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Separating soil respiration components with stable isotopes: natural abundance and labelling approaches

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Due to the potential of forest ecosystems contributing to CO_2 increase as well as to climate change mitigation, forest-atmosphere CO_2 exchange has been intensively studied over last decades. However, the contribution of individual components of belowground carbon pools is still poorly known. In particular, there is no unequivocal means to separate root respiration (autotrophic) from heterotrophic respiration by soil microflora and fauna. Most studies investigating soil respiration disturbed the soil and tried to exclude autrophic respiration. Here we review alternative non invasive methods to separate the two components. Those methods share the application of the stable carbon isotope ^{13}C, using either local changes in natural abundance of $^{13}CO_2$ or artificial labelling of trees with CO_2 enriched or depleted in ^{13}C. We conclude that the applicability of natural stable isotope methods is still limited in forest ecosystems because only in a few cases there are large enough differences in ^{13}C among soil carbon pools (usually due to the earlier presence of a C_4 canopy, which is seldom in forests). On the other hand, artificial labelling with CO_2 either enriched or depleted in ^{13}C is now in a widely used for partitioning soil respiration components. However, recent findings gave clear evidence that measurements of soil CO_2 efflux can be substantially influenced by the return efflux of the abiotic label. Still, especially the combination of Free Air Carbon Enrichment (FACE) with dual $\delta^{13}C$ and $\delta^{18}O$ stable isotope approach has the potential to provide new answers on the response sensitivity of turnover dynamics of the largest belowground soil carbon storage to elevated temperature and CO_2.

Keywords: Carbon stable isotopes, Labelling, Natural abundance, Autotrophic and heterotrophic soil respiration

Introduction

Soil respiration is the largest component of ecosystem respiration (Ryan & Law 2005) and therefore a key element in the carbon source/sink role especially for forest ecosystems. The ongoing increase in both atmospheric temperature and CO_2 content has an enhancing effect on the flux of CO_2 from soil respiration (Heimann & Reichstein 2008). In spite of the increased soil respiration flux with elevated air temperature and CO_2 level, the individual components of soil respiration [autotrophic root respiration, heterotrophic microbial respiration, and litter and soil organic matter (SOM) degradation] remain difficult to quantify. This is a challenging quest because all existing methods (Kuzyakov 2006) bear their respective limitations. Promising new insights have been obtained from the use of stable carbon isotopes (Crow et al. 2006, Millard et al. 2008, Søe et al. 2004), where either: a) natural variability in the abundance of carbon isotopes in different compartments is examined; or b) a labelled $^{13}CO_2$ signal is applied to compartments of the soil carbon cycle.

The aims of this mini review are firstly to present the main idea behind selected stable isotope approaches, namely: a) the method of natural ^{13}C abundance; b) the method of isotopic labelling with ^{13}C-depleted CO_2; and (c) the method of isotopic labelling with ^{13}C -enriched CO_2. The second aim is to evaluate the methods' applicability for the separation of the autotrophic and heterotrophic components of soil respiration in field conditions.

Method of natural abundance

The heavier stable isotope ^{13}C is much less abundant than the lighter ^{12}C.$^{13}C \approx 1.10\%$ of the total carbon (Farquhar & Richards 1984). The isotopic carbon signature of any material is reported in the delta notation ($\delta^{13}C$) with per mil (‰) units, which refers to the ratio between the isotopic $^{13}C/^{12}C$ ratio (R) of the sample to the international reference V-PDB standard (eqn. 1):

