NOTE

Before using this manuscript/paper, please make sure to check and follow the copyright regulations provided at https://www.elsevier.com/journals/catena/0341-8162/guide-for-authors; https://www.elsevier.com/about/policies/copyright; https://www.elsevier.com/about/policies/sharing and there provided links.

The research manuscript/paper provided here (for educational/research and non-commercial purposes) is based on the revised manuscript version of Nov. 2016 and it may slightly differ from the version that was published in CATENA journal in 2017. The following is the information on the published version of this manuscript/paper:

Alina Premrov, Thomas Cummins, Kenneth A. Byrne, Assessing fixed depth carbon stocks in soils with varying horizon depths and thicknesses, sampled by horizon, CATENA, Volume 150, 2017, Pages 291-301, ISSN 0341-8162, DOI: https://doi.org/10.1016/j.catena.2016.11.030. (https://www.sciencedirect.com/science/article/pii/S0341816216305112)

© 2017

This manuscript version is made available under the CC-BY-NC-ND 4.0 license https://creativecommons.org/licenses/by-nc-nd/4.0/

Assessing fixed depth carbon stocks in soils with varying horizon depths 1

- and thicknesses, sampled by horizon 2
- Alina Premrov^{*1}, Thomas Cummins¹, Kenneth A. Byrne² 4 6 First and corresponding author, E-mail: alina.premrov@ucd.ie; Tel.: ++353 (0) 1 716 7561 7 ¹ UCD School of Agriculture and Food Science, University College Dublin, Belfield, Dublin 4, Ireland 8 ² Department of Biological Sciences, School of Natural Sciences, University of Limerick, 10 Limerick, Ireland
- 11

9

3

5

12 Abstract

13 Soil surveys for improving carbon (C) stock estimates frequently involve soil sampling by pre-14 determined regular depth-intervals, in order to enable more convenient computation of soil 15 organic carbon (SOC) stocks. As a result, soil horizons are often neglected in these surveys, 16 although they represent distinct components of the soil profile. When soil-horizon depths and thicknesses vary considerably within the same site, soil sampling by horizon with accompanying 17 18 depth measurements may be more suitable. The main objective in this study was to investigate 19 the potential differences in current SOC stocks in different afforested mineral soils, with varying 20 horizon depths and thicknesses, and that were sampled by soil horizon, by using the trapezoidal 21 SOC stock computing approach, and comparing it to the spline approach. An adaptation of the trapezoidal 22 rule computation approach, enabling relatively simple crude estimations of the fixed depth-interval 23 SOC stocks from horizon data, was developed. Estimations of SOC stocks for 18 sites located on three different afforested mineral soils (Gleys, Podzols and Cambisols, aged \geq 20 years) were 24 25 done for 0-30 cm, 30-60 cm and 0-60 cm fixed depth-intervals, excluding surface organic

layers. The results indicate that the trapezoidal approach is likely to provide cruder estimates of 27 SOC stocks than the spline approach, although no statistically significant differences were observed between the fixed depth-interval SOC stocks (for 0-30 cm and 30-60 cm) when 28 29 computed by the two methods. Both methods showed a significant effect of horizon and soil group on SOC stocks. The soil below the 30 cm depth was estimated to store over 22% of the 30 31 total SOC stocks to 60 cm depth. Gleys showed significantly greater mineral SOC stocks than 32 Podzols, with differences mainly evident in the upper 30 cm, which was observed regardless of 33 the computing methodology used (trapezoidal or spline). The adapted trapezoidal rule 34 computing approach is hoped to facilitate the use of soil-horizon sampling in studies on SOC 35 stocks. 36 37 Keywords: Soil organic carbon stocks, soil horizon, soil depth, forest mineral soil, trapezoidal 38 computing approach 39 40 1. Introduction 41 42 Forest soil surveys which include C stock monitoring are becoming increasingly important due to 43 greenhouse gas emissions reduction targets at national and international level, as well as 44 assessing the role of forest soils in mitigating such emissions. The soil represents the largest terrestrial organic carbon (C) pool, globally estimated at 1115 10⁹-2200 10⁹ tC (Batjes, 1996). 45 The total world soil organic carbon (SOC) stock of the upper 30 cm soil depth is estimated at 46 684.109-724.109 tC, and for the upper 100 cm at 1462.109-1548.109 tC (Batjes, 2014). In 47 particular, forest soil represents an important terrestrial organic C stock. The estimated soil C 48 stock up to 100 cm depth for worlds forests is c. 383 ± 30.10⁹ tC (Pan et al., 2011). According to 49 Jobbágy and Jackson (2000), the global SOC storage of different temperate forests for 0-100 50 cm depth can be estimated in the range 73.109-122.109 tC. Soil surveys, which aim to improve 51 the estimates of soil C stocks, frequently involve soil sampling to 30 cm depth or even less, and 52

26

with soil sampling often performed by pre-determined regular soil-depth intervals (Baritz et al.,
2010; Cools and De Vos, 2010; UNECE, 2006).

55

56 Shallow-depth sampling is often used in soil studies due to difficulties and costs associated with 57 soil sampling at greater depths, as well as due to expectations that deeper soil horizons are 58 more stable and less likely to change over the time although not all studies support this 59 (Harrison et al., 2011). Soil sampling at shallow depths can result in an underestimation of C 60 present in the soil profile (Harrison et al., 2011). Although deeper subsoil horizons are known to 61 have relatively low C content they should still be accounted for in the C-cycle (Rumpel and 62 Kögel-Knabner, 2010). Also, soil sampling is often performed by pre-determined regular soil-63 depth intervals - e.g. at 10 cm depth-intervals, from a soil profile, for forest soils (Stolbovoy et 64 al., 2005; UNECE, 2010). The advantage of such pre-determined regular depth sampling is that 65 it can enable relatively simple computation of a variable of interest, such as SOC stocks to a 66 specific depth (Stolbovoy et al., 2005). This can be done by e.g. soil-depth normalisation (Freier 67 et al., 2010), or by summing the calculated C stocks of the pre-determined regular depth-68 intervals (Lee et al., 2009). As a result of soil sampling by regular depth-intervals, soil horizons 69 are often neglected in SOC stock surveys, even though they represent distinct components of 70 the soil profile.

71

72 In order to increase the accuracy of SOC stock estimations, and clarify the effects of pedogenic 73 processes on the storage of SOC, Wiesmeier et al. (2012) recommend that SOC inventories 74 should have the soil analysis completed by horizon instead of by fixed depth increments. Where 75 the soil-horizon depths and thicknesses vary considerably within the same site, errors in C stock 76 estimations may be generated if the differences in horizon thickness are not taken into account. 77 These errors could be potentially omitted by e.g. excavating more soil pits at different locations 78 within the same site, but this would require more labour-intensive procedures, and would 79 consequently increase the cost of sampling and its duration. Field methods often need to be

adapted in order to reduce the costs and to be feasible within limited project resources.
Furthermore, soil pit excavation can also be especially challenging for forest soils due to
potentially remote locations, rocky, difficult and steep terrain, the presence of coarse roots, and
use of manual methods because of other constraints.

84

In cases when soil-horizon depths and thicknesses can vary within the same site, and when excavation of more soil pits is not an option, sampling by horizon with horizon-boundary depth measurements may be a more suitable approach (Premrov et al., 2014). However, such sampling may require more demanding computation procedures: e.g. due to differences in thicknesses among sampled horizons at different sampling points, separate computations of C stocks for the chosen fixed depth-interval are required, taking into account the horizon thicknesses from sampled points, separately for each site.

92

93 The approach taken in this study was to develop an adaptation of a trapezoidal rule computation 94 by Lord and Shepherd (1993) that would allow relatively simple estimation of fixed depth-interval 95 SOC stocks for soils with varying horizons and depths, and to compare it with the more complex 96 spline computation method based on the equal-area quadratic smoothing spline modelling 97 explained by Bishop et al. (1999). Area-based SOC stocks were to be estimated by adapting the 98 computation approaches in a way that would enable the use of the soil-horizon thicknesses and 99 horizon volume-based C stocks (mass C per volume of soil), and would at the same time also 100 account for varying number of samples obtained for each horizon. The main objective of this 101 work was to investigate the differences in current SOC stocks in different mineral soils with 102 varying horizon depths and thicknesses that were sampled by horizon, by using the adapted 103 trapezoidal SOC stock computing approach, and comparing it with the more complex spline approach. Specific 104 aims were to investigate the potential differences in SOC stock by soil group (in three Irish

- 105 afforested mineral soils: Gleys, Podzols and Cambisols), by horizon, and by soil depth (0–30 cm,
- 106 30–60 cm and 0–60 cm fixed depth-intervals excluding surface organic layers).
- 107

108 2 Materials and methods

109

110 2.1 Field sites and sample specifications

111 Eighteen afforested sites were sampled between March 2014 and March 2015 across the

112 Republic of Ireland (Fig. 1a) as a part of the larger CForRep project (CForRep, 2013;

113 <u>https://www.ucd.ie/cforrep/</u>), with sites being selected from Ireland's National Forest Inventory

114 database (National Forest Inventory, 2012), after pre-screening the database for afforested sites

115 located on selected mineral soils, and aged ≥ 20 years (Premrov et al., 2015). The CForRep.

