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REGULAR ARTICLE

Respiration costs associated with nitrate reduction as estimated by $^{14}CO_2$ pulse labeling of corn at various growth stages

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Abstract Utilization of nitrogen in the form of either nitrate (NO_3^-) or ammonium (NH_4^+) ions may affect the carbohydrate metabolism and energy budget of plants. Recent studies showed that greater expenses of NO_3^- to NH_4^+ reduction mostly occur in the roots and during darkness. Fertilization of corn with ^{15}N-labeled nitrate and ammonium, combined with pulse labeling of plants in a $^{14}CO_2$ atmosphere at the V6 and V8 growth stages, allowed us to evaluate the effect of N form on the CO_2 efflux from soil. NH_4^+ oxidation was inhibited by adding dicyandiamide. In respect to ammonium, nitrate addition increased root-derived CO_2 efflux from corn by 2.6 times at stage V6 and by 1.8 times at stage V8. The time of peak $^{14}CO_2$ efflux from soil also differed between two growing stages: at V6, efflux peaked only on the second day after pulse labeling, while at V8 this occurred within the first 6 h. The strong effect of NO_3^- and NH_4^+ on root respiration requires considering the N form in the soil and the nitrate reduction site location in a plant when modeling soil respiration changes and when separately estimating individual CO_2 sources that contribute to the total soil CO_2 efflux.

Keywords *Zea mays* · Nitrate reduction · ^{14}C pulse labeling · Soil CO_2 efflux · Root-derived respiration · Rhizosphere · N fertilization · C and N cycles

Abbreviations

V6	sixth leaf collar stage
V8	eighth leaf collar stage
SE	standard error
SOM	soil organic matter
PVC	polyvinyl chloride

Introduction

Nitrogen (N) and carbon (C) are elements needed by both lower and higher plants for growth. Nitrogen is lost from top soil by leaching and is also consumed by microbial organisms; it is thus often a limiting factor for plant growth, especially at high soil temperatures and humid climatic conditions. Plants that grow in nitrogen-poor soils have often developed

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mechanisms to cope with the low N supply. These mechanisms include very sensitive uptake systems and the possibility to grow on various N sources. The main N sources include ammonium (NH_4^+) and nitrate (NO_3^-) (Tischner 2000), although recent studies have shown that amino acids are significant organic N sources in some environments (Chapin et al. 1993; Näsholm et al. 2000).

Utilization of N either in the form of nitrate (NO_3^-) or ammonium ions (NH_4^+) may affect the carbohydrate metabolism and energy economy of the plant (Blacquiere 1987). NH_4^+ is fixed by the GS/GOGAT pathway into amino acids (glutamine/glutamate). This incorporation occurs immediately after N uptake in the roots, but no significant amounts of ammonium have ever been recorded in the xylem sap (Tischner 2000). NO_3^- is taken up by roots and is reduced in both roots and shoots of higher plants (Beevers and Hageman 1980). The contribution of each place to nitrate reduction is genetically and environmentally determined. Reduction of NO_3^- to NH_4^+, catalyzed by nitrate and nitrite reductase enzymes, together with subsequent assimilation of NH_4^+, is among the most energy-intensive processes in the plants and can involve additional respiration.

Several studies have concluded that NO_3^- transformation is generated by oxidation of carbohydrates with additional production of CO_2 in the roots and both in shoots and roots during darkness (Aslam and Huffaker 1982; Ninomiya and Sato 1984; Schilling et al. 2006). This transformation, however, has not been confirmed for illuminated leaves, where the reduction to NH_4^+ is linked directly with photosynthetic electron transport and the enzymes that are involved receive the reducing equivalents as electrons from ferredoxin and from NADH or NAD(P)H through the glyceraldehyde-3-phosphate/3-phosphoglycerate shuttle (Atkins et al. 1979; Aslam and Huffaker 1982; Warner and Kleinhofs 1992). The C cost for the reduction to NH_4^+ therefore depends on the location of the reduction site in the plants, with greater expenses for plants in which this occurs largely in the roots. Acid-based neutralization of the hydroxyl (OH^-) ion excess formed as a result of NO_3^- assimilation also has immediate C costs by producing organic acids like malic acids; these costs should be considered (Raven and Smith 1976; Raven 1985). This kind of pH regulation involves additional carbohydrate degradation if performed in roots and is coupled with photosynthetic CO_2 consumption if it occurs in shoots. A portion of neutral product formed in the shoots is however transported to roots and after decarboxylation with subsequent CO_2 evolution results in the OH^- anion excretion to the root medium. The latter process amplifies the difference between two types of N nutrition (NH_4^+ and NO_3^-) in terms of quantity of respired C per unit of assimilated N, but could smooth the difference between two sites of nitrate reduction.

Nonetheless, the contribution of roots and shoots to overall plant nitrate reduction is uncertain. The location of the nitrate reduction site in the plant does not necessary depend on the type of species or cultivars (Schilling et al. 2006; Silveira et al. 2001), but can vary within a single plant. Previous studies confirm that environmental conditions, plant phenology and the quantity of N supply influence changes in the reduction site. However, most of these findings stem from nutrient solution studies, which do not reflect the real plant uptake rates or root and microbial respiration under true soil conditions. NO_3^- translocation from root to shoot depends on the N concentration in the soil. At low NO_3^- concentration, N reduction occurs mainly in the roots, while at higher NO_3^- concentrations N storage and transportation is further adjusted (Atkins et al. 1979, 1980; Oscarson and Larsson 1986). Nitrate uptake by roots and reductase capacity are not equally distributed along a root axis and are not identical during root ontogeny (Cruz et al. 1995; Di Laurenzio et al. 1996). Siebrecht et al. (1995) and Pan et al. (1985) located a high N uptake rate together with the highest reductase activity in the root apical region. Nitrate reductase was low in the older root parts, indicating a possible NO_3^- translocation from these root parts to the shoots. Gojon et al. (1986) reported that roots participate actively in reducing incoming nitrates during the induction process of N uptake, shortly after the fertilization, but that nitrate reduction in roots decreases considerably thereafter.

