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Biology and Fertility of Soils

DOI:
10.1007/s00374-017-1193-1

Published: 01/05/2017

Publisher's PDF, also known as Version of record

Biol Fertil Soils
DOI 10.1007/s00374-017-1193-1

SHORT COMMUNICATION

Different ways in which CO_2 can be released during the turnover of roots in soil

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Received: 22 September 2016 / Revised: 20 February 2017 / Accepted: 7 March 2017

Abstract Here, we investigated how root age and mode of death influenced their subsequent turnover and rate of C loss from soil. Young white-coloured and older pigmented roots of *Cistus monspeliensis* were excised (to simulate death by mechanical severance) or frozen (to simulate death by cell rupture) and immediately buried in soil. CO_2 loss from soil was then measured over time. In a parallel experiment, the rate of CO_2 loss from severed or ruptured roots in the absence of soil was determined. Our results revealed large differences in root chemistry related to age, with young roots having a lower C:N ratio and a greater nutrient content (soluble C, N, P and K). Both root age and mode of death resulted in very different temporal patterns of C release from soil. The amount of C lost from soil followed the series: severed white roots (42.6 ± 3.3 mg C) > ruptured pigmented roots (27.7 ± 0.4 mg C) = ruptured white roots (27.1 ± 0.5 mg C) > severed pigmented roots (10.1 ± 1.0 mg C) > soil only (3.0 ± 0.2 mg C). Therefore, depending on the treatment, 7 to 41% of the total root-derived C was lost as CO_2 over the duration of the experiment. Comparison with soil-free treatments revealed that the CO_2 release from the severed roots buried in soil was not associated with microbial breakdown but caused by root-induced autophagy in an attempt to keep themselves metabolically active. Ruptured roots also induced a rapid loss of CO_2 which we ascribe to the diffusive loss of root solutes into the soil and subsequent microbial mineralization. Surprisingly, the rate of C loss from soil was greater from the severed root tips than those that were ruptured. Our results imply two distinct routes of C loss dependent on how roots die, one which bypasses the microbial community and one which flows through it.

Keywords Apoptosis · Carbon cycling · Heterotrophic respiration · Necromass · Rhizosphere · Rhizodeposition · Carbon sequestration

Introduction

Fine roots contribute approximately 33% of the annual net primary production in terrestrial ecosystems (Jackson et al. 1997). Upon senescence, their turnover is responsible for the formation of stable humic material, the release of nutrients that contribute to soil fertility, whilst also releasing substantial amounts of CO_2 back to the atmosphere. Fine root decomposition is plant species dependent and is regulated by a range of plant factors (e.g. presence of mycorrhizal symbionts, age and physiological state) and edaphic factors (Ghidey and Alberts 1993; Silver and Miya 2001; Redin et al. 2014; Goebel et al. 2011; Han et al. 2015; Tahir et al. 2016). The quantity, quality and lability of root C inputs to soil are also dependent on the way in which roots enter the necromass pool (Jones et al. 2004; van Doorn et al. 2011). At least three distinct pathways for fine root death can be identified:

(1) Type I death: Programmed (vacuolar) root cell death where C and nutrients are re-translocated to other growing areas of the plant prior to metabolic activity ceasing leaving just a nutrient-poor root corpse behind (e.g. occurs when a root has exhausted a patch of soil and is no longer needed or occurs during senescence and seed setting of annual crops; Jones et al. 2004).

Electronic supplementary material The online version of this article (doi:10.1007/s00374-017-1193-1) contains supplementary material, which is available to authorized users.

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Published online: 23 March 2017

(2) Type II death: Necrotic root death, where live roots become excised from the host plant (e.g. severing of during tillage, by meso/macrofauna, during tree windthrow) but still remain structurally intact and metabolically active. As the supply of C from the host plant is cut off, the roots maintain their supply of energy by breaking down all available internal C reserves. This autophagy progressively exhausts all internal soluble and structural C reserves before the roots ultimately die many days or weeks later (Saglio and Pradet 1980). There is no nutrient re-translocation, and the cell corpse (largely insoluble and structural C) remains largely unprocessed.
(3) Type III death: Catastrophic root death where metabolic activity in the root ceases immediately, and all C and nutrients from the roots are free to enter soil (e.g. occurs upon freezing-induced cell lysis, from severe mechanical damage in which the cells rupture; Schaberg et al. 2008; Kreyling et al. 2012; Muliele et al. 2015).

