Contribution of exudates, arbuscular mycorrhizal fungi and litter depositions to the rhizosphere priming effect induced by grassland species

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ABSTRACT

The presence of plants induces strong accelerations in soil organic matter (SOM) mineralization by stimulating soil microbial activity — a phenomenon known as the rhizosphere priming effect (RPE). The RPE could be induced by several mechanisms including root exudates, arbuscular mycorrhizal fungi (AMF) and root litter. However the contribution of each of these to rhizosphere priming is unknown due to the complexity involved in studying rhizospheric processes. In order to determine the role of each of these mechanisms, we incubated soils enclosed in nylon meshes that were permeable to exudates, or exudates & AMF or exudates, AMF and roots under three grassland plant species grown on sand. Plants were continuously labeled with ^{13}C depleted CO_2 that allowed distinguishing plant-derived CO_2 from soil-derived CO_2. We show that root exudation was the main way by which plants induced RPE (58–96% of total RPE) followed by root litter. AMF did not contribute to rhizosphere priming under the two species that were significantly colonized by them i.e. *Poa trivialis* and *Trifolium repens*. Root exudates and root litter differed with respect to their mechanism of inducing RPE. Exudates induced RPE without increasing microbial biomass whereas root litter increased microbial biomass and raised the RPE mediating saprophytic fungi. The RPE efficiency (RPE/unit plant-C assimilated into microbes) was 3–7 times higher for exudates than for root litter. This efficiency of exudates is explained by a microbial allocation of fresh carbon to mineralization activity rather than to growth. These results suggest that root exudation is the main way by which plants stimulated mineralization of soil organic matter. Moreover, the plants through their exudates not only provide energy to soil microorganisms but also seem to control the way the energy is used in order to maximize soil organic matter mineralization and drive their own nutrient supply.

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1. Introduction

Rhizosphere processes contribute almost half of the total CO_2 emissions from the terrestrial ecosystems to the atmosphere at global level (Schimel, 1995). They are also suggested to play a significant role in mediating ecosystem feedbacks to climate change through their effects on net primary productivity, organic matter decomposition, nutrient cycling and carbon (C) storage (Grayston et al., 1997; Kaiser et al., 2010; Cheng et al., 2013). This has led to studies to better understand the magnitude, controls and direction

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of rhizosphere processes over soil organic matter (SOM) dynamics (Dijkstra et al., 2006a,b; Dijkstra and Cheng, 2007; Cheng et al., 2013; Dijkstra et al., 2013; Drake et al., 2013). Despite this increased interest, there are still many uncertainties regarding which processes strongly accelerate SOM turnover in the rhizosphere thereby making the predictions of soils' feedback to climate change contradictory (Davidson and Janssens, 2006).

Rhizodeposition, i.e. the release of root exudates, mucilage and sloughed-off root border cells, constitute significant inputs of labile carbon into soil (Paterson, 2003; Nguyen, 2009). Overall, up to 20% of the net C fixed by plants is released into soil in the form of rhizodeposition during vegetative period (Hütsch et al., 2002). The main component of rhizodeposition is root exudates through which 10–100 times more carbon is released than mucilage and sloughed-off border cells (Nguyen, 2009). The exudates have been found to play important role in soil ecology and plant nutrition. For example, they have been found to enhance mycorrhizal fungal growth (Ratnayake et al., 1978; Elias and Safir, 1987; Tawaraya et al., 1996) thereby helping the plant to explore larger volumes of soil in search of nutrients. Moreover, organic acids present in exudates, help solubilize the insoluble phosphorus (P) in rhizosphere (Moghimi et al., 1978; Lipton et al., 1987; Saleque and Kirk, 1995). This knowledge has even led to the development of novel plant varieties more efficient in using soil P through genetically-engineered enhanced release of organic acids by roots (López-Bucio et al., 2000). Finally exudates have also been suggested to increase the SOM turnover by promoting the microbial activity in the rhizosphere (Hamilton and Frank, 2001; Phillips et al., 2011) although the direct evidence is still lacking (Jones et al., 2004).

