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

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Highlights

- Agricultural subsoils may be more suited to long-term C sequestration than topsoils.
- Sequestration strategies have variable effects on C stocks, depending on soil type.
- Enhanced subsoil C storage can bring ancillary tangible benefits to cropping systems.
- Subsoil sampling and incorporation in models and maps is essential.
- Policies are needed for C sequestration in the whole soil profile, not just topsoils.

17 **ABSTRACT**

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

41 and decline in soil quality. In conclusion, our evidence-based analysis reveals that subsoils
42 offer an untapped potential to enhance global C storage in terrestrial ecosystems.

43 *Keywords:* Chemical stabilization; Greenhouse gas emissions; Organic matter priming;
44 Physical protection; Regenerative agriculture; subsoil carbon.

45

46 **1. Introduction**

47 Soil, a global reservoir of 3000 Pg carbon (C) (Köchy et al., 2015) with a mean age of
48 3100 years (He et al., 2016), has a significant capacity for long-term C storage. However, the
49 extent to which this terrestrial C sink will continue to grow as atmospheric CO₂ concentrations
50 increase remains unclear. Most C in agricultural soils (cropland and pasture) is held in an
51 organic form (soil organic carbon, SOC), which is susceptible to destabilization as a result of
52 changes in land use, management practices and environmental conditions (Guo and Gifford,
53 2002; Davidson and Janssens, 2006). Due to agriculture alone, 133 Gt of SOC has already been
54 lost to the atmosphere in the past two centuries, and the rate of loss is increasing (Sanderman
55 et al., 2017). SOC loss severely impacts soil functions, including water infiltration, nutrient
56 supply and biodiversity, leading to erosion, a decline in soil fertility and a release of greenhouse
57 gases (GHGs - CO₂, CH₄, N₂O) (Don et al., 2011; Tsiafouli et al., 2015). Due to the projected
58 growing demand for food production from already degraded land, intensive agriculture is
59 putting soils at further risk of SOC loss (Johnson et al., 2014; Sanderman et al., 2017). Still,
60 enhanced soil C sequestration of ~1000 additional Pg C is thought to be possible (Lorenz and
61 Lal, 2005).

62 Sequestering organic C in the soil can have multiple benefits, including i) offsetting of
63 anthropogenic C emissions, ii) restoring soil function, iii) improved soil resilience (to erosion,
64 pollution, diseases and drought), iv) increased agricultural productivity and sustainability, and
65 v) greater food security (Lal et al., 2015). Due to these expected benefits, promoting SOC

66 sequestration is of keen interest to both the scientific and policymaking communities. A
67 number of recent analyses suggest that ‘natural solutions’ like sequestering C in soil are
68 economical and ‘no-regrets options’ that could achieve a substantial portion of the negative
69 emissions needed to achieve carbon neutrality (Baker et al., 2020; Sykes et al., 2020). While
70 the recent ‘4 per 1000’ soil C sequestration initiative has drawn both support (Minasny et al.,
71 2017; Rumpel et al., 2020) and criticism (Baveye et al., 2018; de Vries, 2018; Poulton et al.,
72 2018) from the scientific community, this initiative: i) has been an aspiration and definitive
73 step in the direction of direct action to mitigate climate change via soil C sequestration, ii)
74 brought soil C sequestration to extensive scientific, public and political attention, and iii)
75 considers soil below the topsoil, albeit to a maximum depth of 40 cm, in the context of C
76 sequestration.

77 Currently, the practiced measures to limit C loss and/or maximise C retention in
78 agricultural soils are largely targeted to topsoils (Ap horizon; ca. 0-30 cm). This predisposition
79 towards topsoils is confirmed by Yost and Hartemink, (2020) who found the mean soil depth
80 studied in 4 primary soil science journals to be 24 cm between 2004 and 2019. Topsoil C
81 retention strategies include reducing tillage intensity, the addition of organic amendments,
82 growing cover crops, using leys with grazing livestock, agroforestry and restoring of natural
83 vegetation (Smith, 2008), along with a variety of regenerative agriculture practices still being
84 tested. However, topsoil, despite being rich in SOC (per volume of soil), has a relatively low
85 potential to sequester further C (Rumpel and Kögel-Knabner, 2011; Hobley et al., 2017). Due
86 to favourable soil conditions for decomposition, high microbial activity, aeration, large inputs
87 of labile organic matter, and high soil disturbance; topsoils experience high rates of C
88 mineralization and short C residence times (Schlesinger and Andrews, 2000; Fontaine et al.,
89 2007; Salomé et al., 2010). As a result, C retention strategies have had varying results in
90 improving soil C stocks and decreasing soil GHG emissions in the long term (Kirkby et al.,

91 2014; Smith et al., 2014). In addition, as topsoil C sequestration is reversible, changes in land
92 use and management can lead to rapid C loss (Smith, 2008).

93 While deep soil horizons (ca. ≥ 30 cm) are often considered biologically quiescent, deep
94 soil C is responsive to environmental change (Bernal et al., 2016; Hobbey et al., 2017; Slessarev
95 et al., 2020) and comprises the majority of the global soil C pool (Jobbágy and Jackson, 2000).
96 Therefore, to limit C losses and increase C stocks over longer timescales (i.e. 50-1000 years;
97 Piccolo et al., 2018), approaches targeting deeper, low disturbance soil may have the potential
98 to be more successful. The residence time of subsoil (B horizon; ca. ~ 30 -100+ cm) C increases
99 with depth, with C here commonly attaining millennial age (Torn et al., 1997, 2002; Rumpel et
100 al., 2002; Schöning and Kögel-Knabner, 2006). This is confirmed by Shi et al., (2020) who
101 determined the global mean of deep cropland and grassland soil (30-100 cm) to be 3700 and
102 5400 years old by radiocarbon measurements, which is 3.8- and 3.5-fold older than measured
103 in the topsoil (0-30 cm), respectively. As awareness of the potential for subsoils to promote
104 SOC sequestration grows, interest in C dynamics and strategies of sequestration in subsoils
105 have developed (Chabbi et al., 2009; Kautz et al., 2013; Chen et al., 2018). However, how
106 subsoil C is stabilised, enabling this long-term persistence is still not fully understood (Fontaine
107 et al., 2007; Jones et al., 2018) and specific subsoil C sequestration strategies are lacking
108 sufficient evidence and comparative assessment.

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

115 **2. Subsoil carbon**

116 2.1. Subsoil biological, chemical and physical properties

117 In the past, and in early subsoil C models (e.g. RothPC-1, Jenkinson and Coleman,
118 2008), subsoils were essentially thought of and treated as ‘less concentrated’ topsoils, but this
119 general assumption has more recently been dismantled (Salome et al., 2010). Indeed, the
120 differences between the environmental, physico-chemical and biological characteristics of
121 topsoils and subsoils (Rumpel and Koegel-Knabner, 2011) are such that a sound understanding
122 of subsoil processes cannot be directly inferred from our current understanding of topsoils.

123 Because of their high spatial variability at a range of scales (i.e. field, landscape,
124 regional), driven in part by pedology, environment and climate, subsoils are difficult to
125 generalise (Chabbi et al., 2009). To better characterise the diversity, similarities, and
126 differences of top and subsoil horizons, we collected soil profile data of 203 studies across
127 different climates and soil types around the world (Fig. 1, Fig. 2). Details on the search term
128 strategy, selection criteria and spread of soil orders and study locations are presented in the
129 Supplementary Information (Table S1-S2, Fig. S1-S2). We used a topsoil-subsoil boundary of
130 30 cm when categorising the measurements. A numerical boundary was used because studies
131 predominantly sample soil by soil depth intervals (Yost and Hartemink, 2020). This particular
132 depth was chosen as it is commonly the boundary of soil disturbance (reflecting a historical
133 12-inch plough; Davis et al., 2018) in ploughed soils, which was a key criterion in the definition
134 of subsoils in this review. However, this boundary does not well represent all soils. For
135 example, in lower production rain-limited environments where no-tillage practices are often
136 used, the topsoil may be functionally defined as <10 cm deep (Hoyle et al., 2013). To avoid
137 falsely categorising soil horizons and better determine whether measurements belonged to the
138 A or B horizon, we used the authors defined boundaries within the individual studies (see
139 Supplementary Information for more detail).

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

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

155 Generally, the rates of C input to the subsoil are much lower than topsoils, and rates of
156 release back to the atmosphere are also slow, as evidenced by the older age of C at depth (Shi
157 et al., 2020). Why C turnover is slower in the subsoil, then, is likely due to: i) low disturbance
158 from agricultural practices (Lal et al., 2015); ii) proportionally lower SOC and microbial
159 biomass (Fontaine et al., 2007; Salome et al., 2010; Liu et al., 2018), iii) the physical
160 (in)accessibility of microbes to C substrates outside of hotspots (Heitkötter and Marschner,
161 2018; Dove et al., 2020; Salomé et al., 2010); iv) high abundance of available mineral surfaces
162 (e.g. clay and Fe/Al in the Bw horizon) and Ca^{2+} for adsorption and chemical stabilization of
163 C (Mikutta et al., 2006; Rumpel and Kögel-Knabner, 2011); and, v) the prevailing oligotrophic
164 conditions (i.e. low O_2 , N availability, pH etc.) which limit enzyme synthesis (e.g. O_2 -

165 dependent phenol oxidases) and activity (Xiang et al., 2008; Salomé et al., 2010; Shahzad et
166 al., 2018a; Dove et al. 2020) and so microbial activity. These mechanisms underpin the mean
167 residence times of SOC in subsoils, which are typically on the scale of millennia as compared
168 to centuries in topsoils (Shi et al., 2020).

169 At the field scale, subsoils can be characterised by a high degree of spatial heterogeneity
170 due to the presence of larger aggregate structures (e.g. prisms), preferential water flow
171 pathways and root proliferation (White and Kirkegaard, 2010). This can lead to the creation of
172 biological hotspots in subsoils (e.g. biopores; Chabbi et al., 2009; Kautz et al., 2013; Kuzyakov
173 and Blagodatskaya, 2015). These hotspots of degradable substrates and associated microbial
174 activity play an important role in C, N and P transformations in the subsoil (Hoang et al., 2016).
175 Outside of these microsites, the inactivity of microbes may explain the measured long-term
176 stability of SOC (Heitkötter and Marschner, 2018).

