Root exclusion method for separating soil respiration components: Review and methodological considerations

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ABSTRACT

Soil respiration (Rs) is a vital ecosystem process where soil emits carbon dioxide (CO_2) into the atmosphere. Annually, soil emits an estimated 68–101 Pg Carbon (C) into the atmosphere, making it the second-highest terrestrial contributor to carbon fluxes. Since Rs consists of autotrophic and heterotrophic constituents, accurate methods of determining the contribution of each element to total Rs are critical for understanding their differential response to environmental factors and aiding CO_2 emissions reduction. Consequently, the root exclusion (RE) technique combined with manual chamber measurements is frequently employed for Rs partitioning in the field due to its low cost and simplicity. Nevertheless, RE treatments alter the soil environment, leading to bias in respiration measurements. This review aims to elucidate the current understanding of RE [trenching (Tr) and deep collar (DC) insertion techniques] by examining Rs partitioning studies performed in several ecosystems. The present review also discussed methodological considerations when utilizing RE (Tr and DC insertion methods) and the combinations of RE with stable isotopic and modeling approaches. Furthermore, suggested future research directions for improving the Tr and DC insertion methods in RE are included.

Key Words: deep collar insertion, heterotrophic respiration, methodological considerations, microbial respiration, root trenching, soil microbial communities, soil respiration partitioning

INTRODUCTION

Global warming resulting from elevated greenhouse gas (GHG) levels, such as carbon dioxide (CO_2), in the atmosphere has highlighted the importance of understanding ecosystem carbon (C) cycling to control C emissions from terrestrial ecosystems. Soil CO_2 emission from soil respiration (Rs) is the second most significant terrestrial C fluxes source, accounting for 68–101 Pg C yr^{-1} (Jian *et al.*, 2020), which is ten times higher than anthropogenic CO_2 emissions (Friedlingstein *et al.*, 2020).

The Rs, or soil CO_2 efflux, is a crucial ecosystem process in which CO_2 is released from soil into the atmosphere (Luo & Zhou, 2006). The process is commonly divided into autotrophic and heterotrophic components (Bond-Lamberty et al., 2004). Various terminologies have been utilized to denote the autotrophic component that comprises the respiration of plant roots (maintenance and growth) and root-linked microorganisms in the rhizosphere, such as autotrophic (Ra) (Högberg *et al.,* 2001) and root and rhizosphere (Hopkins *et al.,* 2013) respirations. Hereafter, the term Ra represents root respiration (Rr) and root-associated microorganisms in the rhizosphere.

The heterotrophic component, which includes the respiration of microorganisms that decompose organic matter (SOM) from soil and litter, is fascinating considering that SOM-derived CO_2 efflux from

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microbial decompositions contributes directly to C losses in the atmosphere. Simultaneously, CO_2 efflux from Ra is compensated for by fixation during photosynthesis (Agus *et al.*, 2010). Consequently, separating Rs components is crucial to procuring heterotrophic respiration (Rh) estimates, considering that it is a critical factor in determining soil carbon balance and ecosystem emission factors (Hergoualc'h & Verchot, 2014; Murdiyarso *et al.*, 2019).

Generally, partitioning Rs in the field into its autotrophic and heterotrophic components is challenging due to the various biological and ecological processes that regulate them (Baggs, 2006). Moreover, while plant activities and photosynthates supplies to roots influence autotrophic respiration (Kuzyakov & Cheng, 2001), Rh is influenced by soil temperature, moisture, substrate quality, and supply (Davidson & Janssens, 2006).

Plant roots, mycorrhizae, and microbial decomposers contribute differently to Rs in ecosystems at varying soil temperatures and moisture levels, hence allowing CO_2 efflux to respond to the changes in the variables (Kechavarzi *et al.*, 2010; Hursh *et al.*, 2017). For example, Ra could contribute from 5 to over 90% of soil CO_2 efflux, depending on vegetation cover, season, and the Rs partitioning approach (Hanson *et al.*, 2000; Heinemeyer *et al.*, 2007). Nonetheless, Rh is affected by changes in soil microbial biomass, metabolisms under warmer conditions, community compositions, and bacterial-to-fungal ratios, resulting in altered decomposition rates (Classen *et al.*, 2015).

Several methods of separating Rs into its constituents have been developed (Hanson *et al.*, 2000; Kuzyakov, 2006). The approaches could be broadly classified into isotopic (Paterson *et al.*, 2009), such as carbon-13 (^{13}C) natural abundance discrimination and isotopic carbon-14 (^{14}C) mass balance, and nonisotopic, including component integration, root exclusion (RE) by trenching (Tr) or deep collar (DC) insertions, root removal, and gap analysis techniques. Several criteria are considered when determining the best approach to distinguishing Rs components, including disturbance to the ecosystem, application universality in different ecosystems, reproducible and reliable results provisions, ease of use, and cost-effectiveness (Kuzyakov, 2006).

The RE method is one of the most widely used techniques for Rs partitioning owing to its low cost and ease of application. Nevertheless, soil disturbance and cut roots from RE plot installation might increase CO_2 emissions in trenched plots (Hanson *et al.*, 2000; Subke *et al.*, 2006). Conversely, the isotopic ^{14}C mass balance approach only results in minor soil disturbance (Vargas *et al.*, 2011), but it possesses several disadvantages, including a complex experimental setup requiring specific ^{14}C sampling expertise and an expensive, technically challenging radioactive isotope analysis (Carbone *et al.*, 2016). The limitations of any single method could be solved by combining approaches, such as nonisotopic and isotopic (Kuzyakov, 2006). Nonetheless, combined methods are still scarce (Biasi *et al.*, 2012, 2014; Carbone *et al.*, 2016; Comeau *et al.*, 2018).

The Rs partitioning studies with RE via the Tr approach in various ecosystems have gained interest, hence allowing more detailed analyses of experimental limitations. Furthermore, more reports are recording higher soil CO_2 efflux in trenched than non-trenched plots, despite a prolonged stabilization period after Tr, leading to inaccurate Rh estimations. Although the causes have not been thoroughly investigated, the phenomenon is generally attributed to the effects of the RE method, such as severed root decomposition, differences in soil moisture, the rhizosphere priming effect (RPE), and changes in microbial community structures (Lalonde & Prescott, 2007; Comstedt *et al.*, 2011; Drake *et al.*, 2012; Comeau *et al.*, 2016; Kukumägi *et al.*, 2017; Savage *et al.*, 2018; Ishikura *et al.*, 2019).

The distinction between autotrophic and heterotrophic Rs is critical for understanding Rs-related environmental changes and considering an avenue for reducing CO_2 emissions through environmental management (Murdiyarso *et al.*, 2019). Consequently, this review aims to elucidate the current understanding of RE by Tr and DC (RE: Tr/DC) insertion methods by analyzing recent articles on Rs partitioning studies from various ecosystems. The present review also discussed methodological considerations when employing the RE: Tr/DC insertion techniques and the potential of combining approaches (RE with stable isotope and RE with modeling approaches) to overcome the limitations of a single method. Furthermore, this review included future research proposals for improving RE: Tr/DC insertion.

AN OVERVIEW OF RE METHODS FOR PARTITIONING R_s

Hanson *et al.* (2000) suggested that an RE approach refers to any process that indirectly quantifies root respiration by measuring surface soil CO_2 efflux with or without involving roots (no direct root tissue evaluations). The method is divided into root removal, gap analysis, and Tr and DC (Tr/DC)

insertion. This review describes the RE technique with emphasis on the Tr and the DC insertion approaches.

Root removal

The root removal method involves removing roots from the sampling plot soil and placing the soil horizons back in the reverse removal order. During the analysis, barriers could block root growth from the surroundings. Subsequently, root respiration is obtained by determining the difference between measured CO_2 effluxes of the soil with and without root (Hanson *et al.,* 2000). For instance, a study reported not utilizing a barrier to prevent root regrowth in a pit of 0.5 m × 0.5 m and 30 cm deep as the soil CO_2 efflux was measured 2–4 weeks post root removal (Wiant, 1967). Another soil respiration partitioning study performed in the *Fitzroya cupressoides* forests in southern Chile also employed the root removal method, where a PVC collar (40 cm long) was inserted 30 cm into root-free soil to evaluate its Rh. The roots were manually removed before carefully reinstalling the collar into the root-free soil without compacting or mixing the horizons of the soil (Urrutia-Jalabert *et al.,* 2017).

