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Root uptake of N-containing and N-free low molecular weight organic substances by maize: A $^{14}C/^{15}N$ tracer study

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ABSTRACT

Most studies showing potential organic nitrogen uptake were conducted with amino acids. They conclude that, in some ecosystems, amino acids significantly contribute to the N demand of plants and that roots have special transporters to re-uptake amino acids released into the rhizosphere. However, the relevance of the uptake of organic N compounds can only be evaluated by comparing the uptake of N-containing and N-free organic substances. We compared the uptake of alanine, glucose and acetate labelled with ^{14}C by maize. Additionally, the N uptake was estimated by ^{15}N labelled alanine and KNO_3. We found a similar uptake of ^{14}C from alanine, glucose and acetate, amounting for the whole plant less than 1% of ^{14}C input. These results show that maize did not prefer N-containing to N-free organic substances. The uptake of ^{15}N by maize exceeded that of ^{14}C (10- to 50-fold), irrespective of the ^{15}N source. However, plant uptake of nitrate (23.6–35.2% of ^{15}N input) always exceeded the uptake of N from alanine (9.6–28.8%). The uptake of organically bound N by maize growing in soil occurred mainly by transpiration flow – as dissolved organics. The contribution of specific amino acid transporters was minor.

1. Introduction

Nitrogen is the most limiting nutrient in terrestrial ecosystems (Vitousek and Horwath, 1991). Nitrate and ammonium are the dominant N forms available for plants. Thus, mineralization of organic N is usually regarded as necessary before plant uptake (Haider, 1996). This raises the question whether plants are also able to use organic N sources, like amino acids, and whether plants preferentially take them up compared to N-free low molecular weight organic substances (LMWOS), like sugars and organic acids, which are also present in the soil solution (Kuzyakov and Jones, 2006; van Hees et al., 2005). Although these are key substances of metabolism and catabolism of plants, plants lose them to the rhizosphere by secretion and exudation. The intensity of these losses is affected by a wide range of abiotic and biotic factors (Dakora and Phillips, 2002; Kuzyakov and Jones, 2006). Abiotic factors include physical soil structure, nutrient availability, and temperature. Biotic factors include pathogen attack, herbivores, and direct transfer into symbionts (like mycorrhiza).

Jones and Darrah (1996) have shown that maize roots predominantly exude LMWOS by passive diffusion. This is caused by the high concentration gradient between the cytoplasm and the typically 1000-times lower concentrations in the soil solution (mM versus μM range) (Fischer et al., 2007; van Hees et al., 2005; Kielland, 1995). The plasma membrane H^+-ATPase generates an electrochemical potential which further enhances the diffusion of negatively charged exudates (Jones et al., 2004a,b).

Diffusive losses from roots and microbial decomposition of root exudates and dead plant residues are the main sources of LMWOS. In soil, LMWOS are involved in various processes including microbial decomposition, sorption, leaching, and uptake by microorganisms and plants (Jones and Edwards, 1998). Due to multiple factors and their complex interactions in soil, LMWOS transformations in soil are difficult to study (Kuzyakov and Jones, 2006) and the relevance of individual fluxes remains poorly understood.

Assessing the significance of N-containing LMWOS with respect to the plants' N demand requires considering the uptake of

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N-containing and N-free LMWOS. According to Näsholm et al. (2000) and Warren (2006) plants in various ecosystems may cover a part of their N demand taking up organic N (mainly amino acids). This reflects possible adaptation of plants on N_{min} limitation and slow mineralization rates in such ecosystems. At the same time, there is clear evidence that roots have special transporters for active amino acid uptake (Hirner et al., 2006). Furthermore, Walch-Liu et al. (2006) observed enhanced horizontal root growth towards amino acid (glycine) deposits in soil, along with a reduced vertical rooting. Various N-free LMWOS may also be taken up by plants(Xia and Saglio, 1988; Fokin et al., 1993; Kuzyakov and Jones, 2006), but far fewer studies are available compared to those examining amino acid uptake.

Other studies have identified active transporters for sugar uptake in plant roots (Xia and Saglio, 1988; Jones et al., 2004a,b). Jones and Darrah (1993) have shown that neutrally charged sugars previously lost in exudation were recaptured by maize roots. Jones et al. (2005b) demonstrated that plants compete for N-containing and N-free LMWOS especially in organic-matter-rich patches or when their concentration in soil solution is high. However, most studies showing the uptake of amino acids and N-free LMWOS were done in nutrient solutions and their relevance for soil conditions therefore is limited. The importance of organic N uptake by plants under soil conditions for LMWOS can only be proven if the amino acid uptake significantly exceeds that of N-free LMWOS. Significantly higher uptake of N-containing LMWOS compared to N-free LMWOS is an index for preferential uptake mechanisms within plant roots.

Many recent studies showed that besides inorganic N, LMWOS containing N can also be taken up by roots. Some of the studies claimed that uptake of organic N covers a significant part of the N demand of plants.

Uptake of LMWOS only plays an important role within the rhizosphere – a few millimetres surrounding the root. Further from the root LMWOS are decomposed very rapidly by microorganisms or may interact with clay minerals and sesquioxides. Furthermore, the transport of substances via diffusion in soil is only important over a distance of a few millimetres (Kuzyakov et al., 2003). Therefore, studies that inject organic substances in non-rhizosphere soil could underestimate the true potential of plants for LMWOS uptake (Näsholm et al., 2000). However, overestimations are also possible if the amounts of added LMWOS far exceed typical concentrations for soil solution (Jones et al., 2005b; Kuzyakov and Jones, 2006).

