

## Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil

Ahmed, I.U.; Smith, A.R.; Jones, D.L.; Godbold, D.L.

### Forest Ecology and Management

DOI:

[10.1016/j.foreco.2015.07.018](https://doi.org/10.1016/j.foreco.2015.07.018)

Published: 26/07/2015

Peer reviewed version

[Cyswllt i'r cyhoeddiad / Link to publication](#)

*Dyfyniad o'r fersiwn a gyhoeddwyd / Citation for published version (APA):*

Ahmed, I. U., Smith, A. R., Jones, D. L., & Godbold, D. L. (2015). Tree species identity influences the vertical distribution of labile and recalcitrant carbon in a temperate deciduous forest soil. *Forest Ecology and Management*, 359, 352-360.  
<https://doi.org/10.1016/j.foreco.2015.07.018>

#### Hawliau Cyffredinol / General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

#### Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

1 **Tree species identity influences the vertical distribution of labile and recalcitrant**  
2 **carbon in a temperate deciduous forest soil.**

3

4 Iftekhar U. Ahmed<sup>1,\*</sup>, Andrew R. Smith<sup>2</sup>, David L. Jones<sup>2</sup>, Douglas L. Godbold<sup>1</sup>

5 <sup>1</sup>*Institute of Forest Ecology, Universität für Bodenkultur (BOKU), Vienna 1190,*

6 *Austria*

7 <sup>2</sup>*School of Environment, Natural Resources and Geography, Bangor University,*

8 *Bangor, Gwynedd, LL57 2UW, UK*

9

10 Author for correspondence:

11 Iftekhar U. Ahmed

12 Institute of Forest Ecology,

13 Universität für Bodenkultur (BOKU),

14 Vienna 1190,

15 Austria.

16 Corresponding author Tel.: +43 1476541001

17 Corresponding author Fax.: +43 1476544129

18 Corresponding author E-mail: iftekhar.ahmed@boku.ac.at

19 Number of Pages: 31

20 Number of Tables: 2

21 Number of Figures: 5

22 **Abstract**

23 In terrestrial environments, soil organic matter (SOM) is the largest organic carbon  
24 (C) pool. The quantity and quality of organic carbon in soils can be affected by  
25 vegetation through influencing the inputs and outputs of SOM. We examined how  
26 storage and quality of C in SOM were affected by vegetation under grass cover or  
27 single and a polyculture plot of *Betula pendula*, *Alnus glutinosa* and *Fagus sylvatica*.  
28 An acid hydrolysis approach was used to quantify three SOM fractions differing in  
29 biodegradability. Tree species identity and stand composition had no significant effect  
30 on the total amount of C stored in different SOM fractions to a depth of one meter.  
31 However, when examining individual SOM fractions in the upper layers of the soil  
32 profile, significantly more C was stored in the putatively more labile fractions 1 and 2  
33 under *F. sylvatica* and *A. glutinosa*, respectively. In deeper soil layers, the highest  
34 storage of recalcitrant organic C was found under the tree polyculture. The vertical  
35 distribution of these three soil organic C pools was compared to C inputs via  
36 decomposed leaf litter. Our data indicated that in the tree species polyculture,  
37 combining litter inputs of multiple species can have a positive impact on the  
38 accumulation of acid resistant recalcitrant C in deep soil layers in 4 years. This C  
39 fraction has the greatest potential for long-term sequestration.

40

41 **Keywords** Fractionation; Acid hydrolysis; Polyculture; Carbon storage; Tree species  
42 mixture; Decomposition

## 43 **1. Introduction**

44 Soil organic matter (SOM) represents the largest reservoir of terrestrial organic carbon  
45 (C) on Earth, and the organic residues that comprise SOM range from relatively intact  
46 plant or microbial material to highly decomposed humic substances (Rumpel et al.,  
47 2002). Through differences in litter quality, plant species have potential to influence  
48 the storage and dynamics of C in soils, as reported in several previous studies  
49 (Binkley and Valentine, 1991; Hagen-Thorn et al., 2004; Jandl et al., 2007; Leuchner  
50 et al., 2013). However, there is growing evidence that molecular structure is less  
51 important than previously believed as a factor controlling the formation of SOM, and  
52 that the inputs from roots and microbial degradation products are more important than  
53 previously assumed (Schmidt et al., 2011). However, it remains undisputed that these  
54 factors result in a heterogeneous mixture of organic compounds (von Lützow et al.,  
55 2006; Schmidt et al., 2011; Tfaily et al., 2015).

56 To investigate the composition of soil organic matter, SOM can be  
57 fractionated into several pools using a range of techniques (eg. Paul et al., 2001; Weil  
58 et al., 2003; Gregorich et al., 2003; Bajgai et al., 2013). Each of these methods  
59 attempts to isolate soil organic matter pools of different longevity. A common  
60 method used, is to separate pools on the basis of biodegradability (Rovira and Vallejo,  
61 2007). This method separates soil organic matter based on acid solubility into labile  
62 and recalcitrant pools, which are believed to have different turnover times  
63 (McLauchlan et al., 2004). For example, labile C fraction composed of compounds  
64 such as soluble sugars, starch, and other carbohydrates, has been show to play a  
65 dominant role in the evolution of CO<sub>2</sub> from soil due to preferential decomposition and  
66 rapid turnover (Belay-Tedla et al., 2009). In contrast the recalcitrant fraction is  
67 thought to degrade slowly, thus contributing to long term C storage in soils. For

68 example, lignified humus and some physically protected labile SOM can be retained  
69 in soils for several thousand years (Zou et al., 2005; Dungait et al., 2012; Kellner et  
70 al., 2014). However, how the composition of aboveground vegetation affects the  
71 distribution of these pools in soil systems is largely unknown (De Deyn et al., 2008).

72         Tree species may influence soil organic carbon (SOC) stocks through a variety  
73 of mechanisms such as: (i) differences in net primary productivity and the production  
74 of detritus (Montagnini et al., 1993; Hansen et al., 2009), (ii) variation in the quality  
75 and complexity of organic matter input to soils detritus originating from leaf, root and  
76 mycorrhizal biomass (Hagen-Thorn et al., 2004), (iii) variation in the depth and  
77 distribution of roots (Carvalheiro and Nepstad, 1996; Lai et al., 2015), and (iv) by  
78 altering soil invertebrate and microbial populations (Hobbie et al., 2006; Lynch et al.,  
79 2012). Soil organic carbon accumulation may differ in species-diverse communities,  
80 compared to monocultures through variation in biomass inputs to soils, and SOM  
81 transformation processes in the soil (Steinbeiss et al., 2008). In mixed species  
82 communities, species interactions may either increase productivity through resource  
83 use complementarity (Loreau and Hector, 2001; Richards and Schmidt, 2010) or  
84 decrease productivity through inter- or intra-specific competition (Carnus et al.,  
85 2006). Plant community composition in mixtures may also influence long term  
86 storage of soil C through species-specific differences in plant detritus chemical  
87 composition and input rates (Six et al., 2002). In addition, interaction between litter  
88 types in mixed species communities may affect rates of decomposition and turnover  
89 (King et al., 2002). This is particularly true in natural forest ecosystems, where the  
90 litter layer can be comprised of inputs from many species, thus forming more complex  
91 organic substances compared to the litter layer of mono-specific forests. The highly  
92 complex and heterogeneous organic residues found in the SOM of mixed

93 communities have been shown to alter soil C residence times through differences in  
94 biodegradability (Sollins et al., 1996).