Although the root removal technique eliminates the contribution of dead roots to CO_2 production and allows root biomass measurements in research plots, the technique requires a long time and alters soil structure and environmental variables, such as soil temperature and moisture (Wiant, 1967; Urrutia-Jalabert, 2017). Furthermore, plant roots enable soil moisture reduction through evapotranspiration (Leung *et al.,* 2015). Consequently, removing roots and employing a non-permeable PVC collar might lead to waterlogging. Waterlogging inhibits Rh, thus resulting in unusable data (Urrutia-Jalabert *et al.,* 2017). Moreover, utilizing long PVC collars in peatlands and grasslands could lead to waterlogging in the collars after heavy rain (Heinemeyer *et al.,* 2007), suggesting that wet ecosystems (forests, peatlands, grasslands) with poorly drained soils (Urrutia-Jalabert *et al.,* 2017) are more susceptible to the phenomenon.

Gap analysis

Gap analyses provide indirect root respiration estimations by comparing the gaps and forested areas under the crown Rs measurements. Clear-cutting in forest stands and vegetation clippings in grasslands create gaps, which reduce carbohydrate supply into the soil and thus affect the Rs (Brumme, 1995). For example, the Rs of mature beech stands in Germany was compared to the 30 m gaps in the stand installed two years earlier. The measurements were obtained in the gap center. The study documented that the respiration of the living roots accounted for 40% of the Rs (Brumme 1995). In another study, the estimated root respiration of a 10-year-old Japanese cedar wood forest two years after the formation of a relatively small gap (2.5 × 2.5 m) was approximately 50%. The Rs in the report represented the difference between the CO_2 effluxes in the gaps and the forest area (Ohashi *et al.,* 2000).

The gap approach is labor efficient when the gaps have been established in the system or from individual tree death or windthrow. Nonetheless, similar to other RE methods, gap analysis presents the same challenges as Tr, such as increased CO_2 emissions due to dead root decomposition (Toland & Zak, 1994) and soil temperature and moisture alterations (Mayer *et al.,* 2017). Moreover, microenvironmental factors (air and soil temperatures, solar radiation, soil moisture) that enhance microbial activities were found to be different in gaps compared to the neighboring closed canopy stands, suggesting that the gaps potentially possess higher decomposition rates than closed forests (Schliemann & Bockheim, 2014).

Mayer *et al.* (2017) reported that soil temperature and moisture were considerably higher in the gaps of a mixed forest stand than in the control area, and no changes in the potential enzyme activities of the soil were recorded. Consequently, the Rs in the gaps due were not reduced to improved decomposition rates (driven by warmer soil conditions), compensating for the Ra reduction. Furthermore, a rising gap size might result in the rapid growth of understory vegetation due to improved microclimate, such as light and moisture, which could offset gap-induced Rs reduction through the respiration of the new roots of understory vegetation (Pang *et al.,* 2016). Accordingly, approaches to reduce alterations in environmental conditions require consideration. Techniques including employing net-covered frame boxes with clear-cut areas to maintain similar surroundings as the control (Nakane *et al.,*1996), smaller gaps (Ohashi *et al.,* 2000), and applying correction approaches to account for effects of altered soil temperature and moisture on Rs (Wan and Luo, 2003) could be beneficial.

The RE: Tr approach

The Tr method has been widely employed in various ecosystems, including grasslands (Fang *et al.*, 2018; Zhang *et al.*, 2019), tropical (Vijayanathan *et al.*, 2021) and temperate (Jiao and Wang, 2018; Savage *et al.*, 2018) forests, and afforested temperate (Hermans *et al.*, 2022) and tropical (Comeau *et al.*, 2016; Hergoualc'h *et al.*, 2017; Itoh *et al.*, 2017; Ishikura *et al.*, 2018, 2019) peatlands, due to its ease of use. Nonetheless, the Tr approach cuts the C supply to roots and the surrounding rhizospheres (Vogel & Valentine, 2005).

Deep incisions, commonly 20 cm below the rooting zone, are manually conducted with a knife and shovel (Itoh *et al.*, 2017) or mechanically with a chainsaw (Comeau *et al.*, 2016) to sever all roots. The trenched plots are then surrounded by physical barriers of non-permeable materials, including plastic sheets, stainless steel plates, plexiglass, and permeable mesh fabric (1 or 50 μm pore size), to prevent root and mycorrhiza ingrowth (Ishikura *et al.*, 2018; Hermans *et al.*, 2022; Ryhti *et al.*, 2021). Aboveground vegetation in the plots is removed by hand weeding or surface clipping. Herbicides are avoided as they could alter the microbial community of the evaluated soils (Epron, 2009).

Trenched plots in Rs partitioning studies ranged from 30 to 100 cm deep and 0.4 m × 0.4 m–2.5 m × 2.5 m in size (see Table I). Figure 1A illustrates the site installation diagram of trenched plots. Soil CO_2 efflux measurements are typically obtained via the closed chamber method, which involves attaching soil collars to infrared gas analyzers (Comeau *et al.*, 2016; Itoh *et al.*, 2017; Hermans *et al.*, 2022).
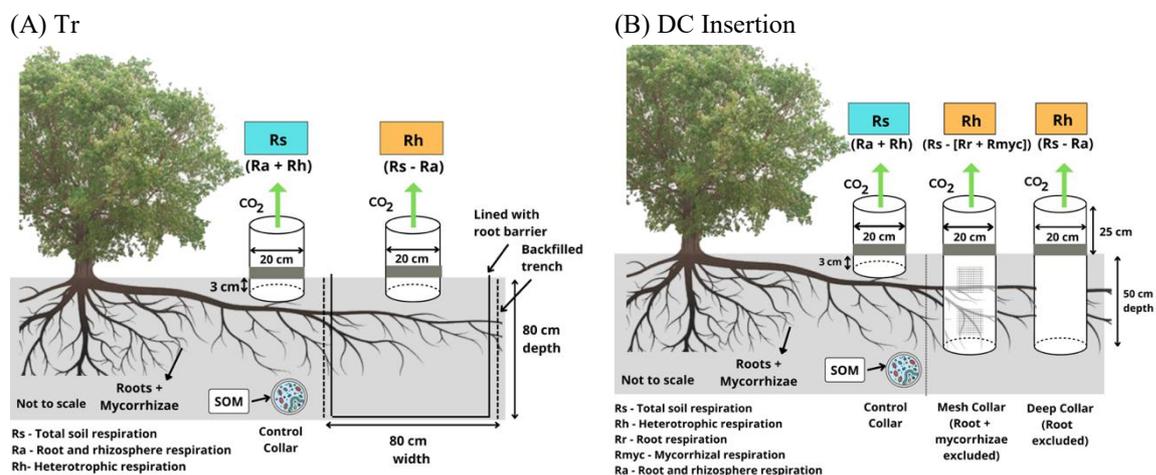
(A) Tr   (B) DC Insertion

Fig.1 Examples of site installation diagrams for (A) Tr and (B) DC insertion
Note: In tr, A 80 cm × 80 cm trenched plot is excavated to a depth of 80 cm and lined with a barrier cloth before refilling with soil. Soil CO_2 efflux is measured with a closed manual or automated chamber within the trenched plot, representing the Rh, while the control collar represents Rs. A DC consists of an open-ended cylinder (20 cm diameter, 50 cm long) driven into the soil profile to cut the roots and act as a barrier for root ingrowth. Another open-ended cylinder (20 cm diameter, 25 cm height) is placed directly on the soil surface above the 50 cm open-ended cylinder. A mesh collar (root + mycorrhiza excluded) is composed of an open-ended cylinder with nylon mesh windows to allow water to flow while preventing root and mycorrhiza ingrowth. Soil CO_2 efflux measured from the DC represents Rh, while the data obtained from the control collar represent Rs

The RE: DC insertion method

The DC insertion is another RE method but with a smaller spatial scale, which involves inserting rigid PVC or stainless steel cylinders into soils to sever roots and cut off carbon supply (Buchmann, 2000; Vogel & Valentine, 2005; Bond-Lamberty *et al.*, 2011; Heinemeyer *et al.*, 2012; ArchMiller & Samuelson, 2016; Jovani-Sancho *et al.*, 2018). The technique is considered a miniature version of Tr and only causes minor disturbances, as no soil digging is required (Subke *et al.*, 2006).

