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

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Abstract

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

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

1. Introduction

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

To investigate the composition of soil organic matter, SOM can be fractionated into several pools using a range of techniques (eg. Paul et al., 2001; Weil et al., 2003; Gregorich et al., 2003; Bajgai et al., 2013). Each of these methods attempts to isolate soil organic matter pools of different longevity. A common method used, is to separate pools on the basis of biodegradability (Rovira and Vallejo, 2007). This method separates soil organic matter based on acid solubility into labile and recalcitrant pools, which are believed to have different turnover times (McLauchlan et al., 2004). For example, labile C fraction composed of compounds such as soluble sugars, starch, and other carbohydrates, has been show to play a dominant role in the evolution of CO_2 from soil due to preferential decomposition and rapid turnover (Belay-Tedla et al., 2009). In contrast the recalcitrant fraction is thought to degrade slowly, thus contributing to long term C storage in soils. For

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

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

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

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

2. Materials and Methods

2.1 Study area

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

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

2.2 Plantation design

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

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

2.3 Positioning of the plots used

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

2.4 Soil collection and sample processing

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

30-40, 40-50, 70-80 and 90-100 cm). A subset of four layers (0-10, 10-20, 40-50 and 90-100 cm) were then used for C fractionation using acid hydrolysis. To ensure representativeness, samples were obtained from each layer, approximately 100 g of soil was collected from each side of the soil pit and mixed to produce a composite 400 g sample. Soils were then air dried, carefully homogenized and sieved to pass through a 2 mm sieve, a 50 g sub-sample was then taken and finely ground using a ball mill (Retsch Mixer Mill MM 200) and passed through a 100 µm sieve prior to acid hydrolysis. Soil pH and electrical conductivity were determined in a 1:2 v/v slurry of soil and distilled water with standard electrodes. Moisture content was determined after drying at 105 °C for 72 h, and organic matter as loss-on-ignition at 450 °C for 16 h. Bulk density was determined using 100 cm^3 cores and corrected for stone content (Rowell, 1994). The clay content of soils was determined by a simplified method combining wet sieving and sedimentation steps, as proposed by Kettler et al., (2001). Soil microbial biomass C of surface soil (0-10 cm) was determined according to the $CHCl_3$ fumigation-extraction method of Vance et al., (1987).

2.5 Leaf and root sample collection

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

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

2.6 Acid hydrolysis of soil and plant materials

Chemical methods allow fractionation of SOC into pools of putative identity. For example in a two-step acid hydrolysis approach, the labile fraction is further divided into two pools- labile fraction 1 that could be putatively identified as polysaccharides, derived from plant and microbial sources, and labile fraction 2 that contains cellulose which is more resistant than fraction 1 (Rovira and Vallejo, 2007). Labile soil C (fraction 1) was extracted from 0.5 g of air dried soil, which was taken from the homogenised 50 g sample described above. The soil was transferred into a sealable Pyrex tube and 15 ml of 2.5 M H_2SO_4 was added and thoroughly mixed. The mixture was heated to 100 °C for 30 min in a digestion block. After cooling the hydrolysed solution was centrifuged at 2695 × *g* for 3 min and the clear supernatant decanted into a fresh glass tube. The residue was washed twice with 15 ml of deionised water and the washings added to the hydrolysate and kept in glass bottles at 4 °C until analysis for C and N using a TOC-V-TN analyzer (Shimadzu Corp., Kyoto, Japan).

To extract a less labile part of the soil C (fraction 2), the unhydrolysed residues were transferred to Pyrex tubes and dried at 60 °C. After cooling, 2 ml of 13 M H_2SO_4 was added, and the tubes were shaken overnight on a horizontal shaker at a speed of 80 strokes min^{-1} at room temperature. Thereafter, 26 ml of deionised water was added to dilute the acid to 1 M and the residues were hydrolysed for 3 h at 100 °C with occasional shaking. After centrifugation at 2695 × *g* for 3 minutes, the clear 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^{nd} 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

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

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

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

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

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

$$M_{mixture} = \left(\frac{4}{10} \times M_{Betula}\right) + \left(\frac{5}{10} \times M_{Alnus}\right) + \left(\frac{1}{10} \times M_{Fagus}\right) \quad \text{Equation 2}$$

2.9 Statistical analysis

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

3. Results

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*.

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

3.3 Total C storage and C pool size of each fraction

The total C storage to a depth of 100 cm in the various plots ranged between 10.2 ± 0.9 under grass to 6.9 ± 0.8 kg C m^{-2} under *F. sylvatica*, with no significant variation between the treatments (Table 1). In Table 1 the pools of C are shown as the total extractable (fraction 1 and fraction 2) and the residual C in fraction 3. The tree species grown in monoculture and polyculture showed no significant difference in total C stocks. We examined the influence of tree species on fraction 3 in upper (0-40 cm) and lower (40-100 cm) region of the soil profile (Fig. 4). In the upper layers no significant differences were found between the treatments, however in the deeper soil layers, the greatest storage of C in fraction 3 was found in the polyculture. The C storage in the polyculture soil at depth was significantly greater compared to the *B. pendula*, *A. glutinosa* and grassland soil, but not statistically different compared to *F. 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

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

4.3 Soil organic carbon and fractionation

Changes in total soil C were only observed in the top 20 cm of soil, below this depth soil C was not different between the tree species or the grassland. Soil C stock change and physical fractionation were investigated at the Bangor site using different but adjacent plots with the same species by Hoosbeek et al., (2011), who found an increