. Based on our experience, short uptake times were used to limit redistribution of the ^{14}C-label post-uptake. Subsequently, the ^{14}C content of the dried roots was determined using an OX400 biological oxidiser (RJ Harvey, Hillsdale, USA) in which the $^{14}CO_2$ evolved was captured in Oxosol scintillant (National Diagnostics, Atlanta, USA). The ^{14}C activity in the scintillant was then determined by liquid scintillation counting using a Wallac 1404 liquid scintillation counter with automated quench correction (Wallac EG&G, Milton Keynes, UK).

Uptake of amino acids and oligopeptides in root tips. For the intact root systems of plants at the 3rd leaf stage, only primary seminal roots (the first-emerged seminal root, morphologically identified by their thicker diameter and dominant growth direction) were selected to ensure uniformity in root type. From each selected primary seminal root, root tips were excised with a sterile scalpel into two distinct segments: 0–1 cm and 1–2 cm from the root apex, corresponding to the primary nutrient-uptake zones. For each plant, 3 replicate segments were collected from each zone, with 3 plants selected per species/treatment group to ensure statistical robustness. These segments were immediately transferred to sterile 1.5 mL Eppendorf tubes containing 0.5 mL of the respective ^{14}C-labelled solutions (as described above). After a 30-min uptake period, root segments were removed from the solution and subjected to a two-step washing procedure: first with deionised water to remove surface-adhered solution, followed by immersion in 0.1 mol/L $CaCl_2$ to displace ^{14}C-labelled compounds retained in the apoplast (Farrar 1985). Segments were then oven-dried at 80 °C for 48 h to constant weight and their ^{14}C activity as described above.

Statistical analysis. All analyses were performed using the R software (version 4.0.3; R Core Team 2019). All data are presented as average values of three replicates ± standard error. Data normality and variance homogeneity were evaluated using the "shapiro.test" and "bartlett.test" functions, respectively. One-way analysis of variance was performed with the "aov" function to identify significant differences (P < 0.05) in the variable's response to crop species. Multiple comparisons were conducted using Tukey's post-hoc test at P < 0.05 with the "agricolae" package. The figures were visualised with the "ggplot2" package.

RESULTS

Comparison of Ala and Ala-derived oligopeptide uptake in rice and wheat roots. Both wheat and rice demonstrated the ability to take up alanine and its peptides, with the uptake of alanine being much greater than all the alanine-derived oligopeptides (Figure 1). Rice showed higher uptake rates of alanine and its peptides than wheat (Figure 1). The rate of root alanine uptake was 3.3 and 2.9 µmol/g DW (dry weight) for rice and wheat, respectively. Alanine absorption

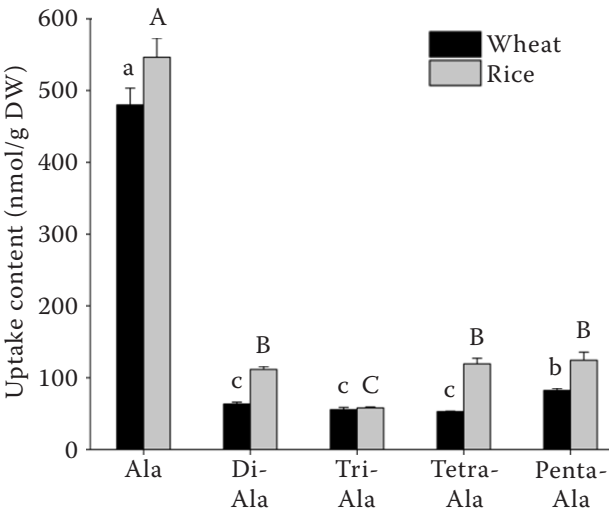

Figure 1. Uptake of ^{14}C-labelled alanine and alanine-containing oligopeptides over a 10 min period across the whole root system of wheat and rice. Values represent means ± standard error (n = 3). Lowercase letters indicate significant differences in wheat root uptake, while uppercase letters indicate significant differences in rice root uptake. DW – dry weight; Ala – alanine; Di-Ala – di-alanine; Tri-Ala – tri-alanine; Tetra-Ala – tetra-alanine; Penta-Ala – penta-alanine