Another issue is the competition and the interaction between plants and microorganisms. Soil microorganisms can compete with plants for N-containing (Hodge et al., 2000) and N-free organic substances (Jones and Edwards, 1998). Moreover, Haller and Stolp (1985) showed that root exudation of low molecular weight organic compounds was a major carbon source for soil microorganisms; this typically increased the microbial population densities in the rhizosphere by 5–50 times compared to root-free soil (Lynch and Whipps, 1990). In this study, we compared the uptake by maize of N-containing and N-free LMWOS labelled with ^{15}N and ^{14}C. Our experiments were based on the following assumptions.

(1) When the percentages of ^{14}C recovered in the plant from N-containing and N-free organic substances are of the same magnitude, the preferential uptake of N-containing LMWOS plays only a minor role in satisfying the plant's N demand.
(2) If the ^{14}C content in the plants is low and no differences between the N-containing and N-free LMWOS treatments are measured, transporters in the roots do not contribute significantly to amino acid uptake into maize in soil.
(3) The amounts of ^{15}N and ^{14}C recovered in the plant and their dynamics can be used to estimate whether uptake was direct or after previous mineralization by soil microorganisms.

2. Material and methods

2.1. Soil sampling

Soil (loamy haplic Luvisol, IUSS Working Group WRB, 2006) was taken from the University of Hohenheim Agricultural Research Station "Versuchsanstalt für Gartenbau", located within the loess plains of the Fildern in Stuttgart, SW-Germany. For this experiment the upper 10 cm of the A_p horizon was sampled, air-dried and sieved (<2 mm). The physico-chemical properties are presented in Table 1.

2.2. Plant growth conditions and experimental system

Seeds of maize (*Zea mays* L. cv. "Amadeo" (KWS Saat AG, Einbeck Germany)) were soaked for 24 h in deionised water and then allowed to germinate in a seed germinator (TCM 207613, Tchibo GmbH, Hamburg, Germany) at 20 °C. After 2.5 d, equally developed plants with one main root axis of approximately 1.5 cm were selected. The seedlings were placed into individual rhizosphere tubes with soil. Control rhizosphere tubes contained soil without plants.

The plant–soil rhizosphere tubes were constructed from polyethylene tubes based on the descriptions of Jones et al. (2005b) and Kuzyakov and Jones (2006) and modified to our purpose. Briefly, the rhizosphere tubes consisted of a 20 cm long, 0.8 cm internal diameter main "rhizotube" section which connected to a 2 cm long, 1.5 cm diameter section. This was used to hold the seed and to prevent mechanical damage (Fig. 1). The rhizosphere tubes were filled with 12 g soil (bulk density of 1.2 g cm^{-3}). The seed was placed above the soil but the soil pressure was simulated by a small plastic barrier (Fig. 1). The main experiment was conducted with three (whole experiment) to five replications (CO_2). This means three replicates for the first four sampling periods (at 0.5, 1.3, 3.2, and 6.2 d) and five replicates for the fifth sampling period (at 9.2 d). This is a total of 17 labelled and planted rhizotubes per treatment. Another 162 planted but unlabelled rhizotubes were placed around and in between the rhizotubes of the main experiment to reduce side effects and to observe ^{14}C release from labelled to unlabelled plants.

After adding the seedlings to the soil, the rhizosphere tubes were put into a growth chamber with day/night temperatures 24 °C/18 °C, 50% relative humidity, and a photoperiod of 12 h and light intensity of 300 μmol m^{-2} s^{-1} (19 klx). The soil was

Table 1
Physico-chemical soil properties

Soil contents	Value
K (CAL-extr.) mg kg^{-1}	250
Mg (CAL-extr.) mg kg^{-1}	160
P (CAL-extr.) mg kg^{-1}	225
pH ($CaCl_2$)	6.5
pH (H_2O)	6.9
eC [μS]	91.5
CEC_{pot} $mmol_c$ kg^{-1}	210
C_{tot} [%]	1.5
N_{tot} [%]	0.14
Content of SOM [%]	2.18
Content of sand [%]	2.9
Content of silt [%]	74.5
Content of clay [%]	22.6

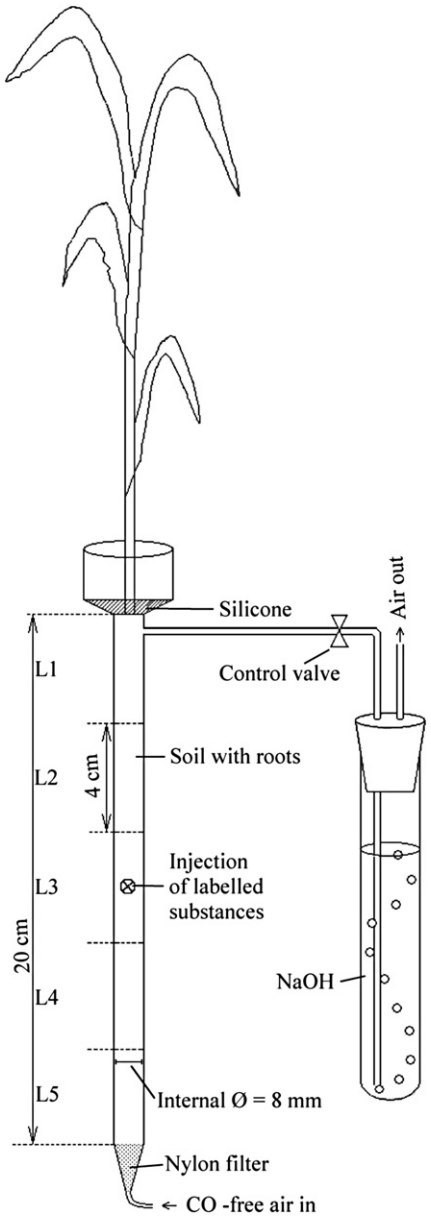

Fig. 1. Schematic representation of the maize rhizosphere tubes into which the $^{14}C/^{15}N$ labelled substances were injected. The substances were injected into the hole (X) in the central soil layer (L3). The same system was used for the root-free soil.