116 project used the general Irish soil classification, where the sites were classified into Podzols and

117 peaty Podzols (Po and peaty Po), Brown Podzolics (BP), Acid Brown Earths (ABE), calcareous

118 Brown Earths (BE) and Gleys (G) (Black et al., 2014; Gardiner and Radford, 1980). The sites

119 were classified individually (on site), and were further re-grouped into the three main soil groups:

120 Gleys, Cambisols and Podzols. Po, peaty Po and BP were grouped as Podzols, ABE were

121 assigned to Cambisols (Reidy et al., 2014), there were no sites with BE in this study, while

122 classification of Gleys remained unchanged. Gleys included sites with Stagnosol and Gleysol

123 soils according to the World Reference Base for Soils (WRB) classification (IUSS Working

124 Group WRB, 2015), or Surface Water Gley and Typical Groundwater Gley, respectively,

125 according to the Irish soil subgroup classification (Reidy et al., 2014). Site locations are

126 presented in Fig. 1a. Six sites were sampled in each soil group giving in total of 18 sampled

127 sites (each site included sampling from a soil pit for bulk density measurements, as well as

128 auger sampling from up to nine points on a 3 x 3 grid; further details are explained in section

129 2.2). The woodland tree species were mainly determined on site but were later classified into

- 130 three major woodland-type categories of coniferous, broadleaf and mixed. Mineral-soil horizon
- 131 designation was also done on-site according to the FAO (2006) guidelines for master horizons,

132 but the specific horizons were later grouped into five major categories [A, E, B, B2 and BC,

133 where B2 refers to the second, often more water-saturated B horizon (e.g. Bg horizon), found in

134 Gleys].

135

136 2.2 Sampling methods

Each site was positioned with a hand GPS (Geographic Positioning System) instrument, and the
slope (in degrees) was measured with a clinometer at the central point. Soil sampling was
performed at nine points (on a 3 x 3 grid over 20 x 20 m; Fig. 1b), up to 60 cm depth, by horizon.
Organic forest-floor and peat horizons were also sampled under the CForRep project, but were
not included in this study on mineral soil horizons.

142

143 Soil samples for bulk-density measurements were collected from each horizon separately for 144 each site, from a soil pit located in the centre of the sampling grid (Fig. 1b, 1c). Bulk-density sampling was performed either with 100 cm³ coring rings, or by the excavation method (i.e. 145 146 excavation of soil material and replacement by sand, adapted from ISO (1998)) where the 147 volume of excavated (replaced) material was determined on-site using fine sand 300-600 µm in 148 size. For bulk-density ring-sampling, a hammering-head for sample rings (Eijkelkamp, The 149 Netherlands) was used to avoid compression. Where possible, the bulk-density sampling was 150 done from the centre of each horizon, from up to three sides of the soil pit (Fig. 1 c). 151 Exceptionally, the two Gley sites in this study had missing bulk-density samples for a single soil 152 horizon; measurements obtained from the most-similar horizon from the same site (of the same 153 soil-horizon category) were used as replacements (i.e. a missing value for a Bg horizon that was 154 under water-table was replaced with the Bg horizon sampled at that same site, but above the 155 water-table; at another site a missing value for Bg(a) horizon was replaced with Bg horizon 156 sampled at that site; details are provided in Fig. 4). Percentage coarse material was estimated on-site, from the soil pit, according to the FAO (2006) guidelines for soil description. 157

158

Horizon samples for SOC analysis were taken with an Edelman auger (Eijkelkamp, The 159 Netherlands), accompanied by the horizon depth measurements (depths to upper and lower 160 161 boundary). Sampling was performed from up to nine sampling points (each arbitrarily located 162 within one cell of the 3 x 3 sampling grid, Fig. 1b). The entire thickness of each soil horizon was 163 sampled at each sampling point, and the samples from each horizon were bulked together into 164 one composite soil sample by horizon, separately for each site. In order to minimize potential 165 cross-contamination between horizons, the individual sampling by horizon from each sampling 166 point was done by first placing each separate augered soil material onto a clean tray. This was 167 done with a special care not to disturb the sequence of the augered material. The material on 168 the tray was then carefully separated into individual soil horizons. All collected soil samples were transported in cool-boxes and stored in a cold-room at 4°C until further laboratory-processing 169 170 and analysis. A total of 510 soil samples were collected from the eighteen afforested sites: 126 samples for bulk-density determination and 384 soil samples from the sampling grids (Fig. 1). 171 Bulking of soil samples from individual sampling points (by horizon, separately for each site) 172 173 resulted in total 50 composite soil-horizon samples.

174

175 2.3 Laboratory methods

176 Bulk-density soil samples were oven-dried at 105°C for > 16 hours, in order to obtain their dry 177 weights (adapted from ISO, 1998). Coarse material (> 2 mm) was separated from fine-earth 178 material (< 2 mm) by dry-sieving, followed by re-drying of coarse material at 105°C for > 16 179 hours, in order to obtain its dry weight. Volume of coarse material was measured by waterdisplacement (by submerging the coarse material into a graduated cylinder filled with water). 180 Processing of composite soil samples involved: bulking of individual horizon soil samples, 181 182 separately for each site (to form a composite sample, in the minority of cases where this was not 183 already done on-site); separation of live biomass; hand-crushing of soil-clods; mixing; and

184 preliminary drying at 40°C. Dry soil was packed and stored at ambient room conditions, to be 185 used for further processing and laboratory analyses. Laboratory sub-sampling was done 186 carefully by hand-mixing and -quartering in several steps (adapted from ISO, 2006). The air-187 moisture content was determined by drying 5 g of sample (fine-earth < 2mm obtained by sieving) at 105°C for > 16 hours until dry (adapted from ISO, 1993). Composite soil samples were 188 189 tested for pH on 2.5 mL fine-earth: 12.5 ml distilled water soil-suspension (adapted from ISO, 190 1995; NSAI, 2011), which showed pH < 6.5. Therefore, it was assumed that the soil from the eighteen sites used in this study did not contain carbonates (ISO, 1995), and the samples were 191 not treated for carbonate-removal. Elemental C analysis on composite soil sub-samples was 192 193 performed on a CE440 Elemental CHN Analyser (Exeter Analytical Inc.), after first 194 homogenising the material by fine milling using Mixer Mill MM200 (Retsch GmbH) and/or 195 Planetary Mono Mill Pulverisette 6 (Fritsch GmbH).

196

197 2.4 Computational methods

The SOC stocks are expressed as volume-based SOC densities, in units of mass of C per volume of soil, and area-based SOC densities, in units of mass of C per area of soil for the specific fixed soil depth-interval. Average horizon thicknesses, including average horizon starting-, ending- and horizon mid-depths were calculated separately for each site from the measurements obtained from the 3 × 3 sampling grid at the site (in continuation the average thickness was named as thickness, and the average mid-depth as mid-depth). The fixed soil depth-intervals were named as follows: "shallow" (0–30 cm); "deep" (30–60 cm), and "total depth" (0–60 cm).

206 2.4.1 Estimating volume-based organic carbon densities for individual horizons

- 207 The volume-based SOC density (SOCVD) of an individual soil horizon was calculated using the
- 208 SOC content (dry mass-based C percentage) measured from the composite horizon sample, the

209 corresponding horizon bulk-density of fine-earth fraction taken from the soil pit [g soil/cm³], and

211 SOCVD = %C/100 × ρ_f × CCF

or

212

213
$$SOCVD = \%C/100 \times (m_{tot} - m_{coarse}) / (V_{tot} - V_{coarse}) \times CCF$$
(1),

214 where SOCVD is the volume-based SOC density of the horizon [gC/cm³]; %C is the C content in 215 the fine earth (< 2 mm) from the composite horizon sample obtained as a dry mass-based 216 percentage C content for oven-dry soil at 105°C (UNECE, 2006; ISO, 1995); 1/100 is the 217 conversion factor for converting percentage C to gC/g soil; ρ_f is a soil bulk-density of fine-earth material < 2 mm [g soil/cm³] [calculated as $(m_{tot} - m_{coarse}) / (V_{tot} - V_{coarse})$]; m_{tot} [g] is the total dry-218 219 mass (at 105°C) of the whole bulk density sample; m_{coarse} [g] is the dry-mass (at 105°C) of 220 coarse fraction (>2mm) obtained after sieving the material from the bulk density sample; V_{tot} [cm³] is the volume of the whole bulk density sample (e.g. 100 cm³ if sampled by core-ring); 221 V_{coarse} [cm³] is the volume of the coarse fraction (> 2 mm) obtained by displacement after sieving 222 223 the material from bulk density samples; CCF is the coarse fraction correction factor, calculated 224 as ((100 - %stones)/100) (Baritz et al., 2010; Kessler et al., 2012), where %stones represent the 225 volume-based percentage of coarse material estimated from the soil pit.