The cylinders function as soil collars, cutting roots, preventing root ingrowth, and providing soil surface contact to measure soil CO_2 fluxes (see Figure 1B). Varying stainless steel cylinder diameters and heights were reported in Rs partitioning studies in tropical peatlands and tropical forests (Takahashi *et al.*, 2011; Melling *et al.*, 2013; Batubara *et al.*, 2019). Moreover, aluminum nets with 1 mm mesh could be placed on the collar openings to prevent litter collection or soil disturbance by animals or mesofauna (da Costa *et al.*, 2018; Jovani-Sancho *et al.*, 2018).

A modified version of the DC approach, the RE with DC (RE:DC) insertion (with root removal), utilizes cylinders with perforated holes or wire mesh of different sizes to prevent root or root-associated mycorrhizal hyphae invasion while allowing moisture flow (Heinemeyer *et al.,* 2007; Wu *et al.*, 2014; Han *et al.*, 2021). Nonetheless, the soil structure is disturbed when employing this method due to the cutting into the soil with a deep collar, removing the soil for manual root removal, and then replacing the soil, as described by the researchers from the Global Ecosystem Monitoring (GEM) Network (Martthews *et al.,* 2014).

TABLE I

Summary of Rs partitioning studies using the RE: Tr/DC insertion approach. Studies quantifying methodological effects such as root decomposition (RD), soil water content (SWC), and applying corrections (Y = yes/ N = no) are mentioned

Ecosystem, Location	Vegetation Type	RE Method	D (cm) /Area	Depth (m)	Stabilization period after RE	Duration	RD (Y/N)	SWC (Y/N)	Correction (Y/N)	Reference
Hemiboreal forest, Estonia	Norway spruce	DC	D = 20	0.45	One month	Two years	Y	Y	Y	Kukumägi et al., 2017
Hemiboreal peatland, Finland	Scots-pine Forest	DC	D = 31.5	0.3	One year	Two years	N	N	N	Minkinnen et al., 2007
Temperate forest, Georgia	Longleaf pine	DC	D = 10	0.6	-	Four months	Y	Y	Y	Archmiller et al., 2016
Temperate forest, UK	Lodgepole pine	DC	D = 10	0.17	-	Nine months	Y	N	N	Heinemeyer et al., 2012
Temperate forest, Canada	Douglas-fir	DC	D = 10	0.5	Two weeks	One year three months	Y	Y	Y	Lalonde et al., 2007
Temperate forest, Sweden	Norway spruce	DC	D = 29.7	0.5	-	One year six months	Y	Y	Y	Comstedt et al., 2011
Tencperate peatland, Ireland	Afforested spruce, pine	DC	D = 15.4	0.3	Four months	One year eight months	N	N	N	Jovani-Sancho et al., 2018
Temperate peatland, UK	Peatland reserve	DC	D = 20	0.2	-	Nine months	Y	N	N	Heinemeyer et al., 2012
Temperate peatland, Finland	Afforested cropland	DC	D = 40	0.3	One year	Three years	N	Y	N	Makiranta et al., 2008
Temperate grassland, UK	Grassland	DC	D =10	0.2	-	Four months	Y	N	N	Heinemeyer et al., 2012
Semi-arid steppe, Mongolia	Grassland	DC	D = 10	0.4	One year	Two years	N	Y	Y	Zhang et al., 2019
Tropical peatland, Sarawak	Primary PSF	DC	D = 20	0.5	14 months	One year two months	N	N	N	Melling et al., 2013
Tropical peatland, Sarawak	OPP	DC	D = 20	0.5	14 months	One year two months	N	N	N	Melling et al., 2013
Tropical peatland, Sarawak	Sago Plantation	DC	D = 20	0.5	14 months	One year two months	N	N	N	Melling et al., 2013
Temperate forest, Japan	Broad-leaved oak	Tr	0.6m x 0.6m	0.4	-	16 months	Y	N	Y	Tomotsune et al., 2013
Temperate forest, USA	Loblolly pine	Tr	1m x 1m	0.45	Five months	Ten months	Y	Y	Y	Drake et al., 2012

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				D						
Temperate montane forest, Austria	Spruce-fir-beech	Tr	2m x 2m	0.6	-	15 months	Y	N	Y	Díaz-Pinés *et al.,* 2010
Temperate forest, Turkey	Oriental beech	Tr	3m x 3m	0.6	Six months	One year six months	Y	Y	Y	Akbas and Tufekcioglu, 2022
Temperate forest, USA	Mixed hardwood	Tr	5 m x 5 m	0.5	Five months	Two years	Y	Y	Y	Savage *et al.,* 2018
Temperate peatland, Scotland	Afforested spruce, pine	Tr	3.5 m x 1.5 m	0.4	Three months	Two years	Y	Y	Y	Hermans *et al.,* 2022
Tropical peatland, Sarawak	OPP	Tr	0..4 m x 0.4 m	0.8	Nine months	Two years	N	N	N	Ishikura *et al.,* 2018
Tropical peatland, Sarawak	Undrained PSF	Tr	0.4 m x 0.4 m	0.8	One year	Two years	N	Y	N	Ishikura *et al.,* 2019
Tropical peatland, Indonesia	Undrained & drained forest	Tr	1 m x 1 m	1.0	Five months	Two years three months	N	N	N	Itoh *et al.,* 2017
Tropical peatland, Kalimantan	Rubber plantation	Tr	1 m x 1 m	1.0	Six months	One year	N	N	N	Wakhid *et al.,* 2017
Tropical peatland, Kalimantan	Primary PSF	Tr	2 m x 2 m	1.0	One year	One year one month	N	N	N	Hergoualc'h *et al.,* 2017
Tropical peatland, Kalimantan	OPP (2 yo)	Tr	2 m x 2 m	1.0	One year	One year one month	N	N	N	Hergoualc'h *et al.,* 2017
Tropical peatland, Kalimantan	OPP (7 yo)	Tr	2 m x 2 m	1.0	One year	One year one month	N	N	N	Hergoualc'h *et al.,* 2017
Tropical peatland, Sumatra	OPP	Tr	2.5 m x 2.5 m	1.0	Six months	One year five months	Y	N	N	Comeau *et al.,* 2016

OPP = oil palm plantation; PSF = peat swamp forest; yo = year old; D = diameter of DC insert.

The RE:DC insertion method is more laborious as additional control treatments are necessary to account for potential soil handling and mixing disturbance effects. The first control treatment, termed 'disturbed', consists of collars inserted into holes, which are then refilled with the handled soil. Next, another row of collars is installed by hammering them into the soil. The step consists of no soil handling, termed 'undisturbed' (Martthews *et al.,* 2014). A detailed comparison of the RE methods (Tr, DC insertion with and without root removal) is listed in Table II.

