



Deep-C storage: Biological, chemical and physical strategies to enhance carbon stocks in agricultural subsoils

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ABSTRACT

Due to their substantial volume, subsoils contain more of the total soil carbon (C) pool than topsoils. Much of this C is thousands of years old, suggesting that subsoils offer considerable potential for long-term C sequestration. However, knowledge of subsoil C behaviour and manageability remains incomplete, and subsoil C storage potential has yet to be realised at a large scale, particularly in agricultural systems. A range of biological (e.g. deep-rooting), chemical (e.g. biochar burial) and physical (e.g. deep ploughing) C sequestration strategies have been proposed, but are yet to be assessed. In this review, we identify the main factors that regulate subsoil C cycling and critically evaluate the evidence and mechanistic basis of subsoil strategies designed to promote greater C storage, with particular emphasis on agroecosystems. We assess the barriers and opportunities for the implementation of strategies to enhance subsoil C sequestration and identify 5 key current gaps in scientific understanding. We conclude that subsoils, while highly heterogeneous, are in many cases more suited to long-term C sequestration than topsoils. The proposed strategies may also bring other tangible benefits to cropping systems (e.g. enhanced water holding capacity and nutrient use efficiency). Furthermore, while the subsoil C sequestration strategies we reviewed have large potential, more long-term studies are needed across a diverse range of soils and climates, in conjunction with chronosequence and space-for-time substitutions. Also, it is vital that subsoils are more consistently included in modelled estimations of soil C stocks and C sequestration potential, and that subsoil-explicit C models are developed to specifically reflect subsoil processes. Finally, further mapping of subsoil C is needed in specific regions (e.g. in the Middle East, Eastern Europe, South and Central America, South Asia and Africa). Conducting both immediate and long-term subsoil C studies will fill the knowledge gaps to devise appropriate soil C sequestration strategies and policies to help in the global fight against climate change and decline in soil quality. In conclusion, our evidence-based analysis reveals that subsoils offer an untapped potential to enhance global C storage in terrestrial ecosystems.

1. Introduction

Soil, a global reservoir of 3000 Pg carbon (C) (Köchy et al., 2015) with a mean age of 3100 years (He et al., 2016), has a significant capacity for long-term C storage. However, the extent to which this terrestrial C sink will continue to grow as atmospheric CO₂ concentrations increase remains unclear. Most C in agricultural soils (cropland and pasture) is held in an organic form (soil organic carbon, SOC), which

is susceptible to destabilization as a result of changes in land use, management practices and environmental conditions (Guo and Gifford, 2002; Davidson and Janssens, 2006). Due to agriculture alone, 133 Gt of SOC has already been lost to the atmosphere in the past two centuries, and the rate of loss is increasing (Sanderman et al., 2017). SOC loss severely impacts soil functions, including water infiltration, nutrient supply and biodiversity, leading to erosion, a decline in soil fertility and a release of greenhouse gases (GHGs - CO₂, CH₄, N₂O) (Don et al., 2011;

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Tsiafouli et al., 2015). Due to the projected growing demand for food production from already degraded land, intensive agriculture is putting soils at further risk of SOC loss (Johnson et al., 2014; Sanderman et al., 2017). Still, enhanced soil C sequestration of ~1000 additional Pg C is thought to be possible (Lorenz and Lal, 2005).

Sequestering organic C in the soil can have multiple benefits, including i) offsetting of anthropogenic C emissions, ii) restoring soil function, iii) improved soil resilience (to erosion, pollution, diseases and drought), iv) increased agricultural productivity and sustainability, and v) greater food security (Lal et al., 2015). Due to these expected benefits, promoting SOC sequestration is of keen interest to both the scientific and policymaking communities. A number of recent analyses suggest that 'natural solutions' like sequestering C in soil are economical and 'no-regrets options' that could achieve a substantial portion of the negative emissions needed to achieve carbon neutrality (Baker et al., 2020; Sykes et al., 2020). While the recent '4 per 1000' soil C sequestration initiative has drawn both support (Minasny et al., 2017; Rumpel and Kögel-Knabner, 2011) and criticism (Baveye et al., 2018; de Vries, 2018; Poulton et al., 2018) from the scientific community, this initiative: i) has been an aspiration and definitive step in the direction of direct action to mitigate climate change via soil C sequestration, ii) brought soil C sequestration to extensive scientific, public and political attention, and iii) considers soil below the topsoil, albeit to a maximum depth of 40 cm, in the context of C sequestration.

Currently, the practiced measures to limit C loss and/or maximise C retention in agricultural soils are largely targeted to topsoils (Ap horizon; ca. 0–30 cm). This predisposition towards topsoils is confirmed by Yost and Hartemink (2020) who found the mean soil depth studied in 4 primary soil science journals to be 24 cm between 2004 and 2019. Topsoil C retention strategies include reducing tillage intensity, the addition of organic amendments, growing cover crops, using leys with grazing livestock, agroforestry and restoring of natural vegetation (Smith, 2008), along with a variety of regenerative agriculture practices still being tested. However, topsoil, despite being rich in SOC (per volume of soil), has a relatively low potential to sequester further C (Rumpel and Kögel-Knabner, 2011; Hobley et al., 2017). Due to favourable soil conditions for decomposition, high microbial activity, aeration, large inputs of labile organic matter, and high soil disturbance; topsoils experience high rates of C mineralization and short C residence times (Schlesinger and Andrews, 2000; Fontaine et al., 2007; Salomé et al., 2010). As a result, C retention strategies have had varying results in improving soil C stocks and decreasing soil GHG emissions in the long term (Kirkby et al., 2014; Smith et al., 2014a). In addition, as topsoil C sequestration is reversible, changes in land use and management can lead to rapid C loss (Smith, 2008).

While deep soil horizons (ca. ≥ 30 cm) are often considered biologically quiescent, deep soil C is responsive to environmental change (Bernal et al., 2016; Hobley et al., 2017; Slessarev et al., 2020) and comprises the majority of the global soil C pool (Jobbágy and Jackson, 2000). Therefore, to limit C losses and increase C stocks over longer timescales (i.e. 50–1000 years; Piccolo et al., 2018), approaches targeting deeper, low disturbance soil may have the potential to be more successful. The residence time of subsoil (B horizon; ca. ~30–100+ cm) C increases with depth, with C here commonly attaining millennial age (Torn et al., 1997, 2002; Rumpel et al., 2002; Schöning and Kögel-Knabner, 2006). This is confirmed by Shi et al. (2020) who determined the global mean of deep cropland and grassland soil (30–100 cm) to be 3700 and 5400 years old by radiocarbon measurements, which is 3.8- and 3.5-fold older than measured in the topsoil (0–30 cm), respectively. As awareness of the potential for subsoils to promote SOC sequestration grows, interest in C dynamics and strategies of sequestration in subsoils have developed (Chabbi et al., 2009; Kautz et al., 2013; Chen et al., 2018). However, how subsoil C is stabilised, enabling this long-term persistence is still not fully understood (Fontaine et al., 2007; Jones et al., 2018) and specific subsoil C sequestration strategies are lacking sufficient evidence and comparative assessment.

In this review, we explore the potential of C sequestration in non-waterlogged subsoils with a specific focus on agricultural lands (cropland and pasture). Firstly, we explore the nature and properties of subsoils and the forms and amounts of C present within them. Subsequently, we review the evidence and different approaches of current subsoil C sequestration strategies, and identify knowledge gaps in the literature. Finally, the challenges facing C sequestration in subsoils are addressed, alongside suggestions of how progress can be made.

2. Subsoil carbon

2.1. Subsoil biological, chemical and physical properties

In the past, and in early subsoil C models (e.g. RothPC-1, Jenkinson and Coleman, 2008), subsoils were essentially thought of and treated as 'less concentrated' topsoils, but this general assumption has more recently been dismantled (Salomé et al., 2010). Indeed, the differences between the environmental, physico-chemical and biological characteristics of topsoils and subsoils (Rumpel and Kögel-Knabner, 2011) are such that a sound understanding of subsoil processes cannot be directly inferred from our current understanding of topsoils. Because of their high spatial variability at a range of scales (i.e. field, landscape, regional), driven in part by pedology, environment and climate, subsoils are difficult to generalise (Chabbi et al., 2009). To better characterise the diversity, similarities, and differences of top and subsoil horizons, we collected soil profile data of 203 studies across different climates and soil types around the world (Fig. 1, Fig. 2). Details on the search term strategy, selection criteria and spread of soil orders and study locations are presented in the Supplementary Information (Table S1-S2, Fig. S1-S2). We used a topsoil-subsoil boundary of 30 cm when categorising the measurements. A numerical boundary was used because studies predominantly sample soil by soil depth intervals (Yost and Hartemink, 2020). This particular depth was chosen as it is commonly the boundary of soil disturbance (reflecting a historical 12-inch plough; Davis et al., 2018) in ploughed soils, which was a key criterion in the definition of subsoils in this review. However, this boundary does not well represent all soils. For example, in lower production rain-limited environments where no-tillage practices are often used, the topsoil may be functionally defined as <10 cm deep (Hoyle et al., 2013). To avoid falsely categorising soil horizons and better determine whether measurements belonged to the A or B horizon, we used the authors defined boundaries within the individual studies (see Supplementary Information for more detail).

As is evident from Fig. 1, subsoil (ca. 30–100+ cm) physical, chemical and biological properties significantly differ to those of topsoils. Physical soil properties, bulk density and clay content, were on average 10 and 22% higher in B horizons, while most biochemical properties were greater in the A horizon. Overall, SOC, TN and MBC were 64, 58 and 48% lower in the B horizon. Importantly, how much properties differ between depths changes when these are split into some of the most agriculturally important soil orders (Fig. 2). The Inceptisol, Alfisol and Mollisol A and B horizons soil property measurements are relatively consistent with each other, apart from a lack of difference in Inceptisol clay content with depth. Ultisol and Oxisols profiles, on the other hand, are more distinct. Bulk density did not differ between soil horizons and CEC was significantly lower in the B horizon of Ultisol and Oxisols.

While the search term strategy was not exhaustive (Table S1), Fig. 1 shows that although several key soil properties involved in C stabilization in subsoils are frequently reported (e.g. pH, SOC, texture, bulk density), other important properties are not (e.g. MBC, CEC, Fe and Al oxyhydroxide content). This lack of reporting of soil quality indicators for subsoils limits our ability to determine the key regulators of deep C storage.

