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Mycorrhizal contribution to soil respiration in an apple orchard

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ABSTRACT

Soil respiration (R_{soil}) partitioning into its components is pivotal to understand C cycling in ecosystems. Notwithstanding the importance of mycorrhizal fungi in the belowground C cycle, their respiration (R_{myc}) is poorly studied in agricultural ecosystems. In this study, we applied a modified trenching method and the ^{13}C natural abundance technique to distinguish R_{myc} from root respiration (R_{root}) and from the respiration due to soil organic matter (SOM) degradation (R_{som}) in an apple orchard (*Malus domestica* Borkh.) located in Northern Italy. Membranes with different mesh size were used to physically exclude roots or mycorrhizae from soil cores with a different $\delta^{13}C$ than the orchard average value. The CO_2 efflux from soil cores was determined with an infrared gas analyser, and isotopic measurements were performed on the soil-emitted CO_2. The different R_{soil} components were determined both by difference and by the isotopic mass balance. Mycorrhizal contribution to soil respiration ($11 \pm 6\%$) was of similar magnitude to that of roots ($12 \pm 4\%$) while R_{som} accounted for $73 \pm 3\%$ of R_{soil}. In presence of apple roots, respiration of SOM and mycorrhizae ($R_{som+myc}$) significantly increased in late summer and autumn, likely because of a priming effect of roots on SOM degradation or to a stimulation of mycorrhizal respiration. Our results suggest that respiration of mycorrhizal fungi can significantly contribute to R_{soil}, and need to be considered to correctly partition soil respiration. Furthermore, as roots increased $R_{som+myc}$, the root exclusion method for soil respiration partitioning may overestimate R_{root}.

1. Introduction

The global carbon dioxide (CO_2) flux from soil to the atmosphere (soil respiration, R_{soil}) represents the second largest C flux between ecosystems and the atmosphere after gross primary productivity (Raich and Schlesinger, 1992). Ecosystem type influences the amount of R_{soil} depending on biotic and abiotic characteristics, which in turn affect the different respiration components. The CO_2 emitted from soil have indeed different origins, traditionally subdivided in autotrophic and heterotrophic (Kuzyakov, 2006). Autotrophic respiration is due to the activity of plant roots (R_{root}), while heterotrophic respiration is due to the decomposition of soil organic matter (SOM) by microbial organisms and soil fauna (R_{SOM}) and to the respiration of root-associated microorganisms, including mycorrhizae, metabolising rhizodeposits from living roots (rhizomicrobial respiration).

In ecological studies, the respiration of mycorrhizal fungi and other root-associated microorganisms is often considered part of R_{root} or, together with R_{root}, as "rhizosphere respiration" (Högberg et al., 2001; Kuzyakov, 2006), mainly because of methodological problems to separate it from autotrophic respiration. The two most commonly used methods to divide the respiration in its autotrophic and heterotrophic components are the physical separation of roots by trenching (Fisher and Gosz, 1986), and the interruption of the phloem flux from shoots to roots by tree girdling (Högberg et al., 2001). These approaches do not allow the separation of R_{root}

Abbreviations: R_{soil}, soil respiration; SOM, soil organic matter; R_{som}, soil respiration due to degradation of soil organic matter; $R_{som+myc}$, soil respiration from microorganisms degrading SOM and mycorrhizal fungi in absence of plant roots; R_{root}, soil respiration due to plant roots, estimated as the difference between R_{soil} and $R_{som+myc}$; R_{root}^*, soil respiration due to plant roots estimated using the isotopic method; R_{myc}, soil respiration from mycorrhizal fungi; $R_{som+myc}^*$, soil respiration from microorganisms degrading SOM and mycorrhizal fungi in presence of plant roots, estimated using the isotopic method; F_{root}, proportion of the soil CO_2 efflux due to roots; $F_{som+myc}$, proportion of the soil CO_2 efflux due to mycorrhizae plus microorganisms degrading SOM; AM fungi, arbuscular mycorrhizal fungi; $\delta^{13}C$, isotopic signature of carbon (‰); AIC, akaike information criterion; MEF, model efficiency.

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from rhizomicrobial respiration, potentially leading to overestimate R_{root} and underestimate heterotrophic respiration (Kuzyakov and Larionova, 2005). A particular modification of the trenching method, using mesh membrane preventing in-growth of roots but permitting the penetration of fungal hyphae, has been proposed and successfully used to estimate the mycorrhizal respiration in grasslands and forests (Heinemeyer et al., 2007; Johnson et al., 2001; Moyano et al., 2008).

Arbuscular mycorrhizal (AM) fungi form a symbiotic association with two third of plants, including many crop and fruit tree species (Fitter and Moyersoen, 1996; Van Geel et al., 2015). In particular, the arbuscular symbiosis has been known in apple trees for many decades (Mosse, 1957), and has been found in the roots of all apple orchards surveys published in literature (Cavallazzi et al., 2007; Miller et al., 1985; Van Geel et al., 2015). The hyphae of arbuscular mycorrhizal fungi can extend up to 30 m per gram of soil (Cavagnaro et al., 2005; Wilson et al., 2009), growing with a rate of 0.7–1 m day^{-1} (Giovannetti et al., 2001). This suggests that mycorrhizal hyphae might play a key role in the process of belowground C cycle (Talbot et al., 2008). The amount of C that is translocated belowground by AM fungal structures varies between 4 and 20% of the total C fixed by the plant (Smith and Read, 2008; Tomè et al., 2015). However, the attempts to quantify the CO_2 released in atmosphere by mycorrhizae are scarce, especially in agro-ecosystems where the fungal C demand is poorly studied (Gianinazzi et al., 2010). Most of the studies have been conducted on annual crops and determined that between 1 and 5% of net photosynthesis is lost by hyphal respiration (Heinemeyer et al., 2006; Jakobsen and Rosendahl, 1990; Moyano et al., 2007). Nevertheless, to our knowledge attempts to distinguish between the root and the mycorrhizal contribution to soil respiration in agro-ecosystems are scarce.