226

227 2.4.2 Estimating soil organic carbon stocks for fixed depth-intervals using an adapted

228 trapezoidal rule approach

- 229 Area-based soil organic carbon stocks (SOCS, tC/ha) for fixed depth-intervals were first
- 230 estimated with a so-called trapezoidal rule computing approach, adapted from Lord and
- 231 Shepherd (1993), who used it for calculating soil-solution nitrate leaching losses. The
- 232 trapezoidal rule was adapted using SOCVD [Eq. 1] expressed as the mass of C per volume of
- soil [gC/cm³], and the corresponding horizon thicknesses [cm], with the inclusion of the
- 234 probability weights (IDRE UCLA, 2016) for each soil horizon, as follows:

235	$SOCS_{a-part I} = 0.5 \times (SOCVD_{a0} + SOCVD_{a}) \times n_a/n \times L_a/2 \times 100$	(2a),
-----	--	-------

236	$SOCS_{a-partII} = 0.5 \times (SOCVD_a + SOCVD_{b0}) \times n_a/n \times L_a/2 \times 100 $ (2b).
237	where 'a' and 'b' subscripts refer to two adjacent horizons; SOCS _{a-part1} is carbon stock
238	computed from horizon start to its mid-depth or $L_a/2$ (Fig. 2a); and SOCS _{a-part II} is stock computed
239	from horizon mid-depth to its end (or the start of the next horizon; Fig. 2a); L_a is thickness of 'a'
240	horizon [cm]; SOCVD _{a0} value [gC/cm ³] refers volume-based soil organic carbon density at the
241	starting depth of 'a' horizon, and SOCVD $_{\rm b0}$ at the starting depth of next 'b' horizon; SOCVD $_{\rm a}$
242	refers to carbon density at horizon mid-depth (obtained from composite 'a' horizon sample); $n_{a}\!/\!n$
243	is the probability weight for horizon/layer 'a' where n _a represents the number of points where the
244	soil horizon/sample 'a' was present out of total number of sampled/augered points n (i.e. n is 9 if
245	all of the points from 3×3 grid were augered; similarly, the probability weight n_b/n applies for next
246	horizon 'b' when computing the carbon stocks for next b-horizon, etc.); 100 is the conversion
247	factor for converting the SOCS units [from gC/cm ² to tC/ha].
248	
249	This adaptation of trapezoidal rule formulae was applied because the horizons were often
250	absent at some sampling locations and present at some other locations at the same site,
251	resulting in varying number of samples obtained for each composite horizon sample that was
252	accounted for in the computation of SOCS by applying the corresponding probability weights

254

253

(IDRE UCLA, 2016).

255 SOCS of whole 'a' horizon was computed by summing SOCS_{a-part I} and SOCS_{a-part I}:

256	$SOCS_a = SOCS_{a-part } + SOCS_{a-part }$	(3),

where $SOCS_a$ is soil organic carbon stock [tC/ha] of whole thickness of 'a' horizon (L_a; Fig. 2).

The estimated SOCS for the total fixed depth-intervals ("shallow", "deep" and "total") were next calculated by summing the SOCS of individual horizons, as follows:

$$SOCS = \sum SOCS_i$$
(4),

where SOCS_i refers to carbon stocks of individual horizons (i.e. SOCS_a, SOCS_b, etc. Eq.3) computed from their thicknesses (Eqs. 2a and 2b) between: 0–30 cm depth for "shallow"; 30–60 cm depth for "deep", and 0–60 cm depth for "total" fixed depth-interval (Fig. 2a). In case if the horizon was cut at the 30 cm depth, SOCS_i of the corresponding horizon-section was included in Eq. 4 in order to compute the SOCS of "shallow" and "deep" fixed depth-intervals (Fig. 2a).

Fig. 2b represents a computing scenario that accounts for organic layers/horizons (not included in this study): organic layers are often thinner than mineral soil horizons and are assumed to have relatively constant SOCS throughout their thickness (e.g. area-based stock values obtained from frame sampling).

271

272 In order to calculate the SOCS of fixed depth-intervals, the SOCVD values at 30 cm and the 273 individual horizon starting- and ending- SOCVD values had to be estimated first, for each site, 274 using linear interpolation (Fig. 2a.) The "starting SOCVD" value of the first mineral horizon at 275 zero depth was estimated by applying linear regression to c. first three horizon SOCVD points (if 276 available) as presented in Fig. 2a, calculated using INTERCEPT Microsoft Excel function. The 277 linear regression was chosen in order to simplify the estimation approach. Although this may 278 result in potential error in the estimation of C-stocks, it was considered that non-linear model 279 application (such as power or logarithmic regression) could also result in estimation errors of the 280 "starting" SOCVD values. This issue may be potentially avoided in case if the "starting" SOCVD 281 values would be known (measured): e.g. by sampling the first few centimetres of the top mineral 282 horizon. A "zero" SOCVD value (Fig. 2a), was assumed for the "ending" SOCVD at the final 283 sampling depth, as the sampling often ceased before reaching the required fixed depth. This 284 assumption was based on an expectation that sampling stopped due to high stone/rock content. 285 It should be noted that this assumption can result in the potential underestimation of C stocks for 286 the last horizon and the "deep" soil depth-interval, which needs to be considered when

interpreting results, especially if the sampling stopped due to other reasons than the highstone/rock content.

289

290 2.4.3 Estimating soil organic carbon stocks for fixed depth-intervals using a spline

291 approach

292 The second approach for estimating area-based soil organic carbon stocks (SOCS, tC/ha) for 293 fixed depth-intervals in this study was an already known spline approach, which is based on the 294 equal-area quadratic smoothing spline modelling, explained by Bishop et al. (1999). Splines were 295 fitted to the data with the help of the "mpspline" function from package "GSIF" (Hengl, 2016) in R (R Core Team, 2015), using average horizon starting and ending depth values for each site, and 296 297 the corresponding SOCVD values from the individiual horizon composite samples (Eq. 1). The parameter lambda (λ) for the "mpsline" function was set to 0.1 as in De Vos et al. (2015). The 298 output obtained was the modelled spline in the form of SOCVD values at 1cm depth-intervals. In 299 order to be able to compare the spline computing approach (dashed curve in Fig. 3) with the 300 301 trapezodial approach ("trapezodial" line in Fig. 3), each spline was next split into separate 302 horizons (or horizon-sections up to 30 and 60 cm fixed depth-intervals). The SOCSs were 303 computed for each horizon/horizon-section after applying the horizon sampling probability 304 weights (as explained in section 2.4.2), and summed up to fixed depth-intervals (30 cm and 60 305 cm depth) according to Eq. 4. SOCSs were computed with approximate integration using 1 cm intervals. The spline-modelled SOCVD value at 1 cm depth was assigned as the "starting 306 307 SOCVD" value at zero depth (Fig. 3), and in case if the sampling ceased before reaching the 308 required fixed depth, the "zero" SOCVD value was assigned to the "ending" SOCVD (as in 309 section 2.4.2).

310

311 2.5 Statistical analysis methods

312 Statistical analysis was performed in R 3.2.3 (R Core Team, 2015) and selected R-packages.

313 Detailed description is provided in sections below.

314

315 2.5.1 Statistical analysis of volume-based soil organic carbon densities

The differences in horizon SOCVD results [gC/cm³] were assessed by applying linear mixed 316 effects (LME) models followed by analysis of variance (ANOVA) (Fox and Weisberg, 2011). 317 ANOVA Type III [Wald x² test], was chosen due to unbalanced horizon data-design (King, 2016). 318 319 The LME models were fitted via maximum likelihood (ML) (Pinheiro et al., 2016) to the natural 320 logarithm (In) transformed SOCVD values (In transformation was used in order to achieve 321 normality and homoscedasticity of the data). Non-weighted and weighed LME models were 322 used, where the fixed weighing was done using the measured number of soil bulk-density 323 samples in order to specify that the residual variance for each horizon SOCVD value was 324 inversely proportional to the number of sampled bulk-density samples per horizon at each site 325 (Bolker, 2015). The "relative" SOCVD standard deviations (computed by standard deviation 326 error-propagation (Caldwell et al., 2016), used in Fig. 4), were not used for LME weighing 327 because they lacked the uncertainty of the SOC component, due to absence of SOC standard 328 deviations from composite soil samples. A random effect was assigned to the site, and fixed 329 effects to the mid-depth, slope [measured in degrees and converted to radians], soil group 330 [Podzols (P), Cambisols (C) and Gleys (G)], and woodland-type [Coniferous, Broadleaf and 331 Mixed]. Variables slope, woodland-type and mid-depth (main effects and interactions) were later 332 excluded from the analysis due to non-significance, and the testing for the potential spatial non-333 independence of sampled sites was done on the final LME model. Different spatial correlation 334 structures were included and evaluated using the Akaike's Information Criterion (AIC). For this, a 335 small random noise (of 0.5 m) was assigned to the measured GPS coordinates (due to 336 coordinates repetition for data measured at the same site), which were first transformed to 337 Universal Transverse Mercator (UTM) coordinates (Bivand et al., 2015). The differences in SOCVD between different soil groups and horizons were further assessed using least-squares 338 339 (LS) means ("Ismeans") pairwise comparison t-tests derived from LME with results averaged

340 over the horizon/soil group using weights proportional to their frequencies in the data-set (Lenth,

341 2016)).