kept moist by adding water from the bottom of the pots 2 –3 times a day to maintain 70% of field capacity. Initially, the rhizosphere tubes were watered with distilled water. Starting from day 1 and extending throughout the experiment, each plant received 17 ml full-strength nutrient solution to maintain the nutrient demands of plants. The fertiliser powder (Flory 3, Euflor, N:P:K:Mg 15:10:15:2, Munich, Germany) was dissolved in water at a concentration of 0.2%, yielding 0.1 g additional nitrate N supply per plant. When roots and associated root hairs had completely occupied the rhizosphere tubes, making it essentially all rhizosphere soil (15 d after transplantation; shoots 12–15 cm long), the soil–root compartment was sealed with a 5 mm layer of non-phytotoxic silicone rubber paste (NG 3170; Thauer and Co., Dresden, Germany). CO_2-free air was pumped into the sealed rhizosphere tubes continuously. To eliminate CO_2 from the incoming air, compartment air was pumped through a 10 M NaOH-filled glass tube (height: 1 m, content: 1 l) as micro-bubbles. The CO_2-free air was essential to trap the soil- and root-derived CO_2 in 1 M NaOH (Fig. 1). Previous experiments have shown that the efficiency of the NaOH traps is higher than 95% (Kuzyakov, unpublished data; Fischer, unpublished data).

2.3. Treatments and labelling

Nine days after planting the germinated seeds, 200 μl of labelled materials were injected at 10 cm rhizosphere tube height (centre of the tube) through a small hole in the plastic wall. The experiment included three treatments.

(a) U–^{14}C–alanine + ^{15}N–alanine;
(b) U–^{14}C–glucose + K$^{15}NO_3$; and
(c) U–^{14}C–acetate of sodium + K$^{15}NO_3$.

These were added to planted and unplanted soils.

The concentration in final soil solution of the LMWOS was 7.5 μM. These amounts were in the range of common LMWOS concentrations from 0.1 to 1000 μM (Jones and Darrah, 1996; Kielland, 1995; van Hees et al., 2005). Detailed information on the substances is presented in Table 2. To minimise side effects, the experimental rhizosphere tubes were put side by side in a square surrounded by a double row of plants that were not used for analysis but were treated in the same way as the experimental plants. Some of the surrounding plants were sampled to estimate plant growth, root growth, rooting depth, and daily water demand.

2.4. Harvesting the soil/rhizosphere tubes

In the subsequent 9.2 d after the injection of the labelled materials into the rhizosphere soil, the CO_2 efflux from the soil–root compartment (i.e. root and microbial respiration) was trapped in 10 ml of 1 M NaOH by pumping the air with membrane pumps (50 ml min^{-1}; Fig. 1). The NaOH flasks were changed twice a day. In addition, the shoot and soil–root compartments were destructively harvested 0, 0.5, 1.3, 3.2, 6.2, and 9.2 d after substrate addition using replicate rhizosphere tubes. The shoots were immediately dried at 65 °C. The soil–root compartments were immediately frozen at −15 °C.

2.5. Analyses

The soil–root compartment of each rhizosphere tube was separated into five zones (L1–L5), each 4 cm in length containing 2.4 g soil as shown in Fig. 1. These parts consisted of a central zone (L3) into which the labelled substances were injected, two adjacent parts (above and below the central part, L2 and L4), and two outer parts (L1 and L5).

The amount of ^{14}C, total carbon (C_{tot}), as well as the amount of ^{15}N and total nitrogen (N_{tot}) was determined separately in roots and soil. The roots were treated similar to Kuzyakov and Jones (2006). Briefly, a frozen soil–root was shaken for 10 s with 9 ml 0.01 M $CaCl_2$. After shaking, the roots were washed in deionised water to

Table 2
Treatments and parameters of the added substances

Parameter	Glucose	Alanine	Acetate
Final LMWOS concentration in soil solution [μM]	7.5	7.5	7.5
Total ^{14}C activity added per rhizosphere tube [kBq]	170.7	150.9	178.0
Final N concentration in soil solution [μM]	13.6	7.5	13.6
^{15}N abundance [%]	54.2	98.8	54.2

"Alanine", "glucose" and "acetate" refer to the treatment rather than to the molecule itself.

remove remaining soil particles. The roots were subsequently dried at 65 °C. After removing the roots, the remaining soil was dried at 65 °C and stored before the analysis.

The ^{14}C activity of the shoots, roots, and soil was determined by liquid scintillation counting (Wallac 1419 liquid scintillation counter, Perkin Elmer Life And Analytical Sciences, Inc., Wellesley, MA, USA) after combustion in a Wösthoff Oxidiser (Wösthoff Carmograph C12, Bochum, Germany) and trapping ^{14}C activity in 20 ml of 1 M NaOH. DOM, the microbial bound fraction, was all determined by liquid scintillation cocktail Rotiszint® eco plus (Carl Roth GmbH + Co. KG, Karlsruhe, Germany). C_{tot} and N_{tot} in solutions were determined with a DIMA-TOC 100 analyser (Dimatec, Essen, Germany).

The ^{15}N abundance of roots and shoots in 2 mg dry matter was determined on a Delta Plus XL isotope ratio mass spectrometer (IRMS, Thermo Finnigan, Bremen, Germany).

2.6. Statistical analysis

The main experiment was conducted with three (whole experiment) to five replications (CO_2). All results were presented as percentage of ^{14}C or ^{15}N input. All data sets were tested with the Kolmogorov–Smirnov-test on normality. An LSD test was used wherever possible. However, where test on homogeneity of variance failed only a non-parametric test can be applied on the data sets. In these cases we used Tamhane T2 and stated this explicitly. The statistical analysis was done with the software SPSS 10.0.1 for windows (SPSS Inc. Chicago, USA).

3. Results

3.1. Recovery of ^{14}C and ^{15}N in the soil–plant system

The total percentage of ^{14}C input recovered in shoots, roots, soil, and CO_2 efflux from soil–root compartment exceeded 80% for all replicates and was between 90% and 105% for more than 80% of the replicates. The highest value in all treatments was found in CO_2 efflux from soil. In the soil we obtained between 20% and 25% of ^{14}C input, whereas the ^{14}C sum in the shoots and roots was <1% (Table 3).

The total percentage of ^{15}N recovered from soil, shoot, and roots ranged between 95% and 106% for all replicates. The highest value in all treatments was found in soil, on average >71%. In the plants we found an average of 17% of ^{15}N recovered (Table 4).

3.2. Plant uptake of ^{14}C and ^{15}N

3.2.1. Plant uptake of ^{14}C label

Total plant uptake of ^{14}C was <1% of ^{14}C input for all three organic substances. Only 0.07–0.17% was found in the roots versus 0.34–0.85% in the shoots (Table 3; Fig. 2). Thus, for all three organic substances – alanine, glucose and acetate – ^{14}C contents in the plant were in the same range.

Table 3
^{14}C recovered in the soil–plant system

	Planted soil			Unplanted soil		
Pool	Alanine	Glucose	Acetate	Alanine	Glucose	Acetate
CO_2	66.1 ± 3.0	57.6 ± 2.8	65.3 ± 2.9	78.1 ± 2.8	66.2 ± 1.8	65.8 ± 2.5
Soil	19.9 ± 2.0	23.6 ± 2.0	20.0 ± 1.8	23.6 ± 1.7	25.8 ± 2.1	17.4 ± 2.7
Root	0.1 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	–		–
Shoot	0.7 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	–		–
Total	~89	~82	~86	~102	~92	~83

Values are means ± SD ($n = 5$). Note: "alanine", "glucose" and "acetate" refer to the treatment rather than to the molecule itself.

Table 4
^{15}N recovered in soil–plant system

	Planted soil		Unplanted soil	
Pool	Alanine	Nitrate	Alanine	Nitrate
Soil	79.0 ± 8.0	74.7 ± 4.6	101.6 ± 2.2	102.6 ± 2.2
Root	5.1 ± 1.1	4.1 ± 0.2	–	–
Shoot	11.8 ± 4.1	24.9 ± 6.6	–	–
Total	~96	~ 03	~102	~103

Values are means ± SD (shoot: $n = 5$; root, soil: $n = 4$). Note: "alanine", "glucose" and "acetate" refer to the treatment rather than to the molecule itself.

No significant differences in ^{14}C uptake into the roots were observed for the three organic substances ($p > 0.05$; Fig. 2). More than half of the roots' ^{14}C activity was found in the central soil–root layer L3, where the labelled substance was injected (alanine: 62 ± 8%, glucose: 69 ± 3%, acetate: 72 ± 4% of total ^{14}C in the roots; Fig. 3).

Although plants were watered from the bottom of the rhizosphere tubes and thus ascending water movement has to be assumed (both in the soil and in the root system) the injected LMWOS apparently descended: the percentage of ^{14}C activity in the roots of all layers combined in L4 is second highest (L3 highest) and higher than in the top layers L2, L1, and the bottom layer L5 (Fig. 3).

Shoot ^{14}C uptake was not significantly different for the three tested LMWOS. The contents ranged from 0.47% to 0.87% ($p > 0.05$). For all three substances the maximum uptake was reached after 3.2 d and ranged between 0.55% and 0.87% ($p > 0.05$); this level was maintained until the next sampling time 3 days later (Fig. 2). We found no ^{14}C activity in the shoots of control (unlabelled) plants grown around and between the labelled plants. Therefore, we conclude that the whole ^{14}C recovered in shoots (Table 3; Fig. 2) was taken as LMWOS from the roots and not by photosynthesis.

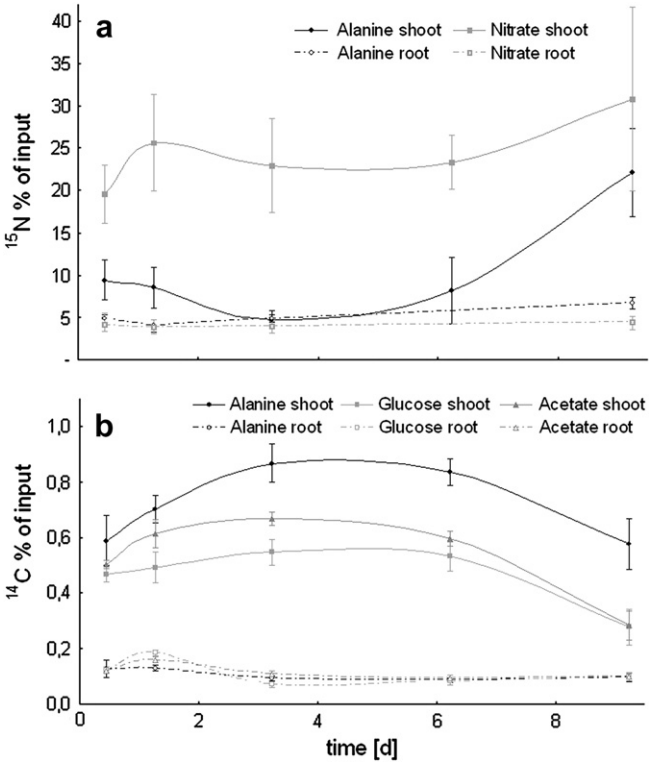

Fig. 2. Dynamics of (a) ^{14}C and (b) ^{15}N derived from LMWOS in the roots and in the shoots. Values are means ± SD ($n = 3$–5).