Based on these results, obtained mainly from nutrient solution experiments, we hypothesized that the form of N supply may significantly affect the amount of CO_2 released by roots during respiration also under true soil conditions. We also expected that the effect of N nutrition on the efflux of recently assimilated CO_2 from soil may change during plant growth stages.

Summarizing the above findings and uncertainties, this study aims to:

a) evaluate the effect of N form (NO_3^- vs. NH_4^+) on CO_2 efflux from soil and root;
b) verify if the relative contribution of roots to the overall plant nitrate reduction process remains stable during plant growing stages;
c) investigate these processes under soil conditions rather than in nutrient solution.

We selected corn (*Zea mays*, L.) as a species whose nitrate reduction sites are located both in the shoots and roots (Pate 1973; Gojon et al. 1986). Corn was labeled twice: on the sixth and eighth leaf collar stage. Plants were placed in $^{14}CO_2$ atmosphere shortly after fertilization with ^{15}N in the form of ammonium or nitrate to separate the effects of N type on recently assimilated ^{14}C from the respired C assimilated earlier and to account for the differences in N uptake rates. We also used soil with and without plants as a control for possible effects of N form on CO_2 from microbial decomposition of SOM.

Materials and methods

Soil

Soil was collected from the Karlshof long-term field experimental station of the University of Hohenheim. Samples, a loamy Haplic Luvisol soil, were taken from the top 10 cm (Ap horizon). Soil was air dried, mixed and sieved through a 5 mm sieve. The soil contained 1.5% C_{tot} and 0.14% N_{tot}, 25 µg g^{-1} N_{min}, 94 µg g^{-1} available K, 16 µg g^{-1} available P, with 2.9% sand, 74.5% silt and 22.6% clay; pH 6.5.

Plants and growth conditions

PVC pots (50 ml) were filled with 50 g of soil each and were used for growing the plants. Another twelve pots were not planted to measure the microbial respiration from bare soil.

Seeds of corn (*Zea mays* L.) were allowed to germinate in the Petri dishes on moist filter paper for 2 days. Germinated seedlings were transplanted to the PVC pots, with one seedling per pot. They were allowed to grow under controlled laboratory conditions with a 12 h/12 h day/night period at a constant day and night temperature of 25±1°C. The photosynthetically active radiation (PAR) intensity was approximately 800 µmol $m^{-1}s^{-1}$ at the top of the plant canopy. A constant day/night temperature was chosen to avoid the effects of changing temperature on CO_2 fluxes. Air relative humidity was maintained at 80%. Soil water content in each pot was maintained gravimetrically at about 60% of its holding capacity by checking its weight daily. Pots with bare soil were incubated at the same conditions. Twenty-four plants which were similar in their growth development and height were chosen for the following treatments.

^{14}C labeling and ^{15}N application

Twenty-four corn plants were equally divided into two groups so that each group contained 12 corn plants. The first group was labeled with $^{14}CO_2$ at the sixth leaf collar stage (V6 corn), while the second group was labeled with $^{14}CO_2$ at the eighth leaf collar stage (V8 corn). Our preliminary findings and the results of Werth and Kuzyakov (2008) show that germinated corn increases its biomass linearly up to 4 weeks after germination. This indicates that the uptake of water and nutrients, including N, at the above-mentioned stages is nearly linear and that the results can therefore be extrapolated within this period.

A day before pulse labeling, the top of each pot was sealed with a silicone paste (NG 3170, Thauer & Co., Dresden, Germany). The seal was tested for air leaks. Before labeling, CO_2 that had accumulated in the soil during the plant's growth was flushed out by pumping air through the soil column.

Three N treatments were applied 4 h before labeling in a $^{14}CO_2$ atmosphere. The treatments were as follows: (a) without added N control treatment; (b) Ammonium treatment with ^{15}N as $(^{15}NH_4)_2SO_4$ and (c) Nitrate treatment with ^{15}N as $K^{15}NO_3$. Four plants of each growing stage were exposed to N treatment. ^{15}N enrichment of each N type was 50 atom %. The amount of ^{15}N applied to a pot was calculated to produce an average concentration of 60 mg of N kg^{-1} soil for each N species added. Dicyandiamide (DCD) at 20 mg kg^{-1} soil was applied in solution with ^{15}N fertilizer to all treatments in order to achieve an effective nitrification inhibition throughout the soil column (in the ammonium treatment) and to balance the side effects of the inhibitor (in the nitrate and

control treatments). Four unplanted pots were fertilized with half the amount of nitrate or ammonium in order to estimate the effects of N fertilization on soil microorganism respiration.

The ^{14}C labeling process has been described in detail by Kuzyakov et al. (1999) and Domanski et al. (2001). Sealed pots with plants were placed in a Plexiglas chamber. $^{14}CO_2$ was introduced into the chamber by adding 1 mL of 5 M H_2SO_4 to a $Na_2{}^{14}CO_3$ (1.5 MBq) solution. This allowed complete evolution of $^{14}CO_2$ into the chamber atmosphere. After a 2-h labeling period the CO_2 from the chamber was trapped using 10 mL of 1 M NaOH solution aiming to remove the remaining unassimilated $^{14}CO_2$. When the chamber was opened, pots with the plants were connected by tubes to an output of membrane pumps. Air was then pumped through every single pot from bottom to top. Another tube was used to connect the pots to a CO_2 trapping tube, filled with 3 mL of 1 M sodium hydroxide (NaOH) solution. The output of the trapping tube was connected to the input of the membrane pump. Therefore, the air containing CO_2 evolved from the soil respiration was circulating in a closed system—from the plant to the soil.

Sampling and analyses

NaOH in the trapping tubes was changed twice a day, i.e. in the morning and in the evening for a period of six days after labeling. The aim was to collect the CO_2 evolved in the rhizosphere during day- and night-periods separately. NaOH traps were analyzed for total trapped CO_2 and for ^{14}C activity (only for planted pots).

The ^{14}C activity was measured in 1 mL aliquots of NaOH with 2 mL of the scintillation cocktail EcoLite$^+$ (ICN) after the decay of chemiluminescence by a liquid scintillation counter (MicroBeta, TriLux).

The total CO_2 efflux from bare soil was estimated by precipitating CO_2 trapped in the NaOH solution with a 0.5 M barium chloride ($BaCl_2$) solution. NaOH was then titrated with 0.1 M hydrochloric acid (HCl) against phenolphthalein indicator (Zibilske 1994).

On the sixth day after each labeling, all the plants were harvested. Each shoot was cut at the base. The lid of the pot was opened and each root-soil column was pulled out of the pot. Roots were carefully washed with deionized water to remove soil particles. Shoots and roots were dried at 70°C, weighed and ground with ball mill (Retsch, Germany) to analyze C_{tot}, N_{tot}, and ^{15}N content. Three grams of soil were taken from each pot, dried at 70°C and ground to analyze the same parameters. Total C and N together with the isotope ratio $^{15}N/^{14}N$ in the plant and soil samples were determined using a Carlo Erba NA 1500 gas chromatograph (Carlo Erba Instruments, Milano, Italy) coupled to an isotope ratio mass spectrometer (Delta plus IRMS 251, Finnigan Mat, Bremen, Germany).

^{15}N concentration in plant material (Eqs. 1 and 2) was corrected for ^{15}N natural abundances by subtracting the natural ^{15}N concentration, obtained from control unfertilized plants. Eventual ^{15}N fractionation during N uptake process was however not considered.

$$\text{at\% } {}^{15}\text{N} = \left({}^{15}\text{N}/\left({}^{14}\text{N} + {}^{15}\text{N}\right)\right) \times 100 \quad (1)$$

$$\mu\text{mol } {}^{15}\text{N dry mass}^{-1} = \text{at\%}/100 \times \mu\text{mol N dry mass}^{-1} \times 1000 \quad (2)$$

Statistics

The experiment was conducted with four replicates. All replicates were analyzed for ^{14}C, ^{15}N, C_{tot}- and N_{tot}-contents in the shoots and roots. ^{14}C data are presented as the percentage of ^{14}C assimilated during exposure of plants to the pulse labeling. All data were analyzed with SYSTAT 11.0 (SPSS Inc.). Effects of different N treatment (no N, NH_4^+-N and NO_3^--N) during plant growth stages and sampling time (day and night) were tested using two-way analysis of variance (ANOVA). We calculated the least significant difference (LSD 0.05) in a post hoc Newman-Keuls test to identify the significant differences between treatments.

Results

Aboveground and belowground plant biomass

No significant differences were observed in the aboveground (AG) biomass of *Zea mays* between N treatments (p=0.62). AG biomass increased by 30%

over time between the first and second labeling (Fig. 1).

Different N fertilizers significantly affected ($p<0.01$) root biomass of V6 corn, with the lowest value being 0.25 g pot^{-1} for NH_4^+-N fertilized plants and the highest being 0.39 g for the control (Fig. 1). In contrast, there was no difference in the root biomass between N treatments for V8 plants. A positive effect of plant age on the root biomass was observed for plants fertilized with N. In the control experiment in which N fertilizers were not added, no effect of time on root biomass was registered.

Dynamics of $^{14}CO_2$ efflux from the root-soil compartment

V6 The peak $^{14}CO_2$ evolution from the root-soil compartment in all three N treatments occurred on the second day of the experiment—between 26 and 30 h after $^{14}CO_2$ pulse labeling (Fig. 2a). The $^{14}CO_2$ emission rate declined rapidly from the maximum of 3.3% d^{-1} for NH_4^+-N and control, and from 5.7% d^{-1} for NO_3^--N of total assimilated ^{14}C to about 1% d^{-1} on day four. There was a clear diurnal dynamic in rhizosphere respiration of recently assimilated C (^{14}C) (Fig. 2a).

The difference in $^{14}CO_2$ respired from soil with plants fertilized by NO_3^--N and NH_4^+-N was highest during the first days after the labeling. No difference was observed in the peak $^{14}CO_2$ values from soils under NH_4^+ and control treatments ($p=0.77$).

Cumulative $^{14}CO_2$ respired by roots and rhizosphere microorganisms during the six days of the experiment reached 5.8% for the control, and 5.4% and 7% for the NH_4^+-N and NO_3^--N treatments, respectively (Fig. 2a). The difference between the two types of applied N was significant ($p<0.001$), but no difference was observed between NH_4^+-N and the control ($p=0.70$).

To consider possible effects of variation in root biomass, the maximal intensity of $^{14}CO_2$ losses from the soil was calculated per unit of root biomass measured six days after labeling (Fig. 3a). The maximum $^{14}CO_2$ emission was chosen for the comparison between the N treatments because it relates more closely to root respiration than the cumulative $^{14}CO_2$ efflux, of which rhizomicrobial respiration makes up a significant part. A strong effect of N fertilization on recently assimilated $^{14}CO_2$ was found ($p<0.001$). A significant difference was also observed between the two types of N applied ($p<0.001$). Taking the control treatment without N as a 100% reference, the respiration losses of ^{14}C from the plant-soil system with corn labeled at the V6 stage amounted to 175% under NH_4^+-N and 220% under NO_3^--N fertilization.

V8 In contrast to the V6 stage, the maximum $^{14}CO_2$ efflux after the second labeling was recorded within the first day, i.e. 8 h after the start of labeling. The $^{14}CO_2$ efflux peaked before the first sampling (Fig. 2b). Soon thereafter, the emission rate declined from the maximum of 3% d^{-1} for the control, 7.8% d^{-1} for NH_4^+-N and 11.9% d^{-1} for NO_3^--N to the lower rate of 1.2 % C d^{-1} on the third day.

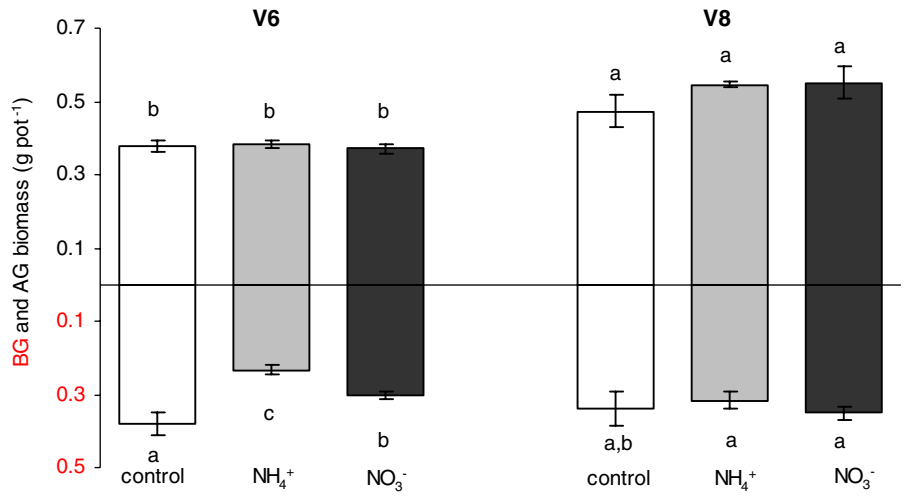

Fig. 1 Aboveground (AG) and belowground (BG) biomass at V6 and V8 *Zea mays* under various N fertilization types (± SE). Letters indicate the significance of the differences between treatments and growth types at $p=0.05$, separately for AG and BG biomass

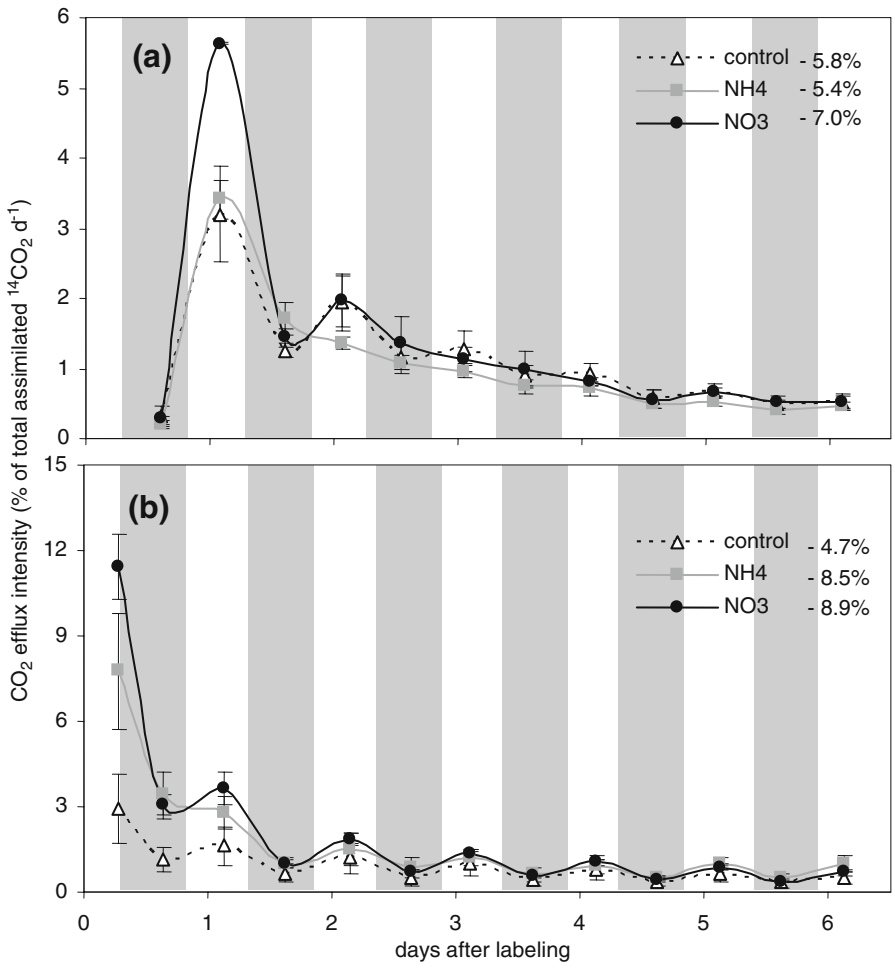

Fig. 2 Effect of N fertilization type on dynamics of $^{14}CO_2$ efflux rate from the soil (± SE) planted with *Zea mays* and labeled at the V6 (**a**) or V8 (**b**) growth stages. Day (white) and night (grey) periods are shown; percentages indicate a cumulative $^{14}CO_2$ efflux at the end of the chase period for each treatment

$^{14}CO_2$ efflux from the soil in all N treatments showed clear diurnal dynamics, with an increase in $^{14}CO_2$ respiration during the day and then a decrease at night.

Similarly to V6 corn, the difference in the quantity of ^{14}C respired from plants under NO_3^--N and NH_4^+-N was highest during the first two days after labeling. The cumulative ^{14}C evolved from roots and rhizosphere microorganisms during six days after the labeling amounted to 4.7% of ^{14}C input in the soil without N fertilization, 8.5% for the NH_4^+-N treatment and 8.9% for the NO_3^--N treatment (Fig. 2b).

The ratio between the maximum $^{14}CO_2$ efflux and root biomass revealed no significant difference between the two types of N applied (Fig. 3a). Losses of ^{14}C from the plant-soil system in the V8 corn, when considering our control as a 100% reference, reached 281% under the NH_4^+-N treatment and 373% under the NO_3^--N treatment ($p<0.01$). The V8 growth stage increased the quantity of respired ^{14}C compared with the younger plants (Fig. 3a).

Pots with unplanted soil were incubated under the same conditions as corn in order to evaluate the effect of N form on microbial respiration. The results show that CO_2 efflux from unplanted soil had no diurnal changes (data not shown), although microbial respiration under NH_4^+-N was slightly higher than that under NO_3^--N. This difference was not statistically significant ($p=0.74$) (Fig. 4).