$$\delta^{13}C = 1000 \cdot \frac{R_{sample}}{R_{standard}} (‰)$$

where a higher $\delta^{13}C$ stands for more heavy stable isotopes than the standard (enrichment) and a lower $\delta^{13}C$ means less heavy stable isotopes than the standard (depletion). It is this ratio that is of particular interest for plant ecology as it is altered / fractionated to a varying degree by the different photosynthetic metabolisms of C_3 and C_4 plants. Because of differences in CO_2 uptake and therefore different rates of diffusive fractionation, tissue of C_3 plants will be isotopically depleted by about -29‰ (Kuzyakov 2006), while C_4 plant tissue shows a depletion of only -13‰ as compared to the standard. Numerous processes (Scartazza et al. 2004) including physiological status and meteorological conditions can influence these averaged values, but still these differences afford an excellent starting-point for separating soil respiration components by natural abundances of isotopes in the case where a C_3 canopy follows a C_4 crop (or *vice versa*) (Yakir & Sternberg 2000). Up to now, this is still an important limitation in the applicability of the natural abundance approach in forests, because C_3 and C_4 substitutions involving trees are rare. In the original forest without C_3 and C_4 substitutions, only small differences in the isotopic signal of soil respiration components were found in laboratory experiments. These differences might be diminished by the great spatial variability of soil efflux isotopic signal found *in situ* (Formánek & Ambus 2004).

Formánek & Ambus (2004) were able to distinguish $\delta^{13}C$ of CO_2 efflux from root-free mineral horizons, root-free humus layer and roots. The $\delta^{13}C$ signatures alone were not sufficient to separate contributions of root respiration and SOM decomposition in the forest soil CO_2 efflux measured in the field. The necessary estimate of root-free respiration profile $\delta^{13}C$ signature was roughly calculated by bulk densities related contribution

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of each layer to the total CO_2 efflux. Therefore, the estimation of 43% of root from total soil respiration could be realistic, but needs to be considered with caution, although it falls into the 29-59% range as reported by other carbon isotopic studies of temperate mixed deciduous forests (Borken et al. 2006, Gaudinski et al. 2000).

Method of isotopic labelling

There are two main labelling processes available: (i) labelling with ^{13}C-depleted CO_2 originating from fossil fuel combustion; (ii) labelling with ^{13}C-enriched CO_2 produced by nuclear processes.

The isotopic signature of air CO_2 is -8.00‰ at present (Bowling et al. 2008), but continuously moves towards more negative values because of fossil C combustion. CO_2 derived from fossil carbon sources, *i.e.*, mineral oil and coal, shows a depleted carbon isotopic signature of about -35.00‰ (Lin et al. 1999), which turns it in an ideal and inexpensive substrate for isotopic labelling of the soil carbon cycle under both field and enclosed conditions. There are many ongoing and already conducted isotopic labelling studies (*e.g.*, Allen et al. 2000) with very promising results, but in the present paper we focus on one particular work by Lin et al. (1999), because this study achieved clear partitioning of three forest soil respiration components (*i.e.*, CO_2 efflux from root, litter, and SOM). In this study, 4-year old Douglas-fir trees were grown under controlled conditions in terracosms with four different combinations of ambient and elevated CO_2 concentrations and temperature. SOM and soil litter originated not from the present trees but from an old growth Douglas-fir forest. In both the elevated and ambient groups, a constant CO_2 concentration was maintained by fumigating the terracosms with tank CO_2. The tank CO_2 fumigation resulted in newly formed plant material such as needles and roots with a $\delta^{13}C$ of -35.77 ± 0.09‰ (± standard deviation) compared to an atmospheric CO_2 $\delta^{13}C$ of -8.55 ± 0.13‰ in the area where SOM as well as the organic litter of the soil in use had formed. With this approach alone, it would have not been possible to separate the three main components of soil respiration and to understand their individual responses to elevated temperature and CO_2, because CO_2 both from SOM and litter degradation carrying the same carbon isotopic signal. Lin et al. (1999) found an elegant solution to this problem: they took advantage of the information in the $\delta^{18}O$ oxygen isotopic signature in the soil respired CO_2 molecule, which is in equilibrium with the respective signature in soil water. When soil water evaporation occurs, there is a diffusive fractionation of the water molecules carrying the two different stable oxygen isotopes ^{16}O and ^{18}O. This isotopic enrichment of ^{18}O takes place only in the upper 10 cm of soil which allows a separation of CO_2 originating from litter degradation and CO_2 from SOM degradation according to their respective oxygen isotopic signature. The disadvantages of this particular approach apply as well for other labelling studies, *i.e.*, FACE. In most cases, the task of maintaining constant conditions results in an elevated CO_2 concentration. This means a major disturbance to the ecosystem carbon flux, because in the supply to microbial communities, the intricate relation between the supply of fresh energy abundant carbon compounds (leaf litter, rhizo-exudates and rhizo-deposition) and old, more nutrient prone soil organic carbon is altered (Paterson et al. 2009). The need to label some components limits the applicability of the method under field conditions, even if a main focus is put on maintaining ambient conditions concerning the CO_2 concentration.