Generally, the rates of C input to the subsoil are much lower than topsoils, and rates of release back to the atmosphere are also slow, as

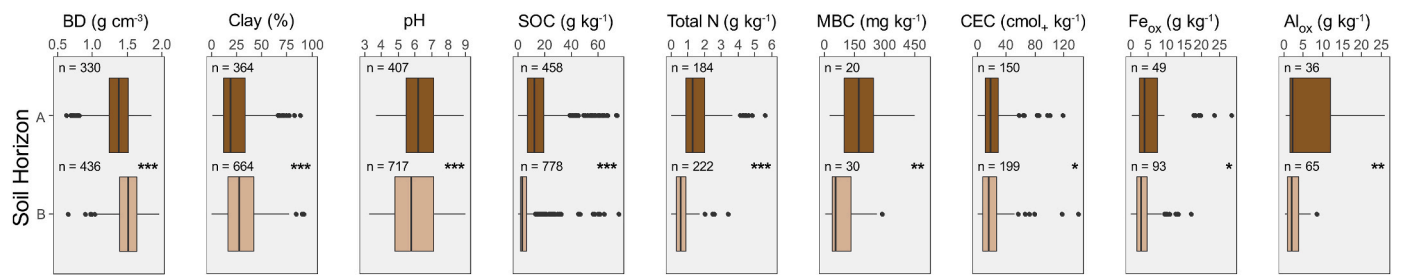


Fig. 1. Measured soil properties of A (ca. 0–30 cm) and B (ca. 30–100+ cm) horizons of agricultural soil profiles. Data was collected from studies ($n = 203$) via a systematic literature search conducted in October 2020. The n in the plots refers to the number of soil profile measurements included in the boxplot. Significance at $p < 0.05$ (*); 0.01 (**); and 0.001 (***). BD is dry bulk density; SOC is soil organic carbon; MBC is microbial biomass-C; CEC is cation exchange capacity; and Fe and Al are oxalate-extractable. See Supplementary Information Section S1.1 above for the search term strategy, selection criteria, data exclusion and conversion and PRISMA diagram.

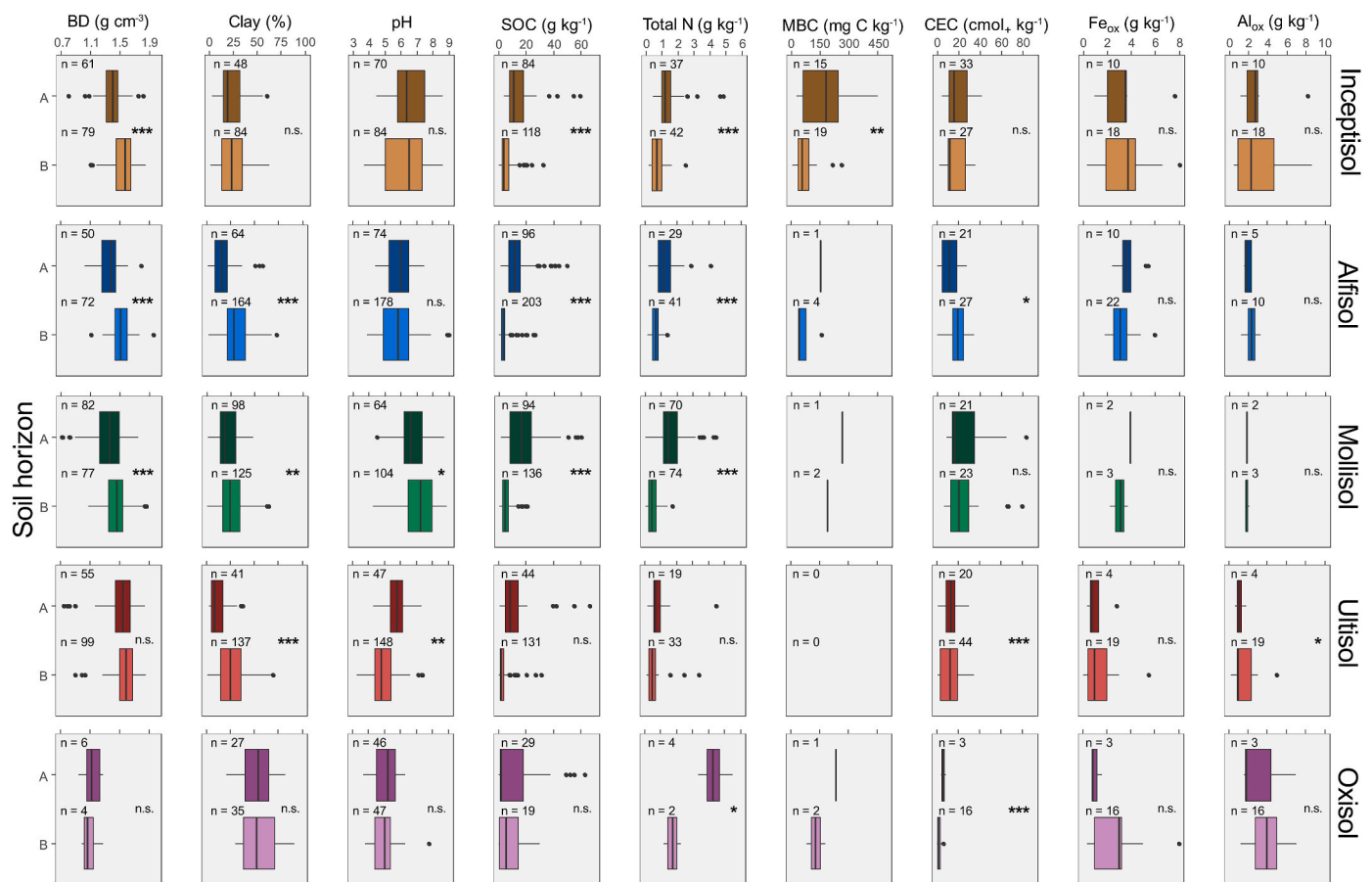


Fig. 2. Measured soil properties of A (ca. 0–30 cm) and B (ca. 30–100+ cm) horizons of agricultural (Inceptisol, Alfisol, Mollisol, Ultisol and Oxisol) soil profiles, ordered by least to most weathered. Data was collected from studies ($N = 188$) via a systematic literature search conducted in October 2020. See the Supplementary Information for the search term strategy, selection criteria, data exclusion and conversion and PRISMA diagram. The n in the plots refers to the number of soil profile measurements included in the boxplot. Significance at $p > 0.05$ (n.s.); $p < 0.05$ (*); 0.01 (**); and 0.001 (***). Where there is no sign the sample size was too small to perform a test. For more information see Supplementary Information BD is dry bulk density; SOC is soil organic carbon; MBC is microbial biomass-C; CEC is cation exchange capacity; and Fe and Al are oxalate-extractable.

evidenced by the older age of C at depth (Shi et al., 2020). Why C turnover is slower in the subsoil, then, is likely due to: i) low disturbance from agricultural practices (Lal et al., 2015); ii) proportionally lower SOC and microbial biomass (Fontaine et al., 2007; Salomé et al., 2010; Liu et al., 2018b), iii) the physical (in)accessibility of microbes to C substrates outside of hotspots (Heitkötter and Marschner, 2018; Dove et al., 2020; Salomé et al., 2010); iv) high abundance of available mineral surfaces (e.g. clay and Fe/Al in the Bw horizon) and Ca^{2+} for adsorption and chemical stabilization of C (Mikutta et al., 2006; Rumpel

and Kögel-Knabner, 2011); and, v) the prevailing oligotrophic conditions (i.e. low O_2 , N availability, pH etc.) which limit enzyme synthesis (e.g. O_2 -dependent phenol oxidases) and activity (Xiang et al., 2008; Salomé et al., 2010; Shahzad et al., 2018a; Dove et al., 2020) and so microbial activity. These mechanisms underpin the mean residence times of SOC in subsoils, which are typically on the scale of millennia as compared to centuries in topsoils (Shi et al., 2020).

At the field scale, subsoils can be characterised by a high degree of spatial heterogeneity due to the presence of larger aggregate structures

(e.g. prisms), preferential water flow pathways and root proliferation (White and Kirkegaard, 2010). This can lead to the creation of biological hotspots in subsoils (e.g. biopores; Chabbi et al., 2009; Kautz et al., 2013; Kuzyakov and Blagodatskaya, 2015). These hotspots of degradable substrates and associated microbial activity play an important role in C, N and P transformations in the subsoil (Hoang et al., 2016). Outside of these microsites, the inactivity of microbes may explain the measured long-term stability of SOC (Heitkötter and Marschner, 2018).

Understanding the influence of microorganisms on deep soil C is crucial because microbial products – including exo-enzymes, extracellular polymeric substances (EPS), and cell wall materials – contribute increasingly to long-lived soil organic matter in deeper soil horizons (Dove et al., 2020; Peixoto et al., 2020; Dwivedi et al., 2017; Sher et al., 2020). Recently, the number of soil microbiology studies focused on subsoils has expanded (e.g. Eilers et al., 2012; Kramer et al., 2013; Li et al., 2014; Brewer et al., 2019; Diamond et al., 2019; Polain et al., 2020). Microbial community composition, biosynthetic potential and metabolic pathways change significantly with depth, with relatively more copiotrophs present in the topsoil and oligotrophs in the subsoil (Fierer et al., 2003; Uksa et al., 2015; Jones et al., 2018; Brewer et al., 2019; Sharrar et al., 2020). The vertical distribution of these microbial groups has been found to be predominantly determined by the availability and forms of C (Fierer et al., 2003; Stone et al., 2014; Fanin et al., 2019). Deep soils are enriched in autotrophic archaea implicated in ammonia oxidation (Brewer et al., 2019) and symbiotrophic fungi (Schlatter et al., 2018) with enzymatic capacities that are distinct from their saprotrophic counterparts. Indications of methylophony and “dark autotrophy” (CO₂ fixation) have also been uncovered in subsoil bacterial genomes (particularly in Chloroflexi) (Brewer et al., 2019; Butterfield et al., 2016). Shifts from protozoa, fungi and Gram-negative bacteria in the topsoil to Gram-positive bacteria (and actinomycetes) with greater depth (Fierer et al., 2003; Stone et al., 2014; Fanin et al., 2019) reflect the required adaptations for survival in deeper soil. Gram-positive bacteria, for instance, are well adapted to subsoils by their ability to sporulate, resilience to harsh environments (i.e. water limited) and preference for older more complex C derived from soil organic matter (SOM; Setlow, 2007; Kramer and Gleixner, 2008). Other strategies, such as storage of internal resources, dormancy and trophic flexibility, found in *Dormibacteraeota* which are particularly abundant in subsoils across the US (Brewer et al., 2019; Lennon et al., 2020), highlight the range of strategies used by microbial groups to overcome the limitations of subsoils.

Agricultural practices have been shown to strongly affect the size, structure and activity of microbial communities in topsoil, however, they appear to have less effect in subsoils where disturbance is lower, and communities seems more resilient. For example, crop type (cotton vs. maize and wheat vs. maize) and time in the cropping cycle has been shown to have relatively little impact on subsoil communities (Polain et al., 2020; Kramer et al., 2013). Inorganic fertilisation, however, can change the microbial community structure throughout the soil profile by topsoil-derived leachates altering the availability of C in the subsoil (Li et al., 2014).

Despite clear differences in microbial communities, microbial competition for C and N can be as intense in the subsoil as in the topsoil (Jones et al., 2018; Diamond et al., 2019). Findings by Jones et al. (2018) suggest that subsoil microbes are more C limited but can rapidly become active and grow upon organic C addition. This is supported by the short lag phase in CO₂ production after the addition of C substrates to subsoils particularly when high amounts of labile C are added (Cressey et al., 2018; de Sosa et al., 2016). Soil N supply also typically decreases with depth (Murphy et al., 1998; Ekelund et al., 2001; Kemmitt et al., 2008; Uksa et al., 2014; Banning et al., 2015) and inorganic N is heterogeneously distributed in the subsoil compared to the topsoil (Taylor et al., 2002; Kuzyakov and Blagodatskaya, 2015). Therefore, limited microbial access to spatially distributed substrates in the subsoil is likely an important factor for SOC accumulation and stabilization

(Preusser et al., 2019).