^{15}N uptake by plants

At both growth stages of *Zea mays*, significantly more ^{15}N was recovered from shoots and roots of plants fertilized with NH_4^+-N than with NO_3^--N (Fig. 5a). The V8 stage significantly decreased the quantity of ^{15}N recovered in the shoots and roots in contrast with

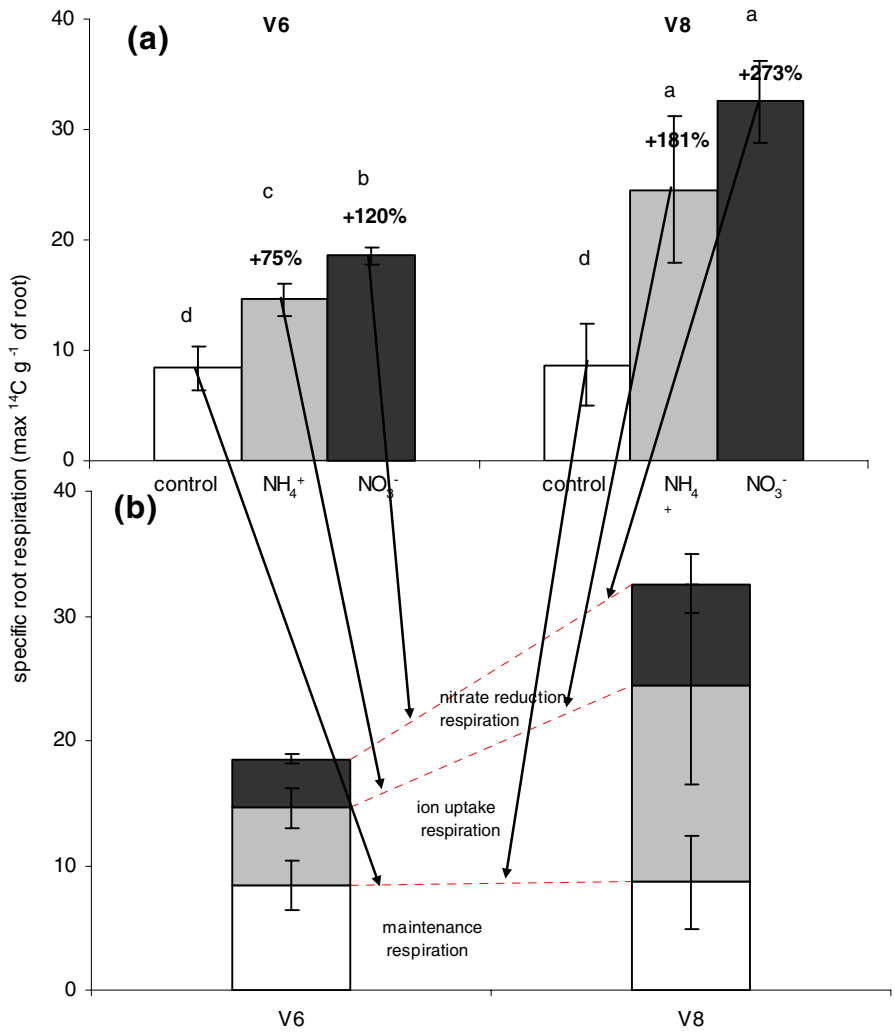

Fig. 3 **a**) Intensity of $^{14}CO_2$ efflux (maximum efflux) respired from root-soil system with V6 and V8 *Zea mays* per unit of root biomass (± SE). Percentages indicate the increase of $^{14}CO_2$ efflux after NH_4^+ or NO_3^- fertilization as related to the $^{14}CO_2$ from control without N; Letters above indicate the significance of the differences between treatments and growth types at p=0.05; **b**) Increase in respiration rates (maximum efflux per unit of root biomass) associated with maintenance, ion uptake and NO_3^- reduction on two growing stages of corn

the V6 plants. The total quantity of ^{15}N incorporated in the shoots and roots of V6 plants was 0.71 mmol g^{-1} dry mass under NH_4^+ -N and 0.45 under NO_3^--N. During the V8 growth stage, only 0.43 mmol g^{-1} dry mass of ^{15}N was recovered under NH_4^+ treatment and 0.34 mmol g^{-1} dry mass under NO_3^- treatment. The difference between N treatments and two growth stages in the quantity of recovered ^{15}N was however lowered to non-significant when accounting for the total N content in shoots and roots (Fig. 5b).

The distribution of N between shoots and roots changed in different plant growth stages. Under NH_4^+-N, 72% of the ^{15}N was recovered in the shoots of V6 corn, whereby 58% was only found in the shoots of V8 corn. Under NO_3^--N the difference between the two stages was less clear: 67% and 62% of the recovered ^{15}N in V6 and V8 plants, respectively, had a shoot origin (Fig. 5).

Maximum $^{14}CO_2$ efflux from plant-soil systems was related to the unit of N absorbed (Fig. 6). ^{14}C respiration from the system with NO_3^--N at stage V6 was about 2.6 times higher than that in plants with NH_4^+-N (p<0.001). At stage V8, the increase in respiration between plants with different N supply was 1.8 (p<0.001).

Discussion

^{14}C-CO_2 efflux from soil

The maximum $^{14}CO_2$ efflux from soil in the V6 corn was found within 26–30 h after labeling versus within

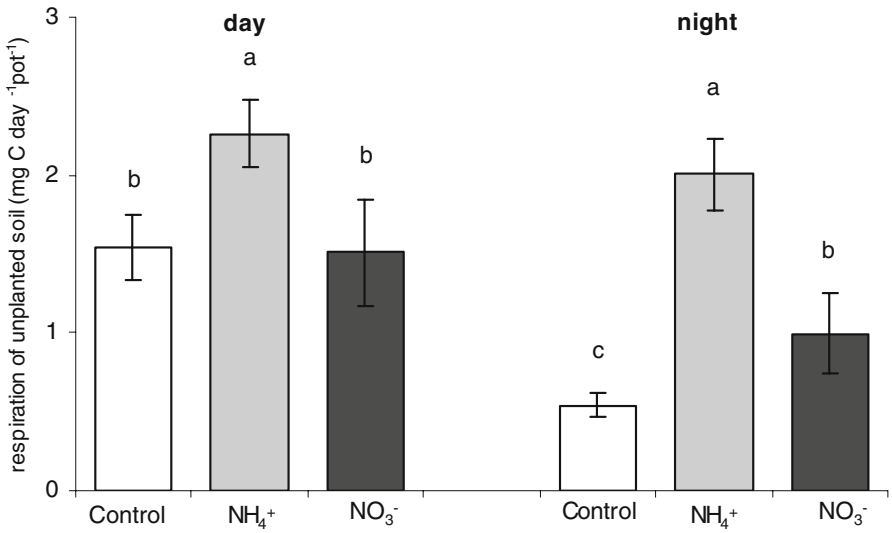

Fig. 4 CO_2 efflux from unplanted pots under various N fertilization (± SE). Letters above columns indicate the significance of the differences between treatments and growth types at $p=0.05$