TABLE II

The features of the RE by Tr and DC insertion (with and without root removal) approaches

Feature	RE method		
	Tr	DC insertion (without root removal)	DC insertion (with root removal)
Site installation	Digging the trench, placing back the soil	Inserting a DC into the soil	• Digging a hole, removing soil and roots, inserting DC, and placing the soil back • Additional soil disturbance control treatment
Disturbance to the soil structure	Great disturbance	Less disturbance (compaction)	Great disturbance (soil mixing and handling)
Amount of work	Large	Relatively small	Relatively small (more laborious than DC insertion)
Advantage	• Simple • Inexpensive	• Simple • Reduced installation time • Can increase replication • More accessible to environmental control (e.g., covers to reduce rain input/litterfall)	• No root decomposition bias • More accessible to environmental control (e.g., covers to reduce rain input/litterfall)
Disadvantage	• Laborious • Must be kept vegetation-free • Possibility of root ingrowth	Possibility of: • Soil compaction • Root ingrowth • Waterlogging within the collars (peatland and grassland)	Possibility of: • Soil compaction • Root ingrowth • Waterlogging within the collars (peatland and grassland)
Application	Various ecosystems	Various ecosystems	Various ecosystems

RE : Tr/DC insertion coupled with automated chamber measurements

Automated chambers are increasingly employed with the Tr technique to categorize Rs into Ra and Rh. Continuous hourly measurements of the technique enable diurnal and seasonal flux variation due to environmental fluctuations detections, which would be overlooked when a manual chamber is applied (Ishikura *et al.,* 2018; Savage *et al.,* 2018; Hoyt *et al.,* 2019; Ishikura *et al.,* 2019;). Hoyt *et al.* (2019) recorded the CO_2 efflux of peat surfaces in conjunction with a trench that was up to 30 cm deep hourly and found a strong diurnal cycle in CO_2 flux and near-surface peat temperature (< 10 cm) that both peaked at midday. The study documented that the diurnal oscillation magnitude was significantly impacted by shading and water table depth (WTD), denoting the limitation of relying on daytime assessments and/or a single correction factor to avoid daytime bias in flux evaluations. The mean daily Rh also exhibited a strong linear association with WTD, while under flooded environments, the Rh was small and constant (Hoyt *et al.,* 2019).

Several studies in tropical peatlands reported that Rh increased with lower WTD (Itoh *et al.,* 2017; Wakhid *et al.,* 2017; Husnain *et al.,* 2014). Nevertheless, one of the drawbacks of automated chambers is poor spatial representation due to power constraints (Savage & Davidson, 2003; Savage *et al.,* 2008) and missing data due to technical issues, such as power loss, which contributed 23% of data loss (Ishikura *et al.,* 2018). Consequently, Gana *et al.* (2016) recommend using manual and automated chamber measurements to compensate for spatial and temporal discrepancies.

CONSIDERATIONS WHEN UTILIZING THE RE: Tr/DC INSERTION TECHNIQUE

In this review, considerations denote the critical factors requiring contemplating when employing the RE method, which is discussed in this section. Moreover, assumptions in the present review refer to the reasons for accepting the measured data as correct or justifications of interpretations while working with obtained data.

Two sets of issues require attention when utilizing the RE: Tr/DC insertion method. First, pre-RE treatment installation and data interpretation post measurements. The RE has been established as causing soil disturbances and severed root decompositions, hence necessitating a stabilization period before procuring measurements. Furthermore, the possibility of root ingrowth into RE plots must be considered, especially if the experiment is prolonged.

The second set of challenges concerning the RE: Tr/DC approach is related to the data of the working experiment. The RE effects (summarized in Table III), such as soil disturbance, severed root decompositions, induction of rhizosphere priming effects (RPE), differences in soil water content, and shift in soil microbial communities could contribute to an elevated CO_2 emission in trenched plots compared to controls, hence leading to an overestimation of Rh. Consequently, this review discussed Rh data correctional steps in severed root decomposition cases. A well-planned experimental design and knowledge and understanding of the strengths and weaknesses of a method would allow the correct interpretation of generated data.

TABLE III

A summary of RE treatment effects (increasing or decreasing Rh) and its regulation

RE treatment effect	Rh (↗□ or ↘□)	Environmental or site-specific regulation
Soil disturbance (soil handling, compaction)	↗□	Increased CO_2 concentration and CO_2 emissions due to compaction were recorded in mineral and peat soils (Conlin & Van den Driessche, 2000; Busman *et al.,* 2021)
Lack of C-input from root exudates and root litter	↘□	-
Lack of root water uptake (increased soil moisture)	↗□ or ↘□	• Potentially more robust in sandy soils and weaker in soils with high water retention and low oxygen diffusivity • More pronounced in dry ecosystems
Residual root decomposition	↗□	Uncertainty in the decomposition of cut roots is site-specific & depends on soil environmental conditions & root biomass

		(Subke *et al.,* 2006; Ishikura *et al.,* 2019).
RPE	↗□	More prominent in C-rich, N-deficient environments, such as peatlands (Hart *et al.,* 1986; Kuzyakov *et al.,* 2000)
Microbial community shift	↗□ or ↘□	The relative contribution of microbial decomposers (fungi and bacteria) to the community differs between different soils in response to environmental factors (Strickland & Rousk, 2010)

Note: ↗□ = increase and ↘□ = decrease

Assumptions

Several assumptions are required in the Tr/DC insert approach to RE (Hanson *et al.,* 2000; Kuzyakov, 2006; Subke *et al.,* 2006; Epron *et al.,* 2009). First, it is assumed that Tr/DC insertion immediately eliminates Rr (Lee *et al.,* 2003); thus, soil CO_2 efflux from Tr/DC insertion plots only represents microbial Rh, while the control (root-intact) plots are attributable to Rs. Accordingly, the Ra is the respiration rate difference between the control and RE plots. Assessing baseline soil CO_2 efflux pre-RE treatment would also ensure no spatial variance between the Tr/DC insertion and control plots and allow soil CO_2 efflux in RE plots to be attributed to the absence of Ra (Epron, 2009).

The second conjecture in the TR/Dc insert approach to RE is that the decomposition of severed roots affect and alter the Rh in the treated plots. During the first few months post trenching, fine roots that decompose quickly would temporarily increase the soil CO2 efflux, while coarse roots that decompose slower would promote soil CO_2 efflux over a more extended time. The time needed for all roots to degrade is unclear and depends on the initial root biomass (Epron, 2009). The RE: Tr/DC insertion approach also presumes that the Rh would not be affected by the lack of C-input from root exudates and litter/residue decomposition.

Soil disturbance

Inserting collars inevitably disturbs soil structure, leading to increased CO_2 emission. In a 15-month RE study, the insertion of 10-cm-diameter cylinders resulted in consistently higher mean respiration rates inside the cylinder than outside (Vogel & Valentine, 2005). The elevated CO_2 emission was attributed to a greater soil bulk density due to soil compaction (Lalonde & Prescott, 2007).

A field study demonstrated that mineral soil compaction increased CO_2 concentration (Conlin &Van den Driessche, 2000), while a laboratory incubation study linked compacted tropical peat soil to a higher CO_2 efflux rate (Busman *et al.*, 2021). Accordingly, soil disturbance should be accounted for, or measurements must be postponed until the soil system stabilizes.

Typically, Tr/DC insertion plots are left in the ground for one to two years to allow the soil to stabilize from the effects of soil disturbance and severed root decomposition before commencing soil CO_2 efflux measurements (Boone *et al.*, 1998; Sulzman *et al.*, 2005; Sayer and Tanner, 2010; Kukumägi *et al.*, 2017; Jiao and Wang, 2018). Nevertheless, data taken years after Tr might underestimate the Rh to total Rs ratio due to a lack of C input from litter (Subke *et al.*, 2006). Consequently, Lang *et al.* (2021) established a disturbance plot similar to an RE plot but without the root ingrowth barriers and control plots to account for the initial pulse of CO_2 from soil disturbance, root decomposition, and increased soil moisture.

Decomposition of severed roots

The time required for the severed roots from RE plot installations to stop respiring and start decomposing is uncertain (Epron, 2009). Severed roots could continue respiring for several months to a year post-Tr by utilizing their carbohydrate reserves (Uchida *et al.*, 1998; Lee *et al.*, 2003; Aubrey and Teskey, 2018). For example, the severed roots in a montane forest were still respiring five months after Tr, which stopped entirely by the tenth month (Díaz-Pinés *et al.*, 2010).