2.2. Subsoil priming of SOM

Soil priming, the short-term mineralization of SOC through the introduction of labile C (Kuzyakov et al., 2000), has different controls in topsoils versus subsoils. These are driven by differences in labile C availability (De Graaff et al., 2014), co-location of decomposers and substrates (Salomé et al., 2010), microbial responses to C inputs (San-aulah et al., 2011), and the frequency of the inputs. However, the occurrence of priming does not mean there is no net SOC storage — in most cases where C is inputted, the resulting net C stock is higher even if some is lost to priming. In addition, due to stimulated microbial growth from priming, microbial products and necromass may accumulate and stabilise in the longer term (‘entombing effect’; Liang et al., 2017) reducing the extent of C loss from priming.

Higher priming C losses have been measured in subsoil compared to topsoil (relative to native soil C content) (Salomé et al., 2010; Hoang et al., 2017; Jia et al., 2017; Meyer et al., 2018), but the opposite response has also been reported (De Graaff et al., 2014).

When OM is added to the subsoil, the strength of priming is likely to be dependent on the C:N ratio of the OM being introduced and the intrinsic nutrient status of the soil (e.g. N and P status; Kuzyakov et al., 2000). If a high C:N material is incorporated, this may temporarily satisfy short-term C demand causing reduced C respiration, however, it is also likely to stimulate microbial growth and induce N mining from native SOM (Jones et al., 2018; Meyer et al., 2018).

A concern for many subsoil priming studies is that experiments are commonly conducted in laboratory conditions that poorly mimic those in the field (e.g. on sieved soil at ambient O₂ concentration) and are known to often overestimate net losses. Physical subsoil disturbance can increase C mineralization by up to 75%, as found in a laboratory incubation study by Salomé et al. (2010). This increase in SOM turnover has been ascribed to (i) improved aeration, (ii) greater physical access to C substrates previously inaccessible or held within aggregates, and (iii) the mining of nutrients from SOM. Many studies also use highly labile C substrates (e.g. glucose) at high dose rates that can drive excessive nutrient limitation. Consequently, the net C loss (priming effects) can be overestimated. Overall, our understanding of the mechanisms and factors involved in subsoil priming remains poor. It is likely that the relative balance between net C losses versus gains may vary on seasonal versus decadal timescales and in response to agronomic management regimes (e.g. subsoil C input, crop nutrient and water use) (Wang et al., 2016). To gain further insight into subsoil C dynamics, the different sources of C that reach the subsoil, mechanisms by which they are stabilised, and realistic *in situ* tests need to be considered in future studies. This will allow interventions to enhance C sequestration in subsoils to be more effectively designed (e.g. timing, placement in the subsoil, frequency of intervention, links to root architecture).

2.3. Subsoil gas emissions

The behaviour and fate of GHGs in subsoils play an important role in subsoil C sequestration (Blagodatsky and Smith, 2012), and overall system C balance. Here we focus on C containing gases, CH₄ CO₂, in the context of C sequestration, although we note that N₂O fluxes (reviewed by Clough et al. (2005)) should also be considered in a holistic analysis of deep soil C sequestration strategies.

Soil CO₂ concentrations are known to increase with depth, despite fluxes decreasing with depth and not contributing substantially to surface fluxes (Davidson and Janssens, 2006; Xiao et al., 2015; Wang et al., 2019). This suggests that CO₂ in the subsoil does not move rapidly to the soil surface and if undisturbed may be entrapped in soil pores and solution, or used by subsoil autotrophs. However, this ‘trapped’ CO₂ is vulnerable; along a subsoil-to-surface CO₂ gradient of >10,000 ppm to atmospheric concentrations, it may only take a few hours to days for CO₂

to diffuse to the atmosphere (e.g. when CO_2 is produced near subsoil macropores or when the water-filled pore space is low; Mencuccini and Hölttä, 2010). Thus, subsoil disturbance could disturb the deep dynamic reservoir of subsoil CO_2 .

Wang et al. (2019) found that while CO_2 concentrations increased with depth following a full inversion of forest subsoil to 60 cm, the soil surface CO_2 flux remained largely unaffected by the highly invasive subsoil disturbances. In the case of enhanced subsoil rooting for C sequestration, plant root uptake of water from the subsoil can lead to increased aeration, greater gas diffusivity, soil shrinkage and the formation of macropores which facilitate migration to the surface (Shaw et al., 2014). Roots can also take up dissolved inorganic C (CO_2 , HCO_3^-) from soil and rapidly transport it through the xylem to the leaves where it can be refixed or released back to the atmosphere (Bloemen et al., 2016). Although the uptake rate of HCO_3^- from soil is generally low, the direct recycling of HCO_3^- produced inside the roots (i.e. from respiration) back to the shoots via the xylem may be significant in reducing CO_2 concentration in soil (Rao et al., 2019). Still, these CO_2 loss effects maybe counterbalanced by the accrual of deep root C inputs; studies of deep-rooted perennial grasses planted in low C soils found no effect of these crops on surface CO_2 fluxes and increases in total soil profile C stocks in some soil types (Bates et al., 2021; Slessarev et al., 2020). Therefore, it is important to consider that different sequestration strategies, such as deep tillage and planting of deeper rooting varieties, may influence the soil-atmosphere flux and the soil CO_2 budget in different ways.

Over a recent decade (2008–2017), global CH_4 emissions from agricultural systems were estimated at 206 Tg y^{-1} ; this represented 56% of the total anthropogenic emissions (Saunois et al., 2020; Jackson et al., 2020). To our knowledge, the fraction of this total contributed by agricultural subsoils has not been estimated. In many well-drained systems, CH_4 produced by methanogens in anaerobic microsites can be consumed by methanotrophs in oxic regions during transit to the surface (Le Mer and Roger, 2001; Wang et al., 2018), suggesting CH_4 dynamics in the soil have limited bearing on any C sequestration outcome.

Very little is known about microbial volatile organic compound (VOC) production and consumption rates in subsoils. VOCs have low-molecular-weights (typically $<250 \text{ MW}$) with high vapor pressures. They can be produced in soil by both microorganisms and plant roots (Peneulas et al., 2014). Like CH_4 , VOCs are both produced and consumed *in situ* (Tassi et al., 2009), suggesting they are unlikely to be relevant to C sequestration.

2.4. Subsoil carbon sources and stabilization

The primary inputs of C to subsoils include: i) root-derived C (both dead roots and living root rhizodeposition); ii) leaching of dissolved organic C (DOC) from the topsoil; iii) delivery of particulate organic matter via bioturbation or leaching, and iv) microbially-derived C (immobilization of CH_4 or volatile organic-C, dark fixation of CO_2). The primary C sources and their stabilization mechanisms in the top- and subsoil are presented in Fig. 3. Roots in the subsoil decompose relatively

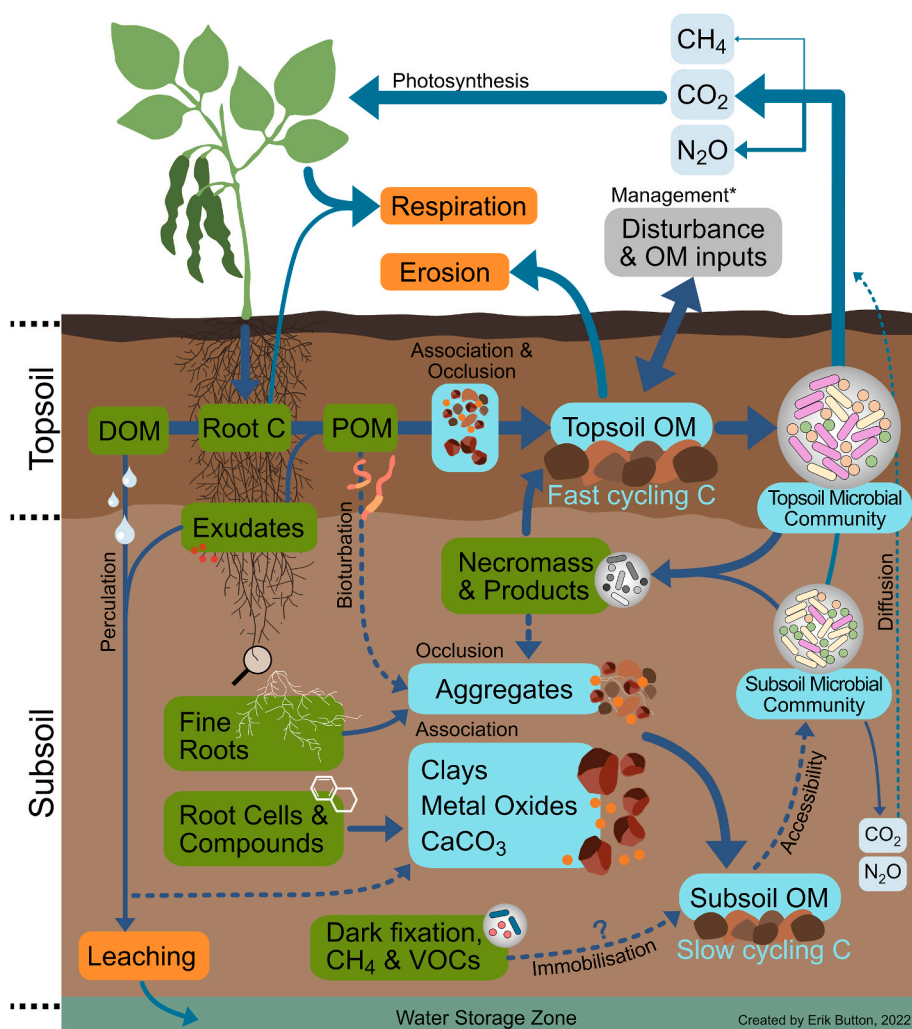


Fig. 3. Conceptual diagram of the top- and sub-soil C cycles, demonstrating the major SOM (soil organic matter) inputs (in green boxes); the primary components determining soil OM persistence (in cyan); agricultural management (in grey box); and losses (in orange boxes and teal arrows) in an arable system. POM is particulate organic matter and DOM is dissolved organic matter. Dashed arrows represent mechanisms that depend on certain soil characteristics to occur or that they occur at very low rates.

*The specific balance between physical disturbance and OM inputs from agricultural management determines the impact on topsoil OM.

slowly, whereas topsoil and detritusphere leachates and root exudates with high C:N ratios and C availability are more easily mineralised. Subsoil microbial biomass has a slower turnover time (Spohn et al., 2016). The C:N ratio declines with soil depth in most agricultural soils (Rumpel and Kögel-Knabner, 2011; Lou et al., 2012), demonstrating that C in the subsoil cycles slower compared to soil nearer the surface.