the first 6 h for V8 corn. This difference in the time lags between C assimilation and its subsequent respiration through the roots in two growth stages could influence the quantity of ^{14}C that was involved to sustain the NO_3^--N reduction process. This underlines the importance of specifying the factors responsible for the observed difference in the lags between C assimilation and root respiration. Physical factors that potentially influence the soil CO_2 production and diffusion rate (Tang et al. 2003; Carbone and Trumbore 2007) were equal on both growth stages of corn: plant growing conditions (soil water content, temperature, PAR) were uniform and the effect of the soil air vertical flow through the soil column was probably negligible because we continuously pumped the air from the bottom to the top of the pot. We therefore conclude that growth-stage-specific differences in the transport of assimilates were responsible for the different lags between C assimilation and utilization of this C by roots for respiration. The difference in the path length cannot explain such changes in the time lag because shoots height was similar in both growth stages. The growth stage could control the metabolic orientation of plants, influencing source (photosynthetically active leaves, which supply a new C)—sink (developing organs of plants, which compete for the new C) interactions (Farrar and Jones 2000; Carbone and Trumbore 2007). The flow of C to sinks depends on the strength of the sink, sink size and growth rate (Dickson 1991; Farrar and Jones 2000). The major energetic costs belowground are the growth of new roots and maintenance of existing ones (Dobrowolski et al. 1990). We observed a significant difference in the root biomass of the two growth stages. Intensively growing roots and higher belowground biomass may accelerate downward transport of assimilates in the case of V8 corn. In contrast, developing shoot cells could better compete for recently assimilated C than roots in the case of younger corn plants. We cannot specify the intensity of N uptake in different phases of the chase period, so we therefore assume that N was taken up uniformly during the first days after pulse labeling, when ^{14}C efflux peaked in both growth stages of corn.

Diurnal changes in $^{14}CO_2$ efflux from planted soil were observed in both growth stages and all three N treatments (Fig. 2). Diurnal CO_2 dynamics have been attributed to day–night variation of soil temperature, based on the assumption that higher daytime soil temperature promotes decomposition of organic compounds in the soil and thus increases CO_2 efflux. However, the high dependence of soil and air temperatures on solar irradiation conceals the fact that larger amounts of easily decomposable substrates are released to the soil during daylight (Fitter et al. 1999). Decomposition processes in the soil are substrate limited (Wardle 1992; Ekblad and Nordgren 2002), which means a higher release of easily decomposable substrates, which is directly linked to photosynthesis. This boosts CO_2 production and results in diurnal CO_2 dynamics (Kuzyakov and Cheng 2001, 2004). Our experimental plants were grown at a constant temperature during day and night times. CO_2 efflux from unplanted soil was indepen-

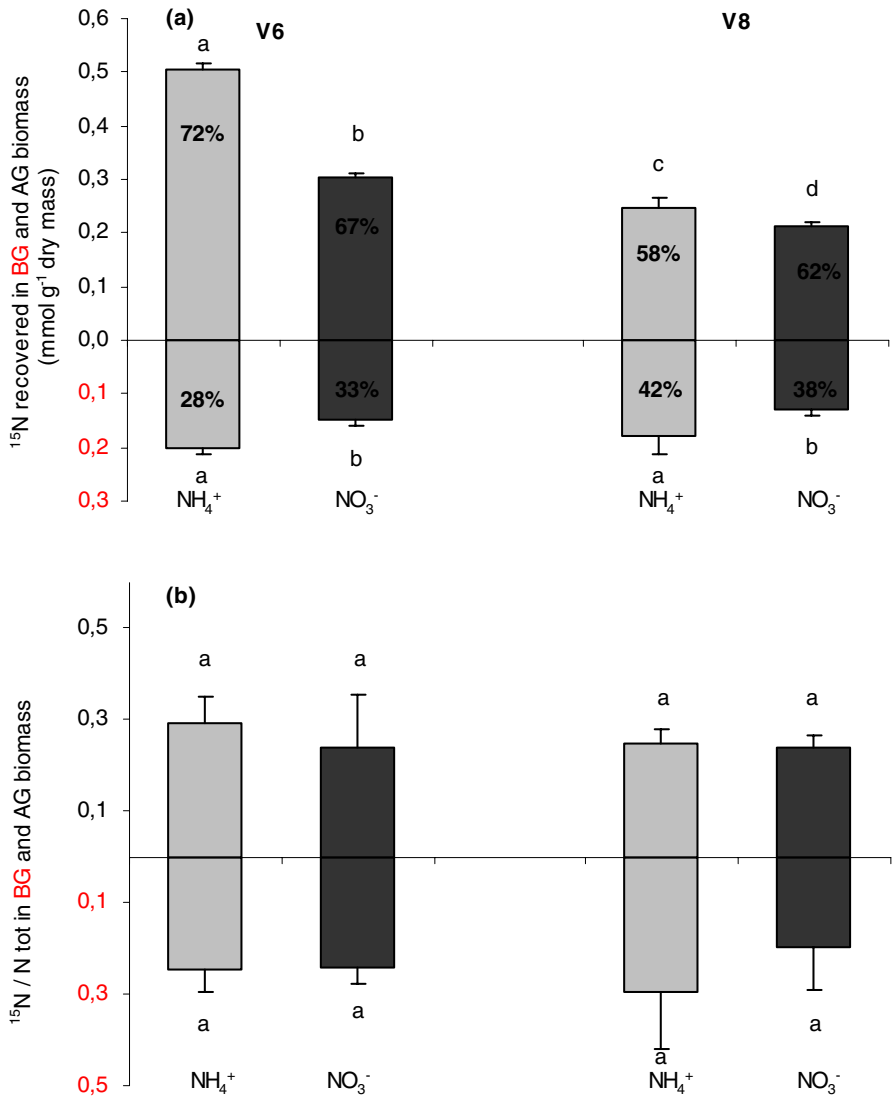

Fig. 5 a)^{15}N amount recovered from shoots and roots at V6 and V8 of *Zea mays* (± SE). Percentages indicate ^{15}N distribution between shoots and roots of plants; **b)** ^{15}N to total N ratio in shoots and roots. Letters indicate the significance of the differences between treatments and growth types at p=0.05, separately for AG and BG biomass

dent of a day/night temperature change (Fig. 4). Accordingly, the daytime increase in $^{14}CO_2$ evolution is attributed to C assimilation during photosynthesis and the subsequent rapid translocation to the roots with an associated signal in the root-derived CO_2. This observation was confirmed also by Kuzyakov and Cheng (2001, 2004).