177 Understanding the influence of microorganisms on deep soil C is crucial because
178 microbial products – including exo-enzymes, extracellular polymeric substances (EPS), and
179 cell wall materials – contribute increasingly to long-lived soil organic matter in deeper soil
180 horizons (Dove et al., 2020; Peixoto et al., 2020; Dwivedi et al., 2017; Sher et al., 2020).
181 Recently, the number of soil microbiology studies focused on subsoils has expanded (e.g. Eilers
182 et al., 2012; Kramer et al., 2013; Li et al., 2014; Brewer et al., 2019; Diamond et al., 2019;
183 Polain et al., 2020). Microbial community composition, biosynthetic potential and metabolic
184 pathways change significantly with depth, with relatively more copiotrophs present in the
185 topsoil and oligotrophs in the subsoil (Fierer et al., 2003; Uksa et al., 2015; Jones et al., 2018;
186 Brewer et al., 2019; Sharrar et al., 2020). The vertical distribution of these microbial groups
187 has been found to be predominantly determined by the availability and forms of C (Fierer et
188 al., 2003; Stone et al., 2014; Fanin et al., 2019). Deep soils are enriched in autotrophic archaea
189 implicated in ammonia oxidation (Brewer et al., 2019) and symbiotrophic fungi (Schlatter et

190 al., 2018) with enzymatic capacities that are distinct from their saprotrophic counterparts.
191 Indications of methylotrophy and “dark autotrophy” (CO₂ fixation) have also been uncovered
192 in subsoil bacterial genomes (particularly in Chloroflexi) (Brewer et al., 2019; Butterfield et
193 al., 2016). Shifts from protozoa, fungi and Gram-negative bacteria in the topsoil to Gram-
194 positive bacteria (and actinomycetes) with greater depth (Fierer et al., 2003; Stone et al., 2014;
195 Fanin et al., 2019) reflect the required adaptations for survival in deeper soil. Gram-positive
196 bacteria, for instance, are well adapted to subsoils by their ability to sporulate, resilience to
197 harsh environments (i.e. water limited) and preference for older more complex C derived from
198 soil organic matter (SOM; Setlow, 2007; Kramer and Gleixner, 2008). Other strategies, such
199 as storage of internal resources, dormancy and trophic flexibility, found in *Dormibacteraeota*
200 which are particularly abundant in subsoils across the US (Brewer et al., 2019; Lennon, 2020),
201 highlight the range of strategies used by microbial groups to overcome the limitations of
202 subsoils.

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

210 Despite clear differences in microbial communities, microbial competition for C and N
211 can be as intense in the subsoil as in the topsoil (Jones et al., 2018; Diamond et al., 2019).
212 Findings by Jones et al. (2018) suggest that subsoil microbes are more C limited but can rapidly
213 become active and grow upon organic C addition. This is supported by the short lag phase in
214 CO₂ production after the addition of C substrates to subsoils particularly when high amounts

215 of labile C are added (Cressey et al., 2018; de Sosa et al., 2018). Soil N supply also typically
216 decreases with depth (Murphy et al., 1998; Ekelund et al., 2001; Kemmitt et al., 2008; Uksa et
217 al., 2014; Banning et al., 2015) and inorganic N is heterogeneously distributed in the subsoil
218 compared to the topsoil (Taylor et al., 2002; Kuzyakov and Blagodatskaya, 2015). Therefore,
219 limited microbial access to spatially distributed substrates in the subsoil is likely an important
220 factor for SOC accumulation and stabilization (Preusser et al., 2019).

221

222 2.2. Subsoil priming of SOM

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

232 Higher priming C losses have been measured in subsoil compared to topsoil (relative
233 to native soil C content) (Salomé et al., 2010; Hoang et al., 2017; Jia et al., 2017; Meyer et al.,
234 2018), but the opposite response has also been reported (De Graaff et al., 2014).

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

239 microbial growth and induce N mining from native SOM (Jones et al., 2018; Meyer et al.,
240 2018).

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

258

259 2.3. Subsoil gas emissions

260 The behaviour and fate of GHGs in subsoils play an important role in subsoil C
261 sequestration (Blagodatsky and Smith, 2012), and overall system C balance. Here we focus on
262 C containing gases, CH₄ CO₂, in the context of C sequestration, although we note that N₂O

263 fluxes (reviewed by Clough et al. (2005)) should also be considered in a holistic analysis of
264 deep soil C sequestration strategies.

265 Soil CO₂ concentrations are known to increase with depth, despite fluxes decreasing
266 with depth and not contributing substantially to surface fluxes (Davidson et al. 2004; Xiao et
267 al. 2015; Wang et al. 2019). This suggests that CO₂ in the subsoil does not move rapidly to the
268 soil surface and if undisturbed may be entrapped in soil pores and solution, or used by subsoil
269 autotrophs. However, this ‘trapped’ CO₂ is vulnerable; along a subsoil-to-surface CO₂ gradient
270 of >10,000 ppm to atmospheric concentrations, it may only take a few hours to days for CO₂
271 to diffuse to the atmosphere (e.g. when CO₂ is produced near subsoil macropores or when the
272 water-filled pore space is low; Mencuccini and Hölttä, 2010). Thus, subsoil disturbance could
273 disturb the deep dynamic reservoir of subsoil CO₂.

274 Wang et al. (2019) found that while CO₂ concentrations increased with depth following
275 a full inversion of forest subsoil to 60 cm, the soil surface CO₂ flux remained largely unaffected
276 by the highly invasive subsoil disturbances. In the case of enhanced subsoil rooting for C
277 sequestration, plant root uptake of water from the subsoil can lead to increased aeration, greater
278 gas diffusivity, soil shrinkage and the formation of macropores which facilitate migration to
279 the surface (Shaw et al., 2014). Roots can also take up dissolved inorganic C (CO₂, HCO₃⁻)
280 from soil and rapidly transport it through the xylem to the leaves where it can be refixed or
281 released back to the atmosphere (Bloemen et al., 2016). Although the uptake rate of HCO₃⁻
282 from soil is generally low, the direct recycling of HCO₃⁻ produced inside the roots (i.e. from
283 respiration) back to the shoots via the xylem may be significant in reducing CO₂ concentration
284 in soil (Rao et al., 2019). Still, these CO₂ loss effects maybe counterbalanced by the accrual of
285 deep root C inputs; studies of deep-rooted perennial grasses planted in low C soils found no
286 effect of these crops on surface CO₂ fluxes and increases in total soil profile C stocks in some
287 soil types (Bates et al., 2021; Slessarev et al., 2020). Therefore, it is important to consider that

288 different sequestration strategies, such as deep tillage and planting of deeper rooting varieties,
289 may influence the soil-atmosphere flux and the soil CO₂ budget in different ways.

290 Over a recent decade (2008-2017), global CH₄ emissions from agricultural systems
291 were estimated at 206 Tg y⁻¹; this represented 56% of the total anthropogenic emissions
292 (Saunio et al., 2020; Jackson et al., 2020). To our knowledge, the fraction of this total
293 contributed by agricultural subsoils has not been estimated. In many well-drained systems, CH₄
294 produced by methanogens in anaerobic microsites can be consumed by methanotrophs in oxic
295 regions during transit to the surface (Le Mer and Roger, 2001; Wang et al., 2018), suggesting
296 CH₄ dynamics in the soil have limited bearing on any C sequestration outcome.

297 Very little is known about microbial volatile organic compound (VOC) production and
298 consumption rates in subsoils. VOCs have low-molecular-weights (typically <250 MW) with
299 high vapor pressures. They can be produced in soil by both microorganisms and plant roots
300 (Peneulas et al., 2014). Like CH₄, VOCs are both produced and consumed *in situ* (Tassi et al.,
301 2009), suggesting they are unlikely to be relevant to C sequestration.

302

303 2.4. Subsoil carbon sources and stabilization

304 The primary inputs of C to subsoils include: i) root-derived C (both dead roots and
305 living root rhizodeposition); ii) leaching of dissolved organic C (DOC) from the topsoil; iii)
306 delivery of particulate organic matter via bioturbation or leaching, and iv) microbially-derived
307 C (immobilization of CH₄ or volatile organic-C, dark fixation of CO₂). The primary C sources
308 and their stabilization mechanisms in the top- and subsoil are presented in figure 3. Roots in
309 the subsoil decompose relatively slowly, whereas topsoil and detritusphere leachates and root
310 exudates with high C:N ratios and C availability are more easily mineralised. Subsoil microbial
311 biomass has a slower turnover time (Spohn et al., 2016). The C:N ratio declines with soil depth

312 in most agricultural soils (Rumpel and Kögel-Knabner, 2011; Lou et al., 2012), demonstrating
313 that C in the subsoil cycles slower compared to soil nearer the surface.

314

315 *2.4.1. Root-derived carbon*

316 Plants may direct up to half of photosynthetically fixed C to roots (Jones et al., 2009)
317 and most subsoil organic carbon (OC) is plant root-derived (Rasse et al., 2005; Suseela et al.,
318 2017). For plants with deep rooting architecture, roots and their products (i.e. exudates and cell
319 sloughing) have substantial potential to enter stabilised subsoil OC pools (Rasse et al., 2006;
320 Kätterer et al., 2011; Suseela et al., 2017), and root litter decomposes more slowly in deep soils
321 (Pries et al., 2018). While this stabilization depends on the soil environment and root
322 physiology (Farrar et al., 2003), three primary root-derived C sources are thought to contribute
323 to stable OC by different mechanisms (Fig. 3): aggregation, root biochemistry and association.