2.4.1. Root-derived carbon

Plants may direct up to half of photosynthetically fixed C to roots (Jones et al., 2009) and most subsoil organic carbon (OC) is plant root-derived (Rasse et al., 2005; Suseela et al., 2017). For plants with deep rooting architecture, roots and their products (i.e. exudates and cell sloughing) have substantial potential to enter stabilised subsoil OC pools (Rasse et al., 2006; Kätterer et al., 2011; Suseela et al., 2017), and root litter decomposes more slowly in deep soils (Pries et al., 2018). While this stabilization depends on the soil environment and root physiology (Farrar et al., 2003), three primary root-derived C sources are thought to contribute to stable OC by different mechanisms (Fig. 3): aggregation, root biochemistry and association.

Due to the major role of biotic processes in soil aggregation, it is often thought less relevant as a C stabilization mechanism in subsoils (Lorenz and Lal, 2005). However, recent studies have revealed aggregation may be as important in the subsoil as it is at the surface (Moni et al., 2010; Sanaullah et al., 2011; Baumert et al., 2018). For example, Moni et al. (2010), found up to 40% of SOC was occluded within aggregates throughout the whole soil profile (>100 cm depth). Sher et al. (2020) found enhanced microbial production of soil-binding extracellular polysaccharides throughout a 1 m soil profile following conversion from annuals to deeper-rooting perennials, and suggest that aggregation is likely an important mechanism in subsoil C protection.

The biochemical composition of primary roots can contribute to their stabilization in soil, particularly those with significant amounts of lignin, tannin or suberin (Rasse et al., 2005). The decomposition of lignin may be slowed through protection via mineral association (Rumpel et al., 2015; Hall et al., 2016; Huang et al., 2019), accelerated by short-term fluctuations in redox states, or gut processing within earthworms (Le Mer et al., 2020). In addition, extracellular enzymes (e.g. those that decompose lignin - phenol oxidase) have been found to largely be stabilised via sorption onto mineral surfaces in subsoils (Dove et al., 2020). While tannin residence time in soil is similar to non-associated lignin (Meier et al., 2008), suberin is a major contributor to SOC with a high potential for long-term stabilization (Rasse et al., 2005; Suseela et al., 2017). In subsoils, Rumpel et al. (2004) found suberin-derived hydroxyalkanoic acids to be preferentially preserved (over lignin) in clay particle fractions. However, despite its important role in root chemistry and SOC stabilization (Suseela et al., 2017), the behaviour and persistence of suberin in subsoils remains poorly understood (McCormack et al., 2015).

Root exudates, organic compounds passively released from roots, can have a multitude of interactions with soil minerals (Farrar et al., 2003). For example, Keiluweit et al. (2015) showed that oxalic acid (a common root exudate) liberates C previously protected by minerals, thus promoting C loss via increased microbial availability. Although exudation varies with plant age and species, measurements of exudation and rhizodeposition suggest up to 7% of net fixed C can be deposited in croplands and 11% in grasslands (Pausch and Kuzyakov, 2018; Jones et al., 2009). Exudates contain sugars, organic acids, amino acids, fatty acids and plant hormones; these are primarily C sources that can be mineralised within hours by the soil microbial community (Rasse et al., 2005; Salomé et al., 2010; Zhalnina et al., 2018). Yet, negatively charged organic acid anions can become fixed on the surface of positively charged Fe and Al (hydr)oxides, protecting them from short-term mineralization (Jones and Edwards, 1998; Oburger et al., 2011). Other root deposits, such as mucilage and EPS, also play an important role as binding agents for aggregate formation (Baumert et al., 2018; Sher et al., 2020). Mucilage is reactive and high in hydroxy groups and

can adsorb to clay particles and organic molecules (Gaume et al., 2000).

Physical separation of decomposers from exudates in subsoils may be one of the key drivers of exudate-C stabilization (Salomé et al., 2010), although very little is known about rhizosphere and root detritusphere microbial communities in subsoils. It is possible that roots also deliver C into subsoils via arbuscular mycorrhizal associations (Sosa-Hernandez et al., 2019). Subsoil arbuscular mycorrhizas are different taxonomically from those in topsoils (Sosa-Hernandez et al., 2018); however, whether they differ functionally requires further research (Wang et al., 2017).

2.4.2. Leaching of dissolved organic C from the topsoil

Dissolved organic carbon (DOC) represents another primary C input to subsoils. During the decomposition of SOM and plant litter, microbes produce nanoparticulate C (nPOC) and DOC (Solinger et al., 2000; van den Berg et al., 2012). DOC consists of a complex array of organic compounds, each with distinct properties, structures, sizes, and sorptive characteristics, and play a significant role in C dynamics, soil formation and pollutant transport (Kaiser and Guggenberger, 2000; Kothawala et al., 2012; Jagadamma et al., 2014). Organic molecules can—depending on the soil hydrology, texture and structure—enter the subsoil and become stabilised in organo-mineral complexes, mineralised, or leached into groundwater or aquatic systems (Fig. 3; Whitmore et al., 2015). Because DOC can be leached to great soil depths and become sorbed to form organo-mineral complexes, DOC is an important source of stabilised C in subsoils (Mikutta et al., 2006; Kramer et al., 2012); multiple studies have observed greater adsorption with increased depth, possibly due to a greater amount of unfilled sorption sites or clay in some soil types (Kaiser and Zech, 2000; Solinger et al., 2000; Jastrow et al., 2007; Moni et al., 2010).

Various stabilization reactions bind DOC to the solid phase (Solinger et al., 2000; Dignac et al., 2017), including van der Waals forces, anion exchange, cation bridging, ligand exchange, hydrogen bonding and physical adsorption, which vary in their importance depending on the functional groups of DOC and the sorbent. Clay particles and Fe and Al (hydr)oxides in the fine fraction (<53 µm) of subsoils are the primary substrates for DOC sorption (Torn et al., 1997; Jobbágy and Jackson 2000; Kaiser and Zech 2000), protecting C for thousands of years (Schöning and Kögel-Knabner, 2006; Shi et al., 2020). Some of these sorption sites can bind C very strongly (through bi- or tri-dentate ligand binding), while others are much weaker (mono-dentate binding or cation bridging). More recent microscopy studies (Müller et al., 2013; Schweizer et al., 2017) reveal that the majority of mineral particles are not colonised by microbes and are largely devoid of OC, which contrasts with older refuted studies (e.g. Guggenberger and Kaiser (2003)).

2.4.3. Delivery of particulate organic matter via faunal bioturbation and leaching

Faunal bioturbation may be an important aspect of subsoil C dynamics (Fig. 3; Wilkinson et al., 2009; Rumpel and Kögel-Knabner, 2011). Soil macro-organisms, such as earthworms, ground-dwelling rodents and termites, directly and indirectly drive both C inputs into and outputs from the top- and subsoil (Bossuyt et al., 2005; Wilkinson et al., 2009; Rumpel and Kögel-Knabner, 2011). By moving, burying and mixing vast quantities of soil and fresh OM, bioturbators have an important role in soil formation, C and N dynamics and shaping the soil environment (Wilkinson et al., 2009).

Anecic earthworms can burrow to soil depths of 1–2 m, occasionally reaching up to 5 m (Lee, 1985). By transporting fresh particulate OM into the subsoil and mixing it with mineral soil, earthworms can contribute to the heterogeneous distribution of subsoil SOC (Don et al., 2008; Rumpel and Kögel-Knabner, 2011), and mediate soil aggregate formation which is associated with SOC stabilization (Six et al., 2004). However, field and lab studies frequently find anecic earthworms induce SOM loss from increased respiration. This is likely due to stimulation of microbial activity within biopores (Banfield et al., 2017) with C-rich labile earthworm mucus and higher O₂ levels (Hoang et al., 2016, 2017).

Earthworms and their casts are known to be hotspots of N_2O emission, as they also contain high mineral N concentrations (Elliott et al., 1991; Lubbers et al., 2013; Nieminen et al., 2015). Finally, Lubbers et al. (2017) found that the topsoil (0–25 cm) SOC content was lower after 2 years in the presence of epigeic and endogeic earthworms, suggesting faunal bioturbation diluted SOC in the topsoil by mixing it with C depleted subsoil. Termites and ants may also increase C transfer to depth either through deposition of necromass, food stores and exudates but also indirectly by creating channels in the soil that fill with water and thus move DOC and POM to depth (Jouquet et al., 2011). These channels may also stimulate aeration and rooting at depth (Banfield et al., 2018) leading to crop yield increases and thus greater C inputs (Kautz et al., 2013).

In addition to faunal bioturbation, particulate organic C (POC) can also be transported downwards in the soil profile by water. In the case of large fragments of SOM, this can occur via macropores while smaller nanoparticulate fragments can be transported through the soil matrix (Li et al., 2019). For example, viruses (ca. 20–100 nm in size) and bacteria (ca. 1–3 μm in size) applied to the soil surface in livestock manure have been measured in subsoils (Krog et al., 2017) and similarly, particles of black C have been shown to move downward in soil profiles (Leifeld et al., 2007; Major et al., 2010).

2.4.4. Microbially derived C

Soil microbial community structure, genomic capacity, and ecophysiology are strongly depth-dependent (Brewer et al., 2019). Understanding the influence of depth on microbial traits is crucial because microbial products – including exo-enzymes, EPS, and cell wall materials – may contribute increasingly to long-lived SOM in deeper soil horizons (Dove et al., 2020; Peixoto et al., 2020; Dwivedi et al., 2017; Sher et al., 2020). While we do not currently have enough data to speculate too much on the persistence of root-derived vs microbe-derived SOM in deep soils, recent evidence suggests that microbially derived necromass is a major contributor to SOC (Zhang et al., 2020; Wang et al., 2021). Wang et al. (2021) estimated that half of SOC under cropland and grasslands is derived from microbial necromass and that it predominantly originates from fungi. In addition, they found that the contribution of microbial necromass to SOC increased with depth in grasslands while the opposite was true in croplands. Overall, the organisms, biosynthetic potential and metabolic pathways of deep soils differ from better-studied shallow soils (Butterfield et al., 2016; Sharrar et al., 2020; Diamond et al., 2019). For example, deep soils are enriched in autotrophic archaea implicated in ammonia oxidation (Brewer et al., 2019) and symbiotrophic fungi (Schlatter et al., 2018) with distinct enzymatic capacities from their saprotrophic counterparts (Miyachi et al., 2020). In addition, deep soil microbes may play a particularly unique role in subsoil C accumulation through immobilization of methane (CH_4) and volatile organic carbon (VOC) or via dark autotrophy (CO_2 fixation).