NH_4^+ versus NO_3^- supply—effect on root respiration

Some studies have confirmed that in the non-green root cells, and in darkness, nitrate reduction is supplied by reducing equivalents from carbohydrate degradation with an additional CO_2 production (Aslam and Huffaker 1982; Ninomiya and Sato 1984). The same process in the leaves during the day, however, is coupled directly with photosynthetic electron transport (Aslam and Huffaker 1982; Atkins et al. 1979; Warner and Kleinhofs 1992) without extra losses of C as CO_2. Following these observations, we expected to observe differences in the quantity of recently assimilated $^{14}CO_2$ respired by roots of *Zea mays* under NO_3^--N or NH_4^+-N nutrition.

At both growth stages, corn respired significantly more $^{14}CO_2$ under NO_3^--N compared to NH_4^+-N nutrition. Theoretically, two CO_2 sources could be responsible for this increase: root respiration and rhizomicrobial respiration. However, some recent studies have shown that the $^{14}CO_2$ efflux after pulse labeling originating from root and rhizomicrobial

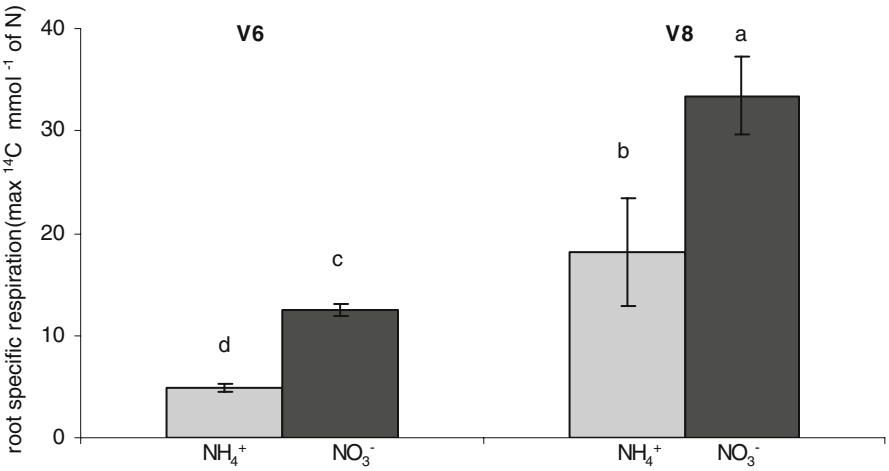

Fig. 6 $^{14}CO_2$ (max efflux) respired from root-soil system at V6 and V8 of *Zea mays* related per unit of total ^{15}N absorbed (± SE). Letters above columns indicate the significance of the differences between treatments and growth types at $p=0.05$

respiration occurs at different times after the labeling (Kuzyakov et al. 1999; Kuzyakov and Domanski 2002). $^{14}CO_2$ from root respiration occurs earlier than $^{14}CO_2$ from microbial respiration by decomposition of root exudates. This is because the latter consists of a chain of successive processes: exudation from the root, intake by microorganisms, and then the respiration by microorganisms. In *Lolium perenne*, for example, the actual root respiration affects the $^{14}CO_2$ efflux curve only during the first 24 h after pulse labeling. Moreover, the maximum effect of exudation on rhizomicrobial respiration predominates in the $^{14}CO_2$ efflux only after about one to two days after pulse labeling (Kuzyakov et al. 2001; Kuzyakov and Domanski 2002). In our study the observed difference in the quantity of $^{14}CO_2$ respired between NO_3^--N and NH_4^+-N treatments or the control fell mainly in the first day after labeling, and no differences between N treatments in the quantity of respired ^{14}C were measured one day after labeling. We therefore assume here that root respiration was mainly affected by N form. Based on the previous findings of the sequence of CO_2 sources after assimilation, and on our observations of the maximum effect of N form during the first day, we conclude that the form of N supply has a strong direct effect on the amount of actual root respiration.

The results of ^{15}N analyses in the shoots and roots demonstrated that at both development stages, corn took up more ^{15}N under NH_4^+-N than under NO_3^--N. This made the difference between two types of N applied in the quantity of respired C even clearer.

Summarizing the above findings, plant nutrition with nitrates increases the autotrophic component of soil CO_2 efflux compared to nutrition with ammonium. We suggest that while modeling or interpreting CO_2 efflux data from soil, and particularly when separating the estimation of individual CO_2 sources contributing to total soil CO_2 efflux, a serious consideration must be given to the form of mineral N in the soil and the location of the nitrate reduction sites. Nitrifiers depend on cytochrome systems for electron transport and ultimately on oxygen (Haynes 1986). Thus, in a well-aerated coarse and medium textured soil (sandy and loamy soils) or soils rich in organic-C, which also improves soil aeration, any N form will be quickly converted to nitrate; irrespectively of the type of N fertilizer applied, plants will absorb most of their N as nitrates. Lower diffusion of ammonium than nitrate in the bulk soil makes the latter also more accessible for plant uptake (Raven et al. 1992). Nitrate uptake by roots will be followed by an additional CO_2 evolution through root respiration due to the extra energy requirements for nitrate reduction and assimilation. The same plant species will respire much less CO_2 growing on fine-textured soils or water-saturated soils. Most of the mineral N will be taken up in the form of ammonium because anaerobic conditions, as well as low temperatures and low pH, do not favor the chemolithotrophic consumption of ammonium (Sprent 1987; Prosser 1989). In this case the rate of CO_2 efflux that originated from the root respiration will certainly be smaller. Singh and Kashyap (2007) demonstrated a marked seasonality in the population size of ammonium- and nitrite-oxidizers and nitrification rates, with maximum activity during the rainy season and minimum during the summer drought. Accordingly, under limited soil water conditions the nitrification

process will also be suppressed and nitrate reductase expenses would vary over the growing season with variation of soil water content. Raven et al. (1992) summarized that in many terrestrial communities nitrification is restricted and ammonium dominates as a N source; this is especially true for communities dominated by perennial plants, at high altitudes and latitudes.