95 Our study determined the vertical distribution of labile and recalcitrant C  
96 fractions in SOM that occurred within the initial four years of forest establishment.  
97 We examined the effects of vegetation on the storage of C fractions in soils under  
98 grass or trees of *A. glutinosa*, *B. pendula*, *F. sylvatica* grown in monoculture, or a  
99 polyculture of the three tree species. *B. pendula* is a light-demanding, early  
100 successional species with fast juvenile growth (Fischer et al., 2002). *A. glutinosa* is an  
101 N-fixing, water-demanding pioneer species, also with high juvenile growth rates  
102 (Braun, 1974). Lastly, *F. sylvatica* is shade tolerant and slow growing when juvenile  
103 (Ellenberg et al., 1991), can persist in the understory, and often dominates late  
104 successional forest. We hypothesized that in the long term SOC storage would be  
105 positively affected by growing trees species selected for contrasting functional traits  
106 in polyculture.

107

## 108 **2. Materials and Methods**

### 109 *2.1 Study area*

110 The BangorDIVERSE experimental site was established at Henfaes Research  
111 Centre, Bangor University, North Wales, UK (53°14' N, 4°01'W) in March 2004 on  
112 two fields with a total area of 2.36 ha. Soils are fine loamy brown earth over gravel  
113 (Rheidol series) and classified as Fluventic Dystrochrept in the USDA system (Smith  
114 et al., 2013a). Soil texture in the 0-10 cm layer was  $48.2 \pm 1.3$  % sand,  $33.6 \pm 0.9$  %  
115 silt and  $18.2 \pm 2.1$  % clay, determined by laser diffraction (Coulter LS particle size  
116 analyser). The soil pH is 5.4 in the 0-10 cm layer increases to 6.3 at 100 cm soil  
117 depth. Soil physicochemical properties are shown in Suppl. Table 1. Climate at the

118 site is classified as hyperoceanic. Mean annual temperature throughout 2005–2008  
119 was 11.5 °C with an annual rainfall of 1034 mm (Campbell Scientific Ltd, Shepshed,  
120 UK).

121

## 122 *2.2 Plantation design*

123 Before tree planting, all vegetation was removed from the fields including the grass  
124 plots and the soil was ploughed and raked. Plots were established in four replicated  
125 blocks of single species or two and three species mixtures of *Alnus glutinosa* L.,  
126 *Betula pendula* Roth., *Fagus sylvatica* L., *Fraxinus excelsior*, *Acer pseudoplatanus*  
127 L., *Castanea sativa* Mill. and *Quercus robur* L. The trees were selected due to their  
128 contrasting shade tolerance, successional chronology and to represent a range of  
129 taxonomic, physiological and ecological types. Two blocks were sited in each field  
130 and the minimum distance between any two plots of the same composition was 35 m.  
131 The size of the plots were 62 m<sup>2</sup> for the grassland, 81 m<sup>2</sup> for single species, and 121  
132 and 196 m<sup>2</sup> for the two species and three species plots respectively. A replacement  
133 series design (with inter-tree spacing constant between treatments) was selected  
134 because of the experiments objective of being realistic in reflecting the practical  
135 realities of how forests comprising monocultures or mixtures of potential canopy tree  
136 species could be established (Jolliffe, 2000). The site was planted with 60 cm saplings  
137 of each species with an inter-tree spacing of 1 m (10,000 stems ha<sup>-1</sup>). A systematic  
138 hexagonal planting design (Aguiar et al., 2001) was used to maximise the mixing  
139 effect so that, in the three species mixture blocks, each tree was surrounded by nearest  
140 neighbours of two-con-specific individuals and one and three individuals of the other  
141 two species, respectively, resulting in each tree having six equidistant neighbours. On  
142 the grassland plots, a grass cover was allowed to regenerate from remnants to form a

143 sward composed of a mixture of *Lolium perenne* L., *Dactylis glomerata* L. and  
144 *Agrostis stolonifera* L. In the work reported here, we used the plots of *A. glutinosa*, *B.*  
145 *pendula* and *F. sylvatica* and a three species polyculture of these species.

146

### 147 2.3 Positioning of the plots used

148 The initial soil organic matter was determined in the top 0-10 cm layer on a 10 × 10 m  
149 grid. The mean SOM content across the fields was ca. 6 %, but across the fields varied  
150 between 4 to 8 % (Fig. 1). Historically, both fields were pasture, but since the 1980s  
151 one field (field 1) was used for small scale forestry experiments, while in 2003 the  
152 other field (field 2) was ploughed and planted with oil seed rape. Two blocks each  
153 containing a replicate single species and mixed species plot as well as the associated  
154 grassland plot, were located on the field used in 2003 for soil seed rape. In the other  
155 field one block was positioned on an area previously planted with *Salix* as short  
156 rotation coppice trial, while the other block was positioned on an area previously used  
157 to grow mainly *Q. robur* and *F. sylvatica* saplings. In 2008, one of the *A. glutinosa*  
158 plots was damaged. To balance the number of replicates, one plot each of the *F.*  
159 *sylvatica*, *B. pendula* and polyculture were removed from the analysis by random  
160 selection, leaving the distribution of plots used as shown in Figure 1.

161

### 162 2.4 Soil collection and sample processing

163 Soil was collected by excavating 100 × 100 × 100 cm pits in the centre of each  
164 grassland plot and each of the three tree species monoculture and tree three species  
165 polyculture plots in September of 2008 (15 pits in total). In the polyculture plots,  
166 samples were collected from a pit at an equal distance from *A. glutinosa*, *B. pendula*  
167 and *F. sylvatica*. Soil samples were collected from seven layers (0-10, 10-20, 20-30,



168 30-40, 40-50, 70-80 and 90-100 cm). A subset of four layers (0-10, 10-20, 40-50 and  
169 90-100 cm) were then used for C fractionation using acid hydrolysis. To ensure  
170 representativeness, samples were obtained from each layer, approximately 100 g of  
171 soil was collected from each side of the soil pit and mixed to produce a composite 400  
172 g sample. Soils were then air dried, carefully homogenized and sieved to pass through  
173 a 2 mm sieve, a 50 g sub-sample was then taken and finely ground using a ball mill  
174 (Retsch Mixer Mill MM 200) and passed through a 100  $\mu\text{m}$  sieve prior to acid  
175 hydrolysis. Soil pH and electrical conductivity were determined in a 1:2 v/v slurry of  
176 soil and distilled water with standard electrodes. Moisture content was determined  
177 after drying at 105 °C for 72 h, and organic matter as loss-on-ignition at 450 °C for  
178 16 h. Bulk density was determined using 100  $\text{cm}^3$  cores and corrected for stone  
179 content (Rowell, 1994). The clay content of soils was determined by a simplified  
180 method combining wet sieving and sedimentation steps, as proposed by Kettler et al.,  
181 (2001). Soil microbial biomass C of surface soil (0-10 cm) was determined according  
182 to the  $\text{CHCl}_3$  fumigation-extraction method of Vance et al., (1987).

183

#### 184 *2.5 Leaf and root sample collection*

185 Fully expanded leaves exposed to full incident light, were collected from the outside  
186 of the crown at upper and middle of the canopy positions. Five trees of each species  
187 were randomly selected and approximately 2 g of leaves were collected and combined  
188 into a composite sample (ca. 10 g) for each species. Root samples were collected  
189 using an 8 cm diameter soil corer. Grassland sward was collected from three  
190 undisturbed locations, washed and the leaves and roots separated before being oven  
191 dried at 80 °C for 24 h. Dried leaves and roots were separately ground using a ball  
192 mill (Retsch Mixer Mill MM 200) and passed through a 100  $\mu\text{m}$  sieve. The total C

193 content of soil and plant materials was determined using a TruSpec<sup>®</sup> CN analyser  
194 (Leco Corp., St Joseph, MI). The leaf and root materials were also analysed by  
195 sequential acids hydrolysis as described below.