324 Due to the major role of biotic processes in soil aggregation, it is often thought less
325 relevant as a C stabilization mechanism in subsoils (Lorenz and Lal, 2005). However, recent
326 studies have revealed aggregation may be as important in the subsoil as it is at the surface
327 (Moni et al., 2010; Sanaullah et al., 2011; Baumert et al., 2018). For example, Moni et al.
328 (2010), found up to 40% of SOC was occluded within aggregates throughout the whole soil
329 profile (>100 cm depth). Sher et al. (2020) found enhanced microbial production of soil-
330 binding extracellular polysaccharides throughout a 1 m soil profile following conversion from
331 annuals to deeper-rooting perennials, and suggest that aggregation is likely an important
332 mechanism in subsoil C protection.

333 The biochemical composition of primary roots can contribute to their stabilization in
334 soil, particularly those with significant amounts of lignin, tannin or suberin (Rasse et al., 2005).
335 The decomposition of lignin may be slowed through protection via mineral association
336 (Rumpel et al., 2015; Hall et al., 2016; Huang et al., 2019), accelerated by short-term

337 fluctuations in redox states, or gut processing within earthworms (Le Mer et al., 2020). In
338 addition, extracellular enzymes (e.g. those that decompose lignin - phenol oxidase) have been
339 found to largely be stabilised via sorption onto mineral surfaces in subsoils (Dove et al., 2020).
340 While tannin residence time in soil is similar to non-associated lignin (Meier et al., 2008),
341 suberin is a major contributor to SOC with a high potential for long-term stabilization (Rasse
342 et al., 2005; Suseela et al., 2017). In subsoils, Rumpel et al. (2004) found suberin-derived
343 hydroxyalkanoic acids to be preferentially preserved (over lignin) in clay particle fractions.
344 However, despite its important role in root chemistry and SOC stabilization (Suseela et al.,
345 2017), the behaviour and persistence of suberin in subsoils remains poorly understood
346 (McCormack et al., 2015).

347 Root exudates, organic compounds passively released from roots, can have a multitude
348 of interactions with soil minerals (Farrar et al., 2003). For example, Keiluweit et al. (2015)
349 showed that oxalic acid (a common root exudate) liberates C previously protected by minerals,
350 thus promoting C loss via increased microbial availability. Although exudation varies with
351 plant age and species, measurements of exudation and rhizodeposition suggest up to 7% of net
352 fixed C can be deposited in croplands and 11% in grasslands (Pausch and Kuzyakov, 2018;
353 Jones et al., 2009). Exudates contain sugars, organic acids, amino acids, fatty acids and plant
354 hormones; these are primarily C sources that can be mineralised within hours by the soil
355 microbial community (Rasse et al., 2005; Salomé et al., 2010, Zhalnina et al., 2018). Yet,
356 negatively charged organic acid anions can become fixed on the surface of positively charged
357 Fe and Al (hydr)oxides, protecting them from short-term mineralization (Jones and Edwards,
358 1998; Oburger et al., 2011). Other root deposits, such as mucilage and EPS, also play an
359 important role as binding agents for aggregate formation (Baumert et al., 2018, Sher et al.
360 2020). Mucilage is reactive and high in hydroxy groups and can adsorb to clay particles and
361 organic molecules (Gaume et al., 2000).

362 Physical separation of decomposers from exudates in subsoils may be one of the key
363 drivers of exudate-C stabilization (Salomé et al., 2010), although very little is known about
364 rhizosphere and root detritosphere microbial communities in subsoils. It is possible that roots
365 also deliver C into subsoils via arbuscular mycorrhizal associations (Sosa-Hernandez et al.,
366 2019). Subsoil arbuscular mycorrhizas are different taxonomically from those in topsoils
367 (Sosa-Hernandez et al., 2018); however, whether they differ functionally requires further
368 research (Wang et al., 2017).

369

370 *2.4.2. Leaching of dissolved organic C from the topsoil*

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

385 Various stabilization reactions bind DOC to the solid phase (Solinger et al., 2000;
386 Dignac et al., 2017), including van der Waals forces, anion exchange, cation bridging, ligand

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

397

398 *2.4.3. Delivery of particulate organic matter via faunal bioturbation and leaching*

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

406 Anecic earthworms can burrow to soil depths of 1-2 m, occasionally reaching up to 5
407 m (Lee, 1985). By transporting fresh particulate OM into the subsoil and mixing it with mineral
408 soil, earthworms can contribute to the heterogeneous distribution of subsoil SOC (Don et al.,
409 2008; Rumpel and Kögel-Knabner, 2011), and mediate soil aggregate formation which is
410 associated with SOC stabilization (Six et al., 2004). However, field and lab studies frequently
411 find anecic earthworms induce SOM loss from increased respiration. This is likely due to

412 stimulation of microbial activity within biopores (Banfield et al., 2017) with C-rich labile
413 earthworm mucus and higher O₂ levels (Hoang et al., 2016, 2017). Earthworms and their casts
414 are known to be hotspots of N₂O emission, as they also contain high mineral N concentrations
415 (Elliott et al. 1991; Lubbers et al 2013; Nieminen et al., 2015). Finally, Lubbers et al. (2017)
416 found that the topsoil (0 - 25 cm) SOC content was lower after 2 years in the presence of epigeic
417 and endogeic earthworms, suggesting faunal bioturbation diluted SOC in the topsoil by mixing
418 it with C depleted subsoil. Termites and ants may also increase C transfer to depth either
419 through deposition of necromass, food stores and exudates but also indirectly by creating
420 channels in the soil that fill with water and thus move DOC and POM to depth (Jouquet et al.,
421 2011). These channels may also stimulate aeration and rooting at depth (Banfield et al., 2018)
422 leading to crop yield increases and thus greater C inputs (Kautz et al., 2013).

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

430

431 2.4.4. Microbially derived C

432 Soil microbial community structure, genomic capacity, and ecophysiology are strongly
433 depth-dependent (Brewer et al., 2019). Understanding the influence of depth on microbial traits
434 is crucial because microbial products – including exo-enzymes, EPS, and cell wall materials –
435 may contribute increasingly to long-lived SOM in deeper soil horizons (Dove et al., 2020;
436 Peizoto et al., 2020; Dwivedi et al., 2017; Sher et al., 2020). While we do not currently have

437 enough data to speculate too much on the persistence of root-derived vs microbe-derived SOM
438 in deep soils, recent evidence suggests that microbially derived necromass is a major
439 contributor to SOC (Zhang et al., 2020; Wang et al., 2021). Wang et al. (2021) estimated that
440 half of SOC under cropland and grasslands is derived from microbial necromass and that it
441 predominantly originates from fungi. In addition, they found that the contribution of microbial
442 necromass to SOC increased with depth in grasslands while the opposite was true in croplands.
443 Overall, the organisms, biosynthetic potential and metabolic pathways of deep soils differ from
444 better-studied shallow soils (Butterfield et al., 2016; Sharrar et al., 2020; Diamond et al., 2019).
445 For example, deep soils are enriched in autotrophic archaea implicated in ammonia oxidation
446 (Brewer et al., 2019) and symbiotrophic fungi (Schlatter et al., 2018) with distinct enzymatic
447 capacities from their saprotrophic counterparts (Miyachi et al., 2020). In addition, deep soil
448 microbes may play a particularly unique role in subsoil C accumulation through immobilization
449 of methane (CH₄) and volatile organic carbon (VOC) or via dark autotrophy (CO₂ fixation).

450 Apart from surface photosynthetic CO₂ fixation and chemoautotrophic fixation, dark
451 anaplerotic (i.e. non-photosynthetic) heterotrophic fixation of CO₂ occurs in a wide range of
452 soils and is linked to the provision of C-skeletons for amino acid synthesis (Yang et al., 2017;
453 Nel and Cramer, 2019). A wide range of soil archaea and bacteria are capable of dark
454 anaplerotic CO₂ fixation in both aerobic and anaerobic conditions (Saini et al., 2011), and
455 produce organic acids. Although the overall contribution of dark fixation is extremely small in
456 topsoils (Ge et al., 2013), dark fixation may be proportionally more important in subsoils,
457 presumably due to C limitations with depth (Šantrůčková et al., 2018), and higher CO₂
458 concentrations. As yet, there are no *in situ* studies of dark CO₂ fixation in agricultural subsoils
459 and it is difficult to critically assess the significance of this process in the overall net C balance
460 of subsoils. In arctic soils, Šantrůčková et al. (2018) found that long term microbial dark
461 fixation of CO₂ corresponded to between 0.016 and 38% of plant C fixation, highlighting the

462 uncertainties regarding the importance of CO₂ fixation in the net soil C balance. The preferred
463 microhabitats and edaphic conditions of microorganisms responsible for dark CO₂ fixation in
464 subsoils are also unknown.

465 While chemoautotrophy (i.e. C fixation from the oxidation of reduced forms of
466 inorganic N and S; NH₄⁺, S²⁻) can be very important in extreme ecosystems (e.g. hydrothermal
467 vents), it is thought to be a relatively minor C fixation process in soil due to the relatively low
468 growth yields of chemoautotrophic organisms and their inability to compete against
469 heterotrophic bacteria. Despite this, chemoautotrophic ammonia-oxidizers and nitrite-oxidizers
470 can be abundant in subsoils (10⁵-10⁸ g⁻¹) suggesting that their role in C fixation should not be
471 discounted (Jones et al., 2018; Tao et al., 2018).

472

473 **3. Enhancing C sequestration in subsoils**

474 Capturing CO₂ from the atmosphere and submitting this to long-term storage in the
475 subsoil as organic C has potential to offset substantial anthropogenic CO₂ emissions and bring
476 a range of other ecosystem service co-benefits. Various approaches to increasing SOC in soils
477 exist, but here we discuss strategies that aim to: i) increase C inputs; ii) reduce C losses; and/or
478 iii) increase C residence time in soil. However, we agree with Olson et al. (2014) that 'true'
479 sequestration is not a transfer of C, but increased C fixation from the atmosphere.

