



Soil organic carbon stability under natural and anthropogenic-induced perturbations

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ABSTRACT

Soil organic carbon (SOC) plays key roles in determining soil properties, plant nutrients, and land-atmosphere carbon exchange, yet is affected by various natural and anthropogenic perturbations such as wildfire, climate change, land use change and pollution. Herein, we review perturbation types and SOC response mechanisms, as well as new technologies to measure SOC stability, highlighting the resistance and resilience of SOC. Although there is great controversy on the nature and structure of SOC, it has been recognized that the SOC stability not only depends on physicochemical characteristics of SOC, but also on soil microenvironment, soil microbial community composition, and types of perturbations. Based on ecosystem stability theories, the change of SOC stability under perturbation can be keeping stable states, changing to an alternative stable states or system collapse. For future studies, we suggest that understanding responses of soil biogeochemical processes to multiple perturbations, well-designed experiments to quantify threshold and uncertainty of SOC, and integration of novel biological approaches besides physicochemical characterizations into SOC stability are needed. This review helps to better understand the process of SOC stabilization and reduce the uncertainties in assessments of global carbon stocks, not only for mitigating the effects of climate change through negative feedbacks but also for maintaining soil functions such as soil fertility, water quality, and resistance to erosion.

1. Introduction: soil organic carbon stability and perturbation

Soil is the largest terrestrial ecosystem carbon pool, where accumulation and stability of organic carbon contributes to soil quality and fertility (Shahriari et al., 2011; Six et al., 2000). Soil organic carbon (SOC) thus plays key roles in determining soil properties, plant nutrients, and land-atmosphere carbon exchange, yet is affected by various natural and anthropogenic perturbations such as wildfire, climate change, land use change or soil pollution. Attributed to the importance of soil carbon in the global carbon cycle and potential feedbacks to climate change, how SOC responds to perturbations is of great interest (Falahatkar et al., 2014; Sistla et al., 2013; Yang et al., 2018a).

Soil organic C stability is defined as the tendency of organic carbon in soil to resist change and/or loss (Doetterl et al., 2016). Recent research on SOC stability has concentrated mainly on the response of SOC in different ecosystems to warming (Davidson and Janssens, 2006), increased atmospheric CO₂ (van Groenigen et al., 2011), nitrogen addition (Neff et al., 2002), and climate extremes (Reichstein et al., 2013). Many researchers have used physical, chemical, or biological approaches, such as spectroscopic methods, isotopic tracing and microbial community analysis, to study the biogeochemical process of SOC

accumulation and loss (Davidson and Janssens, 2006; Schmidt et al., 2011; Trivedi et al., 2013). Recent studies have rapidly improved our understanding of carbon accumulation and decomposition processes in soils, and SOC stabilization mechanisms still requires further in-depth investigations.

The physicochemical structure of SOC had long been considered as the decisive factor of SOC stability. The role of humic substances in stabilization of soil carbon drew attention as early as 1936 (Waksman, 1936). Over the next 70 years, the molecular structure of more SOC components besides humic substance, including light fractions, mineral organic carbon, and soil aggregate have been used for indicating SOC stability (Sollins et al., 1996; Sutton and Sposito, 2005; Zech et al., 1997). Complex chemical composition and molecular characteristics, such as aromatic-, aliphatic-, alkyl aryl-, and biaryl-bonds could protect SOC from decomposition (Zech et al., 1997). Progress in spectroscopic analysis techniques, especially nuclear magnetic resonance spectroscopy, provides information of SOC chemical characteristics at the microscale, leading to more debates and controversies about the nature, structure and stability of SOC (Baveye and Wander, 2019; Sutton and Sposito, 2005; Zaccone et al., 2018). Whether humic substances could be extracted by alkali from soils and be important to SOC stability are

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now under debate (Ohno et al., 2019). Compound-specific isotopic evidence has demonstrated that molecular attributes are insufficient for explaining the persistence of carbon in soils. For example, the mean residence time of lignins or plant lipids that had been suggested to persist in soils, were found to be only 50–100 years, with a faster turnover than the bulk of the organic carbon (Amelung et al., 2008). By contrast, proteins and saccharides that were considered to be labile compounds persisted for decades (Schmidt et al., 2011). Trumbore and Czimczik (2008) proposed that the SOC stability also depended on complex influences from the spatial heterogeneity of biota and environmental conditions, which could alter the likelihood of decomposition. In a seminal review article, Lehmann and Kleber (2015) claimed that the decomposition of SOC did not rely on its physicochemical properties, but on the accessibility of carbon by microbes and enzymes.

The physical stabilization of SOC through soil aggregates is attributed to spatial inaccessibility and reduced oxygen diffusion that protect against decomposition by microbes and enzymes (Sollins et al., 1996). Soil organic carbon can also be stabilized by the interactions between SOC and mineral surfaces which form stable organic–mineral bonds through anion and inner-sphere ligand-exchange reactions (Wiesmeier et al., 2019). Insoluble oxides of Al, Fe (III), Mn (IV) on mineral surfaces could protect SOC from decomposition by forming barriers against enzymatic hydrolysis (Torn et al., 1997). Till now, it is generally accepted that there are three major mechanisms for protecting organic carbon from decomposition, which are biochemical stabilization through the formation of resistant SOC compounds, physical stabilization through microaggregation, and stabilization of SOC by formation of stable organic–mineral bonds on mineral surfaces (von Luetzow et al., 2006). As a result, parameters of carbon physicochemical composition have been selected to represent the carbon stabilization status of soil in coupled climate-carbon models, which study the coupling between climate change and the carbon cycle (Friedlingstein et al., 2006), such as CENTURY (Parton et al., 1987) and ROTH-C (Jenkinson, 1990) models.

