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Root penetration in deep soil layers stimulates mineralization of millennia-old organic carbon

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ABSTRACT

Climate and land-use changes modify plant rooting depth, signifying that organic matter with long residence times in deep soil layers can be exposed to rhizospheres and associated microbial activities. The presence of roots in soils stimulates mineralization of native soil C, via a process termed the rhizosphere priming effect (RPE), which may in consequence lead to loss of soil C. By growing a deep rooting grass, *Festuca arundinacea*, on soil columns and under continuous dual labelling (^{13}C- & ^{14}C-CO_2), we show that root penetration up to 80 cm into a soil profile stimulated mineralization of ~15,000 year-old soil C. The RPE, after normalization with root biomass, was similar along the soil profile indicating that deep C is as vulnerable to priming as surface C. The RPE was strongly correlated with respiration of plant-derived C, and a PLFA marker representative of saprophytic fungi (18:2ω6c) across all soil layers. Moreover, experimental disruption of soil structure further stimulated soil C mineralization. These findings suggest that the slow soil C mineralization in deep layers results from an impoverishment of energy-rich plant C for microorganisms (especially for saprophytic fungi), combined with a physical disconnection between soil C and microorganisms. Based on our results, we anticipate higher mineralization rates of deep millennia-old SOM in response to deeper root penetration which could be induced by changes in agricultural practices and climate.

1. Introduction

During the last decade, the organic carbon stored in deep (> 20 cm depth) soil layers has received increased attention of the scientists concerned with soil feedbacks to climate change as well as the mitigation of global warming (Fontaine et al., 2007; Rumpel and Kögel-Knabner, 2011; Marin-Spiotta et al., 2014; Medlyn et al., 2015; Kaneez-e-Batool et al., 2016). More than 50% of the 2344 Gt C, stored as organic C in soils, is located below 20 cm (Batjes, 1996; Jobbágy and Jackson, 2000). This large pool of deep C is capable of massively altering the global C cycle and climate if its mineralization by soil microorganisms is stimulated in response to global changes. Moreover, ^{14}C dating of soil C has shown that the deep C is often thousands of years old whereas the surface C turns over in decades (Torn et al., 1997; Trumbore, 2000; Jenkinson et al., 2008). This finding has led to many investigations to understand the origin of deep C persistence and to define the conditions of a *quasi*-permanent storage of carbon in soils (Fontaine et al., 2007; Salomé et al., 2010). The applied perspective of these investigations relates to developing technologies to mitigate the rising atmospheric CO_2 and consequent global warming by sequestering C in stable compartments. For example, it has been proposed to use and breed plant species with deep roots to fix atmospheric CO_2 and

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sequester organic carbon in deep soil layers (Hurd, 1974; Lorenz and Lal, 2005; Carter and Gregorich, 2010; Iversen, 2010; Kell, 2011).

The presence of roots in soils stimulates microbial mineralization of native soil C, a process termed the rhizosphere priming effect (RPE) (Kuzyakov et al., 2000; Dijkstra and Cheng, 2007; Cheng et al., 2014). Several processes can contribute to the RPE but the most frequently suggested are co-metabolism and N mining (Fontaine et al., 2003; Kuzyakov, 2010; Allison et al., 2014; Chen et al., 2014). In co-metabolism, the enzymes released by microorganisms during decomposition of plant C (plant litter and rhizodeposits) accidentally interact with native soil organic C accelerating its mineralization. According to mining theory, microorganisms growing on plant C, secrete enzymes decomposing soil organic matter to acquire nutrients. More recently, Keiluweit et al. (2015) suggested that some root exudates can also stimulate carbon loss by liberating organic compounds associated with minerals. The RPE is a major determinant of SOC turnover in surface soils. Increasing the rate of SOC mineralization by up to 400% (Finzi et al., 2015; Shahzad et al., 2015), the RPE can lead to a negative soil C balance in certain conditions (e.g. N poor soils), that is, C input to soil may decrease soil C content (Fontaine et al., 2004a; Dijkstra and Cheng, 2007). Deep C is less exposed to RPE than the surface soil C since most plant roots are concentrated in the surface layer (Fontaine et al., 2007). However, climate and land use changes such as the use of deep ploughing and drought-resistant deep-rooting crop species are modifying the plant rooting depths (Lorenz and Lal, 2005; Schenk and Jackson, 2005; Kell, 2011; Lorenz et al., 2011). Some previously protected deep soil layers are exposed to plant rhizosphere and its cohort of microbial activities possibly leading to RPE. However, the effect of plant rhizosphere on mineralization of deep soil C is unknown.