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

484

485 *3.1. Deeper-rooting phenotypes and perennials*

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

500 By adopting crops that grow an extra 100 cm in depth, Kell (2012) calculates an
501 additional 100 t C ha⁻¹ could be sequestered, corresponding to a 118 ppmv reduction in
502 atmospheric CO₂. Whether these values are accurate is difficult to determine, yet, deeper-
503 rooting undoubtedly increases C entering the subsoil (Liebig, 2005; Omonode and Vyn, 2006;
504 Follett et al., 2012; Ledo et al., 2020), but the benefits thereof may only be apparent in the
505 longer-term (7-10 years), as found by Ma et al. (2000) and Carter and Gregorich (2010). This
506 is because net SOC stock increase is a balance between enhanced root C supply to the subsoil
507 and greater soil respiration (Schmidt et al., 2011; Shahzad et al., 2018b). This is exemplified
508 by the relatively low increase in C in the soil profile (0.07 ± 0.02 g C kg⁻¹ y⁻¹) with time (Fig.
509 4). Figure 4 also demonstrates that higher C gains are more likely in the topsoil (0.13 ± 0.06 g
510 C kg⁻¹ y⁻¹) where root density is greater, and the volume of soil is lower relative to many subsoils

511 $(0.04 \pm 0.02 \text{ g C kg}^{-1} \text{ y}^{-1})$.

512 Perennialisation of annual crops and conversion of annual to perennial crops can
513 enhance rooting depth and architecture, which increases C input into the subsoil (Liebig et al.,
514 2005; Kell, 2011; Powlson et al., 2011). In addition, perennialization means less tillage and the
515 associated C losses, allowing for more C accrual. Slessarev et al. (2020) showed that the
516 increased rooting depths offered by perennial grasses added appreciable soil carbon in sandy
517 and loam soil (although no SOC increase was measurable in clay-rich sites), and deep roots
518 may also lead to changes in site hydrology and the responsiveness of deep soil microbes (Oerter
519 et al., 2021; Min et al., 2021). Aggregation may also increase as a result of greater root biomass
520 found by Sher et al. (2020). A meta-analysis by Ledo (2020) found an 11% increase in the 0-
521 100 cm soil depth following conversion to perennial crops from annuals over a 20-year period.
522 Similarly, Follett et al., (2012) found a 2 t C y^{-1} increase in the 0-150 cm depth following 9
523 years of maize cultivation, where the majority of the increase was below 30 cm. These results
524 are echoed by other studies (Liebig, 2005; Omonode and Vyn, 2006) that found SOC stock
525 gains in the whole soil profile following conversion of annual crops to perennial grasses.
526 However, as Johnston et al. (2016) found, N_2O emissions can increase with perennials and
527 SOC increases in the subsoil can be limited (Ma et al., 2000; Chimento et al., 2014).

528 Apart from breeding deeper rooting varieties, there are several potential avenues for
529 breeding C sequestration desirable traits in crops. For example, enhancing the root release of
530 low molecular weight exudates and extracellular polymeric substances may promote C
531 retention when they become sorbed to mineral surfaces or physically protected (Salomé et al.,
532 2010; Sher et al., 2019). Furthermore, fungi, such as arbuscular mycorrhizal fungi (AMF), can
533 reduce C mineralization by i) their complex C-containing mycelium being less mineralisable,
534 ii) improving root lifespan, iii) enhancing root-derived C protection in aggregates, and iv)
535 outcompeting microbes (mostly bacteria) for N (De Deyn et al., 2008; Bardgett et al., 2014).

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

544

545 *3.2. Organic matter burial in the subsoil*

546 Burial of OM, such as straw, in the subsoil by deep ripping or DP (see 3.1) is a strategy
547 used primarily for amelioration of subsoil compaction, yet it can increase the subsoil C stock.
548 This is supported by data collected from 10 studies (Fig. 4), which show large C gains in both
549 topsoil ($0.5 \pm 0.1 \text{ g C kg}^{-1} \text{ y}^{-1}$) and subsoil horizons ($4.1 \pm 2.5 \text{ g C kg}^{-1} \text{ y}^{-1}$). Burial of large
550 amounts of plant residue or animal waste adds large amount of C to the soil stock ($2.0 \pm 1.1 \text{ g}$
551 $\text{C kg}^{-1} \text{ y}^{-1}$ across the soil profile). While these are remarkable numbers, it is important to
552 remember that a transfer of exogenous C is not the same as C sequestration in terms of C
553 removal from the atmosphere (as defined by Olson et al., 2014). Therefore, amending the soil
554 with large amounts of C will lead to greater C stock. Yet, it is both unlikely that all of the
555 introduced C will remain (Leskiw et al., 2012; Liu et al., 2018) or that it increases C fixation
556 of atmospheric C, meaning the gains in figure 4 are not 'true' C sequestration. In addition, the
557 physical disturbance required to input labile OM at depth may promote access to previously
558 unavailable C (Salomé et al., 2010). This response is confirmed by Shahzad et al. (2018a) who
559 observed increased respiration rates of buried C4 maize litter in C3 subsoil (55-75 cm)
560 compared to the topsoil (0-15 cm). This was attributed to a i) growth in the biomass of subsoil

561 microbes which are more limited by the availability of labile C than in the topsoil; ii) improved
562 co-location of decomposers and substrate, and finally; iii) microbial N mining, as the litter
563 introduced is nutrient-poor (C:N of 21:1) compared to native subsoil OM (C:N of 8.5:1).
564 Therefore, if this is to be pursued as a C sequestration strategy, it is important to ensure
565 additions are driving ‘true’ accrual of C.

566

567 *3.3. Biochar burial*

568 Due to its aromatic structure, biochar is extremely resistant to breakdown (Farrell et al.,
569 2013; Naisse et al., 2015), with commonly <3% of biochar-C decomposing in the first 1-2 years
570 (Major et al., 2010; Jones et al., 2011; Naisse et al., 2015; Wang et al., 2016). This recalcitrance
571 has stimulated interest in its use to store C for climate change mitigation (Das et al., 2014;
572 Smith, 2016). The application of biochar to topsoils has been extensively researched (Song et
573 al., 2016) and surface-applied biochar has limited downward movement potential (Major et al.,
574 2010) To date, there have been relatively few studies on biochar burial in subsoils, particularly
575 at the field scale, however, the limited evidence suggests that it can have positive agronomic
576 benefits when buried at or below 30 cm (Bruun et al., 2014; Iijima et al., 2015). In many cases,
577 however, its C sequestration potential and practicality have been exaggerated. Similar to OM
578 burial, it typically does not cause a truly ‘additive’ C effect, nor net removal of CO₂ from the
579 atmosphere (Chenu et al., 2019) at the landscape scale, and the negative impacts have often
580 been ignored (Jones et al., 2011; Hilber et al., 2017; Baveye et al., 2018). Further agronomic
581 trials are therefore required to critically evaluate subsoil biochar burial as a mechanism to
582 promote long-term C storage.

583

584 *3.4. Iron (hydr)oxide additions*

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

593 Lab-based experiments investigating the association of OC with Fe (hydr)oxides report
594 substantially decreased decomposition from Fe-associated SOC (Jones and Edwards, 1998;
595 Mikutta et al., 2007; Wen et al., 2019). In forest soils, a high proportion of stable OC is bound
596 to iron (Mikutta et al., 2006; Zhao et al., 2016). Furthermore, Porras et al. (2018), found that
597 <0.5% of Fe-associated glucose added to a subsoil decomposed compared to non-associated
598 glucose, with the effect strongest at 50-60 cm depth. As glucose is neutrally charged and does
599 not associate with sorption surfaces, it suggests that an indirect mechanism is involved in
600 suppressing C turnover (e.g. availability of nutrients such P, or mobility of microbes and
601 exoenzymes). Also, this Fe-associated SOC was found to be more resistant than native SOC to
602 increased temperatures (Porras et al., 2018). These promising results, albeit from a handful of
603 lab-based studies, suggest that adding iron or iron-associated OM into subsoils may be an
604 effective strategy for stabilising and sequestering subsoil SOC, respectively. However, a
605 limited evidence base (especially at the field scale) means further research is needed before
606 this strategy can be meaningfully evaluated.

607 The degree of soil C that is saved from mineralization from Fe addition in the field is
608 likely to be dependent on several factors, including the method of subsoil application, the
609 mineral makeup of the soil, the native subsoil C content, soil pH as well as texture and parent

610 material (Button et al., 2022). This method may be particularly relevant in sandy soils where
611 little chemical protection potential exists in the subsoil but of limited relevance to highly
612 weathered soils already rich in Fe. Building a greater evidence base, especially with field
613 studies, will allow for better evaluation of the potential of this strategy.

614

615 *3.5. Clay addition to subsoil*

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

623

624 *3.6. Deep ploughing*

625 Natural instances of soil burial demonstrate that SOC can be stabilised for millennia
626 (Chaopricha and Marín-Spiotta, 2014; Wang et al., 2014a). Mechanical soil inversion
627 techniques, such as deep ploughing (DP), rotary hoe or spading of agricultural land (i.e.
628 mechanical inversion of the soil >30 cm depth), buries more C-rich topsoil and plant residues
629 at depth allowing C accumulation in a 'new' unsaturated C-poor topsoil (Nieder et al., 1995;
630 Alcántara et al., 2017, 2016). However, many disregard DP as a soil management option and
631 suggest that any C input from DP is outweighed by the C lost to respiration (Freibauer et al.,
632 2004; Fontaine et al., 2007; Powlson et al., 2011). Studies that claim this, however, rarely
633 match the timescales at which DP is deemed effective (>10 years), do not fully balance C inputs

634 and CO₂ lost by respiration, lack experimental evidence, or often focus only on CO₂ fluxes and
635 not changes in the C stock.

636 Alcántara et al. (2017) found that 36-48 years after DP (to a depth of 55-90 cm) arable
637 land, SOC stocks increased by 67% compared to a reference subsoil and resulted in
638 substantially lower GHG emissions compared to conventional and zero-tillage management.
639 The specific mechanisms driving the stabilization of the buried topsoil are untested, but lower
640 microbial activity, a physical disconnect between decomposer and substrate and access to
641 unsaturated mineral surfaces deeper in the soil are likely primary drivers (Salomé et al., 2010;
642 Schiedung et al., 2019). More recently, Schiedung et al. (2019) found that 20 years after DP
643 (100-300 cm) total SOC stocks (0-150 cm) increased by 69%, a marked annual C sequestration
644 rate of almost 9 t C ha⁻¹ y⁻¹, but interestingly the ‘new’ topsoil had 36% less SOC than the
645 original topsoil, possibly due to a lower C sequestration capacity. This is supported by
646 Alcántara et al. (2016), who found that ‘new’ topsoils contained 15% less SOC even 3-5
647 decades after DP, suggesting that the capacity of the ‘new’ topsoils to sequester C was low in
648 their study; we expect this is highly context dependent.