Another limitation of the traditional R_{soil} partitioning methods is that they do not consider the possible interactions between the different components. For instance, the decomposition of SOM can be inhibited (Graham et al., 2012) or stimulated (Cheng et al., 2003) by the supply of fresh substrates, such as root litter or the exudates released by roots (Blagodatskaya and Kuzyakov, 2008; Kuzyakov and Larionova, 2005). The contribution of R_{som} to R_{soil} would therefore depend not only on the type and on amount of SOM itself, but also on the growth rate and activity of roots.

In the last years, isotopic techniques have gained increasing attention in soil respiration studies (Dawson et al., 2002). In particular, the ^{13}C natural abundance method is based on the differential discrimination of the heavier C isotope during CO_2 assimilation by plants with different photosynthetic pathways (C_3 and C_4 plants) and has been used to estimate root and microbial respiration under undisturbed conditions (Hanson et al., 2000). Isotopic techniques allow to measure R_{SOM} in presence of plant roots, taking into account the priming effect and all the interactive effects between roots and soil microbes.

Estimating heterotrophic respiration is fundamental to calculate the net ecosystem productivity (NEP) and to understand if agroecosystems act as sinks or sources of C. Even though it has been demonstrated that agroecosystems C fluxes can have the same magnitude of those of forest ecosystems growing in similar climatic conditions (Zanotelli et al., 2013), they received less attention than natural ecosystems. For instance, total soil respiration has been quantified to be around 8 Mg C ha^{-1} y^{-1} in an apple orchard (Zanotelli et al., 2013) and 2.7 Mg C ha^{-1} y^{-1} in a citrus plantation (Iglesias et al., 2013). On average, roughly 5.5 Mg C ha^{-1} each year are released from croplands and other types of agricultural fields (Luo and Zhou, 2006). The autotrophic component in apple orchards has been estimated to be around 35% of total soil respiration (Subke et al., 2006; Ceccon et al., 2011; Panzacchi et al., 2012; Zanotelli et al., 2013).

In this study, we combined the use of mesh membranes for the physical separation of root and extraradical mycorrhizal hyphae, as proposed by Johnson et al. (2001), with the isotopic approach based on ^{13}C natural abundance, to partition R_{soil} and to quantify the contribution of different components (SOM degradation, root respiration, and mycorrhizal respiration) in an apple orchard. In particular, we hypothesized that mycorrhizal fungi provide a considerable contribution to R_{soil} also in a fruit tree ecosystem. Furthermore, the comparison of the partitioning obtained with the two methods allowed us to verify the hypothesis that roots can significantly affect the activity of soil microorganisms such as SOM degrading microbes or mycorrhizae, affecting the estimation of R_{root} performed with traditional methods.

2. Material and methods

2.1. Site description

The experiment was performed in an apple orchard (*Malus domestica* Borkh.) located at Vadena (BZ, South Tyrol, Italy), 46°22′56.06″N, 11°17′18.07″E, at about 220 m above sea level. The climate in the area is warm temperate according to the Köppen–Geiger classification (Kottek et al., 2006). Mean annual precipitations and mean annual temperature measured at the site (Laimburg Weather Station) are 800 mm and 12.4 °C, respectively (1971–2013 reference period). The soil is classified as Calcaric Cambisol according to the FAO Soil Taxonomy, with a sandy–loam texture (Table 1). In the experimental site, apple trees of the cv. Pink Lady on M9 rootstock were planted in 2008, at 1 m distance along the row, and 3 m distance between the rows (alleys). Tree rows have a north-south orientation. The orchard is drip-irrigated, and managed using integrated protocols suggested by the regional government, including periodic herbicide treatments (glyphosate, *N*-(phosphonomethyl) glycine) along the tree rows and periodic cuts of grasses in the alleys. During the experimental period, chemical weeding was stopped to avoid the introduction of chemical substances with unknown effects on the carbon cycle and weeds growing in the experimental plots were manually removed as soon as they appeared.

2.2. Experimental design

The experimental plots were arranged within a 200 × 300 m area of the orchard, following a randomized block design with 5 blocks. Each block was located in a different tree row, and consisted in a 4 × 1 m weed-free area below tree canopies.

Four months before starting the experiment (November 26th, 2012), 3 cylindrical holes (30 cm diameter, 30 cm depth) were

Table 1
Physicochemical characteristics of the native orchard's soil and of the C_4 soil used for the experiment.

Parameter	Unit	Site	
		Orchard soil	C_4 soil
pH (in $CaCl_2$)		7.2	7.0
Texture (USDA classification)		Sandy loam	Sandy loam
Sand	%	70	55
Silt	%	20	35
Clay	%	10	10
Organic C	g kg^{-1}	6.7	24
Organic matter	g kg^{-1}	16	41
Total nitrogen	g kg^{-1}	0.7	2.3
Available P (P–Olsen)	mg kg^{-1}	90	93
Exchangeable potassium	mg kg^{-1}	180	104
Exchangeable magnesium	mg kg^{-1}	120	111
C/N		9.6	10.4
$\delta^{13}C$	‰	−26.2	−23.1