196

#### 197 *2.6 Acid hydrolysis of soil and plant materials*

198 Chemical methods allow fractionation of SOC into pools of putative identity. For  
199 example in a two-step acid hydrolysis approach, the labile fraction is further divided  
200 into two pools- labile fraction 1 that could be putatively identified as polysaccharides,  
201 derived from plant and microbial sources, and labile fraction 2 that contains cellulose  
202 which is more resistant than fraction 1 (Rovira and Vallejo, 2007). Labile soil C  
203 (fraction 1) was extracted from 0.5 g of air dried soil, which was taken from the  
204 homogenised 50 g sample described above. The soil was transferred into a sealable  
205 Pyrex tube and 15 ml of 2.5 M H<sub>2</sub>SO<sub>4</sub> was added and thoroughly mixed. The mixture  
206 was heated to 100 °C for 30 min in a digestion block. After cooling the hydrolysed  
207 solution was centrifuged at 2695 × g for 3 min and the clear supernatant decanted into  
208 a fresh glass tube. The residue was washed twice with 15 ml of deionised water and  
209 the washings added to the hydrolysate and kept in glass bottles at 4 °C until analysis  
210 for C and N using a TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan).

211 To extract a less labile part of the soil C (fraction 2), the unhydrolysed  
212 residues were transferred to Pyrex tubes and dried at 60 °C. After cooling, 2 ml of 13  
213 M H<sub>2</sub>SO<sub>4</sub> was added, and the tubes were shaken overnight on a horizontal shaker at a  
214 speed of 80 strokes min<sup>-1</sup> at room temperature. Thereafter, 26 ml of deionised water  
215 was added to dilute the acid to 1 M and the residues were hydrolysed for 3 h at 100 °C  
216 with occasional shaking. After centrifugation at 2695 × g for 3 minutes, the clear  
217 hydrolysate was removed. The residues were washed twice with distilled water, and

218 the washings added to the hydrolysate and stored at 4 °C until analysis of C and N as  
219 described above. The remaining residual C was fraction 3 and was calculated by  
220 deducting the summed C fractions 1 and 2 from total organic C content of the soil  
221 (Belay-Tedla et al., 2009).

222 Acid hydrolysis of plant material followed the same protocol used for soils  
223 except that the sample size was decreased to 25 mg (Shirato and Yokozawa, 2006).  
224 The plant biomass C: acid ratio was the same as that used in the soil hydrolysis. As  
225 the residues could not be removed by centrifugation, the extracts were separated from  
226 un-hydrolysed residues by filtration using Whatman No. 1 filter papers (GE  
227 Healthcare UK Ltd.). After each hydrolysis, residues were washed twice with distilled  
228 water. Both soil and plant samples were analysed in triplicate.

229

### 230 *2.7 Estimation of C pools throughout the soil profile*

231 The total C stock and the absolute quantity of labile and recalcitrant C in different soil  
232 layers were estimated on area basis using bulk density determination of the different  
233 soil layers after adjustment for stone content. The total C pool size in soil profile (0-  
234 100 cm) was calculated by fitting a 2<sup>nd</sup> order quadratic to the soil C data; predicted  
235 values were then used to interpolate the C content of all soil layers in 10 cm  
236 increments. The actual and in-filled values were then summed to determine total size  
237 of the C pool.

238

### 239 *2.8 Leaf litter decomposition*

240 We studied the decomposition dynamics of leaf litter in each plots using leaf litter  
241 from respective species to examine the impacts of species identity or mixture on  
242 decay rates that affects the quality and quantity of SOC. We used leaves with a natural

243 water content rather than air dried leaves, because firstly, most of the leaves reach the  
244 forest floor as fresh litter in this ecosystem and thus we mimicked the natural process  
245 and secondly, air drying can substantially depressed the initial decay rates, especially  
246 in case of *A. glutinosa* (Taylor, 1998).

247 Litter decomposition rates were determined by mass loss of leaves in 180  
248 nylon mesh bags. Fifteen litter bags (1 mm mesh, 20 cm × 15 cm), containing 5.0 g  
249 litter each, were placed on the forest floor of each tree species monoculture and the  
250 three tree species polyculture plot in July 2009. The bags were deployed at close  
251 contact with mineral soils under the litter layer to include the interaction of soil fauna  
252 activities, especially as the activity of earthworms at the site is high (Scullion et al.,  
253 2014). Litter representing the three species polyculture plots was composed of *B.*  
254 *pendula*, *A. glutinosa* and *F. sylvatica* in the ratios of 4:5:1 based on species  
255 contributions to litter fall baskets within the polyculture plots (Ahmed, 2011). Three  
256 litter bags were harvested after 3, 6, 10, 15 and 21 weeks from each plot. The litter  
257 was cleaned to remove soil particles, and dried at 60 °C for 72 h before weighing. A  
258 sub sample of 0.5-1.0 g was burned at 450 °C overnight, and weighed to determine  
259 ash content. Ash weight was deducted from the total litter weight to account for the  
260 contribution of adhered mineral soil particles to litter mass. The following single  
261 exponential decay model (Equation 1; Olson, 1963) was used fitted to the leaf mass  
262 loss data to compare leaf litter decay between species, where *A* is the initial litter  
263 mass, *k* is the decay rate constant, and *t* is time.

$$\text{mass remaining} = Ae^{-kt} \quad \text{Equation 1}$$

264 To determine the effect of litter mixture on litter decomposition, the mass loss  
265 of the polyculture litter bags was compared with those of a theoretical polyculture  
266 calculated from the mass loss of the single species litter bags. Equation 2 shows the

267 theoretical mixture biomass calculation, where  $M_{species}$  is the mass contributing  
268 towards the mixture. The theoretical basis of this calculation is directly analogous to  
269 the Relative Yield of Mixtures index used to quantify the effects of competition  
270 (Wilson, 1988). The use of Equation 2 in this experiment is comparable with the  
271 Relative Yield Total (Weigelt and Jolliffe, 2003).

272

$$M_{mixture} = \left( \frac{4}{10} \times M_{Betula} \right) + \left( \frac{5}{10} \times M_{Alnus} \right) + \left( \frac{1}{10} \times M_{Fagus} \right)$$

Equation 2

273

274

## 275 2.9 Statistical analysis

276 The BangorDIVERSE experiment was designed as a fully replicated ( $n=4$ ) field  
277 experiment with seven tree species planted in monoculture and mixtures of two and  
278 three species. For this research, three replicate plots each of grass, a single-tree  
279 species or a three tree species polyculture were studied. C pools were compared  
280 across four depths and four species types separately using One-way ANOVA, and  
281 pairwise comparisons made with Tukey's HSD *post hoc* test (SPSS v14.0, SPSS Inc.,  
282 Chicago, IL, USA). Normality was assessed by Shapiro-Wilk test and homogeneity of  
283 variances was determined by Levene's test. Main effects were considered to be  
284 significant at  $P < 0.05$ .

285

## 286 3. Results

287

### 288 3.1 Soil carbon

289 Total soil C content decreased with increasing depth in soils under all species types  
290 and the grassland (Fig. 2). Significant differences in soil C between *F. sylvatica* plots  
291 compared to *B. pendula* plots was observed at top two soil layers, but no differences  
292 in soil C were found in any of the treatments in soil below 20 cm. Soil microbial  
293 biomass C ranged between  $0.56 \pm 0.03$  mg C kg<sup>-1</sup> and  $0.83 \pm 0.08$  mg C kg<sup>-1</sup> in the top  
294 0-10 cm of soil, and the species composition had no significant impact on the  
295 microbial biomass C and N content. In all the plots, the C:N ratio decreased with  
296 increasing soil depth (Suppl. Fig.1), but no statistically significant differences  
297 between any of the treatments at any soil depth were observed. However, the greatest  
298 change in C:N ratio between the top and bottom soil layers was found in the grassland  
299 and the *B. pendula* plots ( $\Delta$  6.1) compared to a change of ca. 3.5 in the *A. glutinosa*,  
300 *F. sylvatica* and polyculture plots.