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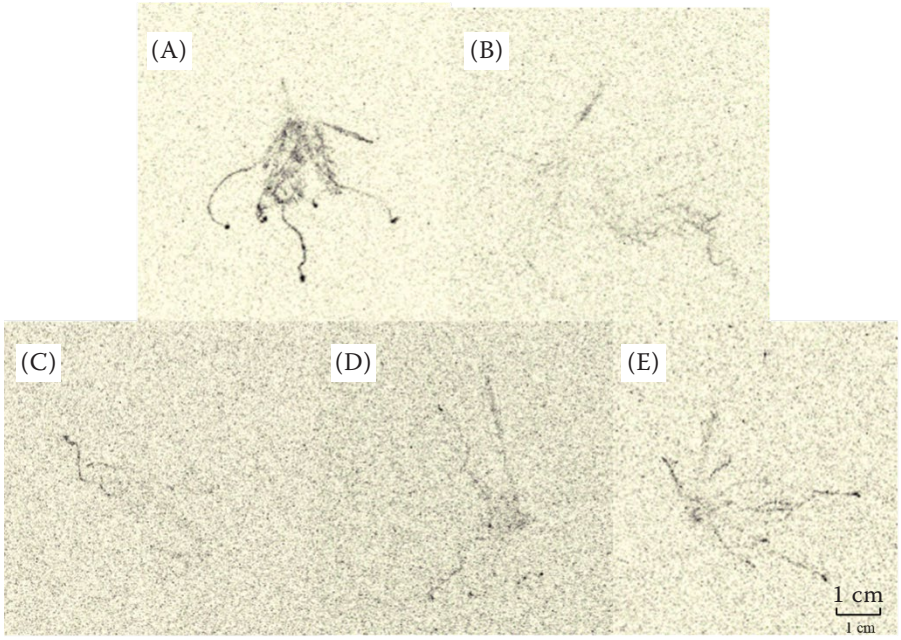

Figure 2. Representative phosphor images of a wheat root after a 10-min substrate incubation in either (A) ^{14}C-labelled alanine; (B) di-alanine; (C) tri-alanine; (D) tetra-alanine, or (E) penta-alanine

in roots was 4–9 times higher in rice and 6–9 times higher in wheat compared to their respective peptide uptake rates. In wheat roots, penta-alanine uptake was significantly higher than all other peptides tested. In contrast, rice roots showed significantly higher uptake of penta-alanine only when compared to tri-alanine. It should be noted, however, that the data is expressed on a molar basis and does not account for the higher amounts of N contained in the peptides. When this was accounted for, the rate of N uptake from penta-alanine was similar to that of alanine.

Spatial root uptake patterns of amino acids and oligopeptides. The distribution of amino acid- and oligopeptide-derived ^{14}C within the roots of wheat and rice is shown in Figures 2 and 3, respectively. Overall, phosphor imaging revealed that the distribution of ^{14}C added as alanine in both crops was present throughout the whole root system. However, higher uptake was apparent in the root tip and hair zone. The distribution of ^{14}C-label also indicated that the uptake of the oligopeptides occurred mainly in the root tips and newly emerging root hair region.

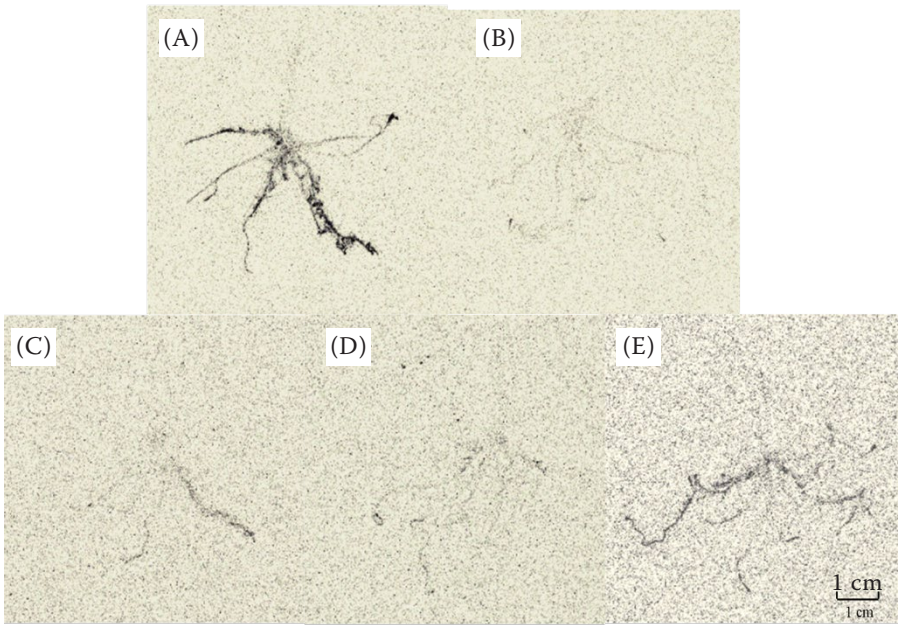

Figure 3. Representative phosphor images of a rice root after a 10-min substrate incubation in either (A) ^{14}C-labelled alanine; (B) di-alanine; (C) tri-alanine; (D) tetra-alanine, or (E) penta-alanine