Diverse mechanisms have been proposed to explain the persistence of deep soil C. Historically, deep C has been viewed as functionally inert presumably because of its protection from microbial decomposition through organo-mineral associations or its existence as recalcitrant chemical structures (Torn et al., 1997; von Lützow et al., 2006). However, resistant chemical structures can persist at decadal timescales only (Kleber et al., 2011). The increasing importance of organo-mineral associations, where organic compounds are associated with reactive mineral phases, in stabilizing SOC down the profile has been demonstrated for a range of soils (Torn et al., 1997; Rumpel and Kögel-Knabner, 2011). However, for certain soils organo-mineral associations do not explain deep soil C persistence (Fontaine et al., 2007). Ewing et al. (2006) proposed that lack of physical disturbance in deep layers retards aggregates from breaking thereby promoting the persistence of deep C since the aggregates deny microorganisms the access to C substrates. Moreover, because most soil C compounds are located in pores inaccessible to microorganisms, it has been proposed that conservation of soil structure induces a spatial disconnection between soil C and microbes thereby slowing down mineralization process (Salomé et al., 2010; Schimel et al., 2011; Dungait et al., 2012). For the sake of simplicity, spatial disconnection and protection of soil C by aggregates will be referred to as “physical protection” in this study. Fontaine et al. (2007) attributed the persistence of deep C to a lack of easily decomposable organic compounds owing to limited roots that supply soil microbes with essential sources of energy (rhizodeposits, litter etc.) in top soil layers, a process named “energy limitation”. In their soil incubation, the mineralization of millennia-old deep C could only operate in the presence of cellulose suggesting that the old C was not an energetically profitable source of C for microorganisms. Different studies, while discussing the origin of deep C persistence, have often considered the processes of physical protection and energy limitation antagonistic at least unrelated (Salomé et al., 2010; Schmidt et al., 2011; Dungait et al., 2012).

The main objective of this study was to determine whether root penetration and exudation in deep soil layers can stimulate mineralization of millennia-old organic carbon, that is, induce a RPE. Moreover, to clarify the processes controlling the persistence of deep soil C, we tested whether energy limitation and physical protection mechanisms together can explain this persistence. The experimental approach consisted of three phases. First, a deep rooting plant species, *Festuca arundinacea,* was sown on undisturbed soil columns after removing the upper 0–10 cm soil layer to favour a deeper root penetration in soil. The grass was grown for 511 days under an atmosphere containing dual-labelled CO_2 (^{13}C & ^{14}C) allowing for the quantification of overall plant activity and the quantification of the RPE. During the second phase, soil columns were sliced in three independent soil layers (surface, intermediate and deep layers) that were subsequently incubated for 7 days. The RPE and ^{14}C age of primed soil C were determined for each soil layer during this incubation. During the third and last phase, soil structure of each layer was disrupted followed by incubation of these disrupted soils. The release of CO_2 induced by soil disturbance was quantified over 79 days.

2. Materials & methods

2.1. Soil sampling

Soil was sampled from a temperate upland grassland located in the environmental research observatory (SOERE) established by the French National Institute for Agricultural Research (INRA) in central France in 2003 (Theix, 45°43′N, 03°01′E). The local climate is semi-continental, with a mean annual temperature of 9 °C and an average annual rainfall of 760 mm. The site has been under grassland for more than 60 years. The soil is a drained Cambisol of 1 m depth. It is a silty, isohumic soil developed from volcano-granitic colluviums with little developed horizons. Moreover, the soil is hydromorphic with moderate permeability in surface. In March 2009, ten intact soil columns of ~9.8 cm diameter were taken within 1 m distance to each other from 0-80 cm depth. The upper 0–10 cm were removed before inserting the core in the PVC tube to remove the existing plants and a large proportion of their fresh litter, which would have confounded the effect of roots' presence on soil C. A percussion core drill equipped with a steel tube that can be opened from sideways was used to extract the soil columns when the soil was relatively wet (soil moisture ~ 30% dry weight; water potential ~ −100 kPa). This method allowed to recuperate entire soil columns and transfer them in PVC tubes (80 cm long, 9.8 cm internal diameter) while preserving the structure of soil columns. The plant experiment was established on these intact soil columns representing 10–80 cm of the soil profile. Two of the ten sampled columns were not transferred in PVC tubes and were cut horizontally in 10–33 cm, 33–56 cm and 56–80 cm layers for determining initial pH, soil SOC contents and isotopic composition ($^{13}C/^{12}C$ ratio). A more detailed description of soil properties and chemical composition of SOC along soil profile of the study site can be found in (Fontaine et al., 2007).

2.2. Experiment

2.2.1. Soil moisture at field capacity

All tubes containing the soil columns were irrigated until the soil columns were water saturated. The soil tubes were then weighed after 48 h of water percolation to determine soil moisture at field capacity. This estimation was used to keep the soil moisture between 75 and 100% of soil field capacity throughout the experiment using an automated drip irrigation method.

2.2.2. Plant establishment and labelling

The experiment was established in the fields of INRA, Clermont Ferrand, France. Four of these tubes containing intact soil columns were sown with *Festuca arundinacea* Schreb. at a density of 2000 seeds m^{-2} and four were kept bare as controls. *F. arundinacea* has one of the deepest roots among plant species commonly found in temperate permanent grasslands (Picon-Cochard et al., 2011; Pagès and Picon-Cochard, 2014). Its roots can easily go to 80 cm of soil depth with 95%