Each mode of death is likely to greatly influence how C and nutrients flow through the soil as well as influencing the size, activity and composition of the soil microbial community. The aim of the study was therefore to elucidate how root age and the mode of death influences root decomposition and thus the rate of C return to the atmosphere. We hypothesise that a Type III cell death would lead to a much more rapid release of CO_2 from soil in comparison to a Type II cell death due to the greater availability of labile C for the soil microbial community. We also hypothesise that in a Type II root death, most of the C release in the early stages is plant-derived rather than soil-microbially derived.

Materials and methods

Plant material

Plants of the dwarf shrub *Cistus monspeliensis* L. were propagated through cuttings and grown in hydroponic culture using half-strength Long Ashton nutrient solution (Hewitt 1966). Plants were grown at 20 °C, with a light intensity (photosynthetically active radiation) of 200 μmol m^{-2} s^{-1} at canopy height and 16 h photoperiod. After 6 months (plant height 40 cm), young white roots (<7 days old) and older highly pigmented (brown) roots (>30 days old) were harvested from the plants. Immediately after excision, sub-samples of each root type were placed into polypropylene microcentrifuge tubes and killed by immersion in liquid N_2 (de Neergaard et al. 2000). The cell sap was then recovered by the centrifugal-drainage procedure outlined in Hill et al. (2008), and the sap analysed for the following properties: total C and N using a Multi N/C 2100S analyser (AnalytikJena, Germany), total free amino acids (TFAA) using the fluorometric *o*-phthaldialdehyde β-mercaptoethanol procedure of Jones et al. (2002), nitrate using the VCl_3 method of Miranda et al. (2001), ammonium using the salicylate-nitroprusside-hypochlorite procedure of Mulvaney (1996) and total soluble phenolics using the Folin Ciocalteu method of Swain and Hillis (1959). The roots were also analysed for the distribution of soluble and structural components (cellulose, hemicellulose, lignin and ash) using an ANKOM2000 automated fibre analyser (Ankom Technology, Macedon, NY; Karcher et al. 2015). Root nutrient content was determined after drying (80 °C, 16 h) by total reflection X-ray florescence spectroscopy using a S2-PICOFOX-TXRF (Bruker, Billerica, MA).

Soil

A sandy clay loam textured soil (Eutric Cambisol) was collected (Ah horizon; 0–10 cm depth) from Abergwyngregyn, North Wales, UK (53° 14′ N, 4° 01′ W). Physical, chemical and biological properties of the soil are detailed in Hill et al. (2008). Immediately after collection, the soil was transferred to the laboratory, sieved (<2 mm) and stored at 5 °C. Soil moisture content was determined by oven drying (105 °C, 16 h).

Root mineralisation

Newly excised white or pigmented roots (3 g, ca. 96 mg C) were placed into individual 50 cm^3 sterile polypropylene tubes. Half the roots were then frozen (−80 °C, 30 min) to instantly kill them. Roots were then mixed with field-moist soil (30 g) to give five treatments: (i) severed white roots plus soil, (ii) severed pigmented roots plus soil, (iii) ruptured white roots plus soil, (iv) ruptured pigmented roots plus soil and (v) soil-only control. There were three replicates of each treatment, and the level of soil disturbance was identical in all treatments. In addition, the four root treatments were also studied in an identical way but in the absence of soil (i.e. root-only controls). All treatments were incubated at 20 °C and CO_2 evolution measured using an automated, multichannel SR1 respirometer (PP Systems, Hitchin, UK). The SR1 respirometer has a built-in humidifier unit which prevents moisture loss from the samples. The CO_2 flux derived solely from roots when buried in soil ($root_{soil}$ CO_2 flux) was estimated by subtracting the background soil respiration ($soil_{only}$ CO_2 flux; unamended controls) from the total CO_2 flux values for each mesocosm.

Statistical analysis

Differences in root chemistry were compared by one-way ANOVA, whilst differences in C mineralisation were compared using a repeated measure ANOVA with post-hoc testing using SPSS v20.0 (SPSS Inc., Chicago, IL). Cumulative CO_2

evolution in the root treatments over the duration of the experiment were compared with a two-way ANOVA with the presence of soil and root type as factors. $P < 0.05$ was used as the cut-off for statistical significance.

Results

Analysis of the two different root types revealed large differences in their chemistry (Table 1). Overall, the white roots had a much higher intrinsic nutrient content (soluble C, N, P and K) and much less structural C by weight, although the lignin concentration did not vary with root age, and the concentration of Ca was much higher in the older roots.