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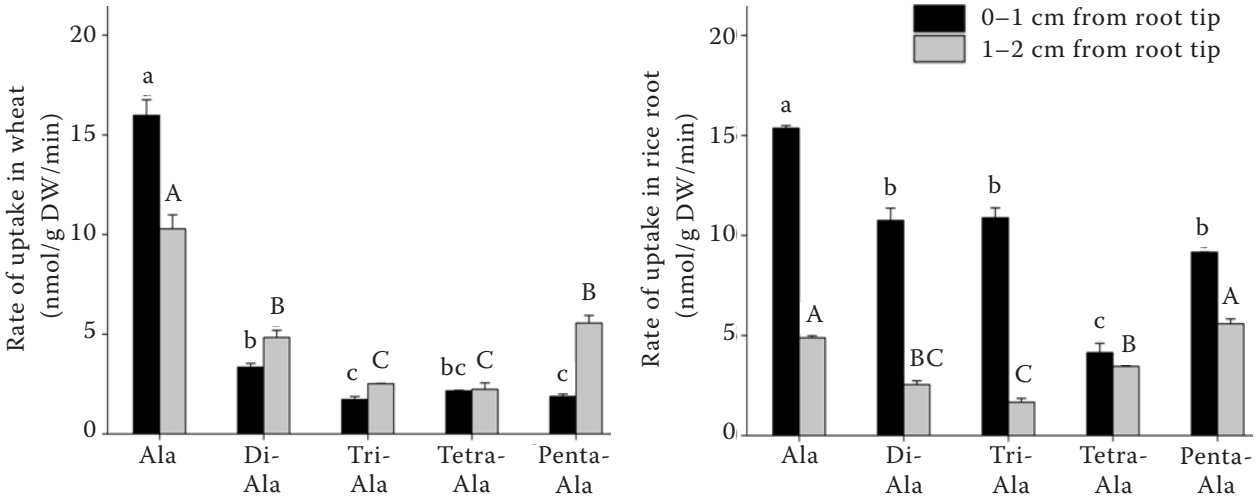

Figure 4. Uptake of ^{14}C-labelled amino acids and oligopeptides by different wheat and rice root sections. Values represent means ± standard error (n = 4). Different lowercase letters indicate significant differences among 0–1 cm root sections, while uppercase letters indicate significant differences among 1–2 cm root sections. DW – dry weight; Ala – alanine; Di-Ala – di-alanine; Tri-Ala – tri-alanine; Tetra-Ala – tetra-alanine; Penta-Ala – penta-alanine

Amino acid and oligopeptide uptake by rice and wheat root tips. To further investigate the spatial differences, the uptake rates of alanine and the different oligopeptides by root tips were calculated (Figure 4). Overall, the uptake rates of alanine were significantly higher than those of their peptides by 1.5–10 times in both crops; however, we also observed differences in peptide uptake in the different root regions. For wheat, the uptake rates of alanine were 16.0 nmol/g DW/min in the 0–1 cm section, much faster (155%) than in the 1–2 cm root sections (Figure 4). However, the uptake of the peptides in wheat was opposite, especially the uptake rates of di-alanine and penta-alanine in the 0–1 cm root section, which were lower by 34% and 69% compared to the 1–2 cm root sections, respectively. For rice, the uptake rates of alanine and its peptides were significantly higher in the 0–1 cm root section than in the 1–2 cm root sections (Figure 4). Moreover, the uptake rates followed Ala > Tri-Ala > Di-Ala > Penta-Ala > Tetra-Ala among 0–1 cm root sections of rice. Overall, peptide uptake rates were 4.1–10.9 nmol/g DW/min in the 0–1 cm root section and 1.7–5.6 nmol/g DW/min in the 1–2 cm root section. Compared with wheat, the absorption rate of peptides was increased by ca. 1–5 times in the 0–1 cm rice roots. However, the absorption rate of peptides was reduced by more than 50% in 1–2 cm rice root sections than in wheat, except for penta-alanine.

DISCUSSION

DON has been shown to represent an important N source in natural habitats where it has been estimated to account for about 88–92% of the total soluble N pool (Qualls and Richardson 2003, Weigelt et al. 2005, Näsholm et al. 2009). Across 26 global sites, encompassing agricultural systems such as irrigated croplands (California site 13) and viticulture (California site 18), the average DON concentration in agricultural topsoils (0–15 cm) was 50.7 ± 5.7 mg N/kg, with significant variability driven by soil type and management (Farrell et al. 2013). Although its significance in agricultural settings is often underestimated (Näsholm et al. 2009, Schmidt et al. 2014, Moreau et al. 2019), numerous research have begun to focus on the importance of the amino acid fraction in plant N acquisition (Chapin et al. 1993, Tegeder and Rentsch 2010, Hill et al. 2011a, b, Farrell et al. 2013, Warren et al. 2023).

The direct uptake of intact proteins by crop plants appears only to be functionally significant when protein is in direct contact with root surfaces (Greenfield et al. 2020). The poor solubility of proteins in soil, strong sorption to the soil's solid phase and low diffusion rates also make them a poor source of N for plants. In contrast, oligopeptides are often highly soluble and can be released into the rhizosphere in significant quantities by the action of proteases generated by the soil microbial community (Jones and Kielland 2012,

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Baraniya et al. 2016). Therefore, the capacity of plants to acquire organic N extends beyond individual amino acids to include oligopeptides, mediated by specialised transporters. Crop species such as wheat possess functional peptide transporters (e.g., TaPTR1) that facilitate the uptake of di- and tripeptides (Hill et al. 2011b).