Most available literature reported that the decomposition of recently severed roots, whether in temperate forests on mineral soil (Ewel *et al.*, 1987; Kelting *et al.*, 1998; Hanson *et al.*, 2000; Lee *et al.*, 2003; Fan *et al.*, 2015, Archmiller and Samuelson, 2016) or tropical peatlands (Melling *et al.*, 2013;

Hergoualc'h *et al.*, 2017; Ishikura *et al.*, 2018), occur between the first two months to a year. Conversely, a few studies found that trenched plots documented significantly increased respiration rates than controls, even after a one to two-year stabilization period, which was often attributed to severed root decomposition (Kukumägi *et al.*, 2017; Savage *et al.*, 2018; Ishikura *et al.*, 2019).

Dead root respiration was still detected two years after a trench was installed in a temperate beech and oak forest and would have likely lasted longer if the study had been extended (Savage *et al.*, 2018). Similarly, a one-year stabilization interval after Tr did not prevent the overestimation of Rh in an undrained tropical peatland forest due to high groundwater levels that hampered dead root breakdown. The inconsistencies in the decomposition of severed roots might be due to site-specificity and dependent on soil environmental conditions and root biomass (Subke *et al.,* 2006; Ishikura *et al.,* 2019).

An increasing number of reports have indicated that the CO_2 released from recently severed roots were substantial and corrected for it by evaluating root biomass decomposition in trenched plots (Ngao *et al.,* 2007; Díaz-Pinés *et al.,* 2010; Varik *et al.,* 2015; Kukumägi *et al.,* 2017; Hermans *et al.,* 2022). Methods for estimating root biomass decomposition include the mesh bag (Hermans *et al.,* 2022), the root ingrowth core (Varik *et al.,* 2015), and the cellulose filter as a proxy (Valentine & Vogel, 2005) approaches.

The initial coarse (over 2 mm) and fine (under 2 mm) and final root biomasses obtained at the end of the experiment are required (Hermans *et al.,* 2022) for CO_2 data corrections. The CO_2 efflux from the decomposition of severed roots (different size classes) could then be calculated with Equation 1 (Ngao *et al*. 2007). The M_1 in the equation could be either measured or calculated by assuming a simple exponential decay function (see Equation 2). Data fits are performed separately for varying root size classes. Accordingly, the CO_2 efflux from severed roots (R_D) decomposition could be subtracted from the CO_2 efflux from trenched plots to obtain the corrected Rh (see Equation 3).

$$R_D = (1 - \alpha_R)(M_0 - M_1) \qquad \text{(Eq. 1)}$$

$$M_1 = M_0 e^{-kt} \qquad \text{(Eq. 2)}$$

$$Rh_{corrected} = Rh_{trenched} - R_D \qquad \text{(Eq. 3)}$$

where α_R represents microbial efficiency, which is the C decomposition constituent during total root C loss and the portion included in soil organic matter, thus the fraction lost as CO_2 is $(1 - \alpha_R)$, M_0 denotes the initial root biomass (g C m^{-2}), M_1 is the remaining root biomass after collection from the field, k denotes the decay constant, and t is the time over which decay was measured.

Hermans *et al*. (2022) documented that the correction of CO_2 efflux from severed root decomposition reduced the heterotrophic contribution from 61 to 38% (a difference of 23%), suggesting that severed root biomass decaying contributes significantly to soil CO_2 efflux two years post trenching. In other studies, CO_2 efflux corrections from fine root decomposition reduced the Rh by up to 14% (Díaz-Pinés *et al.,* 2010) and 15–20% (Kukumägi *et al.,* 2017). Nevertheless, further root turnover corrections increased the Rh by 9–13%, resulting in a 5–8% net reduction. Table IV lists reports of higher soil CO_2 emission in RE plots compared to control plots due to methodological effects and the corrections applied.

Sayer and Tanner (2010) recommended measuring Rs before and immediately after Tr (within seven days) for one occasion measurements as it provides the most accurate estimate of Rh (when Rs was 38% lower in the Tr plots than in the control plots) as the Rs in Tr plots would rise to pre-trenching levels for the next seven months due to severed root decompositions. Moreover, high replication and staggered one-occasion Rs measurements of DC insertion treatments might enable obtaining information on seasonal Rh variations and in response to management regimes (Sayer & Tanner, 2010; ArchMiller & Samuelson*,* 2016; Brown & Markewitz, 2018).

TABLE IV

Summary of studies reporting higher soil CO_2 emission in RE compared to control plots due to methodological effects and the corrections applied

Ecosystem, location	Vegetation	Study duration	Effect of root exclusion	Corrections for RD/SWC/SMT	Reference
Temperate forest, Canada	Douglas-fir	15 months	Higher mean CO_2 efflux in DC insertion throughout the study due to the higher soil BD (due to compaction).	-	Lalonde & Prescott, 2007
Montane forest, Austria	Spruce-fir-beech	15 months	Differences in fine root biomass between the trenched and control plots were approximately 30%	RD: Rh reduced by up to 14%	Díaz-Pinés et al., 2010
Temperate forest, Sweden	Norway spruce	18 months	45% of the respiration in the trenched plots was an artifact of the method	SWC: Rh reduced by 23% RD: Rh reduced by 15%	Comstedt et al., 2011
Hemiboreal forest, Estonia	Silver birch	17 months	SWC was up to 30% higher in the trenched plots compared to the control plots	SWC: Rh/Rs ratio decreased from 13% to 33%	Varik et al., 2015
Hemiboreal forest, Estonia	Norway spruce	Four years	SWC increased by 40% in the trenched plots compared to the control plot Soil pH was higher in the trenched plot compared to the control (pole and mature stand)	SWC: Rh reduced by 23%. RD: Rh reduced by 15–20% Fine root turnover: Rh increased by 9–13% The overall effect of the corrections: Rh decreased 5–8%	Kukumägi et al., 2017
Temperate deciduous forest	Mixed hardwood	Five months	CO_2 efflux in trenched plots exceeded control by 18% (early spring) and 40% (autumn), leading to negative root respiration	RD & SMT: Rh reduced by 11% (2013) and 9% (2014)	Savage et al., 2018
Tropical peatland, Sumatra	Oil palm plantation	Six months	Higher CO_2 efflux in trenched plots with nitrogen fertilization compared to control plots, attributable to the positive priming effect	-	Comeau et al., 2016
Tropical peatland, Sarawak	Undrained PSF	One year	CO_2 efflux in trenched plots exceeded those in control plots for five months despite a stabilization period of one year, attributed to decomposing roots	-	Ishikura et al., 2019

Note: SMT: soil moisture and temperature, SWC: soil water content, RD: residual root decomposition, BD: bulk density, Rh: heterotrophic respiration, Rs: total soil respiration and PSF: Peat Swamp Forest

Root ingrowth

Additional soil CO_2 flux could arise from root invasion from beneath lined trenches (Sayer & Tanner, 2010), depending on the duration of the experiment. Nevertheless, the effect is influenced by

root growth rate and is unlikely to lead to additional CO_2 efflux during a short-term (1–2 years) experiment. For instance, DC inserts evaluated at the end of an RE experiment (1 year 8 months) documented no evidence of root ingrowth (Jovani-Sancho *et al.*, 2018). Conversely, a 20-year RE study recorded root ingrowths in trenched plots (Bowden *et al.*, 2014). Ryhti *et al.* (2021) argued that since pioneer pine roots grow 2–5 cm yr^{-1} (Ding *et al.,* 2021) and that the distance between the trench and the soil collar is over 30 cm, root ingrowths under the mesh fabric of the trenched plots of a boreal forest site in Finland was unlikely to enhance soil CO_2 emissions during the experiment.