301

### 302 3.2 Relative contribution of C fractions to total C concentration

303 The C content of each of the SOM fractions varied between species and between soil  
304 depths. Figure 3 illustrates the distribution pattern of three C fractions in soils under  
305 different plant species and polyculture. In the 0-10 cm layer, fraction 1 was between  
306 22 – 36 % of the total C, this increased to 29 – 52 % at 100 cm depth. The C of  
307 fraction 1 in the *F. sylvatica* plots was significantly ( $P=0.012$  &  $P=0.002$ ) greater  
308 than the soils of *A. glutinosa* plots at top two layers, and at 10-20 cm depth *B.*  
309 *pendula* and the polyculture soils were significantly higher than *A. glutinosa* (*B.*  
310 *pendula*,  $P=0.006$ ; polyculture,  $P=0.035$ ). For fraction 2, except in the grassland plots,  
311 the changes in percentage contribution to the total soil C were less pronounced  
312 compared to fraction 1. Grassland contained a significantly higher percentage of C in  
313 fraction 2 in all soil layers than other plots, except *A. glutinosa*.

314 Fraction 3, the residual C after extraction, representing potentially the most  
315 recalcitrant C was unaffected by soil depth in *F. sylvatica* and the polyculture, but was  
316 significantly lower in the middle layer (40-50 cm) compared to the upper layers in the  
317 *B. pendula* and *A. glutinosa* plots. Species identity and mixture did not significantly  
318 affect the relative contribution of fraction 3 in the top two layers of soil (Fig. 3).  
319 Further down the soil profile, a significantly higher percentage of fraction 3 was  
320 found in polyculture soils than in *B. pendula* ( $P=0.014$ ) and *A. glutinosa* ( $P=0.002$ )  
321 soils at 40-50 cm; and in the 100 cm layer the contribution was higher compared to *A.*  
322 *glutinosa* ( $P=0.013$ ). In both the 40-50 and 100 cm layers, *A. glutinosa* had the lowest  
323 proportion of C within fraction 3 (27 and 34%, respectively).

324

### 325 3.3 Total C storage and C pool size of each fraction

326 The total C storage to a depth of 100 cm in the various plots ranged between  $10.2 \pm$   
327  $0.9$  under grass to  $6.9 \pm 0.8$  kg C m<sup>-2</sup> under *F. sylvatica*, with no significant variation  
328 between the treatments (Table 1). In Table 1 the pools of C are shown as the total  
329 extractable (fraction 1 and fraction 2) and the residual C in fraction 3. The tree species  
330 grown in monoculture and polyculture showed no significant difference in total C  
331 stocks. We examined the influence of tree species on fraction 3 in upper (0-40 cm)  
332 and lower (40-100 cm) region of the soil profile (Fig. 4). In the upper layers no  
333 significant differences were found between the treatments, however in the deeper soil  
334 layers, the greatest storage of C in fraction 3 was found in the polyculture. The C  
335 storage in the polyculture soil at depth was significantly greater compared to the *B.*  
336 *pendula*, *A. glutinosa* and grassland soil, but not statistically different compared to *F.*  
337 *sylvatica*. Both *F. sylvatica* and the polyculture in the lower soil profile had a

338 significantly higher ( $P=0.015$  and  $P<0.001$ ) C storage in fraction 3 compared to the  
339 profile under grass.

340

#### 341 *3.4 Fractionation of litter C inputs*

342 Total C in the leaves and roots of the three tree species was 52 and 53 %,  
343 respectively, significantly greater than the sward comprising the grassland which  
344 contained 44 % ( $P=0.020$ ) and 40 % ( $P=0.002$ ) for leaves and roots, respectively. In  
345 the tree leaves, C extracted from fractions 1 and 2, was similar. In contrast, in the  
346 roots of *F. sylvatica* the C content of fraction 2 was higher than in the other tree  
347 species (Fig. 5). In grass leaves and roots, the highest amount of C was in fraction 1,  
348 and the amount of fraction 3 was only 35 and 37 % of the total C, for leaves and roots,  
349 respectively.

350

#### 351 *3.5 Leaf litter decomposition*

352 During the course of decomposition, mass remaining in leaf litter best fitted a first  
353 order exponential decay model. Decay rate coefficients for the three species grown in  
354 monoculture and polyculture are shown in (Table 2). Overall, and during the first four  
355 sampling intervals (3, 6, 10 & 15 weeks), there was a significant difference in mass  
356 loss between the litter of tree species ( $P<0.001$ ; Table 2). During this period the rate  
357 of mass loss of the single species trees litter was highest in *A. glutinosa*, which was  
358 1.94 and 1.80 times faster than *F. sylvatica* and *B. pendula*, respectively (Table 2). In  
359 the mixed species litter bags there was a dramatic and significant ( $P<0.001$ ) reduction  
360 in mass loss, which was 4.36 times slower than *A. glutinosa* in monoculture.

361

## 362 **4. Discussion**



363

#### 364 4.1 Tree traits

365 The storage and the distribution of organic C in soils are influenced by the quality and  
366 quantity of inputs determined by the integrated effects of species-specific traits  
367 (Schmidt et al., 2011). In this study, we examined three tree species selected due to  
368 their strongly contrasting productivity and functional traits, to accentuate the species-  
369 specific contribution to soil C pools. As a consequence of the trait differences, the  
370 species have different qualities of leaf litter inputs (see below), but also different rates  
371 of fine root turnover and hence root litter inputs (Smith et al., 2013b). The differences  
372 in leaf litter quality were reflected in the initial rates of decomposition, where the  
373 decomposition of *A. glutinosa* was nearly two times faster than the other two species.  
374 We found that decomposition processes of mixed species litter bags were slower than  
375 single species when deployed at our field site. Species-specific interactions during  
376 litter decomposition have been shown to have no effect (Prescott et al., 2000), retard  
377 (Chapman et al., 1988), or enhance decomposition processes (de Marco et al., 2011).  
378 We attribute the reduction in decomposition rates to the combination of a highly  
379 recalcitrant lignocellulose matrix of *F. sylvatica* litter and species-specific secondary  
380 metabolites, such as polyphenols and monoterpenes that inhibit N mineralisation and  
381 species-specific decomposer communities (Hattenschwiler et al., 2005). In addition to  
382 the potential interaction of late successional species litter chemistry in decomposition,  
383 and consistent with the findings of Giertych et al. (2006), we found a higher water-  
384 soluble polyphenolic content in *B. pendula* (20.5 mg L<sup>-1</sup>) compared to *A. glutinosa*  
385 (17.5 mg L<sup>-1</sup>) litter (Ahmed, 2011). The nitrogen content of senesced leaf litter was  
386 30.5, 29.0 and 35.0 g kg<sup>-1</sup> for *F. sylvatica*, *B. pendula* and *A. glutinosa*, respectively,  
387 suggesting that the rapid initial decomposition of *A. glutinosa* was driven by nitrogen

388 availability. However, lignin content did not follow the same species order and was  
389 138, 272 and 338 g kg<sup>-1</sup> for *A. glutinosa*, *B. pendula* and *F. sylvatica*, respectively,  
390 potentially leading to slower decomposition of fraction 3 C for *F. sylvatica* relative to  
391 the other species.