Despite the controversy on the nature and fate of SOC, the consensus was that the soil biotic community and the soil microenvironment were key factors affecting the SOC stability (Jastrow et al., 2007; Schmidt et al., 2011; Yang et al., 2018a). Soil organic C accumulation and decomposition could be controlled by factors such as plant residue quality, spatial accessibility, and soil faunal and microbial community composition (Trumbore and Czimczik, 2008). Recent studies have recognized that SOC stability is an ecosystem property that varies at temporal scale from decades to centuries (Fraterrigo and Rusak, 2008; Schmidt et al., 2011). The rates of plant litter inputs and SOC decomposition by microorganisms depend on the time scale, which leads to changes of SOC stability response over time (Trumbore and Czimczik, 2008). Meanwhile, the sensibility and adaptability of plant and soil microbial community also reflect differences over time with perturbation (Hartmann et al., 2014; Schmidt et al., 2011). Therefore, stabilization mechanism of SOC need to consider biotic factor and time scale besides carbon physicochemical structures.

Presently, we are far from a clear understanding of the mechanisms behind SOC stability, in particular, how natural and anthropogenic perturbations affect organic carbon in soils. Perturbation refers to biological or environmental events that produce successional upset, disaster, stress or disturbance to organisms or systems (Vogl, 1980). In soil ecosystem, perturbation is defined as an effect that causes the soil ecological component to deviate from pre-perturbation state (pre-existing state just before perturbation occurrence, as a reference point), including natural and anthropogenic events (Rykiel, 1985). As the terms perturbation, stress, and disturbance are often used interchangeably, we examine SOC stability studies that use any of these three terms to mean “perturbation”.

The main natural perturbations include wildfire, debris flow, warming, disease and pests, and other meteorological, geological and

hydrological events. Wildfires prevent the accumulation of SOC by altering the physical properties of the soil, soil water content, and the microbial composition (Mack et al., 2011). Meteorological events, such as exceptional rainfalls (Denef et al., 2001) and droughts (Rivest et al., 2015), can change the quantity and quality of SOC input by influencing the biomass and productivity of vegetation and the population dynamics of soil microbes. Geological and hydrological events, such as earthquakes and flooding, can also directly or indirectly change local vegetation, soil, and topography, thus influencing the SOC pools (Cao et al., 2002; Hilton et al., 2011). Disease and pests can result in the exfoliation and death of plants (such as conifers, eucalypt trees and deciduous trees), thus changing the biomass and productivity of local vegetation and ultimately influencing SOC content (Arasa-Gisbert et al., 2018).

In addition to natural perturbation, anthropogenic perturbation has become important for earth's carbon cycling with the increasing impact on soil ecosystems of a growing human population. Types of anthropogenic perturbation associated with SOC stability include land use change, nitrogen deposition, and soil pollution (such as metal contamination and crude oil pollution). Land use change includes urban expansion (Xu et al., 2012), transforming forested land to farmland (Don et al., 2011), grazing (Ayoubi et al., 2014), and agricultural management (e.g., fertilization and tillage practices) (Drosos and Piccolo, 2018). Land use change can influence local SOC inputs and soil redistribution (spatial soil transportation) mainly by altering the local vegetation ecosystem and causing soil erosion (Ayoubi et al., 2012; Doetterl et al., 2016). For example, the conversion from natural to agricultural land use may increase soil erosion and the decomposition of SOC by removing the protective cover of natural vegetation, while the use of fertilizers could induce additional carbon sequestration on agricultural fields (Doetterl et al., 2016). Nitrogen deposition influences atmosphere-SOC cycles by changing litter decomposition, fine root turnover, and soil respiration, ultimately altering the SOC stock (Neff et al., 2002). Acid rain influences the metabolic activity and enzyme activity of soil microorganisms through multifactor interactions (Guo et al., 2003). Metal contamination could inhibit the activities of urease and catalase in soil, thus influencing the accumulation of SOC (Zhang et al., 2016). In areas polluted by organic contamination such as crude oil, oil pollution changes the physical properties and functions of soil, and affects soil microorganism community structure, functional genes, and metabolic processes (Yang et al., 2018a, 2018b). Hydrophobic petroleum components can also affect soil water-retention capacity, gas permeability, and potential adsorption of organic mineral matter (Khanna et al., 2013; Mills et al., 2004), which is likely to affect the formation of soil aggregates and the physical stability of SOC.

Here, we review perturbation types and SOC response mechanisms to gain insights into SOC stability under environmental perturbations, and consider ecological theories to identify general principles underlying SOC stabilization. We highlight the resilience and resistance of SOC and then provide an overview of novel insights and approaches related to SOC stability, which could lead to a new generation of SOC models. We also used a bibliometric analysis to track historical trends of “SOC stability” by the VOSviewer software and suggest future research directions to expand our ability to understand and predict SOC dynamics under perturbations.

2. The response to perturbation and calculation of SOC stability

2.1. The response of SOC stability to perturbation

Based on ecosystem stability theories (Pimm, 1984), the response of SOC stability to perturbation can be classified into three categories: keeping stable states, changing to an alternative stable state and system collapse. These depend on the soil ecosystem property and the type of perturbation it experiences. Fig. 1 qualitatively illustrates the SOC stability response to a perturbation in the soil ecosystem.

