Availability of different nitrogen forms changes the microbial communities and enzyme activities in the rhizosphere of maize lines with different nitrogen use efficiency

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ABSTRACT

We studied how the Lo5 and T250 maize lines, characterized by high and low nitrogen use efficiency (NUE), respectively, modified the microbial biomass, enzymatic activities and microbial community structure in the rhizosphere after exposure to different N forms. The two maize lines were grown for 4 weeks in rhizoboxes allowing precise sampling of rhizosphere and bulk soil with no nutrient additions, and then exposed to with nitric-, ammonium- and urea-N. After N exposure, the plants were inserted back into their original rhizoboxes to allow the root exudates diffusion into the rhizosphere. After 24 h rhizosphere soil were sampled and analyzed. Microbial biomass and soil enzymatic activities were increased after the exposure to different N forms of both maize lines. The plant exposure to different N forms also induced changes in the rhizosphere bacterial and fungal communities composition. Plant responses to the availability of different N forms was a dominant factor regulating activity and composition of the rhizosphere microbial communities, likely due to changes in the rhizodepositions. Therefore different N forms used for fertilization of agriculturally relevant plants such as maize can result in different plant mediated effects on the microbial activity and community structure in the rhizosphere.

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1. Introduction

The rhizosphere, the thin soil layer profoundly modified by plant roots, hosts a larger and more active microbial communities as compared to the bulk soil, sustained by rhizodepositions which include both low molecular weight organic compounds (LMWOCs) such as carboxylic acids, sugars (Hawes et al., 2003) and more complex chemical molecules such as polyphenols (Tomasi et al., 2008), which account for a significant proportion of C fixed by photosynthesis (Uren 2007). Rhizodepositions create favorable conditions for root tip elongation and plant exploitation of soil resources, and they can vary depending on biotic and abiotic soil factors such as soil texture and soil moisture level (Neumann et al., 2009), presence of symbiotic or pathogenic microorganisms (Vivanco and Baluska 2012), presence of toxic compounds (Uren 2007) and nutrient availability (Nguyen, 2003).

It is well-established that different plant species can select different microbial communities in the rhizosphere, also depending on the plant growth stage and season (Berg and Smalla 2009). For example, it has been reported that α-Proteobacteria can be more abundant in the rhizosphere than in bulk soil (McCaig et al., 1998) whereas actinomycetes and γ-proteobacteria can be more abundant in bulk than rhizosphere soil (Heuer et al., 1997; Ulrich et al., 2008). Usually, studies on the activity and diversity of microbial community and biochemical activity in the rhizosphere have been carried out with plants under metabolically resting conditions, i.e. in the absence of specific stimulations, or using systems mimicking the root exudate release from model root surfaces (Baudoin et al., 2003; Renella et al., 2007). Despite it is nowadays possible to determine unculturable microorganisms in rhizosphere and bulk soil, changes in the activity, biomass and structure of the microbial communities in response to nutrient availability in the rhizosphere are still poorly understood. A reliable approach to analyze the active microbial population in complex and dynamic environment as the rhizosphere relies on the simultaneous characterization of both DNA and RNA profiles

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and the comparison of 16S rRNA fingerprint profiles (Griffiths et al., 2000). This approach allows to achieve information on total microbial community structure (DNA fingerprinting) and on active microbial population (RNA-fingerprinting) (Griffiths et al., 2000; Saleh-Lakha et al., 2005).

Nitrogen is the main nutrient limiting plant growth and crop yield (Raun and Gohnson 1999), due to both the inherent plant N use efficiency (NUE), N losses by leaching, run off and volatilization, and to microbial N immobilization in the rhizosphere. While progress on the understanding of the plants mechanisms responsible for NUE have been made using model (Xu et al., 2012) and agriculturally relevant plants (Zamboni et al., 2014), limited information on the changes of microbial community composition, microbial and biochemical activity in the rhizosphere upon increase of N availability are available. For example it is known that several plant species can influence the N availability in soil by releasing microbial nitrification inhibitors in the root exudates (Subbarao et al., 2012). Recently, Zamboni et al. (2014) studied the genetic responses of maize lines Lo5 and T250 characterized by high and low NUE, respectively, and reported that exposure to NO_3^-–N induced a larger genetic response in the high NUE Lo5 (6.2×10^3 transcripts) than in the low NUE T250 (2.0×10^3 transcripts) maize line, with only 368 transcripts shared by these two maize lines. These maize lines have been shown to deplete inorganic N in the rhizosphere with different trends, and presented differences in the microbial communities and enzymatic activities in the rhizosphere (Pathan et al., 2015). Because such genome wide responses were not only limited to genes related to N absorption and organication, but also involved genes responsible for the synthesis of sugars, proteins, secondary metabolites and co-factors, it was reasonable to hypothesize that the N exposure for plant roots resulted in differences in the root exudate profiles of these two plants. Therefore, we hypothesized that different N forms exposure for maize plant should result in different plant responses which could in turn cause short term responses of activity, biomass and structure of microbial communities in the rhizosphere. Moreover, because the plant genetic response was greater in the higher NUE Lo5 than in the low NUE T250 maize line (Zamboni et al., 2014) we also hypothesized that larger microbial stimulation should occur in the rhizosphere of plants with higher NUE and that could also depend on the N form used to induce the plants. To test our hypothesis, we used two maize lines with different NUE, grown in rhizoboxes. In our work we induced the plants with NO_3^-–N, NH_4^+–N and urea, and evaluated the changes in the bacterial and fungal communities composition and enzyme activities of rhizosphere, following the plant responses to the availability of different N forms. Our methodological approach aimed at understating how the plant responses to the various N forms, could influence the enzymatic activities and microbial communities in the rhizosphere.