342

343 2.5.2 Statistical analysis of soil organic carbon stocks

344 The correlations between the area-based SOCSs [tC/ha], slope (in radians) and maximum depth of the soil depth-interval (30 cm for "shallow" and 60 cm for "deep") were first evaluated with the 345 Pearson's correlation coefficient matrix (Harrell et al., 2015). The SOCS results were next 346 assessed by applying the LME models, followed by ANOVA Type II [Wald χ^2 test] (Fox and 347 Weisberg, 2011). LME was fitted via ML (Pinheiro et al., 2016) to the square-root SOCS 348 transformed results. The square-root transformation (SOCS)^{0.5} was used in order to achieve 349 350 normality and homoscedasticity of the data. Analysis was first performed on combined "shallow" 351 and "deep" depth-interval data. A random effect was assigned to the site, and the fixed effects to 352 the soil group, depth-interval, slope, and woodland-type. The differences between "shallow" and 353 "deep" depth-interval SOCSs were assessed after excluding variable woodland-type (due to 354 non-significance). Testing for spatial auto-correlation of SOCS results was done using Moran's I statistics (Paradis et al., 2004), and by inclusion of different spatial correlation structures (as 355 explained in 2.5.1). The differences in the SOCS results between three different soil groups 356 were further assessed separately for "shallow", "deep" and "total depth" data, by applying the 357 358 models separately to each data-set. Differences between depth-interval and soil group SOCSs were assessed by applying LS means ("Ismeans") pairwise comparison t-tests derived from 359 LME models (Lenth, 2016). 360

361

362 2.5.3 Statistical comparison of trapezoidal- and spline- computed soil organic carbon

363 stocks

The SOCS results obtained from trapezoidal and spline computing methods were compared using Welch two sample t-test (performed on transformed (SOCS)^{0.5}, separately for "shallow" and "deep" depth-intervals). In addition, the results were further assessed for separate depth-

367 intervals, as well as for separate soil groups of "shallow" and "deep" depth-intervals, by obtaining 95 % bootstrap confidence intervals of (SOCS)^{0.5} means, based on the approach 368 369 explained in De Vos et al. (2015), which assumes statistically significant differences if 370 confidence intervals do not overlap. However, due to limited number of sites the bootstrap 371 confidence intervals were not used as an independent statistical testing in this study, but they 372 were used merely for checking the findings in conjunction with the earlier statistical t-test. 373 Calculations were performed with the help of function "boot" (with 5000 bootstrap replications) 374 from R package "boot", using the "boot.ci" function set to type "bca" for the adjusted bootstrap 375 percentile interval (Canty and Ripley, 2016; Davison and Hinkley, 1997). 376 377 3 Results and discussion 378 379 3.1 Horizon volume-based soil organic carbon densities Gleys had the greatest average horizon SOCVD: the average SOCVD was 0.029 gC/cm3 for 380 Gleys, 0.027 gC/cm³ for Cambisols, and 0.017 gC/cm³ for Podzols (Table 1). A decrease in 381 SOC density was observed from the top A horizons (0.047 gC/cm³), followed by E and B (0.019 382 gC/cm³ for E and 0.018 gC/cm³ for B), to B2 and BC horizons (0.011 gC/cm³ for B2 and 0.014 383 gC/cm³ for BC; Fig. 4, Table 1). The mid-depth for A horizons ranged from 3.8-13.8 cm, 384

385 followed by E (6.6–26.5 cm), B (12.8–45.2 cm), B2 (29.5–51.4 cm), and BC horizons (41.9–54.8

386 cm). In general, weighted LME models appeared to explain the data better than the non-

387 weighted model (lower AIC). Spatial auto-correlation was not detected, which may be a

388 consequence of a relatively small number of sites (eighteen sites) used in this study. Significant

389 effect on SOCVD results was observed for both horizon and soil group, but not for mid-depth.

390 The interaction mid-depth – horizon was also found to be not significant (p > 0.1, ANOVA Type

391 III Wald χ^2 test). However, in case of absence of the horizon variable, the mid-depth did show a

392 significant effect on SOCVD results. Horizon appeared to explain the data better than mid-depth,

which may be due to variations in horizon depths and thicknesses between different locations,and limited number of sites.

395

The results showed statistically significant difference in horizon SOVCD between Gleys and Podzols (Table 1), but not between the Cambisols and Gleys, or between Cambisols and Podzols. In Ireland Gleys are classified as poorly-drained mineral soils (Radford and Short, 2013), typically located in low-lying wet areas (Fay et al., 2007). If excluding peaty soils, Podzols, on the other hand, are well-drained soils typically located on hill-land areas (Radford and Short, 2013).

402

403 There was a strong effect of horizon (p < 0.0001, Table 1) on SOCVD, where A-horizons (that 404 had highest SOCVD) significantly differed from almost all other horizons. Nevertheless, some 405 other horizons, that were generally considered not to be in direct neighbouring sequence to 406 each-other (within the soil profile), also showed differences in SOCVD (Table 1). Considering 407 that Gleys showed the greatest SOCVD in this study, and that they can be rich in clay, future 408 investigations of the clay-associated organic substances and their potential relation to other 409 factors, such as soil type, soil-physical properties, texture, and management (Leinweber et al., 410 1993) are recommended, in order to obtain further insights into the effect of horizons on SOC 411 storage from this study.

412

413 3.2 Soil organic stocks of fixed depth-intervals

The relationships between the area-based SOCSs results, slope and the maximum depth of soil depth-interval (i.e. 30 cm and 60 cm depth) that were assessed using Pearson's correlation coefficient matrix, showed a significant negative correlation between SOCS values and depth (with decrease in SOCS at greater depth), as expected, but no significant correlation between SOCS and the slope. This result was observed for both SOCSs obtained via trapezoidal and spline

419 approach. A significant effect of slope that was only occasionally derived from LME models fitted 420 to separate soil depth-interval data (Table 2b and 2c) turned to be non significant after excluding 421 a single value for "deep" SOCS with the highest measured slope of c. 0.37 radians (the 422 remaining sites all had slope < 0.21 radians). This indicates that the present study may not have 423 sufficient data for SOCS at higher slopes in order to investigate the effect of slope on soil C 424 storage. The geo-statistical analysis on LME also showed no spatial auto-correlation, most likely 425 due to limited number of sites; this was further confirmed by Moran's I statistics.

426

427 SOC content is generally known to decrease with soil depth. In agreement with this, the "shallow" mean SOCSs observed in this study were significantly greater (p < 0.0001) than the 428 429 "deep" ones (Table 2a). This was observed for both SOCSs obtained using trapezoidal and spline 430 computing approaches. The soil below 30 cm depth could store over 22% of the "total depth" 431 SOCSs (Fig. 5a, 5b). These findings appear to be in general agreement with the total SOC 432 content estimations based on the global soil database by Batjes (1996), who estimated the total 433 of SOC content of the upper 100 cm mineral soil depth-interval at 39-70%, and the SOC content 434 of the upper 30 cm at 58 - 81%. Nevertheless, the results from this study should not be 435 generalised, considering the limited number of sites (eighteen) that has been used, and the 436 maximum total sampling depth of only up to 60 cm.

437

438 The mean "shallow" and "total depth" SOCSs were greatest in Gleys followed by Cambisols and 439 Podzols (Fig. 5), whereas the mean "deep" SOCS values of the three soil groups were relatively 440 close to each other, with the greatest SOCS observed in Cambisols and Gleys, followed by Podzols (Fig. 5). The statistical analysis performed on separate soil depth-interval data 441 442 (separately for "shallow", "deep" and "total depth") showed significant effect of soil group on the 443 "shallow" and on the "total depth" SOCSs (p < 0.05, Table 2b, 2c). However, soil group had a 444 less significant or non-significant effect on the "deep" SOCSs (Table 2b, 2c). Gleys had 445 significantly greater SOCSs than Podzols for both "shallow" (G vs. P: p < 0.05; Table 2b, 2c)

446 and "total depth" depth-intervals (G vs. P: p < 0.05; Table 2b, 2c); whereas the "deep" SOCS 447 results showed no significant differences among the three soil groups (Table 2b, 2c; results derived from LME with inclusion of slope). No significant differences were observed in SOCSs 448 449 (of any depth-intervals) between the Cambisols and Gleys, and Cambisols and Podzols. These 450 findings appear to be in agreement with the results from statistical analysis performed on 451 SOCVD (section 3.2), and were observed regardless of which computing approach was used 452 (trapezoidal or spline). The above results indicate that differences in SOCSs between soil 453 groups are diminishing at greater ("deep") soil depth-interval, which is in agreement with a study 454 by Wiesmeier et al. (2015), who also found no significant differences between different soil 455 classes for deeper soil horizons (C horizons). Furthermore, Wiesmeier et al. (2015) also 456 observed (among others) significantly higher SOC stocks for Gleysols compared to Podzols. 457

458 Despite the fact that this study used relatively approximate estimation of "starting" and "ending" 459 SOCVD values (Fig. 2a) that are required for the computation of fixed depth-interval SOCSs, the results were found to be generally in agreement with the relatively broad range of soil C stocks 460 461 reported in some examples of studies on mineral forest soils. Some examples are: - a study on Irish forested sites to 30 cm depth by Wellock et al. (2011) [66.7-148.8 tC/ha for Podzols, 38.4-462 136.6 tC/ha for Cambisols, and 86.6-164.3 tC/ha for Gleys]; - European forest soil study to 20 463 cm depth by Baritz et al. (2010) [with values for temperate-oceanic to suboceanic climate zones 464 estimated from published figures of c. 53 tC/ha for Podzols, c. 55 tC/ha for Cambisols and c. 65 465 tC/ha for Gleysols]; - a study by Batjes (2014) with estimated C stocks for up to 50 cm depth 466 based on the global soil database [173 tC/ha for Podzols, 69 tC/ha for Cambisols and 97 tC/ha 467 468 for Gleysols].

