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Effects of soil type and composition of rhizodeposits on rhizosphere priming phenomena

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ABSTRACT

Inputs of fresh plant-derived C may stimulate microbially-mediated turnover of soil organic matter (SOM) in the rhizosphere. But studies of such 'priming' effects in artificial systems often produce conflicting results, depending on such variables as rates of substrate addition, substrate composition, whether pure compounds or mixtures of substrates are used, and whether the addition is pulsed or continuous. Studies in planted systems are less common, but also produce apparently conflicting results, and the mechanisms of these effects are poorly understood.

To add to the evidence on these matters, we grew a C4 grass for 61 d in two contrasting soils – an acid sandy soil and a more fertile clay-loam – which had previously only supported C3 vegetation. We measured total soil respiration and its C isotope composition, and used the latter to partition the respiration between plant- and soil-C sources. We found SOM turnover was enhanced (i.e. positive priming) by plant growth in both soils. In treatments in which the grass was clipped, net growth was greatly diminished, and priming effects were correspondingly weak. In treatments without clipping, net plant growth, total soil respiration and SOM-derived respiration were all much greater. Further, SOM-derived respiration increased over time in parallel with increases in plant growth, but the increase was delayed in the less fertile soil. We conclude the observed priming effects were driven by microbial demand for N, fuelled by deposition of C substrate from roots and competition with roots for N. The extent of priming depended on soil type and plant growing conditions.

In a further experiment, we simulated rhizodeposition of soluble microbial substrates in the same two soils with near-continuous additions for 19 d of either C4-labelled sucrose (i.e. a simple single substrate) or a maize root extract (i.e. a relatively diverse substrate), and we measured soil respiration and its C isotope signature. In the more fertile soil, sucrose induced increasingly positive priming effects over time, whereas the maize root extract produced declining priming effects over time. We suggest this was because N and other nutrients were provided from the mineralization of this more diverse substrate. In the less-fertile soil, microbial N demand was probably never satisfied by the combined mineralization from added substrate and soil organic matter. Therefore priming effects were approximately constant over time. We conclude that the chemical nature of putative priming compounds can greatly influence priming phenomena.

1. Introduction

From 10 to 20% of photosynthetic C fixed by plants is released into the rhizosphere as rhizodeposits: that is, soluble exudates, insoluble secretions, rhizosphere C flow and detrital root material (Gregory, 2006; Wichern et al., 2008; Jones et al., 2009). Some of this rhizodeposition is used by microbial communities for respiration and biomass production, and subsequent turnover of the biomass contributes to soil organic matter (SOM) pools. In the process, some part, particularly high-energy compounds present in root exudates, may be used by microbes to accelerate mineralization of existing SOM through so-called priming effects. Such effects are widely observed, but they are poorly understood (Cheng et al., 2014; Zhu et al., 2014). However, in the context of feedbacks to

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environmental change, they may be of equal or greater importance to global C and nutrient cycles as above-ground plant growth.

Priming effects are known to vary with plant type and such variables as canopy photosynthetic rate, plant phenology, root architecture, mycorrhizal symbiosis, and the quality and quantity of root exudates (Dijkstra et al., 2006; Kuzyakov, 2010; Phillips et al., 2012; Shahzad et al., 2015). They also vary with soil type, though this has been less studied (Billings et al., 2010; Kuzyakov, 2010; Paterson and Sim, 2013; Cheng et al., 2014). Important variables are likely to be soil nutrient status, N availability, soil water relations and toxic phenomena, as these can all influence plant growth rates and microbial activity. Priming effects can be either amplified or diminished by nutrient availability. A key hypothesis to explain this is that plant-derived labile C benefits rhizosphere microbes, which in turn can mobilize nutrients from soil organic matter, which in turn benefits the plants (Kuzyakov, 2010; Cheng et al., 2014; Murphy et al., 2015). Hence the interactions between plant and soil variables governing priming effects are complex.

In this study we aimed to add to the evidence on the effects of soil type and the nature of rhizodeposits on rhizosphere priming, and to investigate possible mechanisms to explain the effects. We made experiments with two contrasting soils: both relatively nutrient-poor grassland soils, but one more acidic and with organic matter with a much greater C:N ratio. We used C4 Kikuyu grass (*Pennisetum clandestinum*) as a model plant, which we found would grow well in the two soils in comparison with other C4 grasses. Because our soils contained organic matter derived exclusively from past C3 vegetation, and the C isotope signature of C3 respiration was of the order of 10‰ more negative than C4 respiration, we could use the isotope signature of soil respired CO_2 to partition the respiration into plant and soil sources. Further, we used a periodic grass clipping treatment to vary rhizodeposition: clipping was expected to decrease photosynthetic C fixation, root:shoot partitioning and root exudation. In a parallel experiment with the same two soils, we measured the effect of simulated rhizodeposition with near-continuous additions of mixed-composition C4 substrates.