649 The results from 6 studies of different soils where SOC was measured in the A and B
650 horizons before DP and after 12-48 years after are presented in figure 4 (methods in
651 Supplementary Information). These results demonstrate that i) buried topsoil drives a C
652 increase in the subsoil (0.09 ± 0.2 g C kg⁻¹ y⁻¹); and ii) DP had an overall limited effect on the
653 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
654 strategies for which sufficient data was available. However, 2 important factors were
655 determined for the success of DP. Firstly, the timespan between DP and SOC stock
656 measurement is important (i.e. more time allows for greater accumulation of C in the ‘new’
657 topsoil). Secondly, the location and soil type are crucial to the DP sequestration outcome. DP
658 should not be done when i) soil has high contents of very old SOC, ii) the topsoil is low in C,

659 iii) the soil has a high stone content, iii) steep slopes are present where erosion will be high, or
660 v) subsoils are unfavourable for plant growth (e.g. Al^{3+} and Mn^{2+} toxicity at low pH or Na^+
661 toxicity in alkaline soils). Subsoils with <70% silt content that restrict root growth could benefit
662 from DP (Schneider et al., 2017) and sequester SOC (FAO, 2017). Duplex soils (sand over
663 clay; often with a perched water table at the interface) could also benefit from DP where the
664 new surface soil layer has increased clay content while the subsoil has better drainage.
665 Increased plant production and deeper rooting depth on such soils could lead to greater C
666 sequestration.

667 Mapping the areas with potential for soil inversion and establishing longer-term field
668 studies will be essential to underpin any widespread DP implementation as a management
669 practice for C sequestration. While DP is an expensive process if only used to change SOC
670 profiles, the machinery and additional fuel costs could be offset through increased plant yield
671 in soils where ameliorating subsoil constraints can occur at the same time (e.g. mixing with
672 lime; uplift of subsoil CaCO_3 ; improved aeration, compaction alleviation), or where existing
673 surface soil problems (e.g. herbicide-resistant weed seeds) become buried at sufficient depth
674 to remove this constraint to crop production.

675

676 3.7. Subsoil water table management

677 In most cropping soils, a low water table depth is desirable to promote effective rooting.
678 If the field is not free draining, then subsoil artificial drainage is installed to lower the water
679 table and improve aeration. From a limited number of studies, this physical disturbance and
680 change in conditions has not been shown to have a major effect on GHG emissions (Dobbie
681 and Smith, 2006; Valbuena-Parralejo et al., 2019) and C storage in mineral soils (Mayer et al.,
682 2018). This suggests that artificial drainage may indirectly provide an effective way to deliver
683 C deeper into the soil profile.

684 In contrast to mineral soils, the drainage of agricultural peatlands has resulted in very
685 large net SOM loss rates (equivalent to $12 \text{ t C ha}^{-1} \text{ y}^{-1}$; Taft et al., 2017). This breakdown of
686 SOM also leads to the release of plant-available nutrients making these soils some of the most
687 productive in the world. In many cases these C stores have taken tens of thousands of years to
688 accumulate, however, they are being lost within decades in some cases. This rapid loss of
689 natural capital is fuelled by the removal of anoxic constraints on native SOM decomposition
690 by microbes and mesofauna (Wu et al., 2017). However, at soil loss rates of $1\text{-}2 \text{ cm y}^{-1}$, this
691 practice is clearly unsustainable and mitigation strategies are required to preserve the remaining
692 SOM. Raising the water table, therefore, offers an opportunity in these peat soils to re-establish
693 anoxic conditions and prevent SOM loss from deeper peat layers. If the water table is raised,
694 however, care must be taken not to negatively affect root growth (and thus yields) and also not
695 to create conditions that would be conducive to N_2O and CH_4 release. An experiment where
696 the water table was raised to 30 cm of the soil surface was found to reduce total GHG emissions
697 from 80 to 25 $\text{kg CO}_2\text{-e ha}^{-1} \text{ d}^{-1}$ (Taft et al., 2018). Wen et al (2019) showed the importance of
698 the C/N ratio of cover crop residues on total GHG emissions when raising the water table from
699 50 to 30cm; with vetch (low C/N ratio) resulting increased N_2O and total GHG emissions, and
700 rye (high C/N ratio) resulting in reduced N_2O and total GHG emissions. While proving
701 effective at reducing C losses, raising the water table makes the soil physically unstable,
702 unsuitable for vehicle trafficking and also prone to flooding. This highlights the trade-offs
703 between the effectiveness and practicality of C mitigation options.

704

705 **4. Challenges and opportunities: Looking to the future**

706 *4.1. Genetic engineering - can we modify subsoil rooting?*

707 The demand for food to feed an increasing world population, in a future of climate
708 instability, limited supply of P-rich ore (van Vuuren et al., 2010), and a global imbalance of N

709 fertilizer availability (Springmann et al., 2018), will place additional pressure on agricultural
710 land, with new land clearings causing further SOC loss. At the same time, climate change is
711 affecting food staples variably (Penuelas et al., 2017, Ray et al., 2019). Accelerated
712 improvement to crops to tackle food security is possible with technologies such as gene editing
713 (e.g. CRISPR cas9; Chen et al., 2019) being applied to improve traits, such as yield, disease
714 and salt tolerance, and plant architecture (Energy Futures Initiative, 2020; Lian et al., 2021).
715 The advantage of gene editing is that the genome of a species can be targeted to suppress
716 undesirable traits or turn on and over-express desirable traits (there is no foreign DNA added).
717 This technology is now permitted for application within the agricultural sector in some
718 countries (e.g. USA, Australia) with others still debating its use. In the context of this review,
719 we foresee an opportunity for gene editing to alter root systems (e.g. targeted root exudates)
720 and to change the lignin lattice (composition and structure) to form a less biodegradable plant
721 residue. This potential has already been highlighted in rice and tomato plants using CRIPR
722 cas9 where the production and exudation of strigolactones has been successfully modified to
723 promote plant growth (Butt et al., 2018; Lian et al., 2021). It has also been used as gene editing
724 tool to alter root branching frequency and branching angle in tobacco and rice (Bettembourg et
725 al., 2017; Gao et al., 2018; Kitomi et al., 2020). Lignin is a major component of plant cell walls
726 accounting for 30% of the organic C in the biosphere (Ralph et al., 2004). Its metabolic
727 pathways and function in plants are well characterised; Liu et al. (2018) review the basis for
728 genetic improvement of lignin. Xu et al. (2019) have also demonstrated how CRISPR cas9 can
729 be used to change the secondary metabolite composition in roots while Gasparis et al. (2019)
730 have shown how it can be used to alter a range of root morphology traits. As yet, these
731 technologies have not been harnessed to alter rhizosphere C flow or promote C storage in soil
732 (Energy Futures Initiative, 2020).

733

734 4.2. Climate change - what are the consequences for subsoil SOC stocks?

735 Rising atmospheric CO₂ can increase the growth and grain production of C₃ crops and
736 benefit C₄ crops experiencing drought stress (Fig. 5). Kimball (2016) reviewed 27 years of free
737 -air CO₂ enrichment experiments and found biomass and yield were increased by eCO₂ in all
738 C₃ crop species by 19%, but not in C₄ species except when water was limiting (30%).
739 Conversely, drought stress and rising atmospheric O₃ cause negative impacts on plant
740 production. How climate change will impact subsoil SOC stocks and microbial C utilisation
741 rates is less clear. Elevated atmospheric CO₂ is projected to increase the quantity of C flow to
742 root exudates (Phillips et al., 2010; Fig. 5); however, this may not necessarily translate into an
743 increase in SOC due to a concomitant increase in microbial activity (Keiluweit et al., 2015;
744 Kuzyakov et al., 2019). Pries et al., (2017) found that warming forest soil to 100 cm by 4°C
745 increased respiration of the whole soil profile by 34-37%. Subsoils contributed the majority to
746 this (20-25%) with millennial old C respired. This is echoed by a recent meta-analysis of over
747 100 eCO₂ studies suggesting soil carbon storage declines when plant biomass is strongly
748 stimulated by eCO₂ in forests, however, grassland soils have a large capacity to drawdown CO₂
749 and have increased SOC stocks (Terrer et al. 2021). As well, Baumer et al. (2018) found that
750 increased subsoil exudation caused a 10% increase in SOC, due to a stimulation of fungi.
751 Research is currently lacking to answer the key question - *are subsoil SOC stocks in mineral*
752 *and organic soils secure from climate change?*

753 Extremes in climate events will increase with global warming causing increased
754 frequency of wet-dry cycles (Meehl et al., 2007; Fig. 5). To some extent, subsoils will be
755 buffered from extremes in temperature and moisture due to the overlying topsoil (Wordell-
756 Dietrich et al., 2017; Qin et al., 2019). Gobel et al. (2011) proposed that the soil C balance is
757 sensitive to climate extremes that decrease the wettability of soil and thus increase water
758 repellence. This would increase surface water run-off and cause heterogeneous preferential

759 flow pathways through the subsoil (Fig. 4); causing potentially less plant growth and more
760 spatially variable root growth leading to less subsoil plant root C inputs. Water repellence in
761 both surface and subsoils will also cause the water inside soil pores to form as droplets instead
762 of continuous water films (Goebel et al., 2007). Disconnect in water films, as a consequence
763 of water repellence, will restrict the diffusion of DOC (Or et al., 2007) and nutrients which will
764 limit microbial uptake. This disconnect in water films is likely to also increase the stability of
765 existing SOM against biological enzymatic decomposition thus increasing SOC residence time
766 (Goebel et al., 2007). Water repellence is already widespread globally (Goebel et al., 2011) and
767 is expected to become more frequent. How this alters the SOC balance is uncertain and
768 highlights the need for SOC models to take account of differences in SOM turnover rates in
769 water repellent soils.