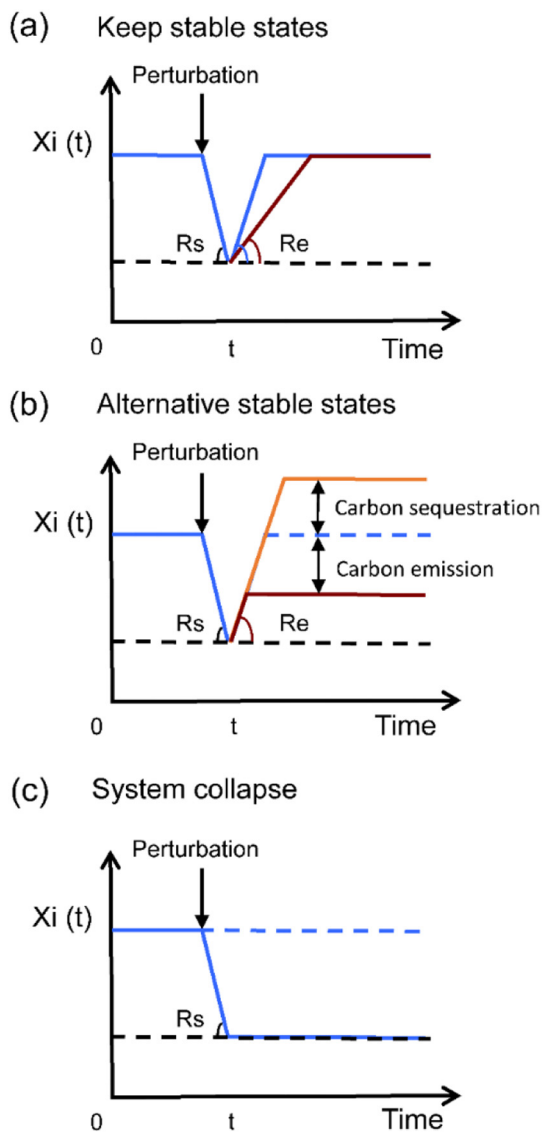


Fig. 1. Base on ecological theory, soil organic carbon (SOC) stability depend on the soil ecosystem property and the type of perturbation it experiences (Ives and Carpenter, 2007). (a) Maintained stable state: SOC stability that returned to its previous level after perturbation. Resistance rate (R_s) and resilience rate (R_e) jointly determine the time (t) it takes for the system to return to its pre-perturbation state. The furthest distance from the equilibrium state is indicated with black dashed lines. (b) Alternative stable states. Pre-perturbation state is shown with blue dashed line. Orange line shows increased SOC stability during carbon sequestration and red line shows decreased SOC stability during carbon emission. (c) System collapse, in which resilience loss leads to system collapse. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

As an ecosystem property, stability can be viewed from two perspectives: resistance (capacity to persist and maintain system state and functions during perturbation, R_s), and resilience (ability to recover from the impact of perturbation, R_e) (McCann, 2000) (Fig. 1). Soil ecosystem resistance and resilience are determined by the co-action of biotic and abiotic factors. Soil organic C may maintain stable states (Fig. 1a), in which SOC indices return to its pre-perturbation state. During this process, resistance rate (quantified by the deviation of system from the pre-perturbation state per unit time after perturbation) and resilience rate (quantified by the reverting of system to the pre-perturbation state per unit time after perturbation) jointly determine the time needed for the system to return its pre-perturbation state. For

SOC stability, many studies have demonstrated that both aboveground plants and underground faunal and microbial communities exhibit tolerance and functional stability to perturbation (Hartmann et al., 2014; Schmidt et al., 2011; Trumbore and Czimczik, 2008). Sistla et al. (2013) reported that warming restructured Arctic tundra but did not change total SOC or nitrogen stocks over the two-decade experimental period in an Alaskan tundra ecosystem. Other researchers have observed that initial increases in soil CO_2 efflux often disappeared within a few years and that total SOC content remained constant (Eliasson et al., 2005; Luo et al., 2001; Melillo et al., 2002). These results could be interpreted as 'acclimation' of the root microbial communities to environmental changes (Davidson and Janssens, 2006). In support of this, genomic information provides evidence that most soil microbial communities possess related genes to relieve stress and acclimate to environmental changes with moderate perturbations (Trivedi et al., 2013).

Soil organic C systems may have alternative stable states (Fig. 1b), in which the final SOC state depends on the pre-perturbation state and the resilience. If the resilience of the system is not strong enough to return to the pre-perturbation state, then SOC stability will decrease through carbon emissions. Many recent studies have demonstrated that natural and anthropogenic perturbations may result in changes to SOC including its physical and chemical structure, soil functional genes and metabolic processes, which ultimately lead to carbon emission (Creamer et al., 2015; Huang et al., 2016; Schuur et al., 2015). Alternative stable states may also occur with positive accumulation of SOC. The 'intermediate disturbance hypothesis' suggests that intermediate levels of perturbation that are not biologically destructive may stabilize the ecosystem (Connell, 1978; Rykiel, 1985). Studies of trees, shrubs, forbs, and grasses have showed that native species diversity and richness may be maximized by moderate disturbance (Mayor et al., 2012; Molino and Sabatier, 2001). The shifts in the stable states of a system could be used to measure SOC stability when alternative stable states occur (Ives and Carpenter, 2007).

Perturbations might also lead to soil ecosystem collapse and loss of resilience (Fig. 1c), if they exceed its catastrophic threshold (tipping point) (Dai et al., 2012). When system collapses and loses resilience, stability can be measured by calculating the resistance of a system to perturbation through time (Ives and Carpenter, 2007). For example, a more resistant SOC system is less affected by perturbation and can withstand greater interference. The inherent characteristics of the system and the intensity of perturbation that the system experiences both determine the stability (Ives et al., 2003).