Apart from surface photosynthetic CO_2 fixation and chemoautotrophic fixation, dark anaerobic (i.e. non-photosynthetic) heterotrophic fixation of CO_2 occurs in a wide range of soils and is linked to the provision of C-skeletons for amino acid synthesis (Yang et al., 2017; Nel and Cramer, 2019). A wide range of soil archaea and bacteria are capable of dark anaerobic CO_2 fixation in both aerobic and anaerobic conditions (Saini et al., 2011), and produce organic acids. Although the overall contribution of dark fixation is extremely small in topsoils (Ge et al., 2013), dark fixation may be proportionally more important in subsoils, presumably due to C limitations with depth (Santrücková et al., 2018), and higher CO_2 concentrations. As yet, there are no *in situ* studies of dark CO_2 fixation in agricultural subsoils and it is difficult to critically assess the significance of this process in the overall net C balance of subsoils. In arctic soils, Santrücková et al. (2018) found that long term microbial dark fixation of CO_2 corresponded to between 0.016 and 38% of plant C fixation, highlighting the uncertainties regarding the importance of CO_2 fixation in the net soil C balance. The preferred microhabitats and

edaphic conditions of microorganisms responsible for dark CO_2 fixation in subsoils are also unknown.

While chemoautotrophy (i.e. C fixation from the oxidation of reduced forms of inorganic N and S; NH_4^+ , S^{2-}) can be very important in extreme ecosystems (e.g. hydrothermal vents), it is thought to be a relatively minor C fixation process in soil due to the relatively low growth yields of chemoautotrophic organisms and their inability to compete against heterotrophic bacteria. Despite this, chemoautotrophic ammonia-oxidizers and nitrite-oxidizers can be abundant in subsoils (10^5 – 10^8 g^{-1}) suggesting that their role in C fixation should not be discounted (Jones et al., 2018; Tao et al., 2018).

3. Enhancing C sequestration in subsoils

Capturing CO_2 from the atmosphere and submitting this to long-term storage in the subsoil as organic C has potential to offset substantial anthropogenic CO_2 emissions and bring a range of other ecosystem service co-benefits. Various approaches to increasing SOC in soils exist, but here we discuss strategies that aim to: i) increase C inputs; ii) reduce C losses; and/or iii) increase C residence time in soil. However, we agree with Olson et al. (2014) that ‘true’ sequestration is not a transfer of C, but increased C fixation from the atmosphere.

The depth of the subsoil that is considered in this review for strategies to have the greatest effect is the soil to approximately 1 m depth (i.e. an ‘impressionable zone’). The volume of this zone is different at each site due to the depth of the B and C horizons, the watertable and presence/absence of a hardpan.

3.1. Deeper-rooting phenotypes and perennials

Use of plants with deep rooting systems, particularly perennials, has been proposed as another method to increase SOC stocks, particularly in subsoils (Paustian et al., 2016). A common concern is that increasing plant C allocation to roots decreases harvestable aboveground biomass (Powelson et al., 2011), however, a review by Kell (2012) concluded that deep roots are unlikely to limit, but may instead promote harvestable biomass. Breeding deeper rooting grass and crop varieties is a less invasive strategy (compared to those discussed above) that has substantial potential in sequestering C in the subsoil of some soil types (Smith, 2004; Kell, 2011, 2012). Deeper roots can yield co-benefits for plant productivity and drought tolerance, including improved plant capture of nutrients (e.g. N, P) and water (Kell, 2012; Lynch and Wojciechowski, 2015; Pierret et al., 2016), as well as higher crop yields (Lilley and Kirkegaard, 2011) and greater resistance to, for example, slope erosion (Dignac et al., 2017). The use of deep-rooting crops can also be readily combined with mechanical interventions to promote access to previously compacted subsoil layers (He et al., 2019) or to the deep placement of fertilisers to promote root proliferation at depth (McEwen and Johnston, 1979).

By adopting crops that grow an extra 100 cm in depth, Kell (2012) calculates an additional 100 t C ha^{-1} could be sequestered, corresponding to a 118 ppmv reduction in atmospheric CO_2 . Whether these values are accurate is difficult to determine, yet, deeper-rooting undoubtedly increases C entering the subsoil (Liebig et al., 2005; Omonode and Vyn, 2006; Follett et al., 2012; Ledo et al., 2020), but the benefits thereof may only be apparent in the longer-term (7–10 years), as found by Ma et al. (2000) and Carter and Gregorich (2010). This is because net SOC stock increase is a balance between enhanced root C supply to the subsoil and greater soil respiration (Schmidt et al., 2011; Shahzad et al., 2018b). This is exemplified by the relatively low increase in C in the soil profile ($0.07 \pm 0.02 \text{ g C kg}^{-1} \text{ y}^{-1}$) with time (Fig. 4). Fig. 4 also demonstrates that higher C gains are more likely in the topsoil ($0.13 \pm 0.06 \text{ g C kg}^{-1} \text{ y}^{-1}$) where root density is greater, and the volume of soil is lower relative to many subsoils ($0.04 \pm 0.02 \text{ g C kg}^{-1} \text{ y}^{-1}$).

Perennialisation of annual crops and conversion of annual to perennial crops can enhance rooting depth and architecture, which

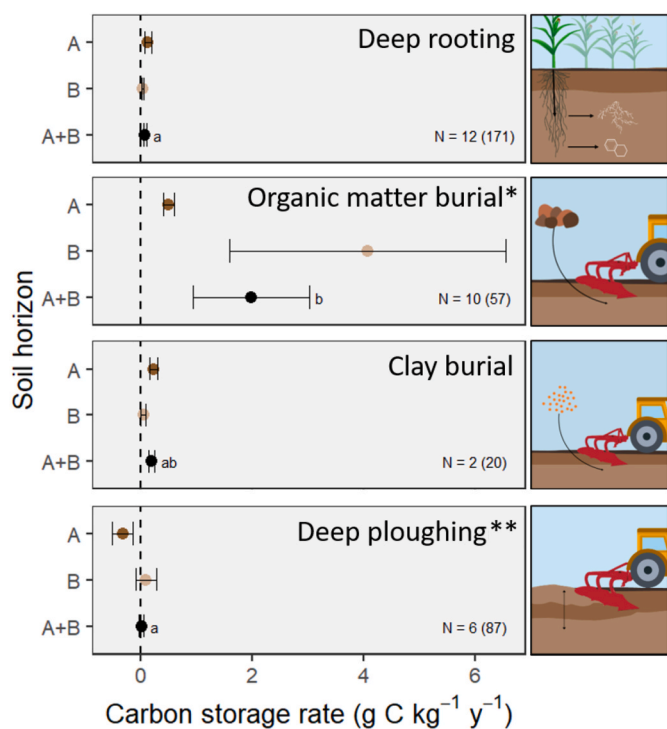


Fig. 4. Means (\pm SEM) of the carbon storage rate in the A and B horizons and the combined A and B horizons (A + B) following different subsoil-targeted C sequestration strategies. The number of studies included (N) is shown in the individual plots and the number of measurements included are in parentheses. Different letters correspond to significant differences between means ($p < 0.05$ Tukey). See the Supplementary Information for the search term strategy and specific inclusion criteria

*Transfer of exogenous C is not the same as C sequestration in terms of C removal from the atmosphere.

**Infrequent deep ploughing (every >10 years).

increases C input into the subsoil (Liebig et al., 2005; Kell, 2011; Powelson et al., 2011). In addition, perennialisation means less tillage and the associated C losses, allowing for more C accrual. Slessarev et al. (2020) showed that the increased rooting depths offered by perennial grasses added appreciable soil carbon in sandy and loam soil (although no SOC increase was measurable in clay-rich sites), and deep roots may also lead to changes in site hydrology and the responsiveness of deep soil microbes (Oerter et al., 2021; Min et al., 2021). Aggregation may also increase as a result of greater root biomass found by Sher et al. (2020). A meta-analysis by Ledo et al. (2020) found an 11% increase in the 0–100 cm soil depth following conversion to perennial crops from annuals over a 20-year period. Similarly, Follett et al. (2012) found a 2 t C y⁻¹ increase in the 0–150 cm depth following 9 years of maize cultivation, where the majority of the increase was below 30 cm. These results are echoed by other studies (Liebig et al., 2005; Omonode and Vyn, 2006) that found SOC stock gains in the whole soil profile following conversion of annual crops to perennial grasses. However, as Johnson et al. (2014) found, N₂O emissions can increase with perennials and SOC increases in the subsoil can be limited (Ma et al., 2000; Chimento et al., 2014).

Apart from breeding deeper rooting varieties, there are several potential avenues for breeding C sequestration desirable traits in crops. For example, enhancing the root release of low molecular weight exudates and extracellular polymeric substances may promote C retention when they become sorbed to mineral surfaces or physically protected (Salomé et al., 2010; Sher et al., 2020). Furthermore, fungi, such as arbuscular mycorrhizal fungi (AMF), can reduce C mineralization by i) their complex C-containing mycelium being less mineralisable, ii) improving root lifespan, iii) enhancing root-derived C protection in aggregates, and iv) outcompeting microbes (mostly bacteria) for N (De Deyn et al., 2008;

Bardgett et al., 2014). While AMF colonisation typically decreases with depth (Bardgett et al., 2014; Lynch and Wojciechowski, 2015), it can be promoted by decreasing the N and increasing the suberin contents in roots (Bardgett et al., 2014). Increasing fine root density promotes the physical occlusion of root C within aggregates (Lynch and Wojciechowski, 2015; Dignac et al., 2017). Also, including traits associated with overcoming subsoil limitations, such as acidity (by e.g. organic acid release) would prove useful in highly weathered tropical soils. While of substantial potential, these breeding avenues are based on theory and are currently experimentally untested.

3.2. Organic matter burial in the subsoil

Burial of OM, such as straw, in the subsoil by deep ripping or DP (see 3.1) is a strategy used primarily for amelioration of subsoil compaction, yet it can increase the subsoil C stock. This is supported by data collected from 10 studies (Fig. 4), which show large C gains in both topsoil (0.5 ± 0.1 g C kg⁻¹ y⁻¹) and subsoil horizons (4.1 ± 2.5 g C kg⁻¹ y⁻¹). Burial of large amounts of plant residue or animal waste adds large amount of C to the soil stock (2.0 ± 1.1 g C kg⁻¹ y⁻¹ across the soil profile). While these are remarkable numbers, it is important to remember that a transfer of exogenous C is not the same as C sequestration in terms of C removal from the atmosphere (as defined by Olson et al., 2014). Therefore, amending the soil with large amounts of C will lead to greater C stock. Yet, it is both unlikely that all of the introduced C will remain (Leskiw et al., 2012; Liu et al., 2018b) or that it increases C fixation of atmospheric C, meaning the gains in Fig. 4 are not ‘true’ C sequestration. In addition, the physical disturbance required to input labile OM at depth may promote access to previously unavailable C (Salomé et al., 2010). This response is confirmed by Shahzad et al. (2018a) who observed increased respiration rates of buried C4 maize litter in C3 subsoil (55–75 cm) compared to the topsoil (0–15 cm). This was attributed to a i) growth in the biomass of subsoil microbes which are more limited by the availability of labile C than in the topsoil; ii) improved co-location of decomposers and substrate, and finally; iii) microbial N mining, as the litter introduced is nutrient-poor (C:N of 21:1) compared to native subsoil OM (C:N of 8.5:1). Therefore, if this is to be pursued as a C sequestration strategy, it is important to ensure additions are driving ‘true’ accrual of C.