392 Plant species identity can also influence the production and distribution of fine  
393 root biomass throughout the soil profile. The effect of species diversity on root  
394 biomass and production is extremely variable with studies showing no effect (Bauhus  
395 et al., 2000), a reduction (Bolte and Villanueva, 2006) or increase (Brassard et al.,  
396 2011). In the species used here, *A. glutinosa* had the highest rate of fine root turnover,  
397 and *F. sylvatica* the highest fine root length in the top 30 cm of soil (Smith et al.,  
398 2013b). However, differences in the rate of fine root turnover were not seen in all  
399 years (Ahmed, 2011). In addition to the influence of plant litter chemistry and species  
400 identity on decomposition, the phenology of leaf and root growth can also influence  
401 belowground processes (Niinemets and Tamm, 2005). Indeed, seasonality has a  
402 particularly strong control on the phenology of grassland species (Steinaker and  
403 Willson, 2008).

404

#### 405 4.2 Organic C storage of soils under different plant species

406 No significance difference in SOC stock (0-100 cm) was observed both between the  
407 tree species, and in comparison to the grassland (Table 1). This is in consistent with  
408 the study of Vesterdal et al. (2008), who reported no significant variation in the soil C  
409 stocks of five European broadleaved tree species, including *F. sylvatica*, after 30 years  
410 of growth. However, we did find a higher organic C concentration in *B. pendula* soil  
411 compared to *F. sylvatica* in the upper two layers of the soil profile (Fig. 2). The  
412 biomass production and subsequent litter fall in *B. pendula* was much higher than the

413 late successional species *F. sylvatica*, and may be the cause of the higher SOC in the  
414 upper soil layers.

415

#### 416 4.3 Soil organic carbon and fractionation

417 Changes in total soil C were only observed in the top 20 cm of soil, below this depth  
418 soil C was not different between the tree species or the grassland. Soil C stock change  
419 and physical fractionation were investigated at the Bangor site using different but  
420 adjacent plots with the same species by Hoosbeek et al., (2011), who found an  
421 increase of 530 g C m<sup>-2</sup> in the top 0-10 cm layer 4 years after planting. In contrast to  
422 our chemical fractionation results, the distribution of coarse, fine and aggregate  
423 particulates were similar between all species and the polyculture. Here we showed  
424 that using chemical fractionation, the organic C content of fraction 1 was significantly  
425 lower in soils under *A. glutinosa* than in *F. sylvatica* in the 0-10 cm soil layer (Fig. 3).  
426 We propose two mechanisms to explain our observed differences in fraction 1 C  
427 content. First, relative to *F. sylvatica*, *A. glutinosa* is poor at translocating nutrients  
428 and carbohydrates during senescence (Lecerf and Chauvet, 2008), and subsequently  
429 the senesced litter of *A. glutinosa* is considered to be of high quality due to thick,  
430 mesophyll rich, leaves with a low C:N ratio. These traits, which are favourable to  
431 grazing by soil organisms and microbial decomposition (Kazakou et al., 2009),  
432 probably resulted in a rapid removal of fraction 1. The influence of litter quality on  
433 decomposition processes was also supported by significantly faster litter mass loss of  
434 *A. glutinosa* than *F. sylvatica* during the first three weeks (Table 2). Second, during  
435 organic matter mineralization and microbial turnover, C not respired as CO<sub>2</sub> is  
436 retained within microbial biomass, or released as dissolved organic carbon (DOC),

437 which then leaches through the soil profile reducing the size of the pool in shallow  
438 soil layers (Currie and Aber, 1997).

439         The percentage of C in fraction 1 increased gradually down the soil profile  
440 under *B. pendula* and *A. glutinosa*, but remained constant under *F. sylvatica* and the  
441 polyculture. Studies using stable C isotopes and radiocarbon have revealed that acid  
442 hydrolysable C, as in fraction 1, as well as mineral associated C are consistently  
443 younger than other fractions (Leavitt et al., 1997), and generally, it is assumed that the  
444 age of soil C increases with depth (Fontaine et al., 2007). Potential sources of the  
445 fraction 1 in the lower soil layers are numerous and include translocation from upper  
446 soil layers with DOC or bioturbation, root exudates (de Graaff et al., 2010), and  
447 priming of more recalcitrant soil organic matter (Rovira and Vallejo, 2007, Kogel-  
448 Knabner et al. 1991) with subsequent release of more labile fractions. Earthworm  
449 activity at the site is high, and the earthworms were shown to have a higher preference  
450 for litter from *B. pendula* and *A. glutinosa* than that from *F. sylvatica* (Scullion et al.  
451 2014).

452         The largest percentage of C in the top soil layer was in fraction 3, and was not  
453 influenced by tree species identity or grass. This is consistent with the findings of  
454 Hoosbeek et al., (2011) who reported that the physical fractionation of particulate  
455 organic matter at the same experiment site and found that tree species identity had no  
456 effect on soil C stabilization processes and microaggregate protection in the upper soil  
457 layers. A caveat of our acid hydrolysis approach to separating C fractions is that  
458 physically protected labile C may be included in fraction 1 (McLauchlan and Hobbie,  
459 2004), but there seems to be broad agreement between the acid hydrolysis and  
460 physical fractionation methods. Similarly, it has also recently be shown that using  
461 acid fractionation schemes more aggressive than the one used here, can lead to de-

462 novo synthesis of non-hydrolysable substances and loss of pure model carbohydrates  
463 (Greenfield et al. 2013).

464 Soils from under grass differed strongly to the tree plots in the distribution of  
465 all three C fractions. Throughout the soil profile, the strong difference between trees  
466 and grass were most likely attributable to a shallow rooting depth and turnover of  
467 non-woody grass roots. In addition, the higher amount of fraction 1 C in grassland soil  
468 might be due to the high quality of grass litter, which contains substantially less lignin  
469 than tree litter, and thus is easily decomposed (Deschaseaux and Ponge, 2001). This is  
470 supported by our analysis of grass leaf and root material, which showed much higher  
471 quantities of fraction 1 C in grass than in tree materials (Fig. 5).

472

#### 473 *4.4 Recalcitrant C in deep soil layers*

474 There were no differences in the percentage of soil C found at 40-100 cm soil depth  
475 between the treatments. Below 40 cm the percentage soil C was less than 1 %.  
476 However, surprisingly, significantly greater quantities of the total soil C were found in  
477 fraction 3 in the three species tree polyculture stands, compared to the single species  
478 stands of *B. pendula* and *A. glutinosa* and the grassland. The higher fraction 3 storage  
479 was found in all three replicate polyculture plots irrespective of previous land-use  
480 (Fig. 1), as was the lower fraction 3 storage in the grassland plots. Similarly, in the  
481 polyculture plots, there were no obvious differences in soil texture, such as high levels  
482 of clay, which could account for the increased fraction 3 storage. The higher fraction  
483 3 storage in polyculture is difficult to reconcile with either C inputs via above- or  
484 below-ground biomass of the different species. By far the biggest difference in  
485 fraction 3 storage, or the percentage of fraction 3 in the total soil C, was found when  
486 the trees were compared to the grassland. This suggests that the differences in fraction

487 3 storage could be related to depth distribution and timing of C inputs, litter quality, or  
488 quantity. A potential mechanism could be a priming effect, where the input of labile  
489 C products into the deep soil layers, possibly via the flow of DOC stimulated  
490 microbial mineralisation of old C (Kuzyakov et al., 2000; Hoosbeek et al., 2004).  
491 Microbial priming of deep soil C was reported by Fontaine et al., (2004) who  
492 demonstrated that fresh inputs of labile C allowed the co-metabolism of old  
493 recalcitrant C by the microbial community at a depth of 60-80 cm. Therefore, the  
494 availability of easily biodegradable compounds through vertical DOC transport into  
495 deep soil layers could be an important factor in mediating the storage of recalcitrant C  
496 in deep soil layers. The chemical composition of DOC is related to the plant litter  
497 from which it is derived (Hansson et al., 2010). Our leaf litter decomposition  
498 experiment showed a large and significant difference between the rate of  
499 decomposition in monoculture and the three species polyculture, which may have  
500 resulted in a greater amount of recently derived C moving down the soil profile and  
501 microbial priming fraction 3 at depth. Again difficult to reconcile with current ideas  
502 is the speed at which these changes must have occurred. However, it must be stressed  
503 that the levels of total C at 100 cm soil depth are very low, enabling detection of small  
504 changes.