2.2. Calculations of resistance and resilience

The definition of stability in ecology are based on a system's ability to defy change (resistance and resilience). Resistance refers to the degree to which a variable changes after a perturbation has occurred. Resilience is defined as the return time to the equilibrium of a system after a perturbation. The metrics of resistance and resilience of ecosystem response to perturbation within a soil stable system can be grouped into three major types of simple stability, relative stability and integrated stability according to their attributes. In the present study, we follow the terminology used by Ingrisch and Bahn (2018) reported in Table 1.

There are several criteria to calculate resistance and resilience including a monotonic increase, identical values for equal perturbations (positive/negative), and undisturbed soil for the reference (Orwin and Wardle, 2004). The ecosystem characteristic, type of perturbation, and ecosystem state were important factors for calculating stability (Ingrisch and Bahn, 2018). Category I stability (simple stability) is calculated by simply comparing the stability indices between disturbed soil and undisturbed soil samples (pre- and post-perturbation). The perturbation impact can be quantified by the change between the pre perturbation state of SOC system and the state after perturbation. The

Table 1

Overview of currently used calculations for resistance and resilience indices, grouped into three categories according to their attribute.

Category	Attribute of stability	Resistance	Resilience	Explanation ^a	Reference
I	Simple stability	$\frac{P_0}{C_0}$	$\frac{P_x}{C_x}$	C_0 measured in the undisturbed soil (control) and P_0 measured in the disturbed soil at time 0 after disturbance (immediately); P_x measured in the disturbed soil at time x after disturbance.	(Kaufman, 1982)
I	Simple stability	$\frac{P_0}{C_0}$	$\frac{P_x}{C_x}$	C_0 , P_0 , and P_x are as above. C_x measured in the undisturbed soil (control) at time x after disturbance.	(Macgillivray et al., 1995)
I	Simple stability	$\left(C_x - \frac{C_x}{P_x}\right) \times 100\%$	$\left(C_x - \frac{C_x}{P_x}\right) \times 100\%$	Parameters are as above.	(Griffiths et al., 2000)
II	Relative stability	$\frac{P_0}{C_0}$	$\frac{P_x - P_0}{C_0}$	Parameters are as above.	(Lloret et al., 2011)
II	Relative stability	$-100\left(\frac{D_0}{C_0}\right)$	$-100\left(\frac{D_x}{C_x}\right)$	$D_0 = C_0 - P_0$; $D_x = C_x - P_x$; C_0 , P_0 , C_x and P_x are as above.	(Banning and Murphy, 2008)
II	Relative stability	$1 - \frac{2 D_0 }{(C_0 + D_0)}$	$\frac{2 D_0 }{(D_0 + D_x)} - 1$	Parameters are as above.	(Orwin and Wardle, 2004)
III	Integrated stability	NC	$\sqrt{\sum_{t=i}^j \frac{(P_x)^2}{C_x}}$	Parameters are as above. NC, not calculated.	(Oneill, 1976)
III	Integrated stability	$\int_0^x f(t) \frac{dt}{x}$	$\int_x^j f(t) \frac{dt}{(j-x)}$	$f(t) = \frac{P_x}{C_x} \times 100$. x and j are time point after disturbance. C_x and P_x are as above.	(Zhang et al., 2010)

^a C is the SOC stability state or process rate of the undisturbed control ecosystem and P is the SOC stability state or process rate of the disturbed ecosystem, at a given point in time since perturbation ($t = x$) whereby $t = 0$ and $t = j$ correspond to the time immediately before and immediately after perturbation, respectively.

smaller impact or change suggested smaller perturbation or more stabilization of the SOC system (Ingrisch and Bahn, 2018). Recently, most SOC stability studies have used this category to quantify a system's capacity to resist and recover from perturbation. However, such approach does not consider the time scale of observation. More complex categories (Category II and III) focus on changes occurring in undisturbed and disturbed soil over time. Category II (relative stability) emphasizes recovery from perturbation and indicates the percentage of pre-perturbation state that has recovered, while category III (integrated stability) emphasizes the perturbation impact and indicates the integrated changes of stability (Ingrisch and Bahn, 2018). Category II and III have been mainly used for calculating the resistance and resilience of microbial community structures, food webs, and enzyme activities in soils (de Vries et al., 2012; Jain et al., 2016; Meola et al., 2014).

3. Indicators and approaches to quantify SOC stability

Changes in SOC are directly estimated in two ways: quantifying the soil total carbon by dry combustion or potassium permanganate oxidation, and measuring soil CO_2 and CH_4 concentrations using an infrared gas analyzer or gas chromatograph (Plante et al., 2011). Changes can also be estimated indirectly based on the turnover rate of carbon by measuring the carbon stable isotopic ratio and using radiocarbon dating, which are both effective tools to investigate SOC dynamics on decadal to millennial timescales (Gaudinski et al., 2000; Hobbey et al., 2013). Here, we detail approaches used to estimate the chemical and physical stability of SOC (Fig. 2). These indicators and approaches all could be used in SOC stability measurement during laboratory incubations and *in situ* experimental observations.

3.1. Chemical stability

Chemical stability of SOC could be analyzed by chemical fractionation procedures, spectroscopic methods, and thermal analysis (Fig. 2a). Many chemical fractionation procedures have been used to generate soil organic fractions and assess the stability of SOC (Fig. 2a). Classic methods for humic substance extraction by alkaline solutions (e.g., NaOH and $\text{Na}_4\text{P}_2\text{O}_7$), followed by acidification with hydrochloric acid, were used to separate humic and fulvic acids (Olk, 2006). The chloroform-fumigation method is used to extract soil microbial biomass carbon (Vance et al., 1987). Acid hydrolysis and potassium permanganate (KMnO_4) oxidation effectively remove labile carbon (sugars, proteins, and amino acids) to determine the resistant fraction (alkyl and

aryl materials) (Blair et al., 1995). Hydrofluoric acid (HF) can be used to destroy the mineral phase and isolate SOC from organo-mineral complexes (Parsons, 1983).