3.3. Biochar burial

Due to its aromatic structure, biochar is extremely resistant to breakdown (Farrell et al., 2013; Naisse et al., 2015), with commonly $<3\%$ of biochar-C decomposing in the first 1–2 years (Major et al., 2010; Jones et al., 2011; Naisse et al., 2015; Wang et al., 2016). This recalcitrance has stimulated interest in its use to store C for climate change mitigation (Das et al., 2014; Smith, 2016). The application of biochar to topsoils has been extensively researched (Song et al., 2016) and surface-applied biochar has limited downward movement potential (Major et al., 2010) To date, there have been relatively few studies on biochar burial in subsoils, particularly at the field scale, however, the limited evidence suggests that it can have positive agronomic benefits when buried at or below 30 cm (Bruun et al., 2014; Iijima et al., 2015). In many cases, however, its C sequestration potential and practicality have been exaggerated. Similar to OM burial, it typically does not cause a truly ‘additive’ C effect, nor net removal of CO₂ from the atmosphere (Chen et al., 2019) at the landscape scale, and the negative impacts have often been ignored (Jones et al., 2011; Hilber et al., 2017; Baveye et al., 2018). Further agronomic trials are therefore required to critically evaluate subsoil biochar burial as a mechanism to promote long-term C storage.

3.4. Iron (hydr)oxide additions

The most important control of C persistence in the soil is believed to

be its association with minerals via sorption (Torn et al., 1997; Lehmann and Kleber, 2015). Of these minerals, Fe and Al (hydr)oxides are widespread in most soils at varying concentrations and have been consistently found to adsorb SOC and increase in concentration with weathering (Kaiser and Guggenberger, 2000; Mikutta et al., 2006; Lalonde et al., 2012). In this review, we focus on Fe (hydr)oxides, due to the large production of iron-containing sludge from the wastewater treatment process (Chen et al., 2015), which could be used for the chemical modification of subsoils.

Lab-based experiments investigating the association of OC with Fe (hydr)oxides report substantially decreased decomposition from Fe-associated SOC (Jones and Edwards, 1998; Mikutta et al., 2007; Wen et al., 2019a). In forest soils, a high proportion of stable OC is bound to iron (Mikutta et al., 2006; Zhao et al., 2016). Furthermore, Porrás et al. (2018), found that <0.5% of Fe-associated glucose added to a subsoil decomposed compared to non-associated glucose, with the effect strongest at 50–60 cm depth. As glucose is neutrally charged and does not associate with sorption surfaces, it suggests that an indirect mechanism is involved in suppressing C turnover (e.g. availability of nutrients such P, or mobility of microbes and exoenzymes). Also, this Fe-associated SOC was found to be more resistant than native SOC to increased temperatures (Porrás et al., 2018). These promising results, albeit from a handful of lab-based studies, suggest that adding iron or iron-associated OM into subsoils may be an effective strategy for stabilising and sequestering subsoil SOC, respectively. However, a limited evidence base (especially at the field scale) means further research is needed before this strategy can be meaningfully evaluated.

The degree of soil C that is saved from mineralization from Fe addition in the field is likely to be dependent on several factors, including the method of subsoil application, the mineral makeup of the soil, the native subsoil C content, soil pH as well as texture and parent material (Button et al., 2022). This method may be particularly relevant in sandy soils where little chemical protection potential exists in the subsoil but of limited relevance to highly weathered soils already rich in Fe. Building a greater evidence base, especially with field studies, will allow for better evaluation of the potential of this strategy.

3.5. Clay addition to subsoil

Similar to the discussion above for Fe, clay addition also has the potential to bind large amounts of SOC. While clay addition has been used to improve SOM and nutrient retention in sandy topsoils (Cann, 2000; Schapel et al., 2019), its addition to sandy subsoils has received less attention. In this scenario, the clay would be added to the soil surface and then incorporated by mechanical soil inversion. While this approach shows promise (Hall et al., 2010; Churchman et al., 2014, Fig. 4), evidence is limited and the practicality and long-term impacts on C storage are not yet known.

3.6. Deep ploughing

Natural instances of soil burial demonstrate that SOC can be stabilised for millennia (Chaopricha and Marín-Spiotta, 2014; Wang et al., 2014a). Mechanical soil inversion techniques, such as deep ploughing (DP), rotary hoe or spading of agricultural land (i.e. mechanical inversion of the soil >30 cm depth), buries more C-rich topsoil and plant residues at depth allowing C accumulation in a 'new' unsaturated C-poor topsoil (Nieder et al., 1995; Alcántara et al., 2016, 2017). However, many disregard DP as a soil management option and suggest that any C input from DP is outweighed by the C lost to respiration (Freibauer et al., 2004; Fontaine et al., 2007; Powlson et al., 2011). Studies that claim this, however, rarely match the timescales at which DP is deemed effective (>10 years), do not fully balance C inputs and CO₂ lost by respiration, lack experimental evidence, or often focus only on CO₂ fluxes and not changes in the C stock.

Alcántara et al. (2017) found that 36–48 years after DP (to a depth of

55–90 cm) arable land, SOC stocks increased by 67% compared to a reference subsoil and resulted in substantially lower GHG emissions compared to conventional and zero-tillage management. The specific mechanisms driving the stabilization of the buried topsoil are untested, but lower microbial activity, a physical disconnect between decomposer and substrate and access to unsaturated mineral surfaces deeper in the soil are likely primary drivers (Salomé et al., 2010; Schiedung et al., 2019). More recently, Schiedung et al. (2019) found that 20 years after DP (100–300 cm) total SOC stocks (0–150 cm) increased by 69%, a marked annual C sequestration rate of almost 9 t C ha⁻¹ y⁻¹, but interestingly the 'new' topsoil had 36% less SOC than the original topsoil, possibly due to a lower C sequestration capacity. This is supported by Alcántara et al. (2016), who found that 'new' topsoils contained 15% less SOC even 3–5 decades after DP, suggesting that the capacity of the 'new' topsoils to sequester C was low in their study; we expect this is highly context dependent.

The results from 6 studies of different soils where SOC was measured in the A and B horizons before DP and after 12–48 years after are presented in Fig. 4 (methods in Supplementary Information). These results demonstrate that i) buried topsoil drives a C increase in the subsoil (0.09 ± 0.2 g C kg⁻¹ y⁻¹); and ii) DP had an overall limited effect on the net C stock (0.004 ± 0.05 g C kg⁻¹ y⁻¹). Based solely on this data, DP is the least effective of the strategies for which sufficient data was available. However, 2 important factors were determined for the success of DP. Firstly, the timespan between DP and SOC stock measurement is important (i.e. more time allows for greater accumulation of C in the 'new' topsoil). Secondly, the location and soil type are crucial to the DP sequestration outcome. DP should not be done when i) soil has high contents of very old SOC, ii) the topsoil is low in C, iii) the soil has a high stone content, iii) steep slopes are present where erosion will be high, or v) subsoils are unfavourable for plant growth (e.g. Al³⁺ and Mn²⁺ toxicity at low pH or Na⁺ toxicity in alkaline soils). Subsoils with <70% silt content that restrict root growth could benefit from DP (Schneider et al., 2017) and sequester SOC (FAO, 2017). Duplex soils (sand over clay; often with a perched water table at the interface) could also benefit from DP where the new surface soil layer has increased clay content while the subsoil has better drainage. Increased plant production and deeper rooting depth on such soils could lead to greater C sequestration.

Mapping the areas with potential for soil inversion and establishing longer-term field studies will be essential to underpin any widespread DP implementation as a management practice for C sequestration. While DP is an expensive process if only used to change SOC profiles, the machinery and additional fuel costs could be offset through increased plant yield in soils where ameliorating subsoil constraints can occur at the same time (e.g. mixing with lime; uplift of subsoil CaCO₃; improved aeration, compaction alleviation), or where existing surface soil problems (e.g. herbicide-resistant weed seeds) become buried at sufficient depth to remove this constraint to crop production.

3.7. Subsoil water table management

In most cropping soils, a low water table depth is desirable to promote effective rooting. If the field is not free draining, then subsoil artificial drainage is installed to lower the water table and improve aeration. From a limited number of studies, this physical disturbance and change in conditions has not been shown to have a major effect on GHG emissions (Dobbie and Smith, 2006; Valbuena-Parralejo et al., 2019) and C storage in mineral soils (Mayer et al., 2018). This suggests that artificial drainage may indirectly provide an effective way to deliver C deeper into the soil profile.

In contrast to mineral soils, the drainage of agricultural peatlands has resulted in very large net SOM loss rates (equivalent to 12 t C ha⁻¹ y⁻¹; Taft et al., 2017). This breakdown of SOM also leads to the release of plant-available nutrients making these soils some of the most productive in the world. In many cases these C stores have taken tens of thousands of years to accumulate, however, they are being lost within decades in

some cases. This rapid loss of natural capital is fuelled by the removal of anoxic constraints on native SOM decomposition by microbes and mesofauna (Wu et al., 2017). However, at soil loss rates of 1–2 cm y^{-1} , this practice is clearly unsustainable and mitigation strategies are required to preserve the remaining SOM. Raising the water table, therefore, offers an opportunity in these peat soils to re-establish anoxic conditions and prevent SOM loss from deeper peat layers. If the water table is raised, however, care must be taken not to negatively affect root growth (and thus yields) and also not to create conditions that would be conducive to N_2O and CH_4 release. An experiment where the water table was raised to 30 cm of the soil surface was found to reduce total GHG emissions from 80 to 25 kg $CO_2\text{-e ha}^{-1} d^{-1}$ (Taft et al., 2018). Wen et al. (2019b) showed the importance of the C/N ratio of cover crop residues on total GHG emissions when raising the water table from 50 to 30 cm; with vetch (low C/N ratio) resulting increased N_2O and total GHG emissions, and rye (high C/N ratio) resulting in reduced N_2O and total GHG emissions. While proving effective at reducing C losses, raising the water table makes the soil physically unstable, unsuitable for vehicle trafficking and also prone to flooding. This highlights the trade-offs between the effectiveness and practicality of C mitigation options.