505 To conclude, our data suggest that even within relatively short time scales  
506 vegetation types and tree species identity and mixtures can influence both  
507 accumulation of soil C in surface layers, but also the storage of more recalcitrant  
508 fractions in deeper soil layers. This may be due to the direct inputs of new C, but also  
509 due to the effects of new C influencing the levels and distribution of old soil C.

510

511 **Acknowledgements**

512 The experimental facility was supported by the INTERREG IVB North-West Europe  
513 project 003A ForeStClim. IUA was supported both by ForeStClim and by the  
514 Forestry Commission UK for part of the work. The authors would like to thank Llinos  
515 Hughes and Mark Hughes of Henfaes Research Farm, Bangor University for their  
516 help in sampling and processing of soils. We would like to thank the two anonymous  
517 reviewers for their very constructive suggestions which were definitely helpful to  
518 improve the quality of the article.

519

## 520 **References**

- 521 Aguiar, F.C., Ferreira, M.T., Moreira, I. 2001. Exotic and native vegetation  
522 establishment following channelization of a western Iberian river. Regul.  
523 River, 17, 509-526.
- 524 Ahmed, I.U. 2006. Leaf decomposition of birch (*Betula pendula*), alder (*Alnus*  
525 *glutinosa*) and beech (*Fagus sylvatica*) grown under elevated atmospheric  
526 CO<sub>2</sub>. Master thesis, SENRGY, Bangor University, Bangor, UK.
- 527 Ahmed, I.U. 2011. Ecosystem carbon dynamics: as influenced by tree species and  
528 mixture in temperate deciduous woodland. PhD dissertation, SENRGY,  
529 Bangor University, Bangor, UK.
- 530 Bajgai, Y., Hulugalle, N., Kristiansen, P., McHenry, M. 2013. Developments in  
531 Fractionation and Measurement of Soil Organic Carbon: A Review. Open J.  
532 Soil Sci., 3(8), 356-360.
- 533 Bauhus, J., Khanna, P.K., Menden, N. 2000. Aboveground and belowground  
534 interactions in mixed plantations of *Eucalyptus globulus* and *Acacia mearnsii*.  
535 Can. J. For. Res., 30, 1886–1894.

536 Belay-Tedla, A., Zhou, X., Su, B., Wan, S., Luo, Y. 2009. Labile, recalcitrant and  
537 microbial carbon and nitrogen pools of a tall grass prairie soil in the US Great  
538 Plains subjected to experimental warming and clipping. *Soil Biol. Biochem.*,  
539 41, 110–116.

540 Binkley, D., Valentine, D. 1991. Fifty-year biogeochemical effects of green ash, white  
541 pine, and Norway spruce in a replicated experiment. *For. Ecol. Manage.*, 40,  
542 13–25.

543 Bolte, A., Villanueva, I. 2006. Interspecific competition impacts on themorphology  
544 and distribution of fine roots in European beech (*Fagus sylvatica* L.) and  
545 Norway spruce (*Picea abies* (L.) Karst.). *Eur. J. For. Res.*, 125, 15–26.

546 Brassard, B.W., Chen, H.Y.H., Bergeron, Y., Paré, D. 2011. Differences in fine root  
547 productivity between mixed- and single-species stands. *Func. Ecol.*, 25, 238–  
548 246.

549 Braun, H.J. 1974. Rhythmus und Grösse von Wachstum, Wasserverbrauch und  
550 Produktivität des Wasserverbrauches bei Holzpflanzen. *Allg Forst-und Jagd-  
551 Ztg* 145, 81–86.

552 Carnus, J., Parrotta, J.A., Brockerhoff, E., Arbez, M., Jactel, H., Kremer, A., Lamb,  
553 D., O’Hara, K., Walters, B. 2006. Planted forests and biodiversity. *J. For.*, 104,  
554 65–77.

555 Carvalheiro, K.D., Nepstad, D.C. 1996. Deep soil heterogeneity and fine root  
556 distribution in forests and pastures of eastern Amazonia. *Plant Soil*, 182, 279–  
557 285.

558 Chapman, K., Whittaker, J.B., Heal, O.W. 1988. Metabolic and faunal activity in  
559 litters of tree mixtures compared with pure stands. *Agric. Ecosys. Environ.*,  
560 24, 33–40.



561 Currie, W.S., Aber, J.D. 1997. Modelling leaching as a decomposition process in  
562 humid Montana forests. *Ecol.*, 78, 1844–1860.

563 De Deyn, G.B., Cornelissen, J. H. C., Bardgett, R.D. 2008. Plant functional traits and  
564 soil carbon sequestration in contrasting biomes. *Ecol. Lett.*, 11, 1-16.

565 de Graaff, M.A., Classen, A.T., Castro, H.F., Schadt, C.W. 2010. Labile soil carbon  
566 inputs mediate the soil microbial community composition and plant residue  
567 decomposition rates. *New Phytol.*, 188, 1055–1064.

568 de Marco, A., Meola, A., Maisto, G., Giordano, M., de Santo, A.V. 2011. Non-  
569 additive of leaf litters in a Mediterranean maquis effects of litter mixtures on  
570 decomposition. *Plant Soil*, 344, 305–331.

571 Deschaseaux, A., Ponge, J.F. 2001. Changes in the decomposition of humus profiles  
572 near the trunk base of an oak tree (*Quercus petraea* (Mattus) Liebl). *European*  
573 *J. Soil Biol.*, 37, 9–16.

574 Dungait, J.A.J., Hopkins, D.W., Gregory, A.S., and Whitmore, A.P. 2012. Soil  
575 organic matter turnover is governed by accessibility not recalcitrance. *Glob.*  
576 *Chang. Biol.*, 18, 1781–1796.

577 Ellenberg, H., Weber, H.E., Düll, R., Wirth, V., Werner, W., Paulissen, D. 1991.  
578 *Zeigerwerte von Pflanzen in mitteleuropa. Scr. Geobot.*, 18, 248.

579 Fischer, H., Bens, O., Huttli, R.F. 2002. Changes in humus form, humus stock and soil  
580 organic matter distribution caused by forest transformation in the North-  
581 Eastern lowlands of Germany. *Forstwiss Cent.*, 121, 322–334.

582 Fontaine, S., Barot, S., Barre, P., Bdioui, N., Mary, B., Rumpel, C. 2007. Stability of  
583 organic carbon in deep soil layers controlled by fresh carbon supply. *Nat.*, 450,  
584 277–281.

585 Fontaine, S., Bardoux, G., Abbadie, L., Mariotti, A. 2004. Carbon input to soil may  
586 decrease soil carbon content. *Ecol. Lett.*, 7, 314–320.

587 Giertych, M.J., Karolewski, P., Zytowski, R., Oleksyn, J. 2006. Differences in  
588 defence strategies against herbivores between two pioneer tree species, *Alnus*  
589 *glutinosa* L. Gaertn. and *Betula pendula* Roth. *Polish J. Ecol.*, 54, 181–187.