469

470 The total depth-interval (0 up to 60 cm) SOC stocks from all eighteen sites in this study were

471 estimated at 122.7 tC/ha (arithmetic mean) if using trapezoidal approach, and c. 125.4 tC/ha if using

472 spline approach. These estimates appear to be lower than SOC stocks for e.g. Belgium forests to 1

473 m depth [c. 153 tC/ha (Lettens et al., 2005)], but greater than the average stocks for forest soils in 474 Bavaria, Germany to about 93 cm depth or parent material [c. 98 tC/ha (Wiesmeier et al., 2012)]. 475 Nevertheless, the sampling depth of only 60 cm (or less) used in this study can still be 476 considered as relatively shallow, and therefore, it can be expected that certain soils in this study 477 could potentially have some additional carbon stored at greater depths. For example, an ICP Forests-based study (including most of the European forests) by De Vos et al. (2015) estimated 478 479 SOC stocks up to 1 m depth at 182 tC/ha for Gelysol, and 121 tC/ha for Podzols (bootstrapped 480 mean), with both being greater than SOC stocks to 60 cm depth estimated for Gleys and Podzols 481 from this study (Table 2b, 2c).

482

3.3 Comparison of trapezoidal and spline computing approaches for estimating soil organic carbon stocks of fixed depth-intervals

485 The results showed that the trapezoidal approach is likely to provide more crude estimates of 486 SOC stocks than the spline approach: it estimated the overall "shallow" SOCSs for c. 4.1 tC/ha 487 less than the spline approach, and the overall "deep" SOCSs for c. 1.3 tC/ha more than the 488 spline approach (Table 2b, 2c). This is not surprising, considering that the trapezoidal approach 489 is assuming a simple linear relationship between horizon SOCVD values in the soil profile (Fig. 490 2a) Nevertheless, the trapezoidal approach has an advantage of being relatively simple (i.e. it 491 can be implemented in Excel) and computationally less demanding. No statistically significant 492 differences were observed between the fixed depth-interval SOCSs computed by the two methods: trapezoidal vs. spline p > 0.5, Welch two sample t-test on "shallow" and "deep" data. 493 This was further confirmed by overlapping of the 95 % bootstrap confidence intervals of 494 (SOCS)^{0.5} means from both computing methods. The overlapping of confidence intervals 495 496 occurred in case of the "shallow" and deep" depth-interval SOCSs, as well as in case of SOCSs 497 of separate soil groups of "shallow", and "deep" depth-intervals. This comparison needs to be 498 interpreted with caution due to limited number of sites used for bootstrapping in this study. 499 Nevertheless, SOCS results obtained from the two computing methods, trapezoidal and spline,

showed the similar significant differences between depth-intervals and soil groups (Table 2b,

501 2c), which indicate that the trapezoidal SOC stock computing approach (despite being cruder)

502 may be suitable when investigating the differences between different SOCSs.

503

504 4 Conclusions

505 This study on organic carbon (C) in soils, with varying horizon depths and thicknesses, 506 demonstrates that sampling by horizon with accompanying depth measurements can enable 507 computational estimations of soil organic carbon (SOC) stocks for fixed soil depth-intervals. The 508 development and application of a simple trapezoidal rule computing approach (adapted from 509 Lord and Shepherd (1993)) is hoped to facilitate the use of soil-horizon sampling in studies on 510 SOC stocks. The comparison of the presented trapezoidal SOC stock computing approach with 511 the more complex spline approach (Bishop et al. 1999) showed that both computational 512 methods can enable estimates of fixed depth-interval SOC stocks to be computed from 513 measurements of mineral soil horizons sampled by auger boring, for the three Irish mineral 514 afforested soils (Gleys, Podzols and Cambisols), aged \geq 20 years. The study showed that 515 horizon and soil group had a significant effect on SOC stocks. The soil below the 30 cm depth 516 was estimated to store over c. 22% of the total upper 60 cm SOC stocks. This result should not 517 be generalised considering that it is based on limited number of sites (eighteen sites) used in 518 this study; nevertheless, it confirms the importance of including the deeper soil horizons in the 519 soil C-cycle. Although the trapezoidal approach is thought to be much more crude than the 520 spline computing approach (and likely to be more suitable for more crude SOC stock 521 estimations), no statistically significant differences were observed between the fixed depth-522 interval SOCSs for "shallow" and deep" data computed by the two methods. Gleys were shown 523 to have significantly greater mineral SOC stocks than Podzols, with differences mainly evident in 524 the upper 30 cm soil depth, which was observed regardless of computing methodology used 525 (trapezoidal or spline).

527	Acknowledgements
528	Authors are grateful to the Irish Department of Agriculture, Food and the Marine for CForRep
529	project funding under the CoFoRD programme, and for providing the access to the data from
530	Ireland's National Forest Inventory Database 2012. Thanks go to William Hamilton, Gero Jahns
531	and Martijn Leenheer for their support with soil sampling, to Urszula Rogozinska, Nicola Murphy
532	and Adam Taylor (UCD) for their help with laboratory sample-handling and -processing, to Ann
533	Connolly (UCD) for her support with CHN analysis, and to all from the UCD, School of
534	Chemistry who provided help with access to the laboratory equipment and instruments. Authors
535	are grateful to Coillte and to all those who provided access to forest sites used in this study.
536	
537	References
538	
539	Baritz, R., Seufert, G., Montanarella, L., Van Ranst, E., 2010. Carbon concentrations and stocks
540	in forest soils of Europe. For. Ecol. and Manag. 260(3), 262-277.
541	Batjes, N.H., 1996. Total carbon and nitrogen in the soils of the world. European J. of Soil Sci.
542	47(2), 151-163.
543	Batjes, N.H., 2014. Total carbon and nitrogen in the soils of the world. European J. of Soil Sci.
544	65(1), 10-21.
545	Becker, R.A. and Wilks, A., 2016. Package 'mapdata' .Original S code by Richard A. Becker and
546	Allan R. Wilks. R version by Ray Brownrigg. URL: https://CRAN.R-
547	project.org/package=mapdata Date accessed: 27/08/2016.
548	Bishop, T.F.A., McBratney, A.B., Laslett, G.M., 1999. Modelling soil attribute depth functions
549	with equal-area quadratic smoothing splines. Geoderma 91, 27-45.
550	Bivand, R., Keitt, T., Rowlingson, B., 2015. rgdal: Bindings for the Geospatial Data Abstraction

551 Library. R package version 1.1-3. https://CRAN.R-project.org/package=rgdal.

552	Bivand, R., Lewin-Koh, N., 2015. maptools: Tools for Reading and Handling Spatial Objects. R
553	package version 0.8-37. URL: https://CRAN.R-project.org/package=maptools. Date
554	accessed: 17/04/2016.
555	Black, K., Creamer, R.E., Xenakis, G., Cook, S., 2014. Improving forest soil carbon models
556	using spatial data and geostatistical approaches. Geoderma 232–234(0), 487-499.
557	Bolker, B., 2015. GLMM worked examples. McMaster University. URL:
558	http://ms.mcmaster.ca/~bolker/R/misc/foxchapter/bolker_chap.html. Date assessed:
559	13/05/2016.
560	Caldwell, J., Vahidsafa, A., NIST/SEMATECH, 2016. Propagation of Error. UC Davis ChemWiki.
561	UC Davis. URL:
562	http://chemwiki.ucdavis.edu/Core/Analytical_Chemistry/Quantifying_Nature/Significant_
563	Digits/Propagation_of_Error. Date assessed:27/03/2016.
564	Canty, A., Ripley, B., 2016. boot: Bootstrap R (S-Plus) Functions. R package version 1.3-18.
565	CForRep, 2013. CForRep: Additions & Refinements to the Irish Forest Carbon Accounting &
566	Reporting Tool. University College Dublin. URL: http://www.ucd.ie/cforrep/. Date
567	accessed: 27/04/2016.
568	Cools, N., De Vos, B., 2010. Sampling and Analysis of Soil. Manual Part X. Manual on methods
569	and criteria for harmonized sampling, assessment, monitoring and analysis of the
570	effects of air pollution on forests UNECE, ICP Forests, Hamburg.
571	Davison, A.C., Hinkley, D.V., 1997. Bootstrap methods and their applications. Cambridge
572	University Press, Cambridge.
573	De Vos, B. et al., 2015. Benchmark values for forest soil carbon stocks in Europe: Results from
574	a large scale forest soil survey. Geoderma 251-252, 33-46.
575	FAO, 2006. Guidelines for soil description - Fourth edition, Rome, Italy.
57 6	Fay, D., Kramers, G., Zhang, C., McGrath, D., Grennan, E., 2007. Soil Geochemical Atlas of
577	Ireland. Teagasc, Environmental Protection Agency.