Effect of growing stages on root respiration

The growth stages of corn differed in the plant ^{15}N content, and in its distribution between shoots and roots. The $^{14}CO_2$ respiration rates also varied between growth stages and different N treatments.

The $^{14}CO_2$ evolved per unit of root mass was greater at stage V8 for both types of N applied. The control treatment, however, showed no increment in root biomass or peak of respired $^{14}CO_2$. The main respiratory costs were associated with maintaining the existing plant material rather than growth of new plant structures (see Figs. 1, 3a and b). After fertilization of corn with N, respiration associated with ion uptake was appended to these basic maintenance costs. The transport of NO_3^-, NH_4^+, K^+, $H_2PO_4^-$, SO_4^{2-} and Cl^- into root cells was facilitated by carrier proteins or maintained through electrochemical gradients produced by pumping protons out of the cells. The active transport was driven by energy in the form of ATP, which is generated through respiration (Leonard 1984; Mengel and Kirkby 1982). This form of respiration is proportional to the one observed under NH_4^+ nutrition of plants (see Fig. 3a and b). The third form of respiration could be distinguished after fertilizing the plants with NO_3^-. Respiration associated with reduction of NO_3^- to NH_4^+ accounted for a significant part of total respiratory losses (Fig. 3a and b). Respiratory costs associated with ion uptake were greater at the V8 stage, although the quantity of N ions absorbed and recovered from plants was less than at stage V6. One explanation for this observation could be a general depletion of all ions in the soil solution with time. In that case, in the later growing stage a plant invests more in its uptake from the soil, in contrast to younger plants that still have a sufficient nutrient supply.

The main difference between two growing stages fell mainly on the respiration rate associated with ion uptake and nitrate reduction (Fig. 3a and b). At the V6 stage, a 2.6-fold increase in the respired recently assimilated $^{14}CO_2$ was observed under NO_3^--N versus NH_4^+-N. At the V8 stage the increase only amounted to 1.8 (Fig. 6). This indicates that root contribution to overall plant nitrate reduction may be especially important during the early phases of plant growth, followed by a reduction of nitrates in the roots with time. Note that the above calculations were done operating with a total amount of recovered ^{15}N. Plants biomass was harvested on the sixth day after fertilization. The distributions of ^{15}N between shoots and roots during that period vary as a result of upward and downward translocation of reduced N.

Pan et al. (1985) and MacKown et al. (1983) showed that root morphological characteristics may play an important role in influencing the location of the nitrate reduction site. Pan et al. (1985) reported a positive relationship between the percent of nitrates reduced in the roots and the number of lateral roots per unit mass. This indicates that the corn root tips play a role in maintaining a higher level of nitrate reductase activity than more mature roots sections. Hence, nitrate partitioning may differ along the root axis: the younger regions may reduce a higher proportion of incoming nitrates than the older ones. The difference in the root biomass between V6 and V8 corn in our experiment could be responsible for the observed difference in the quantity of nitrates reduced in the roots.

Note that some studies (Ashley et al. 1975; Breteler and Cate 1980; Talouizte et al. 1984; Gojon et al. 1986) have found that the root contribution to overall plant nitrate reduction may be important in the early phases of nitrate utilization, i.e. during the induction process that appears just after adding fertilizers and that probably lasts several days. Our experiment shows that the maximum difference in $^{14}CO_2$ respiration between the two types of N fell exactly on this phase—the first hours after ^{15}N addition and uptake. After the induction period ends, the contribution of roots to nitrate reduction generally decreases due to the following possible reasons: 1) the nitrate reductase level in the roots could be depressed by the supply of amino acids from the shoots; 2) the decrease in nitrate uptake after induction phase could be responsible for limiting the nitrate supply to the root nitrate reductase, particularly because of translocation, which could compete with N reduction in the roots (Rufty et al. 1981); 3) both nitrate and ammonium assimilation during the initial period could depress the carbohydrate content of the roots and further curtail the associated

nitrate uptake and reduction (Fiedler and Proksch 1975; Jackson et al. 1976). The above findings indicate that the observed difference in the quantity of respired CO_2 between two types of N could change after the initial phase of N uptake. Such a possibility could be verified in future experiments applying $^{14}CO_2$ pulse labeling techniques.

Conclusion

Various factors can influence the contribution of plants to CO_2 efflux from soil. Known contributing factors include plant species composition, growth stage and environmental factors such as intensity of photosynthetically active radiation and temperature. This study reveals another factor that radically influences the contribution of the autotrophic component of soil respiration to total CO_2 efflux. Pulse labeling of plants in a $^{14}CO_2$ atmosphere and application of 15N fertilizer allowed evaluating the effect of N form (nitrate or ammonium) on recently assimilated CO_2 efflux from the rhizosphere. Nitrate negatively affected the carbohydrate metabolism and energy economy of corn. In respect to ammonium, nitrate nutrition increased root-derived CO_2 efflux up to 2.6 times. However, the absence of the difference between the two components of root-derived $^{14}CO_2$ efflux—ion uptake respiration and nitrate reduction respiration—and lower increase in $^{14}CO_2$ efflux per unit of recovered N for nitrate over ammonium fed plants at the older growing stage could indicate that the root contribution to overall plant nitrate reduction process is not stable during plant ontogenesis. The contribution of roots could be more important during the early phases of plant growth, consequently reducing the C costs for nitrate reduction in more mature plants. All these factors should be taken into account when modeling and interpreting CO_2 efflux data from soil, particularly when separating the estimation of individual CO_2 sources that contribute to the total soil CO_2 efflux.

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