Spectroscopic methods, such as ^{13}C -nuclear magnetic resonance (NMR) spectroscopy, near infrared (NIR), and diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy are relatively advanced techniques that can be used to characterize SOC. ^{13}C NMR spectroscopy can identify and quantify important organic functional groups of SOC with little sample preparation. Soil samples only need to be pre-treated with HF to remove Fe^{3+} and Mn^{2+} from the soil to increase the signal to noise ratio (Li et al., 2017). The ^{13}C NMR spectra can delineate four common chemical shift regions corresponding to alkyl (0–50 ppm), O-alkyl (50–110 ppm), aromatic (110–160 ppm), and carbonyl (160–200 ppm) C atoms. It is possible to further divide some other chemical shift regions, such as dividing O-alkyl C into methoxyl C (50–60 ppm), carbohydrate C (60–90 ppm), and di-O-alkyl C (90–110 ppm); aromatic C into aryl C (110–142 ppm) and phenolic C (142–160 ppm) (Knicker, 2007). NIR and DRIFT can be used to quickly quantify SOC content, and NIR is primarily a molecular technique best suited for rapid *in situ* soil carbon content (Bricklemeyer, 2012). DRIFT can be combined with NMR to quantify alkyl-C, aromatic-C, and carboxyl-C (Rumpel et al., 2001).

As spectroscopic methods could not provide information regarding chemical compounds of SOC, thermal analyses including thermogravimetry, Rock-Eval pyrolysis, and pyrolysis gas-chromatography/mass-spectrometry (GC/MS) has been used for SOC structure analysis at a more detailed level (Leinweber et al., 2008; Plante et al., 2011; Saenger et al., 2013). Thermogravimetry is a technique that records mass changes in SOC as a function of temperature (Plante et al., 2011). Rock-Eval pyrolysis is originally used for obtaining information on amounts of hydrocarbons and types of kerogen present in sedimentary rocks, which also provides information on the composition of the SOC through the Hydrogen and Oxygen Index values (Disnar et al., 2003). In the pyrolysis-GC/MS, pyrolysis products are separated using a pyrolyser by setting different pyrolysis temperatures and then identified by GC/MS. About 131 SOC fractions have been identified and quantified using pyrolysis-GC/MS (Schellekens et al., 2017), including *n*-alkanes, branched aliphatics, *n*-fatty acids, diterpenes, PAHs, indenenes, benzofurans, aromatics, phenols, lignin phenols, N-containing compounds, and carbohydrate products.

For chemical fractionation procedures, spectroscopic and thermal methods, solid soil samples are prepared by air drying, grinding and sieving. Generally, chemical fractionation procedures could extract