Differences in soil moisture in the RE plots

The RE by Tr/ DC insertion could result in differences in soil water content in root-excluded plots due to the inability of severed roots to absorb moisture (Kuzyakov, 2006; Subke *et al.*, 2006). The phenomenon could be exacerbated if the trenched plots are lined with a non-permeable material to prevent root ingrowths (Bond-Lamberty *et al.*, 2011). Increased soil moisture in trenched plots might also elevate Rh as soil moisture is crucial for microbial respiration (Yan *et al.*, 2018). Accordingly, the effect might be more robust in sandy soils and weaker in high water retention and low oxygen diffusivity soils (Epron, 2009). For example, trenched plots in a temperate beech forest in the summer and early autumn recorded up to twice the moisture content of control plots, hence resulting in overestimated Rh (Epron *et al.*, 1999).

Soil moisture differences in trenched plots are more pronounced in dry ecosystems (Hanson *et al.*, 2000; Baldocchi *et al.*, 2006) or during droughts (Savage *et al.*, 2018). For example, a study that installed a DC in a semi-arid grassland in Mongolia observed increased soil moisture content by 37% compared to the moisture outside the collar (Zhang *et al.*, 2019). Meanwhile, water-filled pore space values in trenched and control plots remained consistent throughout a two-year study conducted in an oil palm plantation on a tropical peatland (Ishikura *et al.*, 2018). The Rs was also unaffected despite higher soil water content in trenched plots compared to the control plot in afforested organic soil croplands (Mäkiranta *et al.*, 2008) and a Japanese larch plantation (Qu *et al.*, 2018). Consequently, monitoring soil moisture contents in trenched and control plots during Tr experiments is essential to develop empirical models that could account for the differences between the two groups (Epron, 2009). Nonetheless, only a few studies have considered the artifact in their Rs partitioning investigations (Ngao *et al.*, 2007; Comstedt *et al.*, 2011; Drake *et al.*, 2012; Kukumägi *et al.*, 2017; Zhang *et al.*, 2019).

Rhizosphere priming effects

The priming effect (PE) is defined as significant short-term alterations in SOM turnover resulting from relatively mild soil treatments (Kuzyakov *et al.*, 2000), including the addition of plant residues, dead microbial biomass, and organic and mineral nitrogen (N) fertilizers (Kuzyakov *et al.,* 2000; Kuzyakov, 2002; Kuzyakov, 2010). The rhizosphere priming effect (RPE) describes the PE occurring in rhizospheres (Kuzyakov, 2002). Root exclusion via trenching or DC insertions could induce a positive RPE by adding C-input from decaying roots (Cheng, 2009; Lajtha *et al.,* 2018). The factor could lead to a more notable soil CO_2 efflux in Tr/DC insertion treated compared to control plots, while the absence of a root system in Tr/DC areas reduces Rh due to the lack of soil priming (Cheng, 2009).

A short-term study to quantify the Rh of an oil palm plantation in a tropical peatland post N fertilization found that the soil CO_2 efflux was higher in the N-fertilized Tr plot than the total Rs of the control plot (non-trenched). The higher respiration rates were attributed to positive RPE, where the excess inorganic N in the Tr plots excluded from roots stimulated SOM decomposition and mineralization (Comeau *et al.*, 2016). The results were consistent with a previous report that recorded a more prominent RPE in C-rich, N-deficient environments, such as peatlands (Hart *et al.*, 1986; Kuzyakov *et al.*, 2000).

The N-fertilization in tropical peatlands significantly increased CO_2 emissions (Hatano 2019), considering that N fertilizers not only influence the N pool in the soil but could also impact the C equilibrium in agricultural ecosystems by directly influencing the plant and microbial elements (Russell *et al.* 2009; Mahal *et al.* 2019). Moreover, some soil CO_2 efflux measurements were higher in N fertilizer-treated Tr plots than in a non-trenched plot in a temperate loblolly pine forest, resulting in

negative Ra during a drought. Nonetheless, the unexpected findings were attributed to spatial and temporal variabilities, which recorded occasional low soil CO_2 efflux from a root-intact location paired with a high soil CO_2 efflux from a trenched location (Drake *et al.*, 2012).

While RPE was demonstrated in RE plots with N-fertilizers, the effect only lasted a few days following the fertilizer treatments (Comeau *et al.,* 2016). Over a short term (days to weeks), RPE is primarily relevant to the dynamic change of labile SOM. Nevertheless, the impact of RPE over a longer term (months to decades) on stabilized SOM remains unclear due to the scarcity of long-term experiments (Huo *et al.,* 2017).

The effects of RE on soil microbial communities

Despite the importance of soil microorganisms in SOM decomposition and CO_2 production (Cleveland *et al.*, 2007), the impacts of the RE approach on soil microbial populations have received little attention (Diaz-Pinés *et al.,* 2010; Wei *et al.,* 2015). The RE technique increases C supply through severed root decomposition, with potential impacts on the structure of the microbial communities and, therefore, heterotrophic respiration (Brant *et al.,* 2006; Wu *et al.,* 2019).

A few studies have attempted to elucidate Rh connection with soil microorganisms by correlating field-measured CO_2 emissions with soil microbial parameters (Kutsch *et al.*, 2010), such as microbial biomass, extracellular enzyme activities, and microbial diversity and community structures (abundance and compositions) (Wei *et al.,* 2015; Borden *et al.,* 2021). Traditionally, soil microbial biomass is adopted as a simple means to quantify soil microorganisms as a single entity (Gonzalez-Quiñones *et al.,* 2011) that plays essential roles in organic matter decomposition and nutrient mineralization. In other words, the microbial biomass is the main catalyst of soil biogeochemical processes and an energy and nutrient supply (Tate, 2020). Nonetheless, the 'black box' approach disallows particular microbial species distinctions (Nannipieri *et al.,* 2020).

Extracellular enzymes catalyze organic matter breakdown and mineralization and are commonly analyzed as a response to changing environmental conditions (Kotrozco *et al.,* 2014; Acosta-Martinez *et al.,* 2018). Furthermore, microbial diversity and community structure are essential regulators of various ecosystem functions (Wagg *et al.,* 2014; Delgado-Baquerizo *et al.,* 2016). Changes in microbial diversity (Tardy *et al.,* 2015) and community compositions (Rieke *et al.,* 2022) significantly influenced carbon mineralization in soil, while empirical data revealed that microbial taxonomic and functional attributes could predict soil CO_2 fluxes (Liu *et al.,* 2018; Liu *et al.,* 2020; Rieke *et al.,* 2022).

A positive correlation was observed between soil CO_2 emission and soil microbial biomass in urban woody and grassy soils of Russia (Sushko *et al.,* 2018) and a forest after fire disturbance (Holden & Treseder, 2013). Nevertheless, some reports recorded that soil microbial biomass could increase, remain unaffected, or decrease post-root exclusion. For instance, Wei *et al.* (2015) observed enhanced microbial respiration in trenched plots with high soil microbial biomass in subtropical forests in China, but only in the warm season. In contrast, an RE by Tr in a montane forest exhibited minor effects on microbial biomass after one year, with a slight increase in bacteria five months later, which could be attributed to the beginning of fine root decomposition (Díaz-Pinés *et al.*, 2010).

In a study, a section of a detritus input removal treatment (DIRT) revealed that soil microbial biomass diminished 1–3 years after Tr, depending on the region (Bluhm *et al.,* 2019). The RE treated compared to the control documented lower biomass of Gram-positive and -negative bacteria and fungi and reduced enzyme activities and soil C and N concentrations (Zhu *et al.,* 2021). In a recent Rs partitioning investigation of a riparian agrosystem, Borden *et al.* (2021) reported that microbial diversity was strongly correlated with Rh, however no changes were observed in the abundance and activities of total bacteria and fungi between the trenched and control plots.

The oil microbial community structure determined by phospholipid fatty acid (PLFA) analysis recorded alterations after a year of RE. Nevertheless, no difference between the trenched and the control plots was observed after three years (Bluhm *et al.*, 2019). Another study found that variations in CO_2 effluxes between short-term (nine months) DC insertion treatments (RE and root + mycorrhizal exclusion) of a temperate grassland were not associated with microbial diversity and community structure. Both reports suggested that the microbial communities at the study sites were resilient to short-term soil disturbances (Allison and Martiny, 2008; Heinemeyer *et al.,* 2012).