In this study, we provide direct evidence for plants' uptake of oligopeptides whilst avoiding competitive absorption by soil microorganisms and soil particles through sterile hydroponic conditions. Our results showed that rice roots, like wheat roots (Hill et al. 2011b), have the capacity to capture organic N directly (both as alanine and its oligopeptides) from solution (Figure 1). These results are consistent with previous research demonstrating that plants can directly utilise free amino acids and small peptides as a N source without the necessity of further cleaving peptides into amino acid monomers (Hill et al. 2011a, b, 2012, Farrell et al. 2013, Smith and Chalk 2021). Wheat grown in agricultural soils amended with organic matter has been shown to directly assimilate peptide-derived N, with uptake rates of L-tri-alanine exceeding those of L-alanine by 2–3 fold (Hill et al. 2011b). Notably, alanine absorption exceeded peptide uptake by 3–7-fold in rice and 6–9-fold in wheat. Moreover, the absorption of alanine was significantly higher than its peptides. However, when expressed on an N basis, the uptake of oligopeptides may equal or even surpass the amino acid basis depending on root location and oligopeptide chain length.

We also observed differences in peptide absorption capacity between wheat and rice. Rice roots exhibited a higher absorption rate of peptides than wheat roots, suggesting that peptides may have more potential as a directly utilisable N form. The observed differences in peptide uptake between rice and wheat roots are likely attributed to physiological, genetic, and ecological factors. Amino acid uptake is mediated by various transporters (Lee et al. 2007, Taylor et al. 2015), which are located in the root plasma membrane (Guo et al. 2021, Liu et al. 2024). Specific membrane-localised amino acid transporters, such as amino acid permeases and the lysine-histidine-like transporter subfamily, are involved in amino acid transport in rice and wheat. Previous studies have shown that plant roots possess a range of peptide transporters (Paungfoo-Lonhienne et al. 2009). Existing evidence suggests differential transport mechanisms for peptides of varying lengths: di- and tripeptides are transported by proton-coupled peptide transporters (PTRs), a nitrate transporter subfamily (Tsay et al. 2007, Kohl et al. 2012), while tetra- and pentapeptides utilise oligopeptide transporters (OPTs) and ATP-binding cassette (ABC) transporters. These transporters exhibit distinct expression patterns across root zones and developmental stages; notably, the patterns also indicate that some peptide transporters are not localised to the epidermis and may not be involved in N capture (Paungfoo-Lonhienne et al. 2009, Qiu et al. 2023). However, the coordination of these different transport processes in roots is not yet fully understood (Guo et al. 2021). The ecological adaptation of rice, typically grown in organic matter-rich environments like paddy fields, may also have led it to evolve and differentially regulate its N acquisition mechanisms. Further research is therefore needed to investigate transporter gene expression, protein localisation, uptake kinetics, and genetic variations in peptide transport systems across a range of agricultural plants.

The biochemical advantage of direct peptide uptake extends beyond simple N acquisition efficiency (i.e., accessing N before it is mineralised by the soil microbial community). In contrast to inorganic N, peptide transport delivers multiple N atoms per transport event while simultaneously providing C skeletons, potentially reducing the metabolic cost of amino acid biosynthesis. A critical knowledge gap concerns the fate of peptides following cellular uptake. While our study demonstrates successful peptide transport across root plasma membranes, crop species' subsequent intracellular processing steps remain poorly characterised (Mikkonen et al. 1986, Belonozníková et al. 2023). Peptide hydrolysis may occur in the cytoplasm *via* cytoplasmic peptidases or following vacuolar sequestration, with longer peptides potentially requiring more complex processing machinery (Carter et al. 2004). The rate-limiting step in peptide utilisation, membrane transport *versus* intracellular cleavage, likely varies with peptide length and may explain why shorter peptides often show higher apparent uptake rates. Understanding these post-uptake processes is crucial for determining whether peptide transport truly represents a metabolic advantage over inorganic N sources.

Root age and system components are crucial for nutrient acquisition, with root tips and root hairs being the primary zones for amino acid uptake (Wang et al. 2006, Tegeder 2014, Guo et al. 2021). Further, differences in root hair density, surface area, and membrane permeability may also be important (Cai and Ahmed 2022). Matsumiya et al. (2012) have