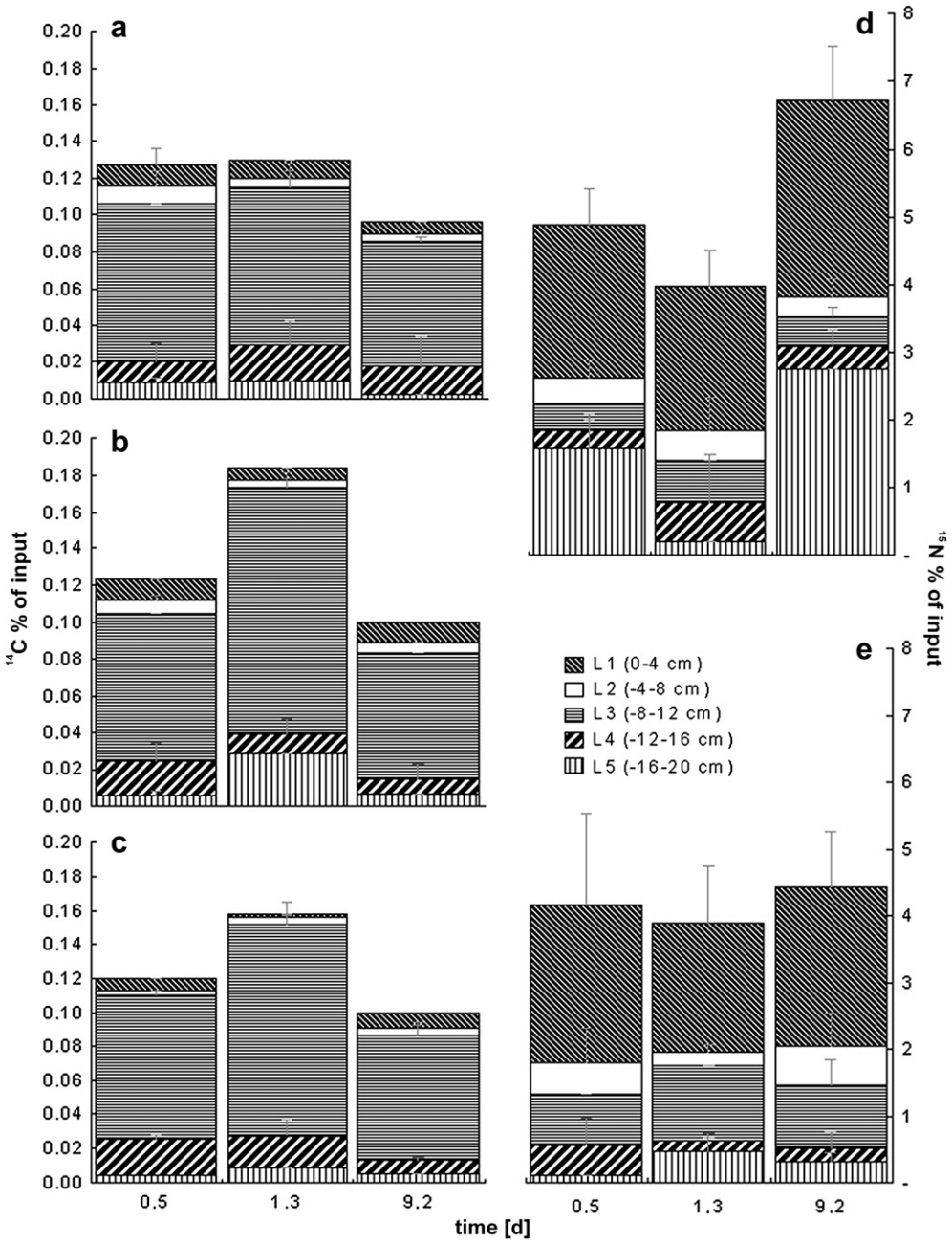

Fig. 3. Percent of ^{14}C and ^{15}N input recovered at five sampling times in the roots of the different soil layers (L1–L5). On the left side, the ^{14}C activities from (a) alanine, (b) glucose, and (c) acetate. On the right side, the ^{15}N amounts from (d) alanine and (e) nitrate. Values are means ± SD ($n = 3$–5).

Contrary to ^{14}C, the ^{15}N% input into the plants averaged 17% for alanine and 29% for nitrate (Table 4). Thus, ^{15}N uptake was two orders of magnitude higher than ^{14}C uptake. This result clearly supports the dominance of inorganic N uptake by maize.

Regardless of the labelled N species injected, both NO_3^- and alanine ^{15}N amounts in the roots were the same (Table 4; $p > 0.05$; Fig. 2). The outer top soil–root layer (L1) contained more than 43% of the total in all root layers (Fig. 3). The highest ^{15}N content in L1 proved the upward movement of ^{15}N transport into the shoots and thus opposite movement to the ^{14}C label. While nitrate ^{15}N peaked for a second time in L3 (17 ± 3%), more alanine ^{15}N was recovered in L5 (24 ± 23%).

The amounts of ^{15}N were significantly higher in shoots than roots throughout the observation period ($p < 0.05$).

3.2.2. Plant uptake of ^{15}N label

Between 6.2 d and 9.2 d, the shoot ^{15}N contents increased for both alanine and nitrate, probably reflecting complete mineralization of alanine and ^{15}N uptake from inorganic products of alanine mineralization. At the last sampling on day 9.2, no significant differences between ^{15}N shoot contents for both substances were observed ($p > 0.05$). However, during the experiment, alanine N uptake was far slower than nitrate N. ^{15}N uptake by the shoots was significantly lower for alanine than for nitrate up to 6.2 d ($p < 0.01$). Thus, the amounts of ^{15}N in the shoots were 2.1–4.8 times higher for nitrate N than for alanine N until 6.2 d (Fig. 2).