The effects of trenching on soil microbial communities could elevate over time. For instance, soil enzyme dehydrogenase activities and soil pH of a hemiboreal forest in Estonia were significantly higher in the trenched compared to control plots after four years of trenching, which affected microbial activities and community compositions (Wang *et al.*, 2019). Kukumägi *et al.* (2017) also reported that the O-horizon was more decomposed in the trenched than in control areas. Consequently, long-term RE by Tr/DC insertion (over two years) is not recommended due to a structural shift in the microbial communities, leading to an enhanced Rh reduction (Kukumägi *et al.*, 2017). A summary of the effects of the RE method on the soil microbial properties relevant to CO_2 emissions is presented in Table V.

Overall, the low correlation between soil microbial properties and increased CO_2 emissions in RE: Tr/DC insertion plots might be due to the scarcity of studies that employ coarse-scale methods of profiling microbial communities. Accordingly, more investigations that utilize available modern molecular approaches (e.g., metatranscriptomics, mRNA-stable isotope probing) are necessary to discover the direct link between CO_2 flux in root-excluded plots to active soil microbial activities at a higher taxonomic resolution than the total microbial community PLFA (Kwon *et al.*, 2019; Nannipieri *et al.*, 2020).

Microbial carbon use (CUE) is becoming a critical microbial parameter to determine if a proportion of mineralized C is respired as CO_2 or assimilated into microbial biomass (Manzoni *et al.*, 2012; Blagodatskaya *et al.*, 2014; Geyer *et al.*, 2016). Since soil microbes with lower CUE are less efficient at incorporating C into biomass, they might possess higher Rh. Alternatively, a higher CUE indicates more plant C is retained as SOM (Manzoni *et al.*, 2012). Consequently, further exploration of microbial community compositions combined with microbial CUE might better interpret higher CO_2 emissions in trenched than control plots, as reported in some studies.

TABLE V

The effects of the RE method on the soil microbial properties relevant to CO_2 emissions

Ecosystem, location	Vegetation	Measurement	Method	Finding	Reference
Montane forest, Austria	Spruce-fir-beech	MCS	PLFA	Soil microbial biomass and MCS were not altered by the end of the second growing season	Díaz-Pinés *et al.*, 2010
Temperate grassland, UK	Grassland	Microbial Diversity	DGGE	Differences in CO_2 emissions among collar treatments were not linked to the diversity and compositions of the bacterial community	Heinmeyer *et al.*, 2012
		Microbial Biomass C	CFE	No significant effects on overall biomass. Nonetheless, repeated disturbance from cutting resulted in organic matter decaying, which altered the microbial C pool and C-use efficiency, and thus the soil CO_2 emission rates	
Hemiboreal forest, Estonia	Norway spruce	Dehydrogenase	Soil enzyme activity	Higher soil enzyme (dehydrogenase) activity and well-decomposed O-horizons in trenched plots compared to control	Kukumägi *et al.*, 2017
		Microbial biomass	SIR	No significant differences in microbial biomass between trenched and control plots	
Deciduous Forest, Germany	Norway spruce, Scots pine, beech	Microbial biomass	SIR	The soil microbial biomass was significantly reduced in trenched plots, after one and three years, depending on the site	Bluhm *et al.*, 2019
		MCS	PLFA	Soil MCS was affected after one year of Tr but recovered within	

				three years	
Riparian agroecosyste ms, Canada	Grass, forest (hardwood, coniferous, mixed)	Microbial diversity	NGS	Microbial diversity showed a stronger correlation with Rh	Borden *et al.,* 2021
		16S rRNA and 18S rRNA genes and transcripts abundance	qPCR and RT-qPC R	Total bacteria and fungi abundance and activity were similar between the control and RE sub-plots	

Note: MCS: microbial community structure, PLFA: phospholipid fatty acid analysis, DGGE: denaturing gradient gel electrophoresis, CFE: chloroform-fumigation extraction; SIR: substrate-induced respiration, qPCR: quantitative polymerase chain reaction, and RT-qPCR: reverse transcriptase quantitative polymerase chain reaction.

COMBINATIONS OF METHODS FOR PARTITIONING Rs

Earlier reviews concluded that no perfect soil respiration partitioning method is available, each having its strengths and weaknesses (Hanson *et al.,* 2000; Kuzyakov *et al.,* 2006; Subke *et al.,* 2006). A study supported the hypothesis by comparing five different soil respiration partitioning techniques in a subtropical secondary forest. The report revealed that no individual approach was satisfactory (Comeau *et al.,* 2018).

Combining two or more methods has been suggested to assess the autotrophic and heterotrophic components of total soil respiration to overcome measurement-specific limitations. In particular, isotopic approaches are considered the most reliable for estimating CO_2 emissions from SOM decompositions (Hanson *et al.,* 2000; Baggs, 2006; Kuzyakov, 2006), given that they result in minimum disturbance to soil structure. Nonetheless, only a few investigations have attempted to combine RE and isotopic methods for Rs partitioning evaluations (^{13}C-labeling and ^{13}C-natural abundance) (Biasi *et al.,* 2012; Risk *et al.,* 2012; Balogh *et al.,* 2016; Zhao *et al.,* 2017).

Modeling techniques have been combined with RE approaches to simulate soil respiration and its components. Modeling methods include statistical models of Rs component responses to environmental variables (Brown & Markewitz, 2018) and process-based biogeochemical models (Wang *et al.,* 2011; Dondini *et al.,* 2017; Robert & Markewitz, 2018). In the subsequent sections of this review, stable isotope-based and combination with RE approaches are briefly described.

Isotopic methods

Quantifying below-ground C due to a translocation between plant and soil is challenging and leads to a total C input to the soil underestimation (Kuzyakov *et al.,* 2001; Bromand *et al.,* 2001). For decades, isotopic techniques have been employed to estimate the relative contribution of plant roots and soil organic matter decompositions to soil respiration. Radioactive ^{14}C or stable ^{13}C is utilized as a tracer of the origin of soil CO_2 efflux in isotopic methods. Several isotopic techniques incorporating various principles were introduced by Kuzyakov (2006), which also highlighted the influence of environmental conditions on the methods chosen.

Separation of heterotrophic respiration from autotrophic respiration *in situ* through isotopic approaches provides quantitative data with the slightest disturbance to the soils and roots and avoids the assumptions of equilibrium in the soil C pool (Hanson *et al.,* 2000; Sakata *et al.,* 2007). Nonetheless, the primary disadvantage of the method is the cost and complexity of the experimental setup, which involves complex analytical instrumentation for radioactive or stable C isotopes (Hanson *et al.,* 2000; Biasi *et al.,* 2012). Furthermore, the partitioning of some soil-plant systems through isotopic methods is limited to the transition of the C_3 to the C_4 ecosystem or *vice versa* (Rochette *et al.,* 1999).

The isotopic technique is classified into pulse, repeated pulse, and continuous labelings, where depending on the mass balance degree, the methods would yield variations of plant C allocations and contributions of root respiration to soil CO_2 efflux (Hanson *et al.,* 2000). Pulse labeling or repeated pulse labeling could be employed to assess the influence of recent incorporation of C on total soil CO_2 efflux (Kuzyakov *et al.,* 2001). Nonetheless, Kuzyakov and Bol (2004) questioned the accuracy of the data obtained through pulse labeling with ^{13}C natural abundance. The concern in the review by Kuzyakov and Bol (2004) was due to the background variation of δ^{13} values, which recorded 10–15%

errors, substance choices, such as slurry and sugar with appropriate decomposition rate, the isotopic discrimination by CO_2 production from different sources were assumed negligible, and the correspondence of the δ^{13} values between two C_3/C_4 sources to calculate the contribution of the third C_3/C_4 source.