590 Greenfield, L. G., Gregorich, E. G., van Kessel, C., Baldock, J. A., Beare, M. H.,  
591 Billings, S. A., Clinton, P. W., Condon, L. M., Hill, S., Hopkins, D. W.,  
592 Janzen, H. H. 2013. Acid hydrolysis to define a biologically-resistant pool is  
593 compromised by carbon loss and transformation. *Soil Biol. Biochem.*, 64,  
594 122-126.

595 Hagen-Thorn, A., Callesen, I., Armolaitis, K., Nihlgard, B. 2004. The impact of six  
596 European tree species on the chemistry of mineral topsoil in forest plantations  
597 on former agricultural land. *For. Ecol. Manage.*, 195, 373–384.

598 Hansen, K., Vesterdal, L., Schmidt, I.K., Gundersen, P., Sevel, L., Bastrup-Birk, A.,  
599 Pedersen, L.B., Bille-Hansen, J. 2009. Litterfall and nutrient return in five tree  
600 species in a common garden experiment. *For. Ecol. Manage.*, 257, 2133–2144

601 Hansson, K., Kleja, D.B., Kalbitz, K., Larsson, H. 2010. Amounts of carbon  
602 mineralised and leached as DOC during decomposition of Norway spruce  
603 needles and fine roots. *Soil Biol. Biochem.*, 42, 178–185.

604 Hattenschwiler, S. 2005. Effects of tree species diversity on litter quality and  
605 decomposition. In: Scherer-Lorenzen M, Korner CH, Schulze ED (eds) *Forest*  
606 *diversity and functions, temperate and boreal systems. Ecological Studies* 176.  
607 Springer-Verlag, Berlin, pp 149.

608 Hobbie, S.E., Reich, P.B., Oleksyn, J., Ogdahl, M., Zytkowskiak, R., Hale, C.,  
609 Karolewski, P. 2006. Tree species effects on decomposition and forest floor  
610 dynamics in a common garden. *Ecol.*, 87, 2288–2297.

611 Hoosbeek, M.R., Lukac, M., Velthorst, E.J., Smith, A.R., Godbold, D.L. 2011. Free  
612 atmospheric CO<sub>2</sub> enrichment did not affect symbiotic N<sub>2</sub>-fixation and soil  
613 carbon dynamics in a mixed deciduous stand in Wales. *Biogeosciences*, 8,  
614 353–364.

615 Hoosbeek, M.R., Lukac, M., van Dam, D., Godbold, D.L., Velthorst, E.J., Biondi,  
616 F.A., Peressotti, A., Cotrufo, M.F., de Angelis, P., Scarascia-Mugnozza, G  
617 .2004. More new carbon in the mineral soil of a Poplar plantation under Free  
618 Air Carbon Enrichment (POPFACE), Cause of increased priming effect? *Glob*  
619 *Biogeochem. Cycles*, 18, GB1040.

620 Jandl, R., Lindner, M., Vesterdal, L., Bauwens, B., Baritz, R., Hagedorn, F., Johnson,  
621 D.W., Minkinen, K., Byrne, K.A. 2007. How strongly can forest management  
622 influence soil carbon sequestration? *Geoderma*, 137, 253–268.

623 Jolliffe, P.A. 2000. The replacement series. *J. Ecol.* 88, 371–385.

624 Kazakou, E., Violle, C., Roumet, C., Pintor, C., Gimenez, O., Garnier, E. 2009. Litter  
625 quality and decomposability of species from a Mediterranean succession  
626 depend on leaf traits but not on nitrogen supply. *Annu. Bot.*, 104, 1151–1161.

627 Kellner H, Luis P, Pecyna MJ, Barbi F, Kapturska, D. 2014. Widespread Occurrence  
628 of Expressed Fungal Secretory Peroxidases in Forest Soils. *PLoS ONE.*,  
629 9(4): e95557.

630 Kettler, T.A., Doran, J.W., Gilbert, T.L. 2001. Simplified Method for Soil Particle-  
631 Size Determination to Accompany Soil-Quality Analyses. *Soil Sci. Soc. Am.*  
632 *J.*, 65, 849-852.

633 King, R.F., Dromph, K.M., Bardgett, R.D. 2002. Changes in species evenness of litter  
634 have no effect on decomposition processes. *Soil Biol. Biochem.*, 34, 1959–  
635 1963.

636 Kogel-Knabner, I., Hatcher, P.G., Zech, W. 1991. Chemical structural studies of  
637 forest soil humic acids: aromatic carbon fraction. *Soil Sci. Soc.Am. J.*, 55,  
638 241-247

639 Kuzyakov, Y., Friedel, J.K., Stahr, K. 2000. Review of mechanisms and  
640 quantification of priming effects. *Soil Biol. Biochem.*, 32, 1485-1498.

641 Lai, Z., Zhang, Y., Liu, J., Wu, B., Qin, S., Fa, K., 2015. Fine-root distribution,  
642 production, decomposition, and effect on soil organic carbon of three  
643 revegetation shrub species in northwest China. *Forest Ecol. Manag.*,  
644 ([doi:10.1016/j.foreco.2015.04.025](https://doi.org/10.1016/j.foreco.2015.04.025))

645 Leavitt, S.W., Follett, R.F., Paul, E.A. 1997. Estimation of slow and fast cycling soil  
646 organic carbon pools from 6N HCl hydrolysis. *Radiocarb.*, 38, 231–239.

647 Lecerf, A., Chauvet, E. 2008. Intraspecific variability in leaf traits strongly affects  
648 alder leaf decomposition in a stream. *Basic App. Ecol.*, 9, 598–605.

649 Leuschner, C., Wulf, M., Bäuchler, P., Hertel, D. 2013. Soil C and nutrient stores  
650 under Scots pine afforestations compared to ancient beech forests in the  
651 German Pleistocene: The role of tree species and forest history. *For. Ecol.*  
652 *Manage.*, 310, 405–415.

653 Loreau, M., Hector, A. 2001. Partitioning selection and complementarity  
654 in biodiversity experiments. *Nat.*, 412, 72–76.

655 Lynch, H.B., Epps, K.Y., Fukami, T., Vitousek, P.M. 2012. Introduced Canopy Tree  
656 Species Effect on the Soil Microbial Community in a Montane Tropical  
657 Forest. *Pacific Science.*, 66(2), 141-150.

658 McLauchlan, K., Hobbie, S.E. 2004. Comparison of labile soil organic matter  
659 fractionation techniques. *Soil Sci. Soc. Am. J.*, 68, 1616–625.

660 Montagnini, F., Ramstad, K., Sancho, F. 1993. Litterfall, litter decomposition and the  
661 use of mulch of four indigenous tree species in the Atlantic lowlands of Costa  
662 Rica. *Agrofor. Sys.*, 23, 39–61.

663 Niinemets, U., Tamm, U. 2005. Species differences in timing of leaf fall and foliage  
664 chemistry modify nutrient resorption efficiency in deciduous temperate forest  
665 stands. *Tr. Physiol.*, 25(8), 1001-1014.

666 Olson, J.S. 1963. Energy storage and the balance of producers and decomposers in  
667 ecological systems. *Ecol.*, 44, 322–331.

668 Paul, E. A., Morris, S. J., Bohm, S. 2001. Determination of soil carbon pool sizes and  
669 turn-over rates: Biophysical fractionation and tracers. In *Assessment Methods*  
670 *for soil carbon*. Lal, R. (ed.). Lewis Publ., Boca Raton, FL. 193-206.

671 Prescott, C.E., Zabek, L.M., Staley, C.L., Kabzerns, R. 2000. Decomposition of  
672 broadleaf and needle litter in forests of British Columbia: influence of litter  
673 types, forest types and litter mixtures. *Can. J. For. Res.*, 30, 1742-1750.

674 Richards, A.E., Schmidt, S. 2010. Complementary resource use by tree species in a  
675 rain forest tree plantation. *Ecol. Appl.*, 20(5), 1237-54.