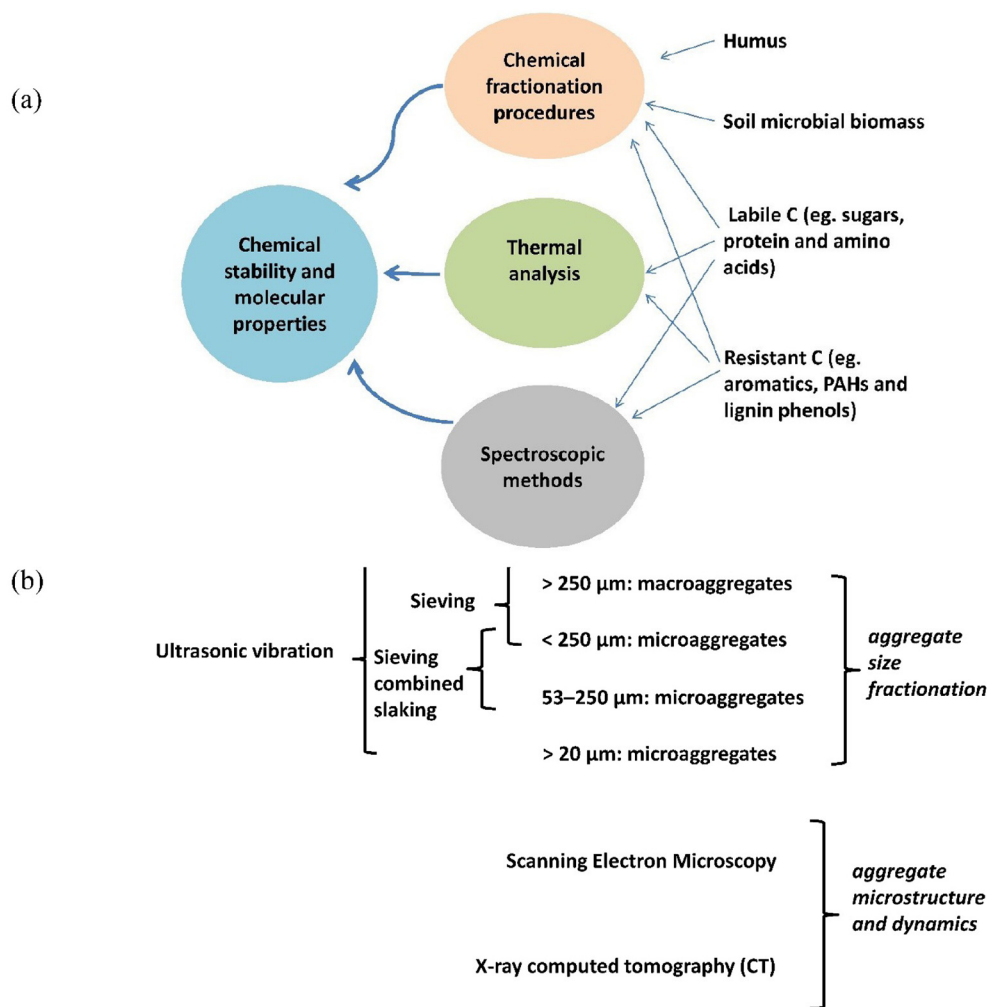


Fig. 2. Soil organic carbon (SOC) intrinsic stability methods that are related to soil physical structure and molecular attributes. (a) Chemical stability methods included chemical fractionation procedures that extract soil organic fractions (e.g., humic, microbial biomass carbon (MBC), labile C, and resistant C) and non-destructive characterization techniques (e.g., thermal analysis and spectroscopy methods). (b) Classic methods of aggregate size fractionation included sieving, sieving combined with slaking, and ultrasonic vibration. Recently, studies applied scanning electron microscopy (SEM) and X-ray computed tomography (CT) to analyze aggregate microstructure and dynamics.

specific components of SOC, such as humic substances, soil microbial biomass carbon and labile carbon. However, most of the extraction schemes are complex, tedious, and of low efficiency, with only a portion (20–60%) of the total SOC being extracted (Mathers et al., 2000). Chemical fractionation procedures often destroy the natural state of SOC, resulting in a loss of information about its stabilization process. The advantages of spectroscopic methods and thermal analyses are that, being non-destructive, they do not alter the original physiochemical structural of SOC (Peltre et al., 2013), providing more information of SOC structural chemistry than chemical fractionation procedures. Although spectroscopic and thermal methods can identify and quantify over a hundred chemical compositions of SOC (Zaccone et al., 2018), they cannot be used to analyze soil microbial biomass carbon and humic substance.

3.2. Physical stability

Soil aggregation is a key physical indicator of SOC stability. According to the aggregate hierarchy theory proposed by Tisdall and Oades (1982), different aggregates within a hierarchical order have different types of organic binding. Organo-mineral microaggregates (< 250 μm) are formed by associations among soil amorphous mineral components, crystalline particles and microbial cells (Tiessen and Stewart, 1988). These microaggregates are temporarily held together as macroaggregates (> 250 μm) by roots and fungal hyphae. As a result, SOC in macroaggregates may readily decompose and SOC in microaggregates is more stable (Tisdall and Oades, 1982).

The methods of aggregate size fractionation are used to isolate

different organic fractions and measure the stability of soil aggregate in many studies (Angst et al., 2017; Le Bissonnais, 2016; Legout et al., 2005). The sieve-based methods often separate aggregates into macroaggregates (> 250 μm) and microaggregates (< 250 μm) through different mesh sizes of sieves. Six et al. (2002) suggested that a fractionation procedure using wet sieving and slaking could obtain microaggregates (53–250 μm). The ultrasonic treatment breaks up macroaggregates and releases microaggregates < 20 μm , but has little effect on aggregates < 2 μm (Christensen, 2001). To further measure the breakdown mechanism of aggregate, Le Bissonnais method could be used for size fractionation with three treatments of fast wetting, slow wetting and wetting stirring (Le Bissonnais, 2016). In addition, aggregate density fractionation could be used to separate aggregate to light and heavy fractions by density cut-off and sonication intensity, helping in clarifying the SOC physical structure in carbon turnover and stabilization (Cerli et al., 2012; Rabot et al., 2018).

Recently, X-ray computed tomography (CT) and scanning electron microscopy (SEM) have been used to analyze the intra-aggregate microstructure and aggregate dynamics in relation to SOC distribution. Papadopoulos et al. (2009) found with X-ray CT that the intra-aggregate pore size distribution did not significantly affect aggregate stability. By using CT and the Pore-Cor network model, Dal Ferro et al. (2012) demonstrated that aggregate porosity and hydrophobicity affect aggregate stability and that SOC stability increased with increasing water retention capacity. Based on recent studies, physical decomposition of residues and the formation of microaggregates might be affected by the ratio of carbon: nitrogen (C:N) of plant residues (Blanco-Canqui and Lal, 2004), lignin concentration (Martens, 2000), soil