4. Challenges and opportunities: Looking to the future

4.1. Genetic engineering - can we modify subsoil rooting?

The demand for food to feed an increasing world population, in a future of climate instability, limited supply of P-rich ore (Van Vuuren et al., 2010), and a global imbalance of N fertilizer availability (Springmann et al., 2018), will place additional pressure on agricultural land, with new land clearings causing further SOC loss. At the same time, climate change is affecting food staples variably (Peñuelas et al., 2017; Ray et al., 2019). Accelerated improvement to crops to tackle food security is possible with technologies such as gene editing (e.g. CRISPR cas9; Chen et al., 2019) being applied to improve traits, such as yield, disease and salt tolerance, and plant architecture (Energy Futures Initiative, 2020; Lian et al., 2020). The advantage of gene editing is that the genome of a species can be targeted to suppress undesirable traits or turn on and over-express desirable traits (there is no foreign DNA added). This technology is now permitted for application within the agricultural sector in some countries (e.g. USA, Australia) with others still debating its use. In the context of this review, we foresee an opportunity for gene editing to alter root systems (e.g. targeted root exudates) and to change the lignin lattice (composition and structure) to form a less biodegradable plant residue. This potential has already been

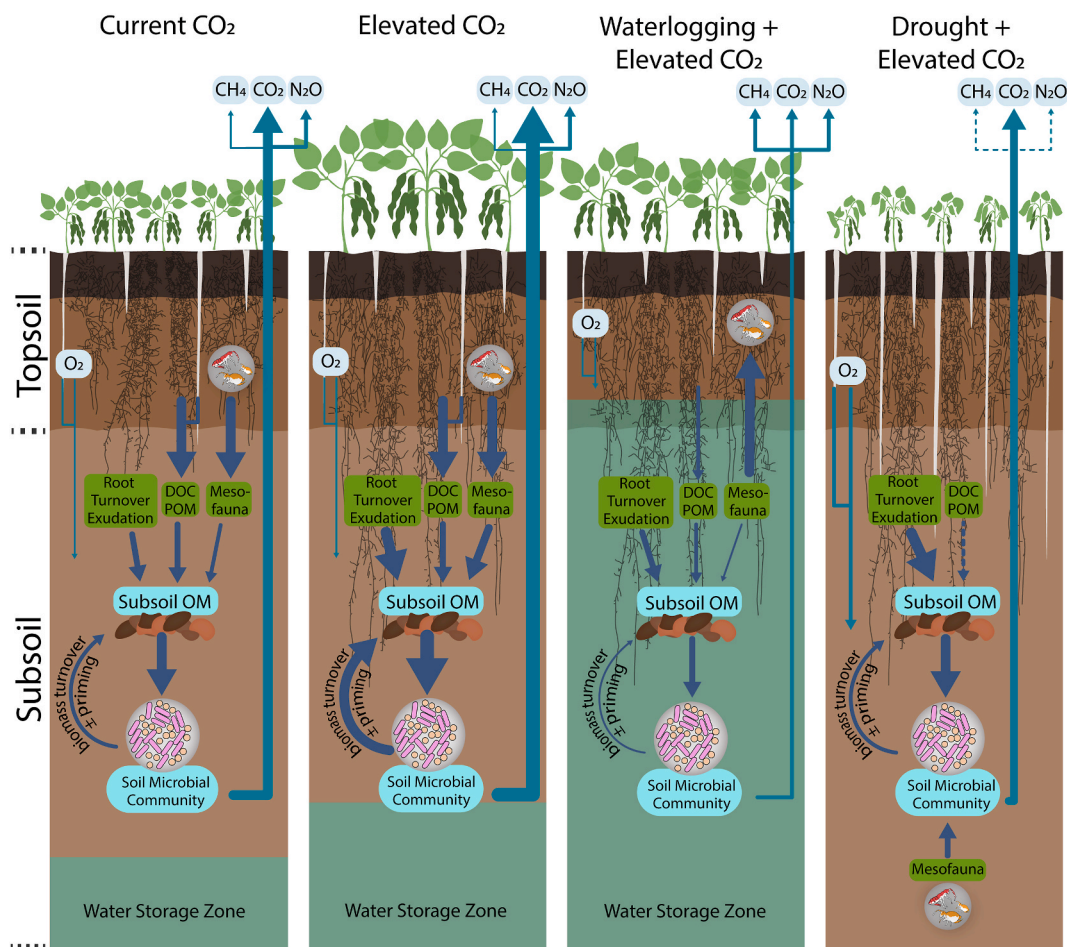


Fig. 5. Schematic representation of how mineral subsoils (i.e. Mollisol, Alfisol) will likely change in response to different climate change scenarios with potential feedbacks in the C and N cycles. Elevated CO₂ will induce plant growth, deeper rooting and more rhizodeposition in the subsoil. This will promote enhanced subsoil microbial activity and may induce subsoil priming of old SOM. The drying in combination with more microbial activity will stimulate more mesofaunal activity and bioturbation at depth. The greater formation of macropores (represented by the white vertical lines extending from the soil surface into the soil) due to greater topsoil drying will promote greater gas exchange and aeration of the subsoil. This will reduce the plant available water wet zone in the soil. Elevated CO₂ in combination with freshwater waterlogging will decrease C turnover and force mesofauna closer to the soil surface. The dashed lines are dependent on water availability, which are in low supply during droughts. This model assumes there are no constraints to deep rooting (e.g. due to excess acidity, salinity or compaction).

highlighted in rice and tomato plants using CRIPR cas9 where the production and exudation of strigolactones has been successfully modified to promote plant growth (Butt et al., 2018; Lian et al., 2020). It has also been used as gene editing tool to alter root branching frequency and branching angle in tobacco and rice (Bettembourg et al., 2017; Gao et al., 2018; Kitomi et al., 2020). Lignin is a major component of plant cell walls accounting for 30% of the organic C in the biosphere (Ralph et al., 2004). Its metabolic pathways and function in plants are well characterised; Liu et al. (2018a) review the basis for genetic improvement of lignin. Xu et al. (2019) have also demonstrated how CRISPR cas9 can be used to change the secondary metabolite composition in roots while Gasparis et al. (2019) have shown how it can be used to alter a range of root morphology traits. As yet, these technologies have not been harnessed to alter rhizosphere C flow or promote C storage in soil (Energy Futures Initiative, 2020).

4.2. Climate change - what are the consequences for subsoil SOC stocks?

Rising atmospheric CO₂ can increase the growth and grain production of C₃ crops and benefit C₄ crops experiencing drought stress (Fig. 5). Kimball (2016) reviewed 27 years of free-air CO₂ enrichment experiments and found biomass and yield were increased by eCO₂ in all C₃ crop species by 19%, but not in C₄ species except when water was limiting (30%). Conversely, drought stress and rising atmospheric O₃ cause negative impacts on plant production. How climate change will impact subsoil SOC stocks and microbial C utilisation rates is less clear. Elevated atmospheric CO₂ is projected to increase the quantity of C flow to root exudates (Phillips et al., 2011, Fig. 5); however, this may not necessarily translate into an increase in SOC due to a concomitant increase in microbial activity (Keiluweit et al., 2015; Kuzyakov et al., 2019). Pries et al. (2017) found that warming forest soil to 100 cm by 4 °C increased respiration of the whole soil profile by 34–37%. Subsoils contributed the majority to this (20–25%) with millennial old C respired. This is echoed by a recent meta-analysis of over 100 eCO₂ studies suggesting soil carbon storage declines when plant biomass is strongly stimulated by eCO₂ in forests, however, grassland soils have a large capacity to drawdown CO₂ and have increased SOC stocks (Terrer et al., 2021). As well, Baumert et al. (2018) found that increased subsoil exudation caused a 10% increase in SOC, due to a stimulation of fungi. Research is currently lacking to answer the key question - *are subsoil SOC stocks in mineral and organic soils secure from climate change?*

Extremes in climate events will increase with global warming causing increased frequency of wet-dry cycles (Meehl et al., 2007, Fig. 5). To some extent, subsoils will be buffered from extremes in temperature and moisture due to the overlying topsoil (Wordell-Dietrich et al., 2017; Qin et al., 2019). Goebel et al. (2011) proposed that the soil C balance is sensitive to climate extremes that decrease the wettability of soil and thus increase water repellence. This would increase surface water run-off and cause heterogeneous preferential flow pathways through the subsoil (Fig. 4); causing potentially less plant growth and more spatially variable root growth leading to less subsoil plant root C inputs. Water repellence in both surface and subsoils will also cause the water inside soil pores to form as droplets instead of continuous water films (Goebel et al., 2007). Disconnect in water films, as a consequence of water repellence, will restrict the diffusion of DOC (Or et al., 2007) and nutrients which will limit microbial uptake. This disconnect in water films is likely to also increase the stability of existing SOM against biological enzymatic decomposition thus increasing SOC residence time (Goebel et al., 2007). Water repellence is already widespread globally (Goebel et al., 2011) and is expected to become more frequent. How this alters the SOC balance is uncertain and highlights the need for SOC models to take account of differences in SOM turnover rates in water repellent soils.

4.3. Microbial survival in subsoils - are there unknown metabolic pathways?

Microbial cells exist in both metabolically active and dormant states. Jones and Lennon (2010) proposed that dormancy contributes to the maintenance of microbial diversity; fast-growing species use energy sources to grow competitively, whereas slow-growing species use greatly reduced metabolism (i.e. anabiosis) to outcompete other species during periods of energy starvation. This enables slower-growing organisms to exist without direct competition with fast-growing species. It is expected that in C-limited subsoils microbial competition for SOC sources required for energy-generation (i.e. electron donors) will be intense. This poses the question - *Do we fully understand microbial metabolic pathways in slow-growing (oligotrophic) species within subsoils and does this impact on C storage?*

The discovery of alternative microbial energy pathways in surface soils suggests more understanding is needed. Research by Greening et al. (2015) highlights that an aerobic heterotrophic acidobacterium uses H₂ oxidation from atmospheric scavenging when organic electron donors are scarce. Hydrogenase-encoding genes were subsequently identified in 51 bacterial and archaeal phyla (Greening et al., 2016). Furthermore, Ji et al. (2017) found atmospheric H₂, CO₂ and CO gases to be energy sources for Antarctic surface soil communities. Atmospheric CO oxidation enables the survival of aerobic heterotrophic bacteria in energy-limited environments and has been found in many species of soil bacteria and archaea (Cordero et al., 2019). These findings highlight that trace gas oxidation may be a general mechanism for microbial persistence in topsoils (Greening et al., 2016). Such exploratory genomic studies warrant investigation in both oxic and anoxic subsoil layers.

4.4. What are the key challenges in studying subsoils and can they be overcome?

Soil sampling depth has largely been driven by interest, practicality, and cost. This is reflected by a majority of C studies focusing on shallow soil layers (<30 cm; Yost and Hartemink, 2020). However, sampling below this in agricultural systems is not that difficult with use of sharpshooter, hammer and semi-mechanical soil corers, while hydraulic probes can be rented cost-effectively for deeper sampling. Indeed, deeper sampling used to be more common (Yost and Hartemink, 2020), so why is sampling becoming shallower? Although there is no current explanation, it could simply be because sampling deeper creates more samples to collect, process and analyse. We would like to argue that sampling deeper is worthwhile and to urge the soil science community not to stop sampling deeper. This is especially important as subsoils are different (Figs. 1 and 2) and changes in SOC stock may vary through the soil profile (e.g. Tautges et al., 2019) which topsoil sampling would miss, resulting in potentially misleading results and interpretation. In the case of *in situ* studies, there are different ways to take samples actively or passively (e.g. subsoil gas sampling systems; see Maier and Schack-Kirchner, 2014), with relative ease and limited soil disturbance which can greatly improve our understanding of the deep soil environment. Nevertheless, *ex situ* studies remain the more common practice (Rumpel and Kögel-Knabner, 2011), however, adequately simulating subsoil environmental conditions (e.g. lack of disturbance, lower O₂ concentrations, different temperature and moisture contents, etc.) is difficult, making these more variable and less appropriate for extrapolation to field conditions.