C mineralisation rates varied quantitatively and temporally between white and pigmented roots, and the mechanism of root death also influenced C mineralisation rates, especially during the first 72 h (Fig. 1a; Fig. S1; $P < 0.001$). Overall, the rate of respiration was low in the unamended soil

Table 1 Major nutrient composition of young white and older pigmented roots of *Cistus monspeliensis* L. Chemical characteristics of the cell sap and soluble components of white and pigmented roots

		Root type	
		White	Pigmented
Cell sap			
Total soluble C	(mg l^{-1})	3093 ± 181a[c]	1085 ± 186b
Total soluble N	(mg l^{-1})	758 ± 34a	231 ± 40b
Free amino acids	(mg l^{-1})	312 ± 16a	25 ± 12b
NH_4^+-N	(mg l^{-1})	62.5 ± 4.6	58.6 ± 1.1
NO_3^--N	(mg l^{-1})	152 ± 7a	100 ± 3b
Soluble phenols	(mg l^{-1})	262 ± 1a	240 ± 2b
Whole root			
Detergent soluble[a]	(% DW)[b]	46.0 ± 0.7a	34.4 ± 0.5b
Hemicellulose	(% DW)	14.2 ± 0.5a	16.3 ± 0.1b
Cellulose	(% DW)	20.7 ± 1.1a	29.7 ± 1.0b
Lignin	(% DW)	18.7 ± 0.5	19.3 ± 0.5
Ash	(% DW)	0.38 ± 0.02	0.32 ± 0.05
Total C	(g kg^{-1} DW)	425 ± 11	410 ± 6
Total N	(g kg^{-1} DW)	44.5 ± 1.3a	28.7 ± 0.1b
C-to-N ratio		9.5 ± 0.1a	14.3 ± 0.2b
Total P	(g kg^{-1} DW)	12.8 ± 0.9a	5.1 ± 0.3b
Total K	(g kg^{-1} DW)	16.8 ± 1.2a	4.7 ± 0.3b
Total Ca	(g kg^{-1} DW)	6.4 ± 0.4a	12.1 ± 0.4b

[a] Includes fats, oils and waxes and soluble cell contents (carbohydrates, lipids, pectin, starch, and soluble proteins)

[b] *DW* dry weight

[c] Means (±SEM, $n = 3$) followed by different letters indicate significant differences between root types at the $P < 0.05$ level. The absence of letters indicates no significant difference

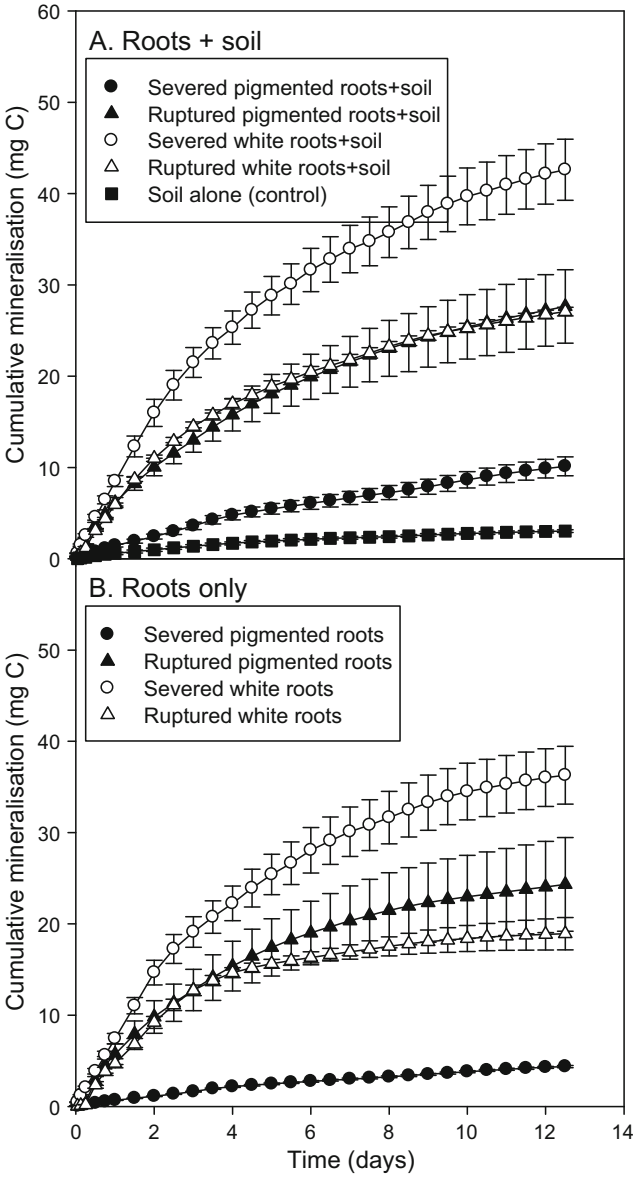

Fig. 1 Cumulative production of CO_2 from soil after burial of either severed or ruptured roots of *Cistus monspeliensis* (**a**), and the production of CO_2 from the same roots in the absence of soil (**b**). The roots were either young (*white*) or old (*pigmented*). Values represent means ± SEM ($n = 3$). Values are expressed per mesocosm to allow direct comparison between treatments (i.e. 30 g soil, 30 g soil + 3 g roots or 3 g roots)