3.2.3. Ratio of plant ^{15}N/^{14}C uptake

The $^{15}N/^{14}C_{shoot}$ ratio was similar for "acetate + nitrate" (53 ± 13.3) and "glucose + nitrate" (58 ± 14.0) but significantly

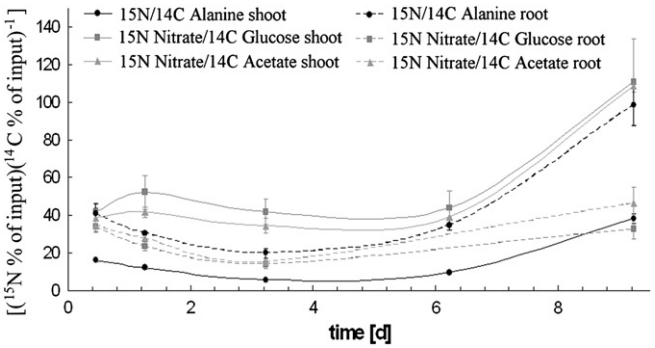

Fig. 4. Dynamics of ^{15}N% of input-to-^{14}C% of input. The *y*-axis shows the multiple of ^{15}N relative to ^{14}C observed in the plant shoot and root. Values are means ± SD ($n = 3$–5). Note that "alanine", "glucose" and "acetate" refer to the treatment rather than to the molecule itself.

narrower for alanine (16 ± 5.8; LSD, $p < 0.001$), while no significant differences were observed for the $^{15}N/^{14}C_{root}$ ratio ($p > 0.05$; Fig. 4).

3.3. Activity of ^{14}C in CO_2 evolved from the soil–root system

The highest proportion of ^{14}C was recovered in CO_2 (57–78%; Table 3), indicating intensive mineralization of added organic substances. In planted treatments, 55 ± 3% of the total ^{14}C was evolved as CO_2 within the first 5 h after addition, rising to 80 ± 3% after 17 h. For unplanted soil the $^{14}CO_2$ recovery increased over the same period from 73 ± 8% to 89 ± 2%. After 2 days, the CO_2 efflux rate was below 1% of ^{14}C input d^{-1} (Fig. 5). The $^{14}CO_2$ activity in alanine treatments was not significantly higher than that in acetate and glucose treatments, indicating similar mineralization rates of alanine and N-free LMWOS ($p > 0.05$). Note, however, that the experiment was not designed to compare the decomposition rates of the three substances. Alanine mineralization was significantly higher in the unplanted than in the planted treatment ($p < 0.05$). For glucose and acetate no significant differences were observed between these two treatments ($p > 0.05$; Table 3; Fig. 5).

3.4. ^{14}C activity and ^{15}N label in soil

For ^{14}C, approximately 21–26% of the initially injected amount was found in the soil of all treatments. For ^{15}N in contrast, nearly 100% of the ^{15}N label was recovered in the soil in unplanted treatments, whereas due to plant uptake, 74 ± 5% of nitrate ^{15}N and 84 ± 8% of alanine ^{15}N were detected in soil with plants (Table 4). While ^{14}C was highest in the central layer L3 and in L2 (50% and 20% of ^{14}C in soil), most of ^{15}N was found in L1 and L5 (16–26%).

4. Discussion

4.1. Experimental conditions

Many studies on LMWOS uptake by plants were conducted without soil. Key studies (i.e. in nutrient solution studies or sand cultures) disregard typical soil microorganisms. Other studies were conducted with LMWOS injected into soil far from the root

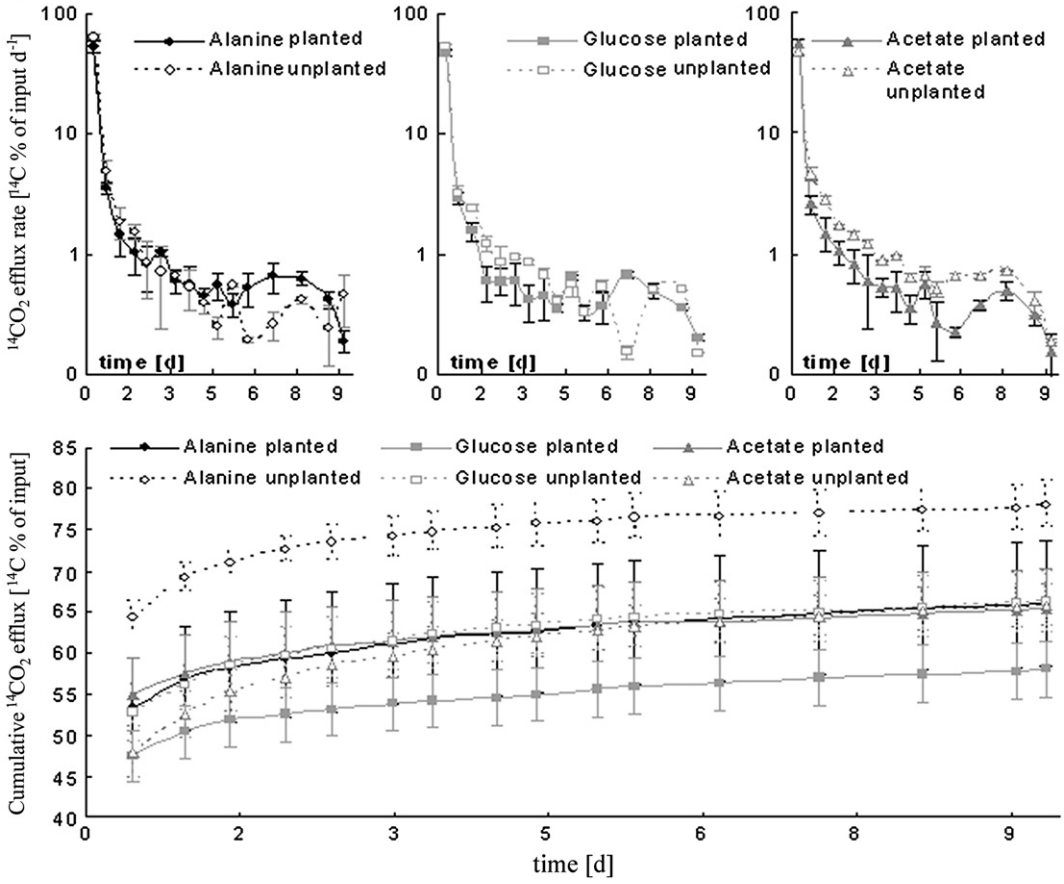

Fig. 5. Mineralization of ^{14}C labelled LMWOS in soil with and without maize roots. Rate of $^{14}CO_2$-evolution (top panels) and cumulative $^{14}CO_2$-evolution from soil (bottom panel) (means ± SD, $n = 5$). Note the logarithmic *y*-axis scale of the three top panels; the *y*-axis on the bottom panel starts at 40%.