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also demonstrated that *Brassica rapa* and *Solanum lycopersicum* root hairs can absorb peptides from a complex organic medium. We found that alanine and oligopeptide uptake occurred throughout the root system, with the highest accumulation seen in the root tip and root hair regions (Figures 2 and 3). Older root segments, characterised by suberisation and reduced transporter expression, may exhibit lower peptide uptake rates than young, actively growing roots. Studies on *Arabidopsis* suggest that peptide transporter genes (e.g., AtPTR5) are predominantly expressed in root tips and young tissues, implying a spatial gradient in uptake capacity (Komarova et al. 2008). Meanwhile, we observed similar segment-specific differences in oligopeptide uptake rates among crop roots: rice exhibited a higher oligopeptide uptake rate in the 0–1 cm root segment compared to the 1–2 cm segment, which is consistent with the pattern observed in their study; in contrast, wheat displayed the opposite trend (Figure 4). This highlights that spatial variations in peptide uptake capacity exist across different root regions in various plant species. The reason may be related to the root morphology. Rice often has a more fibrous root system with a relatively large number of fine roots, while wheat combines seminal and nodal roots. We note that excising roots can alter source-sink relationships for solutes and reduce intracellular peptides and amino acid concentrations; however, major changes usually occur over much longer periods than used here (Brouquisse et al. 1992, Raymond and Smirnoff 2002). The net effect on plant N uptake remains unclear. As occurs in bacteria and yeasts (Sharma et al. 2016, Berg et al. 2023), we also show that the uptake ratio of peptides may vary depending on their chain length. This is supported by studies in other plant tissues (e.g., barley leaf mesophyll; Ramos et al. 2011). It is also likely that the uptake rate will be regulated by the structure and charge properties of the peptides (Berg et al. 2023); however, the importance of this remains poorly understood in plants. These findings collectively highlight the complexity of organic N acquisition mechanisms and the need for continued research to understand better the rate of formation and fate of peptide-N in the rhizosphere.

In conclusion, we acknowledge that the current study possesses several methodological limitations. Firstly, although in line with best practice, the experiments were conducted under sterile controlled conditions, eliminating microbial competition and potentially limiting direct extrapolation to complex natural soil environments. The focus on only wheat and rice limits the generalisability of the observations across plant species and agricultural systems. The analysis of a restricted range of oligopeptides (di-, tri, tetra, and penta-alanine) may also not comprehensively represent the full spectrum of peptides generated in soil and, therefore, uptake mechanisms. Short-term experimental measurements (10–30 min) may also provide an incomplete representation of long-term N acquisition dynamics (e.g., internal metabolic control and transporter feedback). While revealing important insights into peptide transport, significant gaps in knowledge regarding transporter gene expression, protein localisation, and comprehensive uptake kinetics remain. The research did not investigate environmental contextual factors such as N availability, soil type, and plant developmental stage. Further research is required to fully characterise the molecular regulation of peptide transporters, investigate genetic variations across crop species, and examine environmental influences on organic nitrogen acquisition. Understanding these mechanisms may contribute to developing more nitrogen-efficient agricultural approaches.

REFERENCES

Baraniya D., Puglisi E., Ceccherini M.T., Pietramellara G., Giagnoni L., Arenella M., Nannipieri P., Renella G. (2016): Protease encoding microbial communities and protease activity of the rhizosphere and bulk soils of two maize lines with different N uptake efficiency. Soil Biology and Biochemistry, 96: 176–179.

Belonozníková K., Cerny M., Hysková V., Synková H., Valcke R., Hodek O., Krízek T., Kavan D., Vanková R., Dobrev P. (2023): Casein as protein and hydrolysate: biostimulant or nitrogen source for *Nicotiana tabacum* plants grown *in vitro*? Physiologia Plantarum, 175: e13973.

Berg H.Y., Arju G., Becerra-Rodríguez C., Galeote V., Nisamedtinov I. (2023): Unlocking the secrets of peptide transport in wine yeast: insights into oligopeptide transporter functions and nitrogen source preferences. Applied and Environmental Microbiology, 89: e01141–23.

Biernath C., Fischer H., Kuzyakov Y. (2008): Root uptake of N-containing and N-free low molecular weight organic substances by maize: a $^{14}C/^{15}N$ tracer study. Soil Biology and Biochemistry, 40: 2237–2245.

Brouquisse R., James F., Pradet A., Raymond P. (1992): Asparagine metabolism and nitrogen distribution during protein-degradation in sugar-starved maize root-tips. Planta, 188: 384–395.

Cai G.C., Ahmed M.A. (2022): The role of root hairs in water uptake: recent advances and future perspectives. Journal of Experimental Botany, 73: 3330–3338.

https://doi.org/10.17221/229/2025-PSE

Carter C., Pan S.Q., Zouhar J., Avila E.L., Girke T., Raikhel N.V. (2004): The vegetative vacuole proteome of *Arabidopsis thaliana* reveals predicted and unexpected proteins. Plant Cell, 16: 3285–3303.

Chapin F.S., Moilanen L., Kielland K. (1993): Preferential use of organic nitrogen for growth by a non-mycorrhizal arctic sedge. Nature, 361: 150–153.

Drechsler N., Courty P.E., Brulé D., Kunze R. (2018): Identification of arbuscular mycorrhiza-inducible Nitrate Transporter /Peptide Transporter Family (NPF) genes in rice. Mycorrhiza, 28: 93–100.