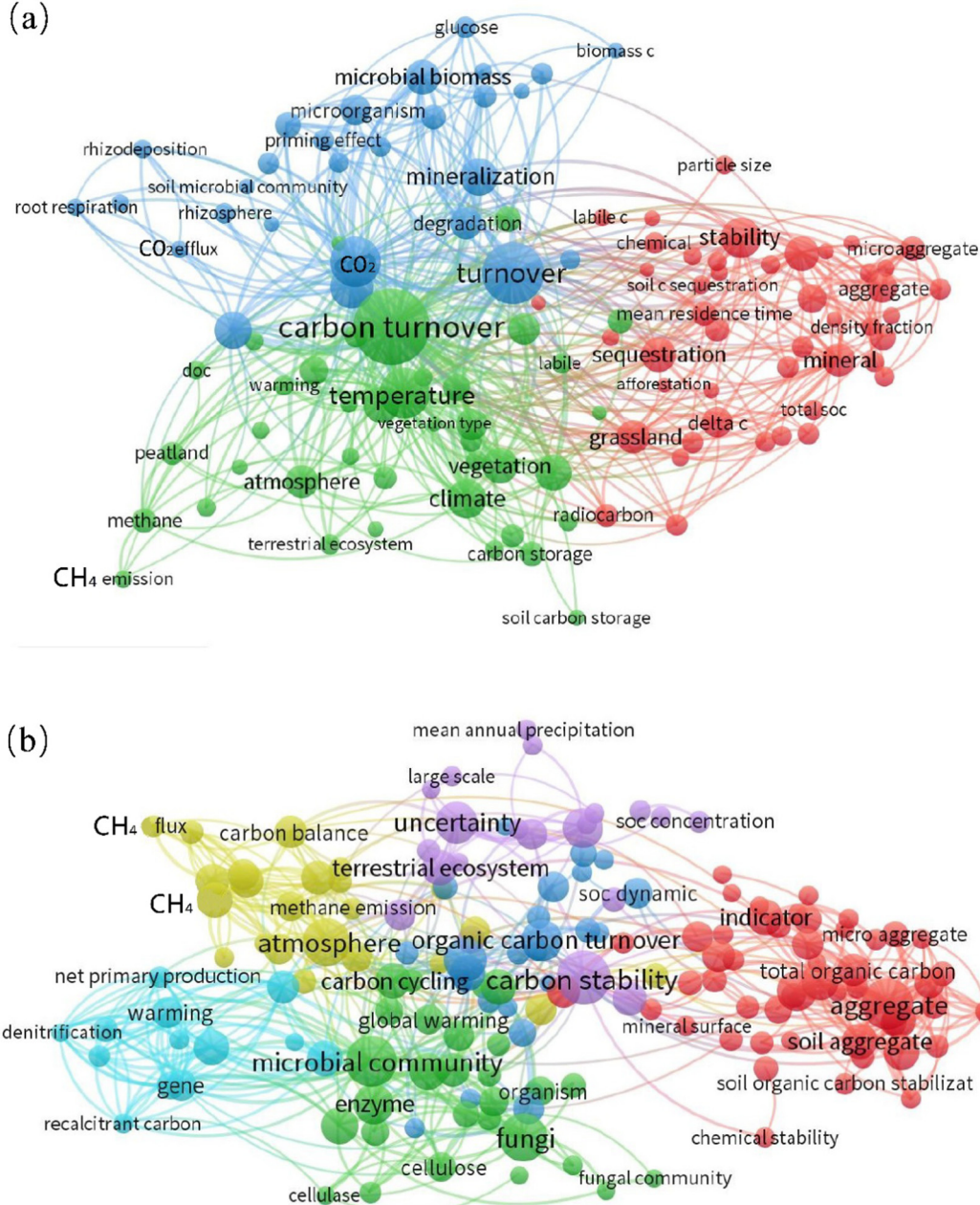


Fig. 3. Occurrence and relevance of keywords. We used an advanced search term for “Topic OR Title: (‘carbon stability’ OR ‘carbon stabilization’ OR ‘carbon turnover’ OR ‘carbon persistence’ AND soil). Timespan: (a) before 2015 (1898-2014) and (b) recent 5 years (2015-2019). Databases: WOS, BIOSIS, CSCD, DRCI, DIIDW, INSPEC, KJD, MEDLINE, RSCI, SCIELO” in Web of Science. The round shape and bold text represents the weight of occurrences of text or terms. The higher the weight of an item, the larger the label and the circle of the item. In order to avoid overlapping labels, some items the label may not be displayed. The same color of the items indicate the cluster of the item belongs and lines between items represent links (Van Eck and Waltman, 2010).

moisture retention capacity (Zibilske and Bradford, 2007), and concentration and mineralogy of clay minerals (Jimenez and Lal, 2006). The integration of 3D image evaluation with CT and SEM have been used to understand the highly heterogeneous intra-aggregate microstructure on the submicron scale, and model biogeochemical reactions of soil interfaces at the pore scale (Dal Ferro et al., 2012; Khan et al., 2012).

While physical, chemical, spectroscopic and thermal approaches help us to much better understand the characterization of SOC stability, single method or proxy only provide limited information of physical or chemical structure changes of SOC with perturbation (Zaccone et al., 2018). Physical chemistry and molecular biology techniques could be combined to assess the stability and dynamics of SOC, and these

indicators could be used to quantify the resistance and resilience of SOC in response to perturbation.

4. Future directions

We used a bibliometric analysis to track historical trends of “SOC stability” by the VOSviewer software (see Fig. 3) and suggested future research directions. Before 2015, the important terms included “carbon turnover”, “CO₂”, “sequestration”, “temperature”, “climate”, “microbial biomass”, “aggregate” (Fig. 3a). Due to the importance of SOC turnover and stability in global CO₂ emissions (van Groenigen et al., 2011), it was not surprising that these words emerged as the highest occurring term within the text corpus, because most studies are geared towards

soil carbon sequestration to mitigate the impact of climate change. In the latest 5 years, the text corpus analysis revealed that the terms “microbial community”, “fungi”, “enzyme”, “gene”, “carbon cycling”, “uncertainty” had the highest relevance score (Fig. 3b). This indicates that recent studies on SOC stability tended to improve the understanding of biological mechanisms, ecological property and uncertainty.

4.1. Biological mechanisms in SOC stability

Soil organisms, including invertebrates and microorganisms, play a vital role in SOC accumulation and decomposition (Tajik et al., 2019; Trivedi et al., 2013). Generally, four biotic strategies are considered to influence SOC dynamics (Ekschmitt et al., 2005): (1) release of exoenzymes by microorganisms to breakdown the organic substrate, (2) mechanical crushing of organic residues by soil fauna, (3) disintegration of soil aggregates and relocation of soil mass by fauna, and (4) carbon fixation in the living biomass. Although biomass carbon of soil microorganisms accounts for a very small portion of the total SOC, it is considered that microbial residues, including murein, chitin, and lipids, are the main sources of stable SOC (Kallenbach et al., 2016).