(0.48 ± 0.06 mg CO_2 kg^{-1} h^{-1}), with much greater CO_2 fluxes seen in the root-amended soils ($P < 0.001$). Over the duration of the experiment (300 h), the total CO_2 flux was significantly different between the soil treatments ($P < 0.001$; Fig. 1) and followed the series:

> Severed white roots (42.6 ± 3.3 mg C) > ruptured pigmented roots (27.7 ± 0.4 mg C) = ruptured white roots (27.1 ± 0.5 mg C) > severed excised pigmented roots (10.1 ± 1.0 mg C) > soil only (3.0 ± 0.2 mg C)

Therefore, depending on the treatment, 7 to 41% of the total root-derived C was lost as CO_2 over the duration of the experiment.

When the CO_2 flux for the two severed root treatments was measured in the absence of soil (i.e. roots only), the respiratory fluxes were extremely similar to those of the root + soil treatments ($P > 0.05$; Fig. 1b, Fig. 2). In contrast, in the two ruptured root-only treatments, the rate of CO_2 loss was initially (0–24 h) much less than the parallel treatment performed in soil ($P < 0.01$ for white roots and $P < 0.001$ for pigmented roots). Following this (24–96 h), the rate of CO_2 efflux became similar in both the soil and soil-free ruptured root treatments. Following this, a major difference was seen between CO_2 efflux in the soil and soil-free treatments but only in the ruptured white root treatment ($P < 0.05$; Fig. 2). A two-way ANOVA showed no significant interaction of soil presence and root type on the total amount of CO_2 produced over the course of the experiment ($F = 0.23$, $P = 0.874$).

Discussion

As expected, tissue chemistry varied substantially with root age. Our findings are consistent with previous studies in which the concentration of low molecular weight solutes (e.g. amino acids, organic acids and sugars), and their rate of turnover inside root tissues was greatest in the most actively growing root regions (Jones and Darrah 1996). The higher major nutrient concentrations (N, P, K) in young white roots in comparison to pigmented roots reported here are also consistent with earlier studies showing nutrient translocation from older roots towards more physiologically active parts of the plant (Goldfarb et al. 1990; Volder et al. 2005). Our study also indicated that older pigmented roots had much greater structural, cell wall-associated C in comparison to younger roots. This brown pigmentation of older roots is concurrent with but not caused by suberisation and has been taken as an indicator of a general decline in root function (Comas et al. 2000; Cruz et al. 2004). Our results indicate that young roots should turnover much faster than older roots, and their lower C:N:P ratio should lead to the more rapid release of nutrients back into the soil.

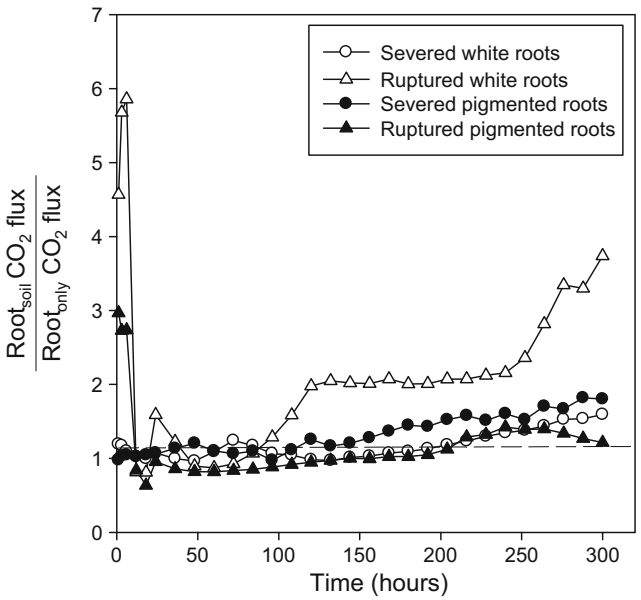

Fig. 2 Ratio of the CO_2 output derived from severed or ruptured *Cistus monspeliensis* roots either in the presence ($root_{soil}$ CO_2 flux) or absence of soil ($Root_{only}$ CO_2 flux). The roots were either young (*white*) or old (*pigmented*). The background soil respiration (i.e. soil without roots present) has been subtracted from the soil + root treatments