surface. The 8 mm diameter of the rhizosphere tubes in our investigation ensured rhizosphere soil conditions (Fig. 1). Considering the distribution of fine roots and root hairs, the maximal distance between the added substances and the root surface was <1 mm. The initial LMWOS concentration (7.5 μM) was calculated for the whole soil solution in the tube (20 cm long). As the labelled substances were injected only into the central layer (L3), the initial concentrations were about 5–8 times higher (about 35–40 μM), which is in the range of LMWOS concentrations commonly found in soils (Kuzyakov and Jones, 2006; Jones and Darrah, 1996; van Hees et al., 2005). Such concentrations of LMWOS in the soil solution imply that no competition shifts for N sources between the microbial population and the roots should be expected.

4.2. Direct uptake

The potential for organic N uptake in most studies was demonstrated using amino acids because they are the dominating N-containing LMWOS in soil (Dennis and Turpin, 1990). The LMWOS uptake hypothesis is supported by Jones and Darrah (1993, 1996), Sacchi et al. (2000), Stubbs et al. (2004) and Xia and Saglio (1988): all reported that plants can re-uptake (up to 90%) of previously exuded LMWOS. Note, however, that most experiments were conducted in hydroponics with small amounts of nutrient solution. As Boddy et al. (2007) found for meadow grasses, we observed low ^{14}C activities in maize (<0.2% of ^{14}C input in the roots and <1% in the whole plant; Fig. 2). Importantly, the ^{14}C uptake was similar for the three LMWOS. The same ^{14}C quantities observed in roots and shoots for the three LMWOS indicate that maize plants did not prefer N-containing to N-free LMWOS. Moreover, the small quantities taken up and the same amounts of N-containing and N-free LMWOS show that, under soil conditions, the contribution of active transport mechanisms for amino acid uptake was minimal. Our conclusion is that, if transporters in roots were found for amino acids (Fischer et al., 1998; Jones et al., 2005a) and sugars (Xia and Saglio, 1988) but not for organic acids (Jones and Darrah, 1995), then the transporters are either very ineffective under soil conditions, or transporters also exist for organic acids, at least for acetate, because ^{14}C contents in the plants of acetate treatments showed the same values as in the alanine and glucose treatments.

Therefore the most plausible explanation is that the major pathway of amino acid and other LMWOS uptake under soil conditions in our experiment was connected to water uptake. The subsequent transport driven by transpiration led to ^{14}C accumulation in the shoots. The root ^{14}C activity could also reflect adsorption of LMWOS on the root surface, or their accumulation at the Casparian strip. This is supported by the relative amounts of ^{14}C found in the different soil layers and the corresponding roots (Fig. 3). Nonetheless, the contributions of both mechanisms are probably minor. The shoot ^{14}C contents prove maximal uptake of intact LMWOS and their transport via xylem. This indicates two possible uptake mechanisms by the roots: (1) the LMWOS may pass the Casparian strips or (2) the LMWOS were taken up by the root tips where the Casparian strips are not formed. In maize the Casparian strips are definitively developed after the first 3 cm behind the root apex. Only within the first 5 mm behind the root apex, however, is access available for soil solutes and subsequent xylem transport possible (Steudle et al., 1993). This root tip region is probably involved in Ca-uptake via the apoplast (Clarkson, 1996); this is also the site of potential pesticide uptake. In this experiment fine roots intensively pervaded the soil in the rhizotubes. Therefore in each rhizotube layer (L1–L5) in the root tips undeveloped Casparian strips were present.

4.3. Mineralization

Our results clearly show that most LMWOS in soils were rapidly mineralized by microorganisms (Fig. 5). In each treatment we found more than 80% of the ^{14}C input as CO_2 within 30 h after addition, suggesting intensive mineralization. Our results are supported by Pinton et al. (2001), who suggested that root-associated rhizosphere and rhizoplane microorganisms continuously metabolize LMWOS. Sugars and amino acids are decomposed within hours (Kuzyakov and Jones, 2006; Pinton et al., 2001). Fast mineralization of materials added and the low ^{14}C activity in the plant (Fig. 2) show that intact uptake of amino acids may be relevant only within 12 h after their release into the soil solution.

We did not measure root and shoot respiration of the plants directly. Kuzyakov et al. (2001) showed that 30–40% of a plant's photosynthetically net fixed C is respired above ground. Kuzyakov et al. (2001) and Swinnen et al. (1995) reported that 10–20% of the C in the roots is released via exudation and root respiration. The highest ^{14}C activity in the roots after alanine addition was 0.13%. Considering at most 20% losses for root exudation, the calculated ^{14}C activity taken up does not exceed 0.3%. The highest ^{14}C activity in the shoot was 0.87% of ^{14}C input in the alanine treatment. Taking into account 40% losses for shoot respiration (Kuzyakov et al., 2001), the calculated activity taken up into the shoot was maximally 1.2%. Subsequently, total plant uptake of alanine was <1.5% of ^{14}C input. Despite the mentioned losses these are maximal values, because the respiratory losses for organic substances taken up by roots were probably much smaller than those of organic substances assimilated by photosynthesis. Either way, the percentage of alanine ^{15}N uptake in the plant was at least 6.5 and up to 110 times higher than the maximum percentage of alanine ^{14}C uptake. Assuming a maximum uptake of 1.5% of alanine ^{14}C and a minimum uptake of 22% of alanine ^{15}N, we calculate that more than 93% of alanine N was taken up as inorganic N after mineralization (Fig. 2).