Farrar J.F. (1985): Fluxes of carbon in roots of barley plants. New Phytologist, 99: 57–69.

Farrell M., Hill P.W., Farrar J., DeLuca T.H., Roberts P., Kielland K., Dahlgren R., Murphy D.V., Hobbs P.J., Bardgett R.D. (2013): Oligopeptides represent a preferred source of organic N uptake: a global phenomenon? Ecosystems, 16: 133–145.

Farrell M., Hill P.W., Farrar J., Bardgett R.D., Jones D.L. (2011): Seasonal variation in soluble soil carbon and nitrogen across a grassland productivity gradient. Soil Biology and Biochemistry, 43: 835–844.

Farzadfar S., Knight J.D., Congreves K.A. (2021): Soil organic nitrogen: an overlooked but potentially significant contribution to crop nutrition. Plant and Soil, 462: 7–23.

Greenfield L.M., Hill P.W., Paterson E., Baggs E.M., Jones D.L. (2020): Do plants use root-derived proteases to promote the uptake of soil organic nitrogen? Plant and Soil, 456: 355–367.

Guo N., Zhang S.N., Gu M.G., Xu G.H. (2021): Function, transport, and regulation of amino acids: what is missing in rice? Crop Journal, 9: 530–542.

Hachiya T., Sakakibara H. (2017): Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. Journal of Experimental Botany, 68: 2501–2512.

Hill P.W., Farrar J., Roberts P., Farrell M., Grant H., Newsham K.K., Hopkins D.W., Bardgett R.D., Jones D.L. (2011a): Vascular plant success in a warming Antarctic may be due to efficient nitrogen acquisition. Nature Climate Change, 1: 50–53.

Hill P.W., Farrell M., Jones D.L. (2012): Bigger may be better in soil N cycling: does rapid acquisition of small L-peptides by soil microbes dominate fluxes of protein-derived N in soil? Soil Biology and Biochemistry, 48: 106–112.

Hill P.W., Jones D.L. (2019): Plant-microbe competition: does injection of isotopes of C and N into the rhizosphere effectively characterise plant use of soil N? New Phytologist, 221: 796–806.

Hill P.W., Marsden K.A., Jones D.L. (2013): How significant to plant N nutrition is the direct consumption of soil microbes by roots? New Phytologist, 199: 948–955.

Hill P.W., Quilliam R.S., DeLuca T.H., Farrar J., Farrell M., Roberts P., Newsham K.K., Hopkins D.W., Bardgett R.D., Jones D.L. (2011b): Acquisition and assimilation of nitrogen as peptide-bound and D-enantiomers of amino acids by wheat. PLoS One, 6: e19220.

Jones D.L., Healey J.R., Willett V.B., Farrar J.F., Hodge A. (2005): Dissolved organic nitrogen uptake by plants – an important N uptake pathway? Soil Biology and Biochemistry, 37: 413–423.

Jones D.L., Kielland K. (2012): Amino acid, peptide and protein mineralization dynamics in a taiga forest soil. Soil Biology and Biochemistry, 55: 60–69.

Jones D.L., Nguyen C., Finlay R.D. (2009): Carbon flow in the rhizosphere: carbon trading at the soil-root interface. Plant and Soil, 321: 5–33.

Kohl S., Hollmann J., Blattner F.R., Radchuk V., Andersch F., Steuernagel B., Schmutzer T., Scholz U., Krupinska K., Weber H., Weschke W. (2012): A putative role for amino acid permeases in sink-source communication of barley tissues uncovered by RNA-seq. BMC Plant Biology, 12: 154.

Komarova N.Y., Thor K., Gubler A., Meier S., Dietrich D., Weichert A., Grotemeyer M.S., Tegeder M., Rentsch D. (2008): AtPTR1 and AtPTR5 transport dipeptides in planta. Plant Physiology, 148: 856–869.

Kuzyakov Y., Xu X.L. (2013): Competition between roots and microorganisms for nitrogen: mechanisms and ecological relevance. New Phytologist, 198: 656–669.

Lee Y.H., Foster J., Chen J., Voll L.M., Weber A.P., Tegeder M. (2007): AAP1 transports uncharged amino acids into roots of *Arabidopsis*. Plant Journal, 50: 305–319.

Liang H., Gao S.J., Qi Z.M., Hu K.L., Xu J.Z. (2021): Leaching loss of dissolved organic nitrogen from cropland ecosystems. Environmental Reviews, 29: 23–30.

Liu K., Li P., Li G., Ma X., Liu M., Liu J., Wu M., Li Z.P. (2023): Long-term fertilization promotes soil organic nitrogen accumulation by increasing the abundance of keystone microbial cluster across aggregates. Applied Soil Ecology, 192: 105086.

Liu L., Yu X.C., Yan Y., He C.X., Wang J., Sun M.T., Li Y.S. (2024): Amino acid transporters on amino acid absorption, transport and distribution in crops. Horticulturae, 10: 999.