Both SOC content and bulk density variability contribute to C stock uncertainty, in turn affecting how large a change in stock can be observed through time or space. In the Western Australian SOC Audit, for example, Holmes et al. (2012) determined that variability in SOC (%) contributed to 84–99% of the uncertainty in C stocks compared to <5% from bulk density. They illustrated that the rapid indirect measurement of bulk density using a gamma-neutron density meter could be used in place of labour-intensive traditional volumetric rings or clod

measurements. Scanlan et al. (2018) have since developed a hand-held 3-dimensional scanning system based on a time-of-flight camera to measure the volume of the void created when using any excavation method (ring, auger, or shovel). This system was shown to measure bulk density accurately and rapidly in soils tested (35–71% gravel content; 0–40 cm depth).

Both near- and mid-infra-red (NIR, MIR) scanning of soil has been shown to provide accurate estimates of SOC content (%) once properly calibrated (Hutengs et al., 2019). In-field scanning of intact soil cores collected to depth provides a rapid means of determining SOC content with the added advantage of also having IR predict SOM fractions required for SOC model initialisation as well as a range of soil properties (e.g. clay %) which are required for C models. These soil layers can then be recovered for additional chemical and biological analysis.

4.5. What is the size of the subsoil C reservoir and how much more can be stored?

To determine how much additional C can be sequestered globally requires that we know how much C is currently present in the soil (Smith et al., 2020). A consensus on the size of the global SOC stock, however, is lacking, with estimates ranging from 500 to 3000 Gt C (Todd-Brown et al., 2013; Scharlemann et al., 2014). This variation in estimates occurs due to variations in model parameters and different soil depths considered in each study (Harrison et al., 2011; James et al., 2014). The alternative, mapping of soils by extensive geochemical sampling projects, has covered the majority of Europe (FOREGS; Salminen, 2005), the USA (NASGLP; Smith et al., 2014b), China (GCB; Wang et al., 2014b) and Australia (NGSA; de Caritat and Cooper, 2011), often also including deep soil samples. While time and resource intensive, this is the best way to get high quality fine-scale data to quantify the global C stock and identify areas where sequestration can be most successful. We encourage colleagues from the Middle East, Eastern Europe, South and Central America, South Asia and Africa to pursue extensive mapping programs.

Smith et al. (2020) argue that to implement C sequestration initiatives, we need more reliable SOC change monitoring, reporting and verification platforms for policymaker support and gaining investments. Similar to the uncertainties of the soil C stock, consensus on how much more C can be sequestered in soil has not been achieved (Minasny et al., 2017; Zomer et al., 2017). This is due to the mentioned issues with stock estimates and differences in the sequestration strategies, land type and how SOC change is measured (Smith et al., 2020). Frequently, the depth of soil C estimation is not stated. As a result, over- or underestimation of the sequestration potential may occur. Current evidence for topsoils suggests that the soil C sink will eventually reach saturation (Solinger et al., 2000; Hoyle et al., 2013; Smith, 2016; Wiesmeier et al., 2015) after approximately 20–80 y of positive C sequestration management (Lal and Bruce, 1999; Minasny et al., 2017; Poulton et al., 2018). As the rate of accumulation is non-linear and decreases soon after it begins, estimated annual C sequestration rates can only realistically be achieved within the short-term. Many studies do not indicate a time within which this rate can be achieved, so their accuracy remains unknown. Furthermore, when management practices targeting greater C accrual in subsoils are discontinued, it is important to consider whether the C sequestered will be susceptible to being lost, and if so at what rate. The recent UN FAO program for the global assessment of C sequestration potential (GSOCseq; FAO, 2019) is a promising new vision that aims to build the international capacity of SOC change monitoring, reporting and verifying which will be essential in moving C sequestration forwards.

4.6. What are the limitations of existing subsoil C simulation models?

Soil C models are essential for predicting SOC sequestration over long timescales (>50 years; Chenu et al., 2018), however, they are only useful if parameterised properly (Dignac et al., 2017). Currently, the

majority of C models (e.g. RothC, Century) are only designed to describe topsoil C dynamics (Smith et al., 1997; Stockmann et al., 2013). Also, the depth of soil that the C stock is estimated to is often not reported in these models, making comparisons between the results of models challenging (Stockmann et al., 2013; Todd-Brown et al., 2013). As surface and subsoil C characteristics and dynamics are substantially different (as demonstrated by Salomé et al., 2010; Sanaullah et al., 2011; Hoyle et al., 2013; Zieger et al., 2018; Qin et al., 2019), model parameterization must be different for subsoils. While subsoil models exist (Table 1), they are relatively recently developed, vary in their description of C, their accuracy and the depth of C they measure. This is mostly because (reliable) estimates for C supply and subsequent transformation rates do not exist for multiple soil layers (Keyvanshokouhi et al., 2019) or are not linked with other factors that affect subsoil C storage (e.g. N availability, carbonate content). While progress in subsoil C models has been made, a greater mechanistic understanding of the specific subsoil C inputs as well as more extensive field-derived data will be required to further optimise existing models (Taghizadeh-Toosi et al., 2014) and be able to reflect potential strategies to increase subsoil C content.

Another aspect that needs consideration in soil C forecasting models is better climate projections for agricultural subsoils (i.e. frequency of wettability). For example, in regions that receive less rainfall, subsoils will become progressively drier, microbial activity will slow and more C may accumulate. This drying may promote feedbacks such as deeper rooting, leading to greater removal of water, changes in nutrient availability which will affect subsoil C dynamics (Fig. 4). In contrast, saturated subsoils may dry out and shift from being anoxic to oxic and thus SOM may decompose faster. As for topsoils (Jiang et al., 2014), there is a clear need to link climate forecast models to C models, preferably with climate models that also incorporate extreme weather events.

Table 1
Models that address different C-related in topsoils and subsoils.

Model name	Function	Additional Information	Reference
–	C estimation	Non-linear mixed effect model for estimation of forest soil (to 2.5 m) C.	James et al. (2014)
–	C estimation	Linear function for topsoil and power function for subsoil (to 0.75 m).	Beem-Miller and Lehmann (2017)
RothPC-1	C turnover estimation	Subsoil (to 1 m) version of topsoil RothC model with 2 new parameters.	Jenkinson and Coleman (2008)
ECOSSE	C turnover estimation	Evaluation of model in predicting SOC dynamics (to 1 m).	Dondini et al. (2016)
ECOSSE	C sequestration estimation	8 year simulation of C and N dynamics (to 0.3 m).	Khalil et al. (2013)
DailyDayCent	C sequestration estimation	Simulation of long-term C storage (to 0.2 m) in agricultural soils with different additions.	Begum et al. (2017)
C-Tool	C sequestration estimation	Simulation of medium - long-term C storage (to 1 m) in agricultural soils.	Taghizadeh-Toosi et al. (2014)
OC-VGEN	C sequestration estimation	Simulation of long-term C storage (to 1.2 m) in agricultural soils with different management scenarios.	Keyvanshokouhi et al. (2019)

4.7. What factors will affect the likely success of subsoil interventions?

The overall success of different interventions to promote subsoil C storage depends on (i) their effectiveness to store C in the long term and the evidence to support this, (ii) their impact on other ecosystem services (including agricultural productivity), (iii) cost of implementation, (iv) practicality, (v) social acceptability, (vi) legislative barriers, and (vii) overall C cost of interventions (i.e. is more C stored than released in the process?). The importance of these is likely to be highly context-specific varying from farm-to-farm and country-to-country. For example, mechanical interventions may not be cost-effective or feasible by smallholder farms, whereas plant-based solutions may be implemented by all landowners. Strategies promoting higher subsoil C storage (e.g. DP, OM burial, deep rooting crops) are likely to improve nutrient cycling and water-holding capacities, leading to increases in crop yield (Gregorich et al., 1994). These win-win-win scenarios (i.e. more C, greater water use efficiency, higher yields) should help promote the adoption of subsoil technologies by farmers. In the case of OM, biochar and Fe additions to the subsoil, mechanical intervention is required. Although not mainstream, most of this equipment is commercially available to allow wide-scale adoption of subsoil technologies should these options gain support. One barrier to overcome in some countries is the legalities surrounding the addition of C-rich wastes to agricultural land. This will need a strong scientific evidence base and may take a long time for legislation to be passed. Lastly, it is known that some farmers can be resistant to change (cultural inertia; Hyland et al., 2016) and that there is resistance to geoengineering approaches to tackle climate change in both the public and scientific community (Robock et al., 2015). In addition, costs, practicalities and training needs may need addressing. Realistically, financial incentives for farmers (e.g. via the carbon market or agri-environment schemes) could be used to promote subsoil C technologies (Siedenburg et al., 2012). There is also a need for policymakers and extension agencies to focus on knowledge exchange and awareness programs, making use of the multiple co-benefits related to adopting pro-subsoil C behaviour (van de Ven et al., 2018).

5. Concluding remarks

Due to their favourable characteristics and conditions, subsoils have a large potential to offset CO₂ emissions by sequestering C. A growing body of evidence suggests that, due to their large volume, less disturbance and more static nature, subsoils have the potential to sequester more C than topsoils, highlighting the importance of undertaking further studies on deep soils. Nevertheless, based on the evidence herein we suggest the best current strategies for enhancing full profile C sequestration success are:

- Use of deeper rooting varieties is a cheap and easy way to enhance the C supply to the subsoil. While tangible benefits may take years to establish, it is likely to be more effective in lighter soils and those not with old C-rich subsoils.
- While additions of OM and biochar to subsoils increase the C stock and can be beneficial, these are unlikely to lead to 'true' C sequestration.
- Addition of iron and clay to subsoils may be effective in very specific soils, but current evidence is not sufficient to recommend their widespread adoption.
- Deep ploughing (DP) can be effective when >10 years apart and in soil that does not have high contents of very old SOC; C-poor topsoil; a high stone content; steep slopes; or unfavourable subsoils for plant growth. Silty and Duplex soils could particularly benefit from DP.
- Water table management can be highly effective in enhancing C sequestration. In mineral soils lowering the water table can allow for greater C delivery in the subsoil, while in organic soils raising it is beneficial to C sequestration – but not for agricultural capability.

Based on the evidence presented, we have also identified 5 key knowledge gaps and priority areas for future research:

1. Improve our understanding of the mechanisms that regulate C stabilization in subsoils and the factors driving long C residence times (e.g. rates of subsoil C supply and loss; stabilization mechanisms of suberin and DOC; sorption of SOC to minerals; role of Ca²⁺ and CaCO₃ in C stabilization, role of microbes in SOC residence time; persistence of microbial necromass; spatial organisation of roots, microbial communities and SOC).
2. Undertake studies that take advantage of space-for-time substitutions, long-term field and chronosequence studies of subsoil sequestration technologies (in isolation or combination). These studies also need to consider the trade-offs between different ecosystem services and the overall effects on soil health as well as their practicality and economic viability.
3. Perennialisation and improvement of deep-rooting traits in crops and grasses that promote greater subsoil C storage (e.g. by harnessing gene-editing technologies; better selection of rhizosphere communities; better *in situ* techniques for studying subsoil root dynamics).
4. Investigations into how climate change, especially changes in moisture status and extreme weather events, will affect subsoil C storage.
5. Use the information gathered above to improve the parameterization of soil profile- and landscape-level models of subsoil C dynamics that allow us to simulate the impact of different land management and future climate scenarios on subsoil C, but also improve global climate models.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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