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Ammonium versus nitrate nutrition of *Zea mays* and *Lupinus albus*: Effect on root-derived CO_2 efflux

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ABSTRACT

Identification of the mechanisms contributing to nitrogen (N) fertilizer-induced changes in CO_2 efflux from soil under agricultural crops has been extremely challenging because of difficulties in separating root and microbial contribution to total CO_2 efflux. In this study we present the evidence that high costs of nitrate reduction result in a strong increase of root-derived respiration and the magnitude of an increase differs between the species with various contribution of shoots and roots to the nitrate reduction process.

Fertilization of *Lupinus albus* and *Zea mays* with nitrate or ammonium and pulse labeling of plants in $^{14}CO_2$ atmosphere allowed evaluation of the effect of N type on total and recently assimilated CO_2 efflux from soil. Addition of nitrate to planted soil increased recently assimilated CO_2 efflux by 168% in *Lupinus albus* (nitrate reduction site – in roots) and by 121% in *Zea mays* (nitrate reduction site both, in shoots and roots) in comparison with control. Ammonium-induced CO_2 increase amounted for 82% in *Lupinus albus* and for 73% in *Zea mays*. Clear diurnal changes in CO_2 efflux from planted soil at constant day/night temperature showed fast response of below-ground processes to photosynthesis. Both approaches for root-derived CO_2 assessment: ^{14}C pulse labeling and difference of CO_2 from planted and unplanted soil showed similar results: the form of N supply and the location of the nitrate reduction site have a strong significant effect on the amount of root-derived CO_2 respiration.

1. Introduction

The nitrogen (N) requirements of plants can be met by both nitrate (NO_3^-) and ammonium (NH_4^+) ion assimilation (Lasa et al., 2002). Utilization of nitrogen in either form may affect the carbohydrate metabolism and energy economy of the plant (Blacquiere, 1987). NO_3^- ions can be accumulated in vacuoles, and so most plant species can transport nitrates to leaves for reduction and assimilation and are able to tolerate high nitrate concentrations without any sign of toxicity. However, NH_4^+ salts absorbed by the plant must be rapidly metabolized into organic nitrogen compounds as many plants tolerate few or no excess ammonium ions (Barker et al., 1996; Chaillou et al., 1994). So almost all NH_4^+ ions are assimilated in roots. This difference in the site for N assimilation leads to a difference in the demand of carbon (C) skeletons, which are provided in part by the phosphorylating cytochrome (TCA) cycle, and hence to a difference in the respiration rate (Lasa et al., 2002).

However, there are still active debates on the effect of the N source on root respiration, as attempts to explain it experimentally have led to arguable results supporting different hypotheses. Some authors suggest that, when compared to NO_3^- nutrition, NH_4^+ nutrition stimulates the rate of root respiration, attributing this increase to the stimulation of alternative pathway activity (Barneix et al., 1984; Blacquiere, 1987; Lasa et al., 2002). There are two pathways involved in respiration: the phosphorylating cytochrome and the non-phosphorylating alternative pathway. The physiological role of the latter is not clear but several authors suggest that this alternative pathway could avoid the overreduction of the electron transport chain and the subsequent production of reactive oxygen species (Purvis and Shewfelt, 1993). Thus, this pathway could allow oxidation of TCA cycle reductant, maintaining TCA cycle carbon flow for provision of biosynthetic intermediates for NH_4^+ ion assimilation.

On the other hand, NO_3^- coming to the plant before assimilation have to be firstly reduced to NH_4^+, and this process, together with assimilation, is among the most energy-intensive processes in plants, in some cases followed by an additional CO_2 evolution (Atkins et al., 1979; Aslam and Huffacker, 1982; Ninomiya and Sato, 1984; Warner and Kleinhofs, 1992; Blacquiere, 1987; Tischner, 2000). The process proceeds in two steppes: conversion of NO_3^- to NO_2^- and the following conversion of NO_2^- to NH_4^+. In illuminated

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leaves, these processes are coupled to photosynthetic electron transport. However, in roots and during darkness, reducing equivalents are generated by oxidation of carbohydrates with subsequent evolution of CO_2 (Aslam and Huffacker, 1982; Ninomiya and Sato, 1984).

Depending on the species, the site of NO_3^- reduction could be located in shoots or roots (Andrews, 1986; Oaks and Hirel, 1985; Pate and Layzell, 1990; Schilling et al., 2006; Silveira et al., 2001; Vuylsteker et al., 1997). By this property, plants are divided into three groups: species reducing NO_3^- predominantly in roots, species reducing NO_3^- predominantly in shoots, and those that do both. The C costs for reduction of NO_3^- to NH_4^+ depend on the site of nitrate reduction in plants.