676 Rovira, P., Vallejo, V.R. 2002. Labile and recalcitrant pools of carbon and nitrogen in  
677 organic matter decomposing at different depths in soils an acid hydrolysis  
678 approach. *Geoderma.*, 107, 109–141.

679 Rovira, P., Vallejo, V.R. 2007. Labile and recalcitrant and inert organic matter in  
680 Mediterranean forest soils. *Soil Biol. Biochem.*, 39, 202–215.

681 Rowell, D.L. 1994. *Soil science methods and applications*. Pearson, London

682 Rumpel, C., Kogel-Knabner, I., Bruhn, F. 2002. Vertical distribution, age and  
683 chemical composition of organic carbon in two forest soils of different  
684 pedogenesis. *Org. Geochem.*, 33, 1131–1142.

685 Schmidt, M.W.I., Torn, M.S., Abiven, S., Dittmar, T., Guggenberger, G., Janssens,  
686 I.A., Kleber, M., Kögel-Knabner, I., Lehmann, J., Manning, D.A.C.,  
687 Nannipieri, P., Rasse, D.P., Weiner, S., Trumbore, S.E. 2011. Persistence of  
688 soil organic matter as an ecosystem property. *Nat.*, 478, 49–56.

689 Scullion, J., Smith, A.R., Gwynn-Jones, D., Jones, D.L., Godbold, D.L. 2014.  
690 Deciduous woodland exposed to elevated atmospheric CO<sub>2</sub> has species-  
691 specific impacts on anecic earthworms. *App. Soil Ecol.*, 80, 84–92.

692 Six, J., Conant, R.T., Paul, E.A., Paustian, K. 2002. Stabilization mechanisms of soil  
693 organic matter, implications for C-saturation of soils. *Plant Soil*, 241, 155–  
694 176.

695 Smith, A.R., Lukac, M., Bambrick, M., Miglietta, F., Godbold, D.L. 2013a. Tree  
696 species diversity interacts with elevated CO<sub>2</sub> to induce a greater root system  
697 response. *Glob. Chang. Biol.*, 19, 217–228.

698 Smith, A.R., Lukac, M., Hood, R., Miglietta, F., Godbold, D.L. 2013b. Elevated CO<sub>2</sub>  
699 enrichment induces a differential biomass response in a mixed species  
700 temperate forest plantation. *New Phyt.*, 198, 156-168.

701 Sollins, P., Homann, P., Caldwell, B.A. 1996. Stabilization and destabilization of soil  
702 organic matter, mechanisms and controls. *Geoderma*, 74, 65–105.

703 Shirato, Y., Yokozawa, M. 2006. Acid hydrolysis to partition plant material into  
704 decomposable and resistant fractions for use in the Rothamsted carbon model.  
705 *Soil Biol. Biochem.*, 38, 812–816.

706 Steinaker, D.F., Wilson, S.D. 2008. Phenology of fine roots and leaves in forest and  
707 grassland. *J. Ecol.*, 96(6), 1222-1229.

708 Steinbeiss, S., Temperton, V.M., Gleixner, G. 2008. Mechanisms of short-term soil  
709 carbon storage in experimental grasslands. *Soil Biol. Biochem.*, 40, 2634–  
710 2642.

711 Taylor, B.R. 1998. Air drying depresses rates of leaf litter decomposition. *Soil Biol.*  
712 *Biochem.*, 30, 403-412.

713 Tfaily, M.M., Chu, R.K., Tolic, N., Roscioli, K.M., Anderton, C.R., Pasa-Tolic, L.,  
714 Robinson, E.W., Hess, N.J., 2015. Advanced Solvent Based Methods for  
715 Molecular Characterization of Soil Organic Matter by High-Resolution Mass  
716 Spectrometry. *Anal. Chem.*, 87, 5206–5215.

717 Vance, E.D., Brookes, P.C., Jenkinson, D.S. 1987. An extraction method for  
718 measuring microbial biomass C. *Soil Biol. Biochem.*, 19, 703–707.

719 Vesterdal, L., Schmidt, I. K., Callesen, I., Nilsson, L. O., Gundersen, P. 2008. carbon  
720 and nitrogen in forest floor and mineral soil under six common European tree  
721 species *For. Ecol. Manage.*, 255, 35–48.

722 von Lütow, M., Kögel-Knabner, I., Ekschmitt, K., Matzner, E., Guggenberger, G.,  
723 Marschner, B., Flessa, H. 2006. Stabilization of organic matter in temperate  
724 soils, mechanisms and their relevance under different soil conditions – a  
725 review. *Euro. J. Soil. Sci.*, 57, 426–445.

726 Weigelt, A., Jolliffe, P. 2003. Indices of plant competition. *J. Ecol.*, 91, 707–720.

727 Wilson, J.B. 1988. Shoot competition and root competition. *J. Appl. Ecol.*, 25, 279–  
728 296.

729 Zou, X.M., Ruan, H.H., Fu, Y., Yang, X.D., Sha, L.Q. 2005. Estimating soil labile  
730 organic carbon and potential turnover rates using a sequential fumigation-  
731 incubation procedures. *Soil Biol. Biochem.*, 37, 1923–1928.

732

### 733 **Figure Legends**

734

735 **Fig. 1** Plot positions overlaid on to a krigged plot of initial soil organic matter  
736 content determined in the top 0-10 cm layer on a 10 × 10 m grid across the two  
737 fields of the BangorDIVERSE experimental site. In field 1 the gray overlay  
738 marks the approximate extend of the short rotation coppice trial. Coloured  
739 boxes represent plots of *A. glutinosa* (green), *B. pendula* (yellow) and *F.*  
740 *sylvatica* (blue) in monoculture, and a three species tree polyculture (orange),  
741 or a grassland (red).

742

743 **Fig. 2** Vertical distribution of soil organic carbon from under monoculture or  
744 polyculture stands of *B. pendula*, *A. glutinosa*, and *F. sylvatica*, or a grassland.  
745 Symbols show means ± SE ( $n=3$ ), statistically significant differences ( $P<0.05$ )  
746 are denoted by a superscript asterisk.

747

748 **Fig. 3** The contribution of fractions 1, 2 and the residual fraction 3 to the total C pool  
749 in different soil layers from under monoculture and a three species polyculture  
750 of *B. pendula*, *A. glutinosa* and *F. sylvatica*, or a grassland. Shown are means  
751 ± SE ( $n=3$ ), statistically significant differences are denoted by a superscript  
752 asterisk (\*  $P<0.05$ , \*\*  $P<0.01$  and \*\*\*  $P<0.001$ ).

753

754 **Fig. 4** The total soil pool ( $\text{kg C m}^{-2}$ ) of fraction 3 as determined by sequential acid  
755 extraction in a 1 m deep soil profile under *B. pendula*, *A. glutinosa*, *F.*



756 *sylvatica* and grassland. Bars show means  $\pm$ SE ( $n=3$ ). Bars not followed by  
757 similar indices are statistically significant ( $P<0.05$ ).

758

759 **Fig. 5** C fractions in leaves and roots of *B. pendula*, *A. glutinosa*, *F. sylvatica* and a  
760 grassland as determined by sequential acid hydrolysis. Values shown are  
761 expressed as the percentage of total C. Shown are means  $\pm$  SE ( $n=4$ ). Bars  
762 with same indices are not statistically significant ( $P<0.05$ ).

763

764 **Suppl. Fig. 1** Relationships between clay content and (a) total soil organic C, (b)  
765 labile C fraction 1 and (c) recalcitrant C (fraction 3) for soils under  
766 monoculture and a three species polyculture of *B. pendula*, *A. glutinosa* and *F.*  
767 *sylvatica*, or a grassland.