- 578 Fox, J., Weisberg, S., 2011. An {R} Companion to Applied Regression, Second Edition.
- 579 Thousand Oaks, CA, Sage. URL: http://socserv.socsci.mcmaster.ca/jfox/Books/Companion.
 580 Date accessed: 18/06/2016.
- 581 Freier, K.P., Glaser, B., Zech, W., 2010. Mathematical modeling of soil carbon turnover in
- natural Podocarpus forest and Eucalyptus plantation in Ethiopia using compound
 specific δ13C analysis. Glob. Change Biol. 16(5), 1487-1502.
- 584 Gardiner, M.J., Radford, T., 1980. Soil Associations of Ireland and Their Land Use Potential.
- 585 Explanatory Bulletin to Soil Map of Ireland. National Soil Survey of Ireland. Soil Survey
 586 Bulletin No. 36. An Foras Talúntais (The Agricultural Institute) Dublin.
- 587 Harrell, F.E., (Jr.), with contributions from Dupont C. and many others, 2015. Hmisc: Harrell
- 588 Miscellaneous. R package version 3.17-1. URL: https://CRAN.R-

589 project.org/package=Hmisc. Date assessed: 27/03/2016.

- Harrison, R.B., Footen, P.W., Strahm, B.D., 2011. Deep Soil Horizons: Contribution and
 Importance to Soil Carbon Pools and in Assessing Whole-Ecosystem Response to
- 592 Management and Global Change. For. Sci. 57(1), 67-76.
- 593 Hengl, T., 2016. GSIF: Global Soil Information Facilities. R package version 0.5-3. URL:

594 https://CRAN.R-project.org/package=GSIF Date accessed: 27/08/2016.

- 595 IDRE UCLA, 2016. UCLA Statistical Consulting Seminars and Workshops. Seminars and
- 596 Workshops, Spring and Summer 2016. Introduction to Survey Data Analysis in Stata 9.
- 597 UCLA: Statistical Consulting Group. IDRE, UCLA Institute for Digital Research and
- 598 Education. URL:http://www.ats.ucla.edu/stat/stata/seminars/svy_stata_intro/default.htm 599 Date accessed: 27/03/2016.
- 600 ISO, 1993. Soil quality Determination of dry matter and water content on mass basis -
- 601 Gravimetric Method. ISO 11465:1993. International Organization for Standardization602 (ISO).

603	ISO, 1995. Soil quality – Determination of organic and total carbon after dry combustion
604	(elementary analysis). ISO 10694:1995. International Organization for Standardization
605	(ISO).
606	ISO, 1998. Soil quality - Determination of dry bulk density. International Organization for
607	Standardization (ISO).
608	ISO, 2006. Soil quality - Pretreatment of samples for physico-chemical analysis. ISO
609	11464:2006(E). International Organization for Standardization (ISO).
610	IUSS Working Group WRB, 2015. World Reference Base for Soil Resources 2014, update
611	2015. International soil classification system for naming soils and creating legends for
612	soil maps. World Soil Resources Reports No. 106, Food and Agriculture Organization of
613	the United Nations (FAO), Rome.
614	Jobbágy, E.G., Jackson, R.B., 2000. The vertical distribution of soil organic carbon and its
615	relation to climate and vegetation. Ecol. Applications 10(2), 423-436.
616	Kessler, M., Hertel, D., Jungkunst, H.F., Kluge, J., Abrahamczyk, S., Bos, M., Buchori, D.,
617	Gerold, G., Gradstein, S.R., Köhler, S., Leuschner, C., Moser, G., Pitopang, R., Saleh,
618	S., Schulze, C.H., Sporn, S.G., Steffan-Dewenter, I., Tjitrosoedirdjo, S.S., Tscharntke,
619	T., 2012. Can Joint Carbon and Biodiversity Management in Tropical Agroforestry
620	Landscapes Be Optimized? PLoS ONE 7(10), e47192.
621	King, W.B., 2016. R Tutorials. Coastal Carolina University. URL:
622	https://ww2.coastal.edu/kingw/statistics/R-tutorials/unbalanced.html. Date assessed:
623	13/05/2016.
624	Lee, J., Hopmans, J.W., Rolston, D.E., Baer, S.G., Six, J., 2009. Determining soil carbon stock
625	changes: Simple bulk density corrections fail. Agric., Ecosyst. and Environ. 134(3-4),
626	251-256.
627	Lettens, S., Van Orshoven, J., van Wesemael, B., De Vos, B., and Muys, B. 2005. Stocks and
628	fluxes of soil organic carbon for landscape units in Belgium derived from heterogeneous
629	data sets for 1990 and 2000. Geoderma 127, 11-23.

- 630 Leinweber, P., Reuter, G., Schulten, H.-R., 1993. Organo-mineral soil clay fractions in
- fertilization experiments: mineralogy, amounts and quality of organic matter and
 influence on soil properties. Appl. Clay Sci. 8(4), 295-311.
- Lenth, R.V., 2016. Least-Squares Means: The {R} Package {Ismeans}. Journal of Statistical
 Software 61(1), 1-33.
- Lord, E.I., Shepherd, M.A., 1993. Developments in the use of porous ceramic cups for
 measuring nitrate leaching. J. of Soil Sci. 44(3), 435-449.
- National Forest Inventory, 2012. Ireland's National Forest Inventory Database 2012. Department
 of Agriculture, Food and the Marine, Ireland.
- NSAI, 2011. Soil improvers and growing media determination of pH. I.S. EN 13037:2011 The
 National Standard Authority of Ireland (NSAI).
- 641 Pan, Y., Birdsey, R.A., Fang, J., Houghton, R., Kauppi, P.E., Kurz, W.A., Phillips, O.L.,
- 642 Shvidenko, A., Lewis, S.L., Canadell, J.G., Ciais, P., Jackson, R.B., Pacala, S.W.,
- 643 McGuire, A.D., Piao, S., Rautiainen, A., Sitch, S., Hayes, D., 2011. A Large and

644 Persistent Carbon Sink in the World's Forests. Science 333(6045), 988-993.

- Paradis, E., Claude, J., Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R
 language. Bioinformatics 20, 289-290.
- 647 Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D., R Core Team, 2016. _nlme: Linear and
- 648 Nonlinear Mixed EffectsModels_. R package version 3.1-125. URL: http://CRAN.R-

649 project.org/package=nlme. Date accessed: 18/06/2016.

- Premrov, A., Cummins, T., Hamilton, W., Byrne, K.A., 2014. Field sampling for refining soil
 organic carbon stock and stock change estimations for Irish forested mineral soils,
- 652 SSSE 2014: The Science and Solutions for a Sustainable Environment Conference
- 652 SSSE 2014: The Science and Solutions for a Sustainable Environment Conference
 653 2014, University College Dublin, Ireland.
- 653 2014, University College Dublin, Ireland.
- Premrov, A., Cummins, T., Hamilton, W., Byrne, K.A., 2015. Accomplished field sampling for
 refining soil organic carbon stock and stock change estimations for Irish forested

656 mineral soil, SOM 2015 - 5th International Symposium on Soil Organic Matter

657 Göttingen. Germany.

- 658 R Core Team, 2015. R: A language and environment for statistical computing. R version 3.2.3.
- R Foundation for Statistical Computing, Vienna, Austria. URL: https://www.R-project.org.
 Date accessed: 02/01/2016.
- Radford, T., Short, I., 2013. Great Soil Groups and their suitability to forestry. Teagasc, Forestry
 Development Unit. ULR:
- http://www.teagasc.ie/forestry/docs/research/Teagasc_soil_groups_and_forestry.pdf.
 Date accessed: 09/09/2013.
- 665 Reidy, B., Simo, I., Spaargaren, O., Creamer, R.E., 2014. Irish Soil Information System.
- 666 Correlation of the Irish Soil Classification System to World Reference Base 2006
 667 system. (2007-S-CD-1-S1). Final Technical Report 8., Environmental Protection
 668 Agency, Ireland.
- Rumpel, C., Kögel-Knabner, I., 2010. Deep soil organic matter—a key but poorly understood
 component of terrestrial C cycle. Plant Soil 338(1), 143-158.
- 671 Stolbovoy, V., Montanarella, L., Filippi, N., Selvaradjou, S., Gallego, J., 2005. Soil Sampling
- 672 Protocol to Certify the Changes of Organic Carbon Stock in Mineral Soils of European673 Union, Luxembourg.
- UNECE, 2006. IIIa Sampling and Analysis of Soil: Annex 1 Methods for Soil Analysis update
 2006 United Nations Economic Commission for Europe (UNECE). Convention on Long Range Transboundary Air Pollution.
- 677 UNECE, 2010. Manual Part X: Sampling and analysis of soil, United Nations Economic
 678 Commission for Europe (UNECE). Convention on Long-Range Transboundary Air
- 679 Pollution, International Co-operative Programme on Assessment and Monitoring of Air
- 680 Pollution Effects on Forests (ICP Forests).
- Wellock, M.L., LaPerle, C.M., Kiely, G., 2011. What is the impact of afforestation on the carbon
 stocks of Irish mineral soils? For. Ecol. and Manag. 262(8), 1589-1596.

