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

3

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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 CO₂ 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 ± 1.3 % sand, 33.6 ± 0.9 %
115 silt and 18.2 ± 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 m² for the grassland, 81 m² for single species, and 121
132 and 196 m² 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⁻¹). 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 μm 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 cm³ 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 CHCl₃ 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 μm sieve. The total C

193 content of soil and plant materials was determined using a TruSpec[®] 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 H₂SO₄ 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 H₂SO₄ was added, and the tubes were shaken overnight on a horizontal shaker at a
214 speed of 80 strokes min⁻¹ 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 2nd 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 ± 0.03 mg C kg⁻¹ and 0.83 ± 0.08 mg C kg⁻¹ 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 (Δ 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 ± 0.8 kg C m⁻² 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⁻¹) compared to *A. glutinosa*
385 (17.5 mg L⁻¹) litter (Ahmed, 2011). The nitrogen content of senesced leaf litter was
386 30.5, 29.0 and 35.0 g kg⁻¹ 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⁻¹ 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⁻² 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 CO₂ 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

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519

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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 \pm 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 \pm 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 (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.