770

771 *4.3. Microbial survival in subsoils - are there unknown metabolic pathways?*

772 Microbial cells exist in both metabolically active and dormant states. Jones and Lennon
773 (2010) proposed that dormancy contributes to the maintenance of microbial diversity; fast-
774 growing species use energy sources to grow competitively, whereas slow-growing species use
775 greatly reduced metabolism (i.e. anabiosis) to outcompete other species during periods of
776 energy starvation. This enables slower-growing organisms to exist without direct competition
777 with fast-growing species. It is expected that in C-limited subsoils microbial competition for
778 SOC sources required for energy-generation (i.e. electron donors) will be intense. This poses
779 the question - *Do we fully understand microbial metabolic pathways in slow-growing*
780 *(oligotrophic) species within subsoils and does this impact on C storage?*

781 The discovery of alternative microbial energy pathways in surface soils suggests more
782 understanding is needed. Research by Greening et al. (2015) highlights that an aerobic
783 heterotrophic acidobacterium uses H₂ oxidation from atmospheric scavenging when organic

784 electron donors are scarce. Hydrogenase-encoding genes were subsequently identified in 51
785 bacterial and archaeal phyla (Greening et al., 2016). Furthermore, Ji et al. (2017) found
786 atmospheric H₂, CO₂ and CO gases to be energy sources for Antarctic surface soil
787 communities. Atmospheric CO oxidation enables the survival of aerobic heterotrophic bacteria
788 in energy-limited environments and has been found in many species of soil bacteria and archaea
789 (Cordero et al., 2019). These findings highlight that trace gas oxidation may be a general
790 mechanism for microbial persistence in topsoils (Greening et al., 2016). Such exploratory
791 genomic studies warrant investigation in both oxic and anoxic subsoil layers.

792

793 4.4. What are the key challenges in studying subsoils and can they be overcome?

794 Soil sampling depth has largely been driven by interest, practicality, and cost. This is
795 reflected by a majority of C studies focusing on shallow soil layers (<30 cm; Yost and
796 Hartemink, 2020). However, sampling below this in agricultural systems is not that difficult
797 with use of sharpshooter, hammer and semi-mechanical soil corers, while hydraulic probes can
798 be rented cost-effectively for deeper sampling. Indeed, deeper sampling used to be more
799 common (Yost and Hartemink, 2020), so why is sampling becoming shallower? Although there
800 is no current explanation, it could simply be because sampling deeper creates more samples to
801 collect, process and analyse. We would like to argue that sampling deeper is worthwhile and
802 to urge the soil science community not to stop sampling deeper. This is especially important as
803 subsoils are different (Fig. 1 and 2) and changes in SOC stock may vary through the soil
804 profile (e.g. Taughtes et al., 2019) which topsoil sampling would miss, resulting in potentially
805 misleading results and interpretation. In the case of *in situ* studies, there are different ways to
806 take samples actively or passively (e.g. subsoil gas sampling systems; see Maier and Shack-
807 Kirchner, 2014), with relative ease and limited soil disturbance which can greatly improve our
808 understanding of the deep soil environment. Nevertheless, *ex situ* studies remain the more

809 common practice (Rumpel and Kögel-Knabner, 2011), however, adequately simulating subsoil
810 environmental conditions (e.g. lack of disturbance, lower O₂ concentrations, different
811 temperature and moisture contents, etc.) is difficult, making these more variable and less
812 appropriate for extrapolation to field conditions.

813 Both SOC content and bulk density variability contribute to C stock uncertainty, in turn
814 affecting how large a change in stock can be observed through time or space. In the Western
815 Australian SOC Audit, for example, Holmes et al. (2012) determined that variability in SOC
816 (%) contributed to 84-99% of the uncertainty in C stocks compared to <5% from bulk density.
817 They illustrated that the rapid indirect measurement of bulk density using a gamma-neutron
818 density meter could be used in place of labour-intensive traditional volumetric rings or clod
819 measurements. Scanlan et al. (2018) have since developed a hand-held 3-dimensional scanning
820 system based on a time-of-flight camera to measure the volume of the void created when using
821 any excavation method (ring, auger, or shovel). This system was shown to measure bulk density
822 accurately and rapidly in soils tested (35-71% gravel content; 0-40 cm depth).

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

829

830 *4.5. What is the size of the subsoil C reservoir and how much more can be stored?*

831 To determine how much additional C can be sequestered globally requires that we know
832 how much C is currently present in the soil (Smith et al., 2020). A consensus on the size of the
833 global SOC stock, however, is lacking, with estimates ranging from 500 to 3000 Gt C (Todd-

834 Brown et al., 2013; Scharlemann et al., 2014). This variation in estimates occurs due to
835 variations in model parameters and different soil depths considered in each study (Harrison et
836 al., 2011; James et al., 2014). The alternative, mapping of soils by extensive geochemical
837 sampling projects, has covered the majority of Europe (FOREGS; Salminen et al., 2005), the
838 USA (NASGLP; Smith et al., 2012), China (GCB; Wang et al., 2014b) and Australia (NGSA;
839 Caritat and Cooper, 2011), often also including deep soil samples. While time and resource
840 intensive, this is the best way to get high quality fine-scale data to quantify the global C stock
841 and identify areas where sequestration can be most successful. We encourage colleagues from
842 the Middle East, Eastern Europe, South and Central America, South Asia and Africa to pursue
843 extensive mapping programs.

844 Smith et al. (2020) argue that to implement C sequestration initiatives, we need more
845 reliable SOC change monitoring, reporting and verification platforms for policymaker support
846 and gaining investments. Similar to the uncertainties of the soil C stock, consensus on how
847 much more C can be sequestered in soil has not been achieved (Minasny et al., 2017; Zomer et
848 al., 2017). This is due to the mentioned issues with stock estimates and differences in the
849 sequestration strategies, land type and how SOC change is measured (Smith et al., 2020).
850 Frequently, the depth of soil C estimation is not stated. As a result, over- or underestimation of
851 the sequestration potential may occur. Current evidence for topsoils suggests that the soil C
852 sink will eventually reach saturation (Solinger et al., 2000; Hoyle et al., 2013; Smith, 2016;
853 Wiesmeier et al., 2015) after approximately 20 to 80 y of positive C sequestration management
854 (Lal and Bruce, 1999; Minasny et al., 2017; Poulton et al., 2018). As the rate of accumulation
855 is non-linear and decreases soon after it begins, estimated annual C sequestration rates can only
856 realistically be achieved within the short-term. Many studies do not indicate a time within
857 which this rate can be achieved, so their accuracy remains unknown. Furthermore, when
858 management practices targeting greater C accrual in subsoils are discontinued, it is important

859 to consider whether the C sequestered will be susceptible to being lost, and if so at what rate.
860 The recent UN FAO program for the global assessment of C sequestration potential (GSOCseq;
861 FAO, 2019) is a promising new vision that aims to build the international capacity of SOC
862 change monitoring, reporting and verifying which will be essential in moving C sequestration
863 forwards.

864

865 *4.6. What are the limitations of existing subsoil C simulation models?*

866 Soil C models are essential for predicting SOC sequestration over long timescales (>
867 50 years; Chenu et al., 2018), however, they are only useful if parameterised properly (Dignac
868 et al., 2017). Currently, the majority of C models (e.g. RothC, Century) are only designed to
869 describe topsoil C dynamics (Smith et al., 1997; Stockmann et al., 2013). Also, the depth of
870 soil that the C stock is estimated to is often not reported in these models, making comparisons
871 between the results of models challenging (Stockmann et al., 2013; Todd-Brown et al., 2013).
872 As surface and subsoil C characteristics and dynamics are substantially different (as
873 demonstrated by Salomé et al., 2010; Sanaullah et al., 2011; Hoyle et al., 2013; Zieger et al.,
874 2018; Qin et al., 2019), model parameterisation must be different for subsoils. While subsoil
875 models exist (Table 1), they are relatively recently developed, vary in their description of C,
876 their accuracy and the depth of C they measure. This is mostly because (reliable) estimates for
877 C supply and subsequent transformation rates do not exist for multiple soil layers
878 (Keyvanshokouhi et al., 2019) or are not linked with other factors that affect subsoil C storage
879 (e.g. N availability, carbonate content). While progress in subsoil C models has been made, a
880 greater mechanistic understanding of the specific subsoil C inputs as well as more extensive
881 field-derived data will be required to further optimise existing models (Taghizadeh-Toosi et
882 al., 2014) and be able to reflect potential strategies to increase subsoil C content.

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

892

893 *4.7. What factors will affect the likely success of subsoil interventions?*

894 The overall success of different interventions to promote subsoil C storage depends on
895 (i) their effectiveness to store C in the long term and the evidence to support this, (ii) their
896 impact on other ecosystem services (including agricultural productivity), (iii) cost of
897 implementation, (iv) practicality, (v) social acceptability, (vi) legislative barriers, and (vii)
898 overall C cost of interventions (i.e. is more C stored than released in the process?). The
899 importance of these is likely to be highly context-specific varying from farm-to-farm and
900 country-to-country. For example, mechanical interventions may not be cost-effective or
901 feasible by smallholder farms, whereas plant-based solutions may be implemented by all
902 landowners. Strategies promoting higher subsoil C storage (e.g. DP, OM burial, deep rooting
903 crops) are likely to improve nutrient cycling and water-holding capacities, leading to increases
904 in crop yield (Gregorich et al., 1994). These win-win-win scenarios (i.e. more C, greater water
905 use efficiency, higher yields) should help promote the adoption of subsoil technologies by
906 farmers. In the case of OM, biochar and Fe additions to the subsoil, mechanical intervention is
907 required. Although not mainstream, most of this equipment is commercially available to allow

908 wide-scale adoption of subsoil technologies should these options gain support. One barrier to
909 overcome in some countries is the legalities surrounding the addition of C-rich wastes to
910 agricultural land. This will need a strong scientific evidence base and may take a long time for
911 legislation to be passed. Lastly, it is known that some farmers can be resistant to change
912 (cultural inertia; Hyland et al., 2016) and that there is resistance to geoenvironmental approaches
913 to tackle climate change in both the public and scientific community (Robock et al., 2015). In
914 addition, costs, practicalities and training needs may need addressing. Realistically, financial
915 incentives for farmers (e.g. via the carbon market or agri-environment schemes) could be used
916 to promote subsoil C technologies (Siedenburg et al., 2012). There is also a need for
917 policymakers and extension agencies to focus on knowledge exchange and awareness
918 programs, making use of the multiple co-benefits related to adopting pro-subsoil C behaviour
919 (van de Ven et al., 2018).