A very interesting example of isotopic labelling with enriched $^{13}CO_2$ is the study of Subke et al. (2009) conducted on stand scale in a naturally regenerated *Pinus silvestris* forest in northern Sweden. Subke et al. (2009) pulse-labeled (3h) the trees in two chambers (each 200 m^3) with highly enriched $^{13}CO_2$ and monitored the resulting $^{13}CO_2$ soil efflux with a combination of deep, root free and surface collars over a period of 6 days. In this setup, the isotopic ^{13}C signature of deep collars excluded plant derived CO_2. The authors were able to separate the initial physical $^{13}CO_2$ flux caused by the direct tracer diffusion from the atmosphere and the later occurring biological $^{13}CO_2$ flux caused by root respiration of photosynthates (biotic label return). Approximately 2-3 days after pulse labelling, only in deep collars $^{13}CO_2$ returned from the initial peak to near natural abundance, while in surface collars, $^{13}CO_2$ likely from the respiration of labelled assimilates allocated below-ground, increased again and reached the maximum ca. 3.5-4 days after the pulse. In spite of the separation of autotrophic pulse-derived $^{13}CO_2$ tracer from the difference between surface collars and deep collars, the experiment was primarily designed to separate abiotic and biotic tracer returns. However, the method has the potential for separating the autotrophic and heterotrophic part of soil CO_2 efflux, if the experimental design allows plant photosynthesis to use only the labelled ^{13}C-enriched CO_2 source.

Conclusions

In this mini review, we compared the use of natural abundance and labelled carbon isotope methods in partitioning forest soil autotrophic and heterotrophic respiration components. The natural abundance carbon isotope method has the potential of separating forest soil respiration components, although its applicability is limited to situations where overplanting from C_4 to C_3 plants occurred. On forest sites with an exclusive C_3 vegetation history, the individual soil components must be physically isolated (disturbing the carbon cycle). In addition, natural $\delta^{13}C$ signatures show high spatial variation allowing only for a rough partitioning of soil respiration. The difficulty of relying on the existing C_4 SOM can be overcome by the tank fumigation with ^{13}C-depleted or ^{13}C-enriched CO_2. The newly formed organic material with depleted or enriched ^{13}C signatures enables to distinguish the root autotrophic respiration from heterotrophic respiration of litter, roots and SOM formed before labelling with natural abundance $^{13}CO_2$. However, also long-term fumigation experiments require an exclusion of the roots for a separation of the components of autotrophic soil respiration. A step further to non-invasive separation of autotrophic and heterotrophic components was achieved by the combination of long-term fumigation with dual $\delta^{13}C$ and $\delta^{18}O$ stable isotope tracers. Isotopic methods advanced from the disturbance of carbon cycle to a less invasive approach achieved by the combination of the former accomplishments. If a virtually undisturbed partitioning of the forest soil respiration components into fast and slow carbon turnover would be possible, it would enable better opportunities to study the effects of both elevated temperature and CO_2 concentration onto the balance of long-term soil carbon storage.

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