Our data demonstrated that severed roots exhibited high rates of CO_2 efflux from soil. Almost all the additional CO_2 released from the severed roots immediately after burial in soil, however, could be attributed CO_2 loss from the roots themselves, rather than by breakdown of the roots by the soil microbial community. This is consistent with visual observation of root integrity by James et al. (1993) and Bingham and Rees (2008) who showed that excised roots continue to respire for up to 30 days after excision. Initially, respiration is fuelled by stored sugars (e.g. glucose, starch and sucrose; 0–12 h), after which point-free amino acids reserves get catabolised and used in respiration. When these labile C pools are depleted, a general rise in autoproteolytic activity is observed. Although the roots are undergoing progressive autophagy, evidence suggests that plasma membrane integrity is maintained for weeks after excision and that no large exudative flux of organic C and N solutes occurs during this time (Saglio and Pradet 1980; Fig. S2). In contrast to Bingham and Rees (2008), however, we showed a significant loss of NH_4^+ into the soil from the severed roots after excision (Fig. S2). We ascribe this to the excess production of NH_4^+ during proteolysis and the need to remove it from the cytosol to prevent it reaching toxic levels (Brouquisse et al. 1992, 1998). No such loss of amino acids or NO_3^- into the soil was observed.

In contrast to roots which can remain alive after excision, roots exposed to low freezing temperatures undergo a catastrophic and immediate death as a result of ice crystal growth and disruption of the cell's membranes and cytoskeleton (Wesley-Smith et al. 2015). At this point, CO_2 efflux can only occur due to a residual loss of HCO_3^- from the cells and CO_2 produced by the rhizosphere microbial community. Our results showed that CO_2 efflux from the ruptured roots in the absence of soil was initially very low; however, this rapidly increased over 12 h suggesting that this was due to the growth or activation of microbes present on the rhizoplane and endorhizosphere. When buried in soil, this pulse of CO_2 release from the ruptured roots was immediate, consistent with the rapid loss of low molecular weight solutes from the roots into the soil by passive diffusion and their rapid consumption and mineralization by a C-limited microbial community (Hill et al. 2008).

The roots used here were grown in hydroponic culture and consequently no mycorrhizal symbionts were present. Further, the experiments were undertaken with fresh bulk soil which may have possessed a different microbial community from that naturally present in the Cistus rhizosphere. Under natural conditions, both of these represent additional factors that can influence the rate of root senescence and C loss from soil, particularly for older roots (Eissenstat et al. 2000). Theoretically, the addition of labile root C may induce either negative or positive priming of soil organic matter turnover. Based on our previous measurements of priming in the same soil using ^{14}C-labelled organic matter and a range of organic materials (e.g. biochar, wood ash, compost, manure and shoot residues), however, these responses are normally very much lower (ca. −10 to +15% changes in basal respiration) than the large positive CO_2 responses observed here (Fig. 1; Jones et al. 2011; Reed et al. unpubl.). We therefore expect that the contribution of priming to the overall CO_2 fluxes observed here will be very low.

Conclusions

This study clearly shows that root age and mode of death results in very different temporal patterns of C release from soil. Contrary to expectation, the rapid release of CO_2 from soil after root excision could not be attributed to the microbial breakdown of root tissues. Our evidence, alongside that of others, suggests that this pulse of CO_2 release is more related to root-induced autophagy in which the root attempts futilely to keep itself metabolically active. Similarly, roots which underwent an immediate death by freezing also resulted in a rapid loss of CO_2 from soil. In this case, however, we ascribe this loss to the diffusion of solutes from ruptured cells into the soil which are subsequently mineralized by the soil microbial community. Surprisingly, the rate of C loss from soil was greater from the senescing excised root tips than those that died immediately. This suggests a flow of C out of the soil which largely bypasses the soil microbial community. Severed older roots were much less metabolically active and more resistant to microbial degradation than either young roots or older ruptured roots, leading to a greater persistence of C in the soil. From our findings, it is clear that more work is needed to characterise and quantify the different ways in which roots die and turnover in soil and how this subsequently impacts upon long-term soil C storage and nutrient cycling. In addition, a greater attention should be paid to the role of mycorrhizal symbionts and endorhizosphere microbial communities in the root decomposition process. Finally, more long-term studies under field conditions are needed to complement laboratory experiments such as those performed here.

Acknowledgements We would like to thank Dr. Paul Baker, Gordon Turner, Jonathan Roberts and Sarah Chesworth for the technical support. We also thank Douglas Godbold for the scientific input and the choice of plant species. Funding was provided by project ENV4970694 funded under the European Union FP4-ENV 2C programme.

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