920

921 **5. Concluding remarks**

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

- 928 • Use of deeper rooting varieties is a cheap and easy way to enhance the C supply to the
929 subsoil. While tangible benefits may take years to establish, it is likely to be more
930 effective in lighter soils and those not with old C-rich subsoils.
- 931 • While additions of OM and biochar to subsoils increase the C stock and can be
932 beneficial, these are unlikely to lead to 'true' C sequestration.

- 933 • Addition of iron and clay to subsoils may be effective in very specific soils, but current
934 evidence is not sufficient to recommend their widespread adoption.
- 935 • Deep ploughing (DP) can be effective when >10 years apart and in soil that does not
936 have high contents of very old SOC; C-poor topsoil; a high stone content; steep slopes;
937 or unfavourable subsoils for plant growth. Silty and Duplex soils could particularly
938 benefit from DP.
- 939 • Water table management can be highly effective in enhancing C sequestration. In
940 mineral soils lowering the water table can allow for greater C delivery in the subsoil,
941 while in organic soils raising it is beneficial to C sequestration – but not for agricultural
942 capability.

943

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

- 946 1. Improve our understanding of the mechanisms that regulate C stabilization in subsoils
947 and the factors driving long C residence times (e.g. rates of subsoil C supply and loss;
948 stabilization mechanisms of suberin and DOC; sorption of SOC to minerals; role of
949 Ca^{2+} and CaCO_3 in C stabilization, role of microbes in SOC residence time; persistence
950 of microbial necromass; spatial organisation of roots, microbial communities and
951 SOC).
- 952 2. Undertake studies that take advantage of space-for-time substitutions, long-term field
953 and chronosequence studies of subsoil sequestration technologies (in isolation or
954 combination). These studies also need to consider the trade-offs between different
955 ecosystem services and the overall effects on soil health as well as their practicality and
956 economic viability.

- 957 3. Perennialisation and improvement of deep-rooting traits in crops and grasses that
958 promote greater subsoil C storage (e.g. by harnessing gene-editing technologies; better
959 selection of rhizosphere communities; better *in situ* techniques for studying subsoil root
960 dynamics).
- 961 4. Investigations into how climate change, especially changes in moisture status and
962 extreme weather events, will affect subsoil C storage.
- 963 5. Use the information gathered above to improve the parameterization of soil profile- and
964 landscape-level models of subsoil C dynamics that allow us to simulate the impact of
965 different land management and future climate scenarios on subsoil C, but also improve
966 global climate models.

967

968

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978

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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.

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.

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).

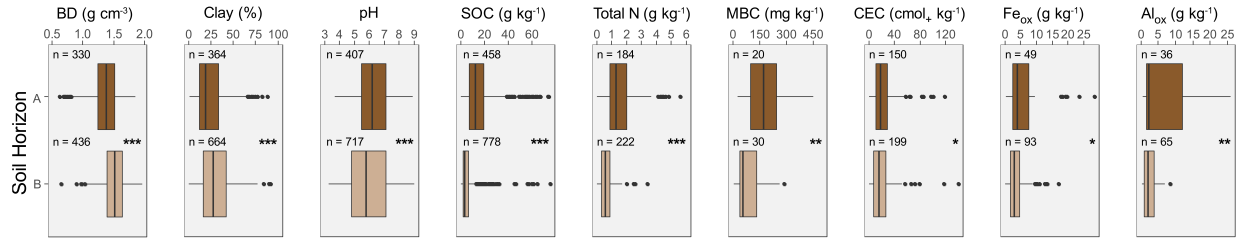
Fig. 5 Schematic representation of how mineral subsoils (i.e. Mollisol, Alfisol) will likely change in response to different climate change scenarios with potential feedbacks in the C and N cycles. Elevated CO_2 will induce plant growth, deeper rooting and more rhizodeposition in the subsoil. This will 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).

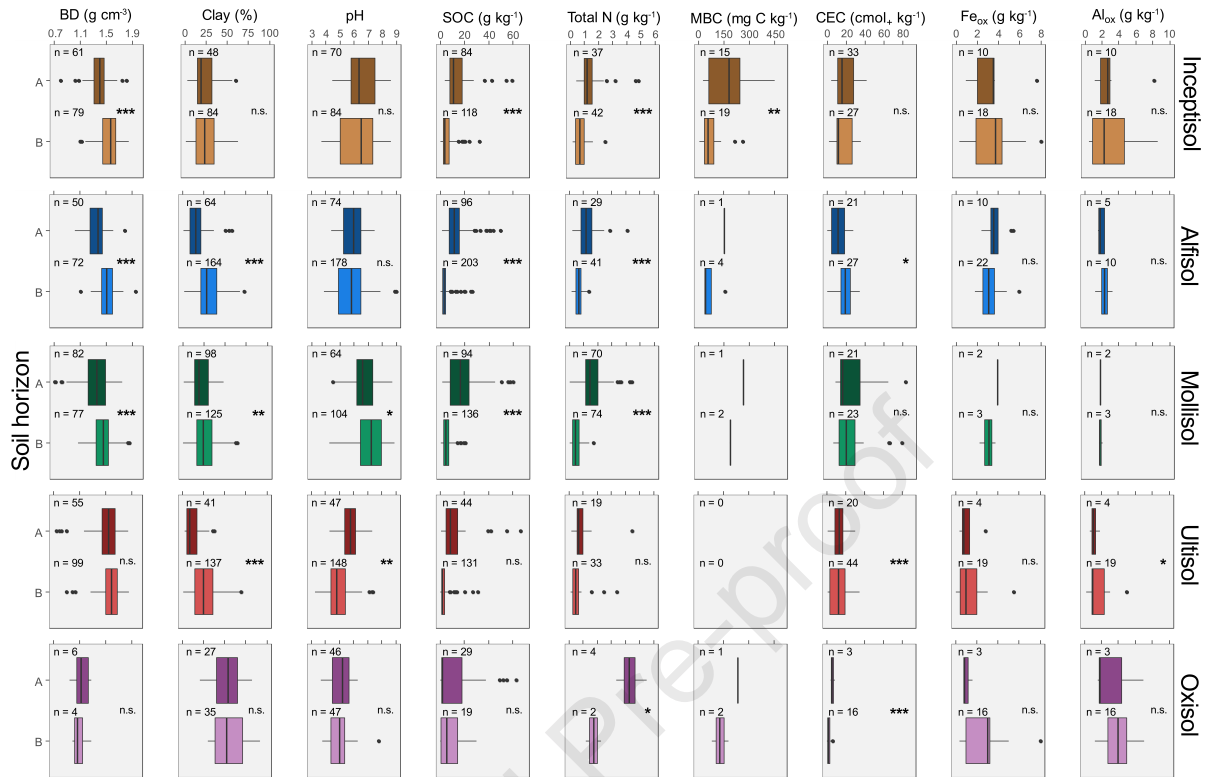
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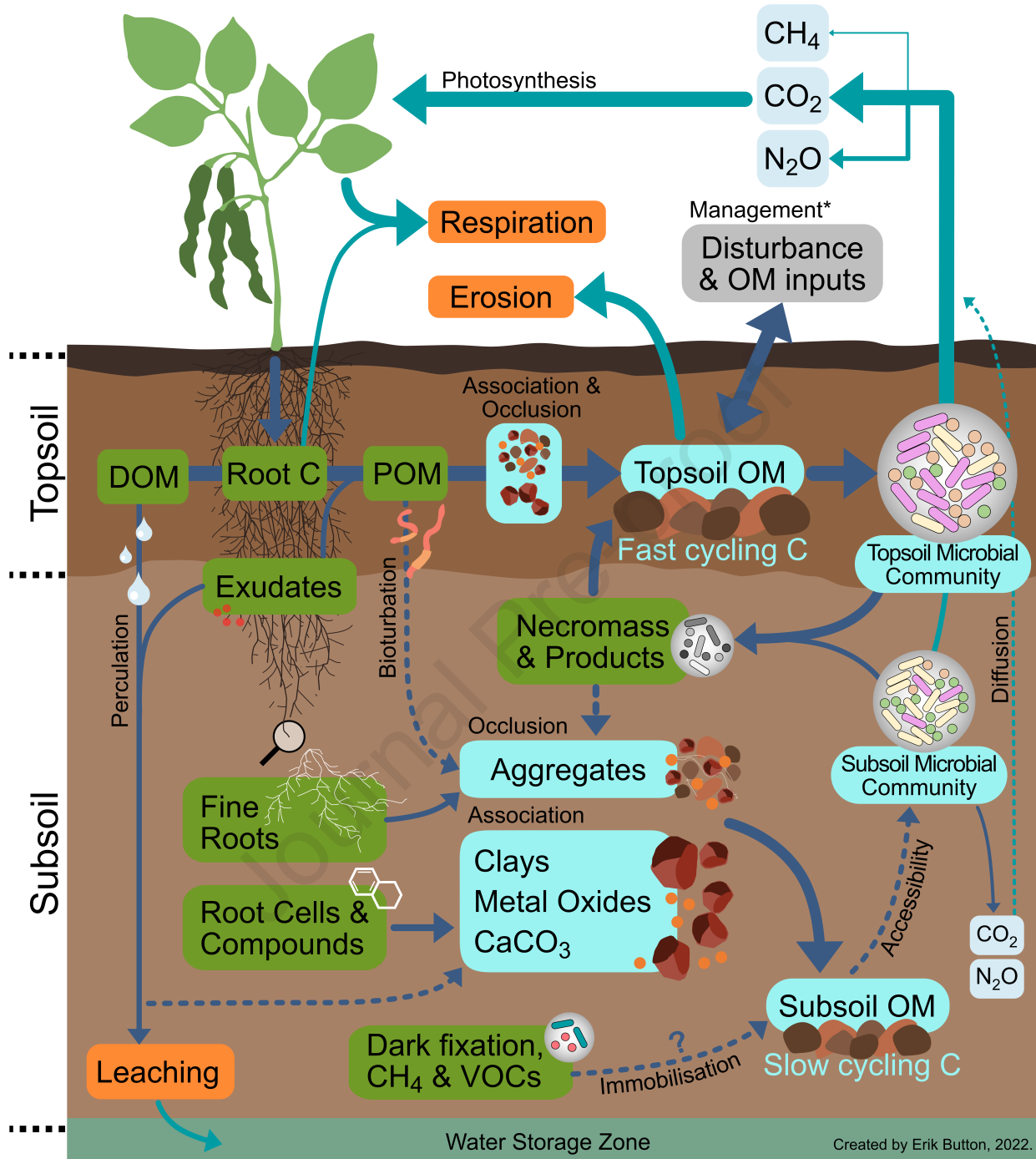
Table 1. Models that address different C-related in topsoils and subsoils.