683	Wiesmeier, M., Spörlein, P., Geuß, U., Hangen, E., Haug, S., Reischl, A., Schilling, B., von
684	Lützow, M., and Kögel-Knabner, I. 2012. Soil organic carbon stocks in southeast
685	Germany (Bavaria) as affected by land use, soil type and sampling depth. Glob.
686	Change Biol. 18, 2233-2245.
687	Wiesmeier, M., Lützow, M.v., Spörlein, P., Geuß, U., Hangen, E., Reischl, A., Schilling, B., and
688	Kögel-Knabner, I. 2015. Land use effects on organic carbon storage in soils of Bavaria:
689	The importance of soil types. Soil and Tillage Res. 146, Part B, 296-302.

Highlights

- ► An approach for estimating fixed depth SOC stocks from horizon data is presented.
- ▶ Trapezoidal approach is likely to provide cruder estimates than spline method.
- ► Horizon and soil group had a significant effect on SOC stocks in both methods.
- SOC stock differences by soil group were mainly evident in the upper 30 cm.
- ▶ Demonstrated approach aims to facilitate horizon sampling use in SOC stock studies.

Tables & Table Captions

Table 1 Mean SOCVD and horizon mid-depth along with soil group (a) and horizon (b) comparisons using pairwise t-test derived from LME applied to In-transformed SOCVD [significant variables and contrasts are highlighted in bold]

				(a)					
ANOVA Ty	pe III.			Back- transformed	Arithmetic		LS means pa	airwise comp	arison ^d
Wald x ² tes		Soil group ^a	Mean ∣In(SOCVD)∣	mean SOCVD	mean SOCVD ± SD [♭] [gC/cm ³]		Comparing soil groups	t-ratio _{df17} e	p-value
Call			± SD	[gC/cm ³]		<u>n</u>	0.00		> 0.05
Soil group _{df2}	< 0.05	P	4.360 ± 0.840	0.013	0.017 ± 0.014	17	C vs. G.	-0.90	> 0.65
Horizon df4	< 0.0001	С	3.824 ± 0.686	0.022	0.027 ± 0.016	16	C vs. P	1.91	> 0.17
		G	3.915 ± 0.930	0.020	0.029 ± 0.025	17	G vs. P	2.70	< 0.04
				(b)					
			•	Back-					
				transformed	Arithmetic				
	Mean		Mean	mean	mean				p-value
	mid-depth		In(SOCVD)	SOCVD	SOCVD ±		Comparing		[t-statistics
Horizon	± SD ^a [cm]		± SD	[gC/cm ³]	SD ^b [gC/cm ³]	n	horizons		df26 ^e]
Α	9 ± 2.2		[3.125] ± 0.363	0.044	0.047 ± 0.019	13	A vs. B, J	A vs. B2,	< 0.0001
E	14 ± 8.5		4.291 ± 0.941	0.014	0.019 ± 0.019	5	A vs. BC	,	
В	31 ± 7.8		4.166 ± 0.538	0.016	0.018 ± 0.008	20	B vs. B2;		< 0.05
B2	44 ± 7.9		4.727 ± 0.736	0.009	0.011 ± 0.007	6	A vs. E; B vs	E. BC:	> 0.05
BC	49 ± 5.4		4.638 ± 0.978	0.009	0.014 ± 0.016	6	B2 vs. E; BC		_

NOTE: ^a P - Podzols, C - Cambisols, G - Gleys; ^b SD standard deviation without inclusion of error propagation; ^c derived from nonweighted/weighted LME applied to In(SOCVD), with inclusion of fixed effects (soil group, horizon) and random effect (site); the applied LME was also used for testing the potential presence of SAC; ^d results (derived from LME) are averaged over the horizon/soil group using weights proportional to their frequencies in the data-set, and with the Tukey method p-value adjustment; ^e df - degrees of freedom. Table 2 Mean SOCSs for fixed depth-intervals and different soil groups along with comparisons between different main effects, derived from LME models applied to square-root transformed SOCSs (calculated by adapted trapezoidal- and spline- approach): (a) analysis on combined "shallow" and "deep" depth-interval data; (b) analysis on separate fixed depth-interval data computed by adapted trapezoidal approach; (c) analysis on separate fixed depth-interval data computed by spline approach [significant variables and contrasts are highlighted in bold]

			(a) Combined	d "shallow" and	l "deep" data				
ANOVA Type	II,		•	Back-	Arithmetic		LS means pa	airwise co	mparison ^g
Wald χ^2 test ^a (Tr & Sp) ^c				transformed	mean				
Main		Depth-	Mean	mean SOCS	SOCS ± SD ^e			t-rațio	
_effect ^f p-	-value	interval ^c	(SOCS) ^{0.5} ± SD	[tC/ha]	[tC/ha]	n	Dp vs. Sh	т . df17	p-value
Depth- <	0.0001	Sh (Tr)	9.150 ± 2.781	83.7	91.0 ± 50.46	18	Tr	-7.33	<0.0001
interval _{df1}		Dp (Tr)	5.342 ± 1.824	28.5	31.7 ± 20.29	18			
Soil <	0.005	Sh (Sp)	9.342 ± 2.871	87.3	95.1 ± 53.64	18	Sp	-7.18	<0.0001
group df2		Dp (Sp)	5.205 ± 1.867	27.1	30.4 ± 20.29	18			
	(b)	Separate fixe	d depth-interval o	lata computed	with adapted trap	ezoid			
ANOVA Type	II,		·	Back-	Arithmetic		LS means pa	airwise co	mparison ^g
Wald χ ² test ^b				transformed	mean				
			Mean	mean SOCS	SOCS ± SD ^e		Comparing	t-rațio	
Main effect [*]	p-value	Soil group ^d	(SOCS) ^{0.5} ± SD	[tC/ha]	[tC/ha]	n		df14	p- value
			"Shallow	" [0-30 cm] dept	h-interval				
Soil group df2									
	<0.005	Р	7.130 ± 2.397	50.8	55.6 ± 29.92	6	C vs. G	-0.87	0.668
Slope _{df1}	>0.05	С	10.004 ± 1.456	100.1	101.8 ± 30.08	6	C vs. P	2.08	0.130
		G	10.317 ± 3.320	106.4	115.6 ± 66.99	6	G vs. P	2.74	0.040
			" "Deep" [30-60 cm] dept	n-interval				
Soil	h								
group df2	0.044 ^h	Р	4.394 ± 1.505	19.3	21.2 ± 13.93	6	C vs. G	-0.72	0.758
Slope df1	<0.05	С	5.864 ± 1.966	34.4	37.6 ± 25.64	6	C vs. P	1.54	0.310
	>0.05 ^{h,i}	G	5.766 ± 1.886	33.28	36.2 ± 18.68	6	G vs. P	2.10	0.125
			"Total dep	th" [0–60 cm] de	pth-interval		1		
Soil									
group df2	<0.05	Р	8.503 ± 2.329	72.3	76.8 ± 35.93	6	C vs. G	-0.93	0.633
Slope df1	<0.05	С	11.661 ± 2.040	136.0	139.4 ± 48.24	6	C vs. P	2.13	0.119
	>0.05'	G	11.848 ± 3.710	140.4	151.8 ± 82.19	6	G vs. P	2.84	0.033

ANOVA Ture	11	(-/		Back-	nputed with splin Arithmetic		-	inuico co	mnoricong
ANOVA Type	п,						LS means pairwise compari		
Wald χ ² test ^b		_		transformed	mean				
			Mean	mean SOCS	SOCS ± SD [®]			t-ratio	
Main effect [™]	p-value	Soil group ^d	(SOCS) ^{0.5} ± SD	[tC/ha]	[tC/ha]	n	Comparing	df14	p- value
			"Shallov	v" [0-30 cm] dep	th-interval				
Soil group df2	•				•			•	·
	<0.005	P	7.248 ± 2.426	52.5	57.4 ± 31.30	6	C vs. G	-1.13	0.514
Slope df1	>0.05	С	10.025 ± 1.403	100.5	102.1 ± 28.98	6	C vs. P	1.97	0.157
		G	10.755 ± 3.457	115.7	125.6 ± 71.31	6	G vs. P	2.89	0.030
			"Deep"	[30-60 cm] dept	th-interval		1		
Soil	•		•	• • •	•				
group df2	>0.05	Р	4.514 ± 1.740	20.4	22.9 ± 17.24	6	C vs. G	-1.01	0.580
Slope df1	>0.5	С	5.395 ± 1.963	29.1	32.3 ± 24.04	6	C vs. P	0.86	0.672
		G	5.704 ± 2.015	32.5	35.9 ± 20.41	6	G vs. P	1.79	0.210
			"Total dep	oth" [0-60 cm] de	epth-interval		1		
Soil	•								·
group df2	<0.05	Р	8.674 ± 2.474	75.2	80.3 ± 41.0 8	6	C vs. G	-1.25	0.445
Slope df1	< 0.05	C	11.462 ± 1.921	131.4	134.5 ± 44.92	6	C vs. P	1.84	0.193
	>0.05 ⁱ	G	12.224 ± 3.813	142.4	161.5 ± 86.17	6	G vs. P	2.90	0.029

NOTE: ^a derived from LME applied to (SOCS)^{0.5} [slope was significant on combined data, if all data were included]; ^b derived from LME applied to (SOCS)^{0.5}; ^c Sh - "shallow", Dp - "deep", Tr - adapted trapezoidal computing approach; Sp - spline computing approach; ^d P - Podzols, C - Cambisols, G - Gleys. ^e SD standard deviation without inclusion of error propagation; ^f df - degrees of freedom; ^g results derived from model are averaged over the levels of soil/depth, with the Tukey method p-value adjustment (for soil); ^h close to 'border significant'; ⁱ significant if including all deep SOCS data, but non-significant if excluding one value for deep SOCS at the highest measured slope (the exclusion of the value appeared not to change the outcome of post-hoc soil group contrast-significance/non-significance).