Ma Q., Kuzyakov Y., Pan W., Tang S., Chadwick D.R., Wen Y., Hill P.W., MacDonald A., Ge T., Si L., Wu L. (2021): Substrate control of sulphur utilisation and microbial stoichiometry in soil: results of ^{13}C, ^{15}N, ^{14}C, and ^{35}S quad labelling. ISME Journal, 15: 3148–3158.

Ma Q., Yao R., Liu X., Ma Q., Tang S., Zhou J.P., Wu L., Jones D.L. (2025): Effect of elements availability on the decomposition and utilization of S-containing amino acids by microorganisms in soil and soil solutions. Plant and Soil, 509: 347–361.

Mahboob W., Yang G., Irfan M. (2023): Crop nitrogen (N) utilization mechanism and strategies to improve N use efficiency. Acta Physiologiae Plantarum, 45: 52.

Mao J.H., Wang J.S., Liao J.Q., Xu X.L., Tian D.S., Zhang R.Y., Peng J.L., Niu S.L. (2025): Plant nitrogen uptake preference and drivers in natural ecosystems at the global scale. New Phytologis, 246: 972–983.

Matsumiya Y., Taniguchi R., Kubo M. (2012): Analysis of peptide uptake and location of root hair-promoting peptide accumulation in plant roots. Journal of Peptide Science, 18: 177–182.

https://doi.org/10.17221/229/2025-PSE

Mengel K., Schneider B., Kosegarten H. (1999): Nitrogen compounds extracted by electroultrafiltration (EUF) or $CaCl_2$ solution and their relationships to nitrogen mineralization in soils. Journal of Plant Nutrition and Soil Science, 162: 139–148.

Mikkonen A., Begbie R., Grant G., Pusztai A. (1986): Intracellular-localization of some peptidases and alpha-mannosidase in cotyledons of resting kidney bean, *Phaseolus vulgaris*. Physiologia Plantarum, 68: 75–80.

Mokhele B., Zhan X.J., Yang G.Z., Zhang X.L. (2012): Review: nitrogen assimilation in crop plants and its affecting factors. Canadian Journal of Plant Science, 92: 399–405.

Moran-Zuloaga D., Dippold M., Glaser B., Kuzyakov Y. (2015): Organic nitrogen uptake by plants: reevaluation by position-specific labeling of amino acids. Biogeochemistry, 125: 359–374.

Moreau D., Bardgett R.D., Finlay R.D., Jones D.L., Philippot L. (2019): A plant perspective on nitrogen cycling in the rhizosphere. Functional Ecology, 33: 540–552.

Murphy D.V., MacDonald A.J., Stockdale E.A., Goulding K.W.T., Fortune S., Gaunt J.L., Poulton P.R., Wakefield J.A., Webster C.P., Wilmer W.S. (2000): Soluble organic nitrogen in agricultural soils. Biology and Fertility of Soils, 30: 374–387.

Näsholm T., Huss-Danell K., Högberg P. (2001): Uptake of glycine by field-grown wheat. New Phytologist, 150: 59–63.

Näsholm T., Kielland K., Ganeteg U. (2009): Uptake of organic nitrogen by plants. New Phytologist, 182: 31–48.

Oburger E., Jones D.L. (2018): Sampling root exudates – mission impossible? Rhizosphere, 6: 116–133.

Paungfoo-Lonhienne C., Schenk P.M., Lonhienne T.G.A., Brackin R., Meier S., Rentsch D., Schmidt S. (2009): Nitrogen affects cluster root formation and expression of putative peptide transporters. Journal of Experimental Botany, 60: 2665–2676.

Perchlik M., Tegeder M. (2017): Improving plant nitrogen use efficiency through alteration of amino acid transport processes. Plant Physiology, 175: 235–247.

Qiu D.Y., Hu R., Li J., Li Y., Ding J.R., Xia K.F., Zhong X.H., Fang Z.M., Zhang M.Y. (2023): Peptide transporter OsNPF8.1 contributes to sustainable growth under salt and drought stresses, and grain yield under nitrogen deficiency in rice. Rice Science, 30: 113–126.

Qualls R.G., Richardson C.J. (2003): Factors controlling concentration, export, and decomposition of dissolved organic nutrients in the Everglades of Florida. Biogeochemistry, 62: 197–229.

Ramos M.S., Abele R., Nagy R., Grotemeyer M.S., Tampé R., Rentsch D., Martinoia E. (2011): Characterization of a transport activity for long-chain peptides in barley mesophyll vacuoles. Journal of Experimental Botany, 62: 2403–2410.

Raymond M.J., Smirnoff N. (2002): Proline metabolism and transport in maize seedlings at low water potential. Annals of Botany, 89: 813–823.

Reeve J.R., Smith J.L., Carpenter-Boggs L., Reganold J.P. (2009): Glycine, nitrate, and ammonium uptake by classic and modern wheat varieties in a short-term microcosm study. Biology and Fertility of Soils, 45: 723–732.