As microbial community structure and activity could impact the process of SOC decomposition, recent ecosystem models have started to incorporate microbial communities (Creamer et al., 2015). Wieder et al. (2013) created a new soil biogeochemistry module to improve global soil carbon predictions in the Community Land Model (CLM) by modelling microbial processes. Trivedi et al. (2013) reviewed microbial modulators of soil carbon storage and how data related to microbial composition and activities can be incorporated into mechanistic and predictive models. The contribution of microorganisms to SOC might have been underestimated in previous researches, thus it's necessary to consider biological factors in conceptual and quantitative SOC models and integrate new microbiological techniques in the future study of SOC stability.

4.2. SOC stability uncertainty and thresholds

For resistance and resilience, soil ecosystems need a balance between stabilizing and destabilizing forces. If these forces are balanced through natural feedback loops, the SOC pool will naturally retain its stable state (Fraterrigo and Rusak, 2008). During the growing season, gross primary productivity and litter input increase, thus soil often shows net carbon uptake in summer. However, in spring, autumn, and especially winter, soil carbon loss might occur due to seasonal variations without perturbation (Schuur et al., 2009; Sistla et al., 2013). The succession of soil communities and the oscillating densities of predators and prey could also lead to the natural variation of the SOC pool (Ekschmitt et al., 2005). Schuur et al. (2009) found that soil CO₂ flux from tundra soil to the atmosphere ranges from −40 to 80 (g C m^{−2}) in different seasons. Another study in northeast Spain showed that total carbon and glomalin (i.e. a glycoprotein produced by mycorrhizal fungi that may bind aggregates) varied significantly in different seasons (Emran et al., 2012). The overlay of natural variation of SOC and impact of perturbation, result in the uncertainty of SOC stability.

The key issue is whether natural and/or anthropogenic perturbation leads to irreversible damage by pushing the soil ecosystem over critical thresholds. When these thresholds are crossed, the system cannot maintain its natural feedback loops and a loss of resilience can occur. This loss of resilience can lead to the degradation of soil ecological function, and prevent soil recovery (Kleinen et al., 2003). The critical threshold depends on the population dynamics of underground and aboveground species, intrinsic soil properties, and the frequency and severity of the perturbation (Ingrisch and Bahn, 2018). Other studies have investigated soil microbial resistance and resilience thresholds to perturbation, such as during a drought (Rivest et al., 2015), simulated rainfall (Steenwerth et al., 2005), and heavy metal pollution (Mertens

et al., 2007). However, the exact tipping points of SOC stability thresholds and uncertainty to different types of perturbation remain unclear. Therefore, assessing these critical thresholds in future studies is needed.

4.3. The response of SOC stability to multiple perturbations

Many soil ecosystems are disturbed by multiple natural and/or anthropogenic events (Moreno-Mateos et al., 2017; Vinebrooke et al., 2004). Multiple types of perturbation occurring simultaneously often have diverse impacts on SOC stability compared to a single type of perturbation, resulting in complex responses. For instance, warming can accelerate the decomposition of SOC (including light fractions, heavy fractions and mineral organic carbon) (Fang et al., 2005; Knorr et al., 2005). However, there is evidence suggesting that rising temperatures restructure Alaskan tundra ecosystems without changing SOC storage due to increasing nitrogen availability, plant biomass, and dominance of woody plants (Sistla et al., 2013). In New South Wales, Australia, Creamer et al. (2015) examined the effects of litter addition and higher temperature on SOC decomposition in woodland ecosystems and found that these may increase long-term stability of SOC.

Soil organic C stability variations are often only considered with a single type of perturbation in many incubation experiments, which may lead to inconsistencies between laboratory-based studies and field observations. Studies estimating carbon loss in soil heating experiments have detected major increases in carbon decomposition rates with increasing temperature (Oberbauer et al., 2007). However, *in situ* observations by measuring changes in the ¹³C/¹²C ratio and total SOC content revealed that SOC stability does not vary with temperature (Giardina and Ryan, 2000; Schmidt et al., 2011; Sistla et al., 2013). An explanation for the discrepancy may be that other factors (e.g., moisture, change of plant type, and other sources of perturbation) act alongside warming to alter long-term SOC stability (Giardina and Ryan, 2000), which is rarely reflected in short-term laboratory studies. There are other multiple and compounding perturbations, such as firing with global warming in forest and pasture, land use change with agricultural management, which could appreciably influence the ecosystem and cause the change of SOC stability (Knicker, 2007; Mack et al., 2011). Understanding the response of soil biogeochemical processes to multiple types of perturbation will therefore be vital for accurate predictions of global SOC dynamics.

5. Conclusions

Based on ecosystem stability theories, SOC stability responses to perturbations as either keeping stable states, changing to an alternative stable states or system collapse. The understanding of SOC stability with perturbations has evolved using more advanced characterization techniques. Relatively new non-destructive characterization techniques such as spectroscopic methods, thermal analysis, X-ray computed tomography (CT) and scanning electron microscopy (SEM) could provide SOC fine chemical and structural characteristics as well as SOC dynamics at the submicrometer scale. To accurately quantify changes in SOC stability, the resistance and resilience of SOC can be calculated based on the pre- and after-perturbation state of SOC and the temporal scale of perturbation impact. We suggest that biological mechanism need to be considered in the future research in addition to physical and chemical processes, threshold and uncertainty of SOC stability, impact of multiple and compounding perturbations. This will advance our understanding of SOC stabilization processes and reduce the uncertainties in assessments of global carbon stocks.

Declaration of Competing Interest

All the authors have read and approved the manuscript. There are

no conflicts of interest to declare.

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