Kuzyakov and Larionova (2005) suggested four methods of partitioning root-derived CO_2 effluxes with pulse labeling technique; isotope dilution, model rhizodeposition, $^{14}CO_2$ dynamic efflux modeling, and exudate elution. The study found that the isotope dilution and $^{14}CO_2$ efflux dynamic modeling techniques recorded similar R_R and R_H estimations, while the model rhizodeposition and exudate elution approaches over- and underestimated the R_r and R_h. The report concluded that CO_2 δ^{13} and microbial biomass were the best methods when plants were continuously labeled in a $^{13}CO_2$ or $^{14}CO_2$ atmosphere.

Continuous labeling with $^{14}CO_2$ or $^{13}CO_2$ is commonly employed under laboratory or field conditions comparable to the life span of a plant (Hanson *et al.,* 2000) since plants are continuously exposed to the atmosphere at a fixed ^{14}C or ^{13}C ratio. Consequently, with a known value of the newly assimilated amount of carbon in the atmosphere, continuous labeling is more suitable for total C transferred by plants to soil estimations and belowground C pools labeling and investigating the soil organic C losses, as CO_2 is being compensated by root C input (Meharg, 1994; Kuzyakov, 2006). Nevertheless, continuous labeling under field parameters was deemed unsuitable, considering the requirement for maintaining a constant isotope ratio over an extended time and controlling the temperature and moisture parameters within the labeling chamber is complex and costly (Kuzyakov, 2006).

The ^{13}C natural abundance technique is another isotopic approach that offers more advantages over the other methods, considering that all C pools in plants are labelled. Moreover, the method is non-invasive and does not involve handling radioactive materials (Rochette *et al.,* 1999). The ^{13}C natural abundance technique is commonly applied to growing C_4 plants on C_3 soil, or *vice versa* and is estimated based on the $\delta^{13}C$ value of the soil CO_2 (Kuzyakov, 2006). Free air CO_2 enrichment (FACE) is another isotopic technique paired with a shifted C isotopic composition of added CO_2 that generates differences in the $\delta^{13}C$ of roots- and SOM-evolved CO_2 (Søe *et al.*, 2004; Kuzyakov, 2006). Nevertheless, the FACE experiment is only suitable for plants grown at CO_2 concentrations higher than the present atmosphere.

The RE and stable isotopic methods

Biasi *et al.* (2012) examined an isotope technique based on ^{13}C pulse-chase labeling with the conventional root trenching approach to assess the fractional contribution of Rh to overall Rs in Finland boreal peatlands cultivated with reed canary grass. The two-pool isotope mixing model (Hanson *et al.,* 2000) employed to separate respiration sources revealed that 50% of the total CO_2 originated from Rh compared to 70% through a root trenching experiment, which was possibly overestimated. Nevertheless, the study cautioned that as root cutting was necessary to obtain the $\delta^{13}C$ values of autotrophic respiration, the method was not entirely non-invasive. Consequently, further studies are required to demonstrate the potential of ^{13}C pulse labeling.

Utilizing more than one experimental technique to evaluate soil C losses from grasslands is preferable. For example, a study employing trenching and isotopic methods based on ^{13}C natural abundance to separate Rs into autotrophic and heterotrophic factors in a *Robinia pseudoacacia* L. plantation in southern Taihang mountains, China, recorded that the contribution of Ra in either approach was similar. Surprisingly, the report suggested that the experimental error produced by the trenching approach was insignificant compared to the isotopic technique (Zhao *et al.,* 2017).

A comparative study of RE and isotopic approach in dry grassland by Balogh *et al.* (2016) found that the measured CO_2 effluxes and the isotopic signals documented similar results regarding component responses, with the Ra recording the most significant decrease in response to drought and the relative contribution of Rh to Rs rose during soil drying and was the highest during drought. A combination of various approaches might reduce uncertainties in estimating Rs component contributions (Risk *et al.,* 2012). Accordingly, $\delta^{13}C$ stable isotopic signal and soil CO2 efflux in RE plots measurements to estimate the relative contributions of soil respiration constituents necessitates more attention in future investigations.

Wang *et al.* (2011) combined Ra and Rh field observations obtained via the RE method with a process-based FOREST-DNDC model to predict soil respiration and its elements from a subtropical conifer forest in China. The findings demonstrated that, although there was a slight underestimation of the simulated Ra value due to overestimation of the field-measured Ra values, the simulated Rs and components were comparable to the results from the RE method.

Dondini *et al.*. (2017) compared monthly Rh values simulated by the ECOSSE model with those obtained from automated chamber measurements on RE plots and discovered a considerably strong association between them. The correlation coefficients reported ranged from 0.81 to 0.96 for three vegetations. Nonetheless, the model underestimated the flux values during spring and summer (warm weather). The study concluded that the ECOSSE model combined with continuous measurements of Rh over RE plots aids in evaluating the performance of models that simulate Rh at site levels.

A process-based biogeochemical model, DAYCENT, combined with RE, was employed to quantify the proportion of Rh to Rs in southern loblolly pine plantations over an annual cycle. The findings revealed that the technique performed poorly compared to statistical models that utilized soil characteristics, such as microbial biomass, temperature, and moisture, which explained 75% of the Rh variability. Furthermore, the DAYCENT model only simulated CO_2 efflux of the top 20 cm of soil and did not account for Ra from the roots below the depth, which are drawbacks of the approach. Consequently, this model might work in an agricultural system but would perform poorly in pine plantations and larger forest ecosystems (Robert & Markewitz, 2018).

Combining RE with different modelling approaches demonstrated comparable, underestimated, or differed significantly simulated and measured Rh. Nevertheless, the RE and modelling combinations could offer the most precise method for estimating Rs and its components, as isotopic methods could be costly, time-consuming, and inappropriate in all ecosystems and study sites.

CONCLUSION AND RECOMMENDATIONS FOR FUTURE RESEARCH

Although the RE method is simple, its methodological concerns are frequently overlooked, resulting in biased estimates of autotrophic and heterotrophic Rs contributions. Methodological issues also influenced the Tr and DC insertion procedures. Nonetheless, the DC insertion technique is advantageous in terms of lower soil disturbance effects and greater control of environmental variation due to the small collars. Conversely, DC insertions increase soil compaction risk, thus leading to increased respiration rates.

The higher overall CO_2 emissions in trenched compared to control plots are attributable to residual root decomposition, soil moisture differences, and RPE resulting from enhanced C-inputs from dead roots. Although a few studies revealed a weak correlation between soil microbial properties and increased CO_2 emissions, changes in microbial communities should not be ruled out. Nevertheless, the RE method is still preferred for soil respiration partitioning studies due to its simplicity, provided that methodological effects are accounted for a more accurate data acquisition. Consequently, this review recommends the following improvements to the Rs partitioning method.

1. Studies involving Rs partitioning utilizing the RE: Tr/DC insertion method should consider methodological effects, such as the decomposition of cut roots and differences in soil moisture in trenched plots, to estimate the Rs components accurately (Savage *et al.,* 2018).

2. The Rs component results require interpretation with caution due to the possibility of positive RPE influence when employing the Tr method with N addition. More long-term studies are required to determine the combined effects of Tr and N fertilization on soil CO_2 emissions, especially in ecosystems with low N levels. (Comeau *et al.,* 2016).

3. Long-term (over two years) RE: Tr/DC insertion is not recommended due to microbial community structural shift, which resulted in increased or decreased Rh (Kukumägi *et al.,* 2017). Furthermore, the possibility of root reinvasion from the outside trenched plot through the root barrier or below the trenched plot could lead to an overestimated Rh (Borden *et al.,* 2014; Savage *et al.,* 2018).

4. More studies on the active soil microbial community response to Tr effects/plant input removal, their species composition in various ecosystems, and vegetation cover are necessary. In particular, assays in microbial community composition with C use efficiency are needed to explain the presence of higher Rh in trenched plots compared to non-trenched plots.

5. A minimum of two methodological approaches to Rs partitioning investigations (such as combining the RE method with isotopic modelling approaches) are preferable to compensate for the methodological bias of a single strategy and, thus, improve confidence in result interpretations. Furthermore, the Rh measurements over RE plots with automated and manual chambers enable the detection of diurnal and seasonal flux variations while accounting for spatial differences.

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