The ratio between ^{15}N and ^{14}C (as percent of ^{15}N and ^{14}C input) observed in roots or shoots shows which portion of the added substances was taken up in organic form. A ratio close to 1 indicates intact amino acid uptake, while a wide ratio supports mineralization and subsequent uptake of mineralized N by the plant. As the ^{14}C uptakes into plants were not significantly different (mentioned above: direct uptake) between the three organic substances (Fig. 2), the $^{15}N/^{14}C_{plant}$, $^{15}N/^{14}C_{root}$, and $^{15}N/^{14}C_{shoot}$ ratios are indicators for the uptake of the different N forms (Fig. 4). The $^{15}N/^{14}C_{root}$ ratios during the first 30 h are within the same range (30–40) for the three substances. In contrast to the nitrate treatments, where $^{15}N/^{14}C_{root}$ uptake ratios remained in the same range (23–47) throughout the observation, the alanine $^{15}N/^{14}C_{root}$ uptake ratio increased significantly from 31–41 to 99 after 9.2 d. Since Jackson et al. (1989) mentioned that plants can compete for organic-compound-derived N after mineralization, nitrification and release after microbial decomposition, and Jones et al. (2004b) showed that LMWOS can be transported into microbial cells, we suggest that microorganisms took up most of the alanine ^{15}N that was subsequently excreted as NH_4^+ or released after microbial turnover. Although plants can compete with microorganisms for NH_4^+, they compete better and more effectively for NO_3^- (Jackson et al., 1989). The $^{15}N/^{14}C_{shoot}$ ratios for the nitrate treatments were on average 3.4 (range: 2.4–7.7) times higher than those of the alanine treatment. This difference reflects direct and quick nitrate uptake. Nitrate is a very mobile anion that diffuses faster than amino acids in most soils (Jones et al., 2005a; Wilson et al., 1988). Hence, it can easily be moved by mass flow to the roots (Nye and Tinker, 1977). However, Jackson et al. (1989) showed that microorganisms are better competitors for inorganic N sources and are much more effective than plants. This means that added ^{15}N label is likely to cycle within the microbial biomass several times before it is taken up by plant roots. Our findings support the theory

of Jackson et al. (1989) that microorganisms are the better short-term competitors for N, but that plants will win out in the longer term. Previously mineralized alanine ^{15}N was released into the soil as inorganic N, which maize took up. This was demonstrated by the continuously increasing $^{15}N/^{14}C_{shoot}$ ratio over the 9.2d-period (Fig. 4). This was supported by Hodge et al. (2000) who summarize that the rapid turnover of soil microorganisms allows plants to capture plant-available N species released from microorganisms.

Due to the small amount of soil in our rhizotubes, drying–rewetting events driven by transpiration and watering alternated 2–3 times a day. Such dynamics can disrupt microbial cells and thus reduce soil microorganisms' competitiveness (Clein and Schimmel, 1994). This in turn would improve the plants' competitiveness for N. Furthermore, enhanced microbial-cell lyses by these drying–rewetting cycles mean less microbial competition for inorganic N (Hodge et al., 2000). Our plants probably acquired inorganic N faster than under real field conditions due to reduced microbial competition and particularly because LMWOS were injected directly into the rhizosphere.

The decreases of the ^{15}N contents in the shoots (Fig. 2) and also of the $^{15}N/^{14}C_{shoot}$ ratios – from 30 h until 6.2 d after the start of the experiment both in the nitrate and in the alanine treatments (Fig. 4) – were due to the following two reasons.

(A) The most alanine (and nitrate) was taken up by microorganisms during the first hours after labelling. A few days later, microbial turnover released high amounts of mineral ^{15}N into the soil solution. Subsequently, the plants took up this mineral ^{15}N, thus increasing the $^{15}N/^{14}C_{root}$ and $^{15}N/^{14}C_{shoot}$ ratios after 9.2 d.

(B) The quantity of nitrate uptake depends on the amino acid concentrations in the phloem sap. Translocation of amino acids from older to younger leaves represses nitrate uptake via the roots because amino acid concentrations in the phloem sap increase. As soon as the high demands of younger shoot development and the ending translocation reduce the phloem sap's amino acid concentration, nitrate uptake increases again (Imsande and Touraine, 1994). Leaf senescence (cotyledons) occurred during the time when no further ^{15}N uptake was detected.

4.4. Conclusions

We agree with Jones et al. (2005a) that evidence for amino acid N acquisition as major plant N acquisition pathway is still lacking under field conditions. We are aware that the results of our experiment in rhizotubes cannot be transferred directly to field conditions. In the rhizotubes only rhizosphere soil was present. However, under field conditions, the microbial uptake and decomposition of LMWOS in the root-free soil is much more important compared to our rhizotube conditions. Therefore, we expect that the total uptake of organic N by maize under field conditions will be even lower than those observed in this experiment under controlled conditions.

The uptake of N-containing and N-free LMWOS, in this experiment, was of a similar small magnitude. Thus, young maize plants under soil conditions do not preferentially take up N-containing LMWOS. This indicates that the contribution of amino acid uptake into maize to cover the plants' N demand is of minor importance. We suggest that LMWOS uptake as a whole occurred mainly by transpiration flow. Thereby, the contribution of specific LMWOS transporters was minimal. Quick mineralization of the LMWOS showed that soil microorganisms were highly effective competitors for these resources.

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