The mineralization of SOM is accelerated under living plants when compared to unplanted controls due to stimulation of soil microbes (Helal and Sauerbeck, 1984; Liljeroth et al., 1994; Kuzyakov and Cheng, 2001) — a phenomenon known as the rhizosphere priming effect (RPE). It has been suggested that exudates from roots and root-associated mycorrhizae provide energy-rich substrates to rhizosphere microbes thereby enabling them to secrete extracellular enzymes responsible for the accelerated SOM decomposition (Clarholm, 1985; Hamilton and Frank, 2001). However, lab incubations testing the effect of various components of exudates on SOM mineralization have reported contradictory results. Briefly, the additions of isotopically labeled sugars, amino acids and organic acids induced positive or negative priming effects (Hamer and Marschner, 2005a, 2005b; Blagodatskaya et al., 2007; Ohm et al., 2007) or did not have any effect even if the microbial activity was stimulated (De Nobili et al. 2001; Salomé et al. 2010). This lack of effect is explained by r-strategist microorganisms that only use these easily degradable substrates (Fontaine et al., 2003; Blagodatskaya et al., 2007; Ohm et al., 2007). Despite their association with roots of about 80% of terrestrial plant species, the role of arbuscular mycorrhizal fungi (AMF) in the RPE remains unknown. The catabolic capabilities of root-associated AMF are generally considered low compared to those of soil decomposers (Read and Perez-Moreno, 2003; Talbot et al., 2008), suggesting they probably play a minor role in rhizosphere priming. However, they have been found to participate in the degradation of plant litter (Hodge et al., 2001; Leigh et al., 2009; Cheng et al., 2012), suggesting that their catabolic capability has been underestimated. Finally, the RPE induced by plants may arise from the supply of litter to soil decomposers since this type of organic matter systematically induces the priming effect in incubation studies (Nottingham et al. 2009; Pascault et al. 2013). Knowledge of the contribution of exudates, AMF and root litter depositions to RPE is fundamental to predicting plant effects on soil C cycling under changing climates.

Plant species differ in terms of total labile C inputs through rhizodeposition into the soil thereby inducing varying stimulation of soil microorganisms and RPE (Dijkstra et al., 2006a,b). Moreover, a specific plant species can shape a specific structure of microbial community (Grayston et al., 1997; Germida et al., 1998; Broeckling et al., 2008) by controlling the quality and quantity of rhizodeposition into the rhizosphere (Grayston et al., 1997; Broeckling et al., 2008). Therefore it is important to study the mechanisms of rhizosphere priming under different plant species and linking the variation in RPE with the soil microbial community structure shaped by a certain plant species.

The aim of this study was to determine the role of exudates and AMF in rhizosphere priming, the relative importance of exudates, AMF and roots in determining the RPE and mechanisms by which each of these induce changes in the RPE. The effect of exudates on SOM dynamics was disentangled from that of mycorrhizae and root-litter deposition by using meshes of different pore sizes under monocultures of three grassland species namely Lolium perenne (Lp), Poa trivialis (Pt) and Trifolium repens (Tr). Continuous ^{13}C labeling of plants was used to distinguish soil-derived (R_s) and plant-derived respiration (R_p). The rhizosphere priming effect was calculated as the difference between R_s from planted soils and from control bare soil. We hypothesized that exudates and root litter would induce strong priming effects by favoring the growth of microbes and AMF would not have any positive effect on RPE.

2. Materials and methods

2.1. Soil sampling and conditioning

The soil was sampled from an upland grassland located in the environmental research observatory (ORE) established by French National Institute for Agricultural Research (INRA) in central France in 2003 (Theix, 45° 43′N, 03°01′E). The soil is a drained Cambisol developed from a granitic rock. The soil was taken from 10 to 40 cm soil profile. The upper 10 cm that is rich in fresh C was removed given that respiration of this pre-existing unlabeled fresh C cannot be separated from that of recalcitrant SOM. Moreover the presence of plants can modify the decomposition of fresh C (Personeni and Loiseau, 2004). The soil properties were: pH 6.3 ± 0.23, clay $21 \pm 2.1\%$, soil organic carbon (SOC) 17 ± 0.28 g kg^{-1} soil and SOC δ^{13}C -26.4 ± 0.02‰. This soil was enclosed in small PVC cylinders (height 1.5 cm, diameter 5 cm) whose sides were sealed by the mesh of three different pore sizes. The pore size 0.45 μm was meant to only allow the entry of exudates excluding the mycorrhizae and roots in a living rhizosphere (exudates treatment). Whereas, the pore sizes 30 and 1000 μm would also permit the entry of mycorrhizae and roots (mycorrhizae & roots) respectively. From now on, these soil-containing cylinders will be called soil compartments.

2.2. Establishment of monocultures

Three grassland species i.e. L. perenne (Lp), P. trivialis (Pt) and T. repens (Tr), that were previously found to induce variable priming effects (Shahzad et al., 2012), were selected for this experiment. PVC pots (20 cm high, 7.8 cm internal diameter) were filled with sand (pH 7) whereas four soil compartments each containing about 15 g of equivalent dry soil were placed vertically in each pot (Fig. 1). In August 2010, twelve pots were sown by each of the three plant species representing four replicates for each pore size of mesh (0.45, 30, 1000 μm). Four pots containing soil compartments (1000 μm mesh) were kept bare as control soil. An automated drip irrigation method was used for water supply and all pots were water-saturated whenever the soil moisture decreased to $75 \pm 5\%$ of the soil field capacity. The near-field capacity conditions were maintained to avoid the artifact of drying-rewetting cycles that may increase CO_2 release from soil (Schimel et al., 2011).