2. Materials and methods

2.1. Soils

The soils were collected from 0 to 5 cm depth at two locations: (1) a surface water gley soil, Brockhurst series, at Temple Balsall, Warwickshire, England (276559N, 420189E), sampled in March 2012 (hereafter referred to as the clay soil); and (2) a brown sand, Cottenham series, at Shuttleworth College, Bedfordshire, England (243867N 514421E), sampled in May 2012 (hereafter referred to as the sandy soil). Both sites were under long-term C3 grasses, the sandy soil with C3 bracken, and both with no fertilizer applications for at least 5 years. The soils were air-dried and sieved (<6 mm) after removing recognisable plant fragments. The properties of the sieved soils were (a) clay soil: clay loam texture, pH (KCl) 5.5, organic C 46.2 g kg^{-1}, total N 4.9 g kg^{-1}, C:N ratio 9.4; and (b) sandy soil: loamy sand texture, pH (KCl) 3.8, organic C 64.8 g kg^{-1}, total N 4.2 g kg^{-1}, C:N ratio 15.4. The pH of the sandy soil was raised to 5.0 by adding 4 g kg^{-1} of powdered $CaCO_3$, moistening to field capacity and leaving for over 12 months, by which time all the $CaCO_3$ had reacted with the soil (i.e. there was no residual effect on the $\delta^{13}C$ of soil respiration).

2.2. Experiment A: actual rhizodeposition

2.2.1. Experimental design

The experiment was conducted in a glasshouse at Cranfield University, Bedfordshire, UK from May to July 2014. Mean day- and night-time temperatures in the glasshouse during the experiment were 28.5 ± 0.6 and 15.0 ± 0.3 °C, respectively. The experiment consisted of the two soils, planted or not with Kikuyu grass (*Pennisetum clandestinum* Hochst. ex Chiov, obtained from Barenbrug Holland B.V., Nijmegen, Netherlands) which was either clipped or unclipped. Soil CO_2 efflux and its $\delta^{13}C$ were measured periodically, and destructive harvests were made at the midpoint and end of the experiment to determine plant growth.

Sieved soil (<6 mm) was moistened to 60% of water holding capacity and packed into 300 mm long, 103 mm internal diameter PVC tubes to 250 mm depth with 1.5 and 1.4 kg soil per pot for the clay and sandy soils, respectively. The base of each pot was capped with a fabric mesh to block roots. Soil moisture was maintained at 60% of water holding capacity throughout the experiment by daily watering to weight. Seeds of Kikuyu grass were sown at a rate of 20 seeds per pot, 2–3 mm below the soil surface in a circular band approximately 30 mm from the edge of the pots. A single application of 0.16 g pot^{-1} of water soluble fertilizer (36-0-12 NPK + trace elements; Vitax, Leicester, UK) was made shortly after germination. A gas sampling chamber made of 100 mm long, 46 mm ID PVC pipe was placed over the bare soil in the middle of the pots and pushed down to 3 cm depth. The top of the chamber was fitted with a PVC cap containing gas-tight inlet and outlet ports with three-way Luer-lock stopcocks.

A total of 20 pots were prepared for each soil. Four replicates from each treatment were destructively sampled at 48 days after planting (DAP) and the remaining pots at 61 DAP. In the clipped treatments, clipping was carried out weekly starting at 31 DAP, and was sufficient to reduce plant height to 3 cm above the soil. Clippings were retained for analyses. Soil CO_2 efflux and its $\delta^{13}C$ were measured every few days from 21 DAP as described in Section 2.2.2. At harvests, the gas sampling chamber was removed from the pot and any soil adhering to it was removed and added to the bulk soil. In the planted pots, shoots were cut at soil level, placed in paper bags and oven-dried at 100 °C for 24 h to determine shoot biomass. The soil and roots were transferred to a large tray and roots were extracted by hand and shaken lightly to remove loosely-attached soil. The roots were then thoroughly washed with deionised water and oven-dried to determine root biomass. A portion of the fresh roots was retained for measuring the $\delta^{13}C$ of root respiration (Section 2.2.2). Soil samples were analysed within 1 d of sampling for total C and N using an elemental analyser (Elementar Vario EL, Hanau, Germany) and for microbial biomass by fumigation extraction (Vance et al., 1987). Prior to microbial biomass measurements, the soil was re-sieved (<6 mm) and recognisable plant fragments were removed. This procedure will not capture very fine plant material and loose cells, but this will be a small part of the measured microbial biomass, as is recognised in the wide use of fumigation-extraction in rhizosphere studies.

2.2.2. Soil respiration and $\delta^{13}C$ measurements

For respiration measurements, the gas sampling chamber was connected to a closed loop (1/8 inch ID BEV A-line tubing) containing a diaphragm pump (Charles Austen DA1 SE1, Byfleet, UK), a column of soda lime to scrub CO_2 from the air, and an infrared gas analyser (IRGA; Licor LI-820, Lincoln NE, USA). Air was circulated at approximately 1 l min^{-1} for 2 min, which was sufficient to reduce the chamber CO_2 mixing ratio to <1 μmol mol^{-1}. The CO_2 scrubber was then bypassed, and the CO_2 emitted from the soil surface over the subsequent 15 min was measured. The soil respiration flux F_S (μmol C m^{-2} soil s^{-1}) was calculated from the measured rate of change in CO_2 concentration in the microcosm headspace in the final 2 min of the post-scrubbing period using the equation