Figure Captions

Figure 1 Schematic presentation of site locations and soil sampling approach (a) site locations - not to scale; (b) composite soil sampling (per horizon) by arbitrary-choosing the sampling point within each sampling grid cell; (c) bulk-density soil sampling from a central soil pit

Note: (a) schematic illustration of the country-border was created with the help of R 3.2.3 (R Core Team, 2015) and R packages "maptools" (Bivand and Lewin-Koh, 2015) and "mapdata" (Becker et al., 2016); (c) presented is one side of soil pit (at central grid-location V), sampling included up to three-sides of soil pit

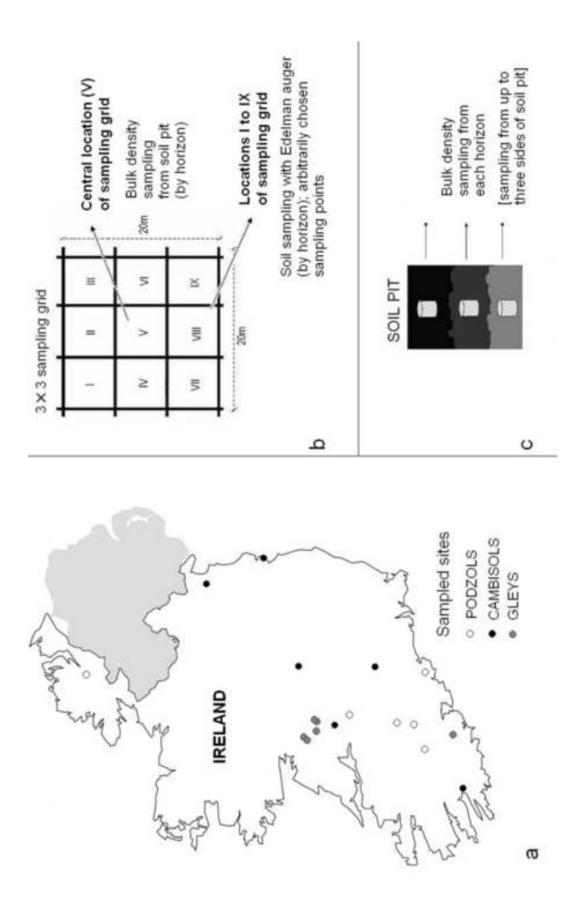
Figure 2 Schematic presentation of the adapted trapezoidal rule calculation approach for estimating soil organic carbon stocks (SOCS) in units of mass C per area for required soil horizons and fixed depth-intervals [fictive example]: (a) simplified C stock estimation approach for mineral soil horizons (labelled with a, b, c, etc.), including crude linear regression estimation of the "starting" SOCVD at zero depth (used in this study); (b) scenario that accounts for organic layers/horizons (not included in this study)

Figure 3 Schematic presentation of the modelled spline (dashed curve, obtained from the spline approach) fitted to the horizon volume based soil organic carbon density (SOCVD) data with accompanying average horizon starting- and ending-depths, and the "trapezoidal" line (obtained from adapted trapezoidal approach) for estimating soil organic carbon stocks (SOCS) for fixed depth-intervals [fictive example]

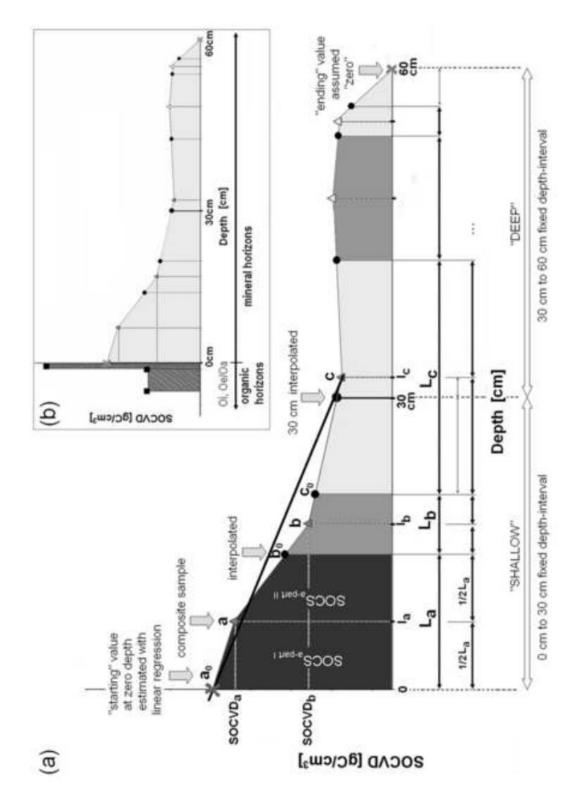
Figure 4 Horizon volume-based soil organic carbon density (SOCVD) and corresponding horizon average mid-depth for Irish afforested Podzols, Cambisols and Gleys

Figure 5 Mean soil organic carbon stocks (SOCSs) of fixed depth-intervals for Irish afforested Podzols, Cambisols and Gleys (six sites per soil group): (a) computed by trapezoidal approach, (b) computed by spline approach

Figures



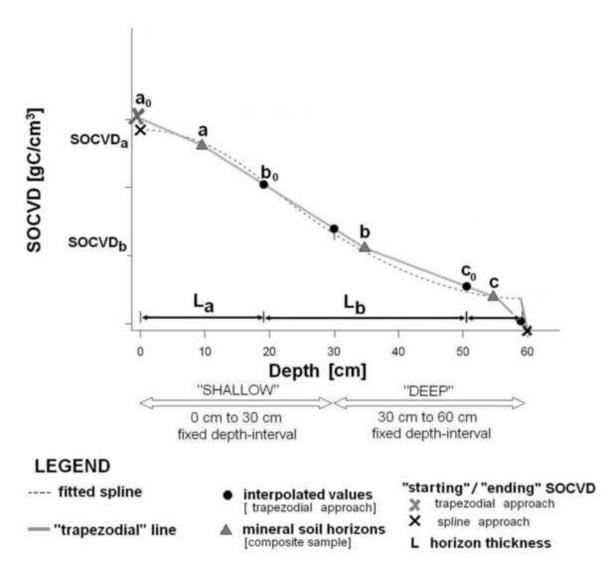


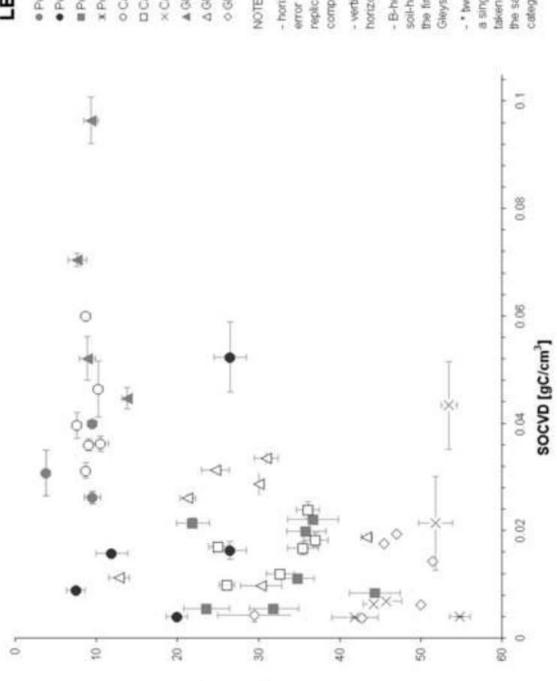


LEGEND

- I horizon "mid depth" L horizon thickness △ mineral soil horizons ▲ [linear regression applied to ▲ points]
- SOCVD volume based soil organic C density interpolated values
- SOCS calculated soil (mineral horizons) organic C stocks
- X "starting" / "ending" SOCVD organic horizon C stocks organic horizons







Mid-depth [cm]

- horizontal error bars - "relative" standard - vertical error bars - standard error of soilthe first B sol-horizons in the sol-profile of - B-horizons labelled with no. 2 refer to the replication (SOCVD value represents one soil-hortzons of B-category found below error obtained from bulk-density sample [Bw, Bg, Bg(a)*] (Bh. Bs, Bsg) [Bw2, Bg2*] [Ea, Eag] horizon average "mid-depth" (W) [H (MB) [AP] composite sample), D Cambisots-B × Cambisots-BC LEGEND o Cambisols-A o Gleysols-B2 x Podzols-BC A Gleysols-A ∆ Gleysols-B · Podzols-A Podzols-B Podzols-E Gleysols, NOTE

- * two sites had missing bulk-densities for a single soll-horizon (measurements were taken from the next similar soll-horizon at the same site of the same soll-horizon category).