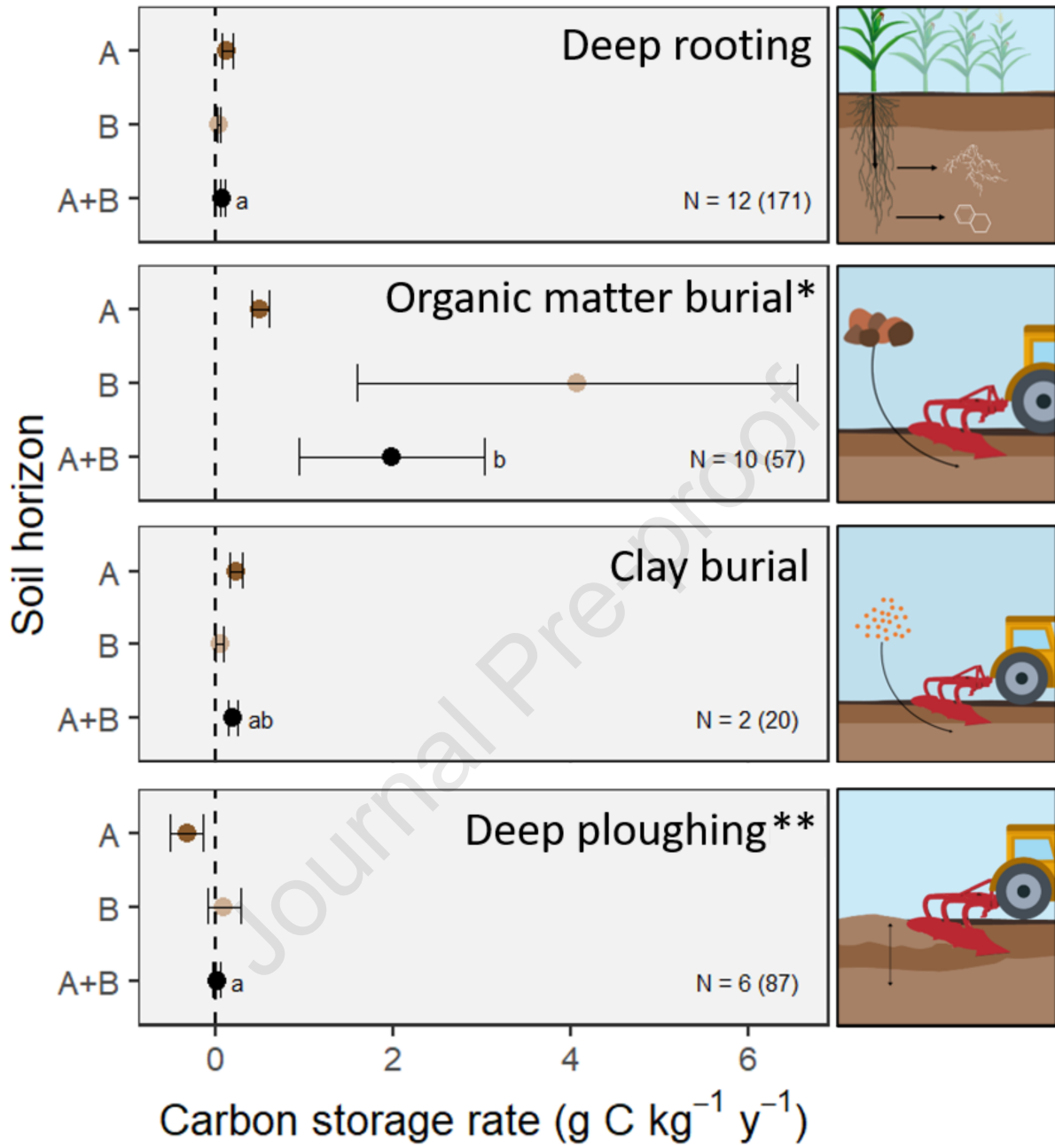
Model name	Function	Additional Information	Reference
-	C estimation	Non-linear mixed effect model for estimation of forest soil (to 2.5 m) C.	James et al. (2014)
-	C estimation	Linear function for topsoil and power function for subsoil (to 0.75 m).	Beem-Miller and Lehmann (2017)
RothPC-1	C turnover estimation	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)

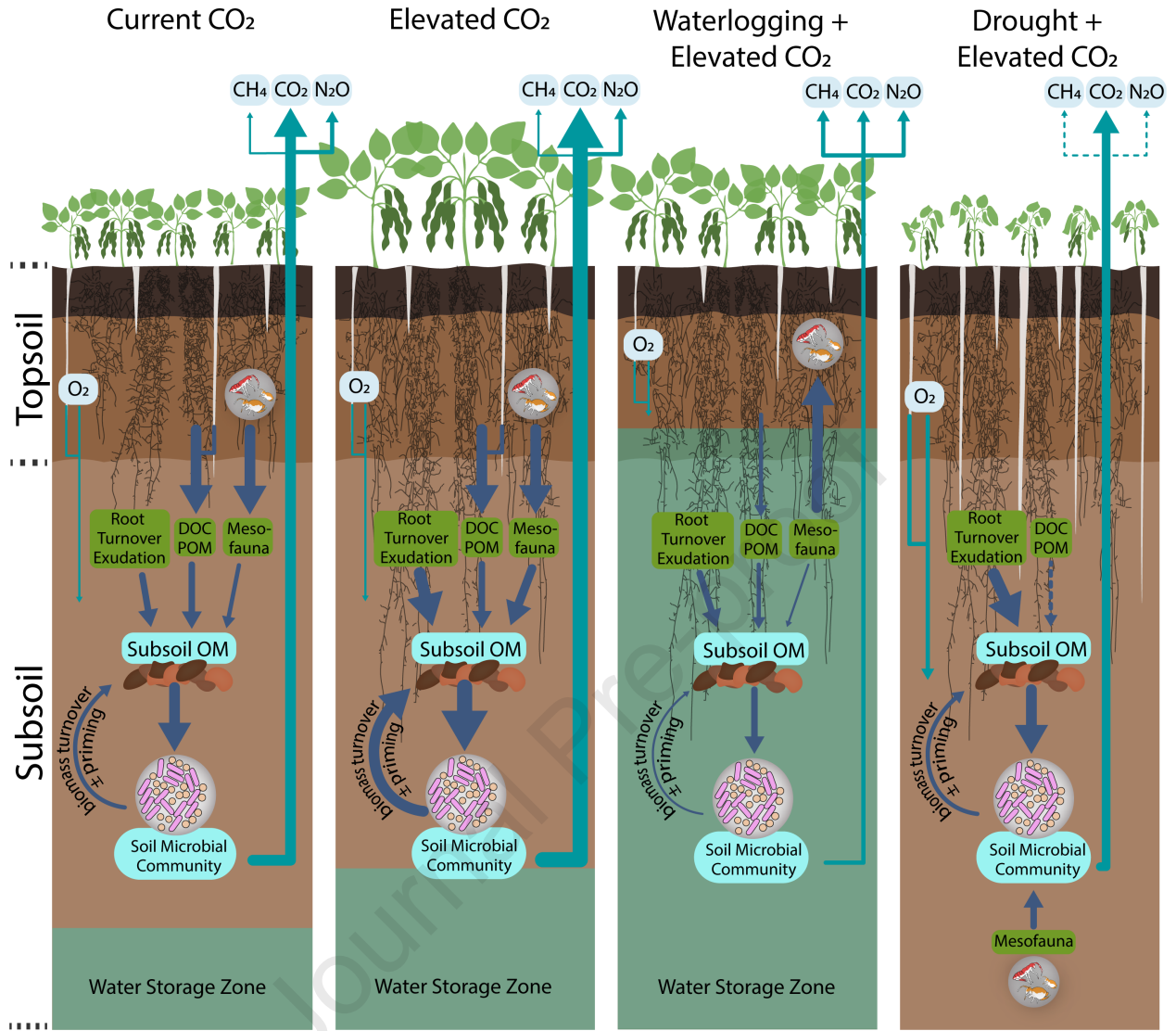


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1 **Highlights**

- 2 • Agricultural subsoils may be more suited to long-term C sequestration than topsoils.
- 3 • Sequestration strategies have variable effects on C stocks, depending on soil type.
- 4 • Enhanced subsoil C storage can bring ancillary tangible benefits to cropping systems.
- 5 • Subsoil sampling and incorporation in models and maps is essential.
- 6 • Policies are needed for C sequestration in the whole soil profile, not just topsoils.

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Declaration of interests

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.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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