Ros G.H., Hoffland E., van Kessel C., Temminghoff E.J.M. (2009): Extractable and dissolved soil organic nitrogen – a quantitative assessment. Soil Biology and Biochemistry, 41: 1029–1039.

Sainju U.M., Ghimire R., Pradhan G.P. (2019): Nitrogen fertilization I: impact on crop, soil, and environment. In: Rigobelo E.C., Serra A.P. (eds.): Nitrogen Fixation. London, Intertech Open Ltd.

Schmidt S., Näsholm T., Rentsch D. (2014): Organic nitrogen. New Phytologist, 203: 29–31.

Schimel J.P., Chapin F.S.III. (1996): Tundra plant uptake of amino acid and NH_4^+ nitrogen *in situ*: plants compete well for amino acid N. Ecology, 77: 2142–2147.

Sharma N., Aduri N.G., Iqbal A., Prabhala B.K., Mirza O. (2016): Peptide selectivity of the proton-coupled oligopeptide transporter from *Neisseria meningitidis*. Journal of Molecular Microbiology and Biotechnology, 26: 312–319.

Shen Z.Y., Qiu J.L., Hong Q., Chen L. (2014): Simulation of spatial and temporal distributions of non-point source pollution load in the Three Gorges Reservoir Region. Science of the Total Environment, 493: 138–146.

Smith C.J., Chalk P.M. (2021): Organic N compounds in plant nutrition: have methodologies based on stable isotopes provided unequivocal evidence of direct N uptake? Isotopes in Environment and Health Studies, 57: 333–349.

Soper F.M., Paungfoo-Lonhienne C., Brackin R., Rentsch D., Schmidt S., Robinson N. (2011): *Arabidopsis* and *Lobelia anceps* access small peptides as a nitrogen source for growth. Functional Plant Biology, 38: 788–796.

Tang S., Ma Q., Marsden K.A., Chadwick D.R., Luo Y., Kuzyakov Y., Wu L., Jones D.L. (2023): Microbial community succession in soil is mainly driven by carbon and nitrogen contents rather than phosphorus and sulphur contents. Soil Biology and Biochemistry, 180: 109019.

Tang S., Pan W., Zhou J., Ma Q., Yang X., Wanek W., Marsden K.A., Kuzyakov Y., Chadwick D.R., Wu L., Gregory A.S., Jones D.L. (2024): Soil nitrogen and phosphorus regulate decomposition of organic nitrogen compounds in the Rothamsted experiment. Soil Biology and Biochemistry, 196: 109502.

Taylor M.R., Reinders A., Ward J.M. (2015): Transport function of rice amino acid permeases (AAPs). Plant and Cell Physiology, 56: 1355–1363.

Tegeder M. (2014): Transporters involved in source to sink partitioning of amino acids and ureides: opportunities for crop improvement. Journal of Experimental Botany, 65: 1865–1878.

Tegeder M., Masclaux-Daubresse C. (2018): Source and sink mechanisms of nitrogen transport and use. New Phytologist, 217: 35–53.

Tegeder M., Rentsch D. (2010): Uptake and partitioning of amino acids and peptides. Molecular Plant, 3: 997–1011.

Tsay Y.F., Chiu C.C., Tsai C.B., Ho C.H., Hsu P.K. (2007): Nitrate transporters and peptide transporters. FEBS Letters, 581: 2290–2300.

https://doi.org/10.17221/229/2025-PSE

Wang H., Inukai Y., Yamauchi A. (2006): Root development and nutrient uptake. Plant Science, 25: 279–301.

Warren N.D., Hobbie E.A., Chen J.E., Smith R.G. (2023): Relative uptake of organic and inorganic nitrogen by common weed species. Weed Science, 71: 470–477.

Weigelt A., Bol R., Bardgett R.D. (2005): Preferential uptake of soil nitrogen forms by grassland plant species. Oecologia, 142: 627–635.

Wilkinson A., Hill P.W., Farrar J.F., Jones D.L., Bardgett R.D. (2014): Rapid microbial uptake and mineralization of amino acids and peptides along a grassland productivity gradient. Soil Biology and Biochemistry, 72: 75–83.

Wu J.S., Kurten E.L., Monshausen G., Hummel G.M., Gilroy S., Baldwin I.T. (2007): NaRALF, a peptide signal essential for the regulation of root hair tip apoplastic pH in *Nicotiana attenuata*, is required for root hair development and plant growth in native soils. The Plant Journal, 52: 877–890.

Xiu B., Liang S.K., He X.L., Wang X.K., Cui Z.G., Jiang Z.J. (2019): Bioavailability of dissolved organic nitrogen and its uptake by *Ulva prolifera*: implications in the outbreak of a green bloom off the coast of Qingdao, China. Marine Pollution Bulletin, 140: 563–572.

Ye J.Y., Tian W.H., Jin C.W. (2022): Nitrogen in plants: from nutrition to the modulation of abiotic stress adaptation. Stress Biology, 2: 4.

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