In this study we use the term "root-derived CO_2" for the sum of actual root respiration and CO_2 derived from microbial activity in the immediate vicinity of the root (rhizomicrobial respiration) and "SOM-derived respiration" for CO_2 evolved after microbial decomposition of soil organic matter in root free soil. We selected maize and lupine since the two species have different sites of nitrate reduction: *Zea mays* reduces half of the NO_3^- in shoots and half in roots and *Lupinus albus* reduces the major part of the NO_3^- in roots (Pate, 1973). The objective of the present work was to confirm or refute that feeding lupine and maize with NH_4^+ reduces root-derived efflux from soil compared to feeding with NO_3^-. Three nitrogen treatments were applied to each species: nitrate fertilizer, ammonium fertilizer, and a control treatment without any N fertilizer. A nitrification inhibitor was used to prevent microbial conversion of NH_4^+ to NO_3^- in soil. Pulse labeling of plants in a $^{14}CO_2$ atmosphere was applied to quantify the effect of both fertilizers on recently (^{14}C) and total assimilated C. The difference between total CO_2 efflux from the plant-soil system and microbial respiration from bare soil incubated at the same conditions was compared with the results of the principal method of labeling for root-derived CO_2 quantification.

2. Materials and methods

2.1. Soil

The soil, a loamy Haplic Luvisol, was taken from the top 10 cm (Ap horizon) of the Karlshof long-term field experimental station of the University of Hohenheim. Soil samples were air dried, mixed and passed through 5 mm sieve. The soil contained 1.5% C_{tot} and 0.14% N_{tot}, with 2.9% sand, 74.5% silt and 22.6% clay; its pH was 6.5.

2.2. Plants and growth conditions

Centrifuge tubes of 50 ml were filled with 50 g of soil each and were used for growing the plants. Twenty four pots remained unplanted to measure microbial respiration from bare soil.

Seeds of maize (*Zea mays* L.) and lupine (*Lupinus albus* L.) were germinated on moist filter paper in Petri dishes for 2 days. Germinated seedlings were transplanted to the PVC pots, with one seedling per pot, and were grown under controlled laboratory conditions with a 12 h/12 h day/night period at a constant day and night temperature of $25 \pm 0.5\,^{\circ}C$, and with a photosynthetically active radiation (PAR) intensity of approximately 800 $\mu mol\ m^{-1}\ s^{-1}$ 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. During the experiment, soil water content in each pot was maintained gravimetrically at about 60% of the available field capacity by checking its weight daily. Before the labeling, the weakest plants were eliminated and only twenty-four plants similar in development and height were chosen for the following treatments. Pots with bare soil were exposed to the same incubating conditions.

2.3. ^{14}C labeling and N application

Two species were labeled with ^{14}C: 12 plants of maize were chosen and labeled in the morning on the 20th day after germination; 12 plants of lupine were labeled on the 36th day after germination.

One day before the labeling, the top of each pot was sealed with a silicone paste (NG 3170 from Thauer and Co., Dresden, Germany). The seal was tested for air leaks. Pumping the air through the soil column flushed out the CO_2 accumulated in the soil during the plant's growth.

Three nitrogen treatments were applied 4 h before ^{14}C labeling: (a) a nitrate treatment, with ^{15}N as $K^{15}NO_3$; (b) an ammonium treatment, with ^{15}N as $(^{15}NH_4)_2SO_4$; and (c) a control variant without any added nitrogen. Four plants of each species were exposed to each N treatment (^{15}N enrichment 50 atom %). Dicyandiamide (DCD) at 20 mg kg^{-1} soil was applied in solution with ^{15}N fertilizer to all the 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). The amount of ^{15}N applied to a pot was calculated to produce an average concentration of 60 mg of N kg^{-1} for each N species added. Four unplanted pots were fertilized with half amount of nitrate or ammonium to estimate the effect of N fertilization on respiration of soil microorganisms.

The ^{14}C labeling process has been described in detail by Kuzyakov et al. (1999) and Kuzyakov and Cheng (2001) and Domanski et al. (2001). Briefly, sealed pots with plants were put in a plexiglas chamber, $^{14}CO_2$ was introduced to 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, trapping of CO_2 from the chamber through 10 mL of 1 M NaOH solution was started to remove the remaining unassimilated $^{14}CO_2$. Then the chamber was opened. Pots with the plants were connected to an output of membrane pumps by tubes: air was pumped through every single pot from bottom to top. Another tube was connecting each pot 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-soil system to the trapping solution to the membrane pump and back to the plant-soil system.

2.4. Sampling and analyses

NaOH in the trapping tubes was changed for the first time 6 h after the labeling and then twice a day, in the morning and in the evening, for 6 days after the labeling, with the aim of collecting CO_2 evolved in the rhizosphere during day- and night-periods. NaOH traps were analyzed for total carbonate content and for ^{14}C activity.

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). Total assimilated $^{14}CO_2$ was determined as a difference between the $^{14}CO_2$ added to the labeling chamber and the $^{14}CO_2$ recovered from the solution with the remaining unassimilated $^{14}CO_2$.

To estimate total CO_2 efflux from the soil, CO_2 trapped in NaOH solution was precipitated with a 0.5 M barium chloride ($BaCl_2$) solution and then NaOH was titrated with 0.1 M hydrochloric acid (HCl) against phenolphthalein indicator (Zibilske, 1994).

On the 6th 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