2. Materials and methods

2.1. Soil, plants, rhizobox set up and soil sampling

The sandy clay loam soil, classified as a Eutric Cambisol (FAO), was sampled from the Ap horizon (0–25 cm) from an experimental farm under conventional maize crop regime located at Cesa (Tuscany, Central Italy). The soil had the following properties: 32.1% sand, 42.2% silt, 25.7% clay, $10.8 \, g \, kg^{-1}$ total organic C (TOC), $1.12 \, g \, kg^{-1}$ total N and $6.45 \, g \, kg^{-1}$ total P. The soil was sieved at field moisture (<2 mm), after removing visible plant material and immediately used for filling the two soil compartments of the rhizoboxes before the plantlet insertion in the plant compartment.

The plants used for the experiments were the maize (*Zea mays* L.) inbred lines Lo5 and T250 with high and low NUE, respectively

(Balconi et al., 1997; Zamboni et al., 2014). Maize seeds were germinated in a closed chamber at 25 °C, and the seedlings were inserted into the plant compartment of the rhizoboxes, enclosed by $0.22 \, \mu m$ mesh nylon tissue, avoiding the soil passing into in the plant compartment. Then plants were grown in climatic chambers with 16:8 light/dark period, $200 \, \mu E \, m^{-2} \, s^{-1}$ light intensity and of $22/25 \,^\circ C$ for the dark and light periods, and a relative humidity of 70%. Twelve rhizoboxes were prepared for each of the two maize lines. Plants were manually watered with distilled H_2O to avoid nutrient additions and the Lo5 and T250 maize lines were grown for 21 and 28 d, respectively, the growth periods allowing the full colonization of the plant compartment by plant roots without nutrient starvation. The N content of the soil solution was monitored by Rhizon® probes into the soil in contact with the plant compartment; this measurement allowed to evaluate the maize plants NUE and to prevent plant starvation. At the end of the growth period (t0), three out of twelve rhizoboxes for each plants were destructively sampled for immediate analysis of chemical properties, microbial biomass, enzyme activities, and microbial community structure of the rhizosphere and bulk soil. The used rhizoboxes allowed precise sampling of the 0–2 mm soil layer adhering to the plant compartment, considered as the rhizosphere, whereas the soil at distance greater than 22 mm was considered as bulk soil.

For testing the responses to plant metabolic effects with different N forms, the plant compartment of each maize line were taken out from the rhizoboxes and immersed into sterile glass beakers containing sterile solutions of 0.1 M NH_4SO_4, 0.1 M KNO_3 or 0.1 M urea, and $200 \, \mu E \, m^{-2} \, s^{-1}$ light intensity, for 4 and 8 h for the LO5 and T250 maize lines, respectively, prepared in three replicates from each N form. After the N exposure, the plant compartments were thoroughly washed with sterile deionized H_2O and inserted back into their original rhizoboxes, by ensuring the full contact with soil. Under the adopted experimental conditions the urea hydrolysis could be considered as negligible, and the plants were mainly absorbing intact urea. The rhizoboxes with the N induced plants were incubated for 24 h in climatic chambers under the same light, radiation, temperature and humidity conditions described above. After 24 h both rhizosphere was sampled as previously described. Since preliminary experiments showed that microbial biomass, enzymatic activities and microbial community structure of the bulk soil were not influenced after the N forms exposure, only the bulk soil of plants exposed to H_2O (t0) and sampled after 24 h after their reinsertion into the rhizoboxes were showed in the present paper.

2.2. Analysis of soil microbial biomass and enzyme activities of the rhizosphere and bulk soil

The NH_4^+–N and NO_3^-–N concentrations of the rhizosphere solution were analyzed by ion selective electrodes (Crison) on soil solution extracted using the Rhizon® probes.

Soil microbial biomass was determined by the ATP content according to Ciardi and Nannipieri (1990). Arylesterase activity was determined as described by Zornoza et al. (2009). Acid and alkaline phosphomonoesterase activities were assayed according to Tabatabai and Bremner (1969), and phosphodiesterase activity as reported by Browman and Tabatabai (1978). β-glucosidase activity was assayed according to Tabatabai (1982). All hydrolase activities were determined at 37 °C for 1 h, followed by centrifugation at 6000 g at 4 °C, and quantification of the p-nitrophenol (p-NP) released by the enzyme activities determined at by spectrophotometry at 400 nm wavelength (Lambda 2, Perkin-nElmer). Urease activity was determined according to Nannipieri et al. (1974); the released NH_4^+–N was extracted with 1 M KCl and quantified at 660 nm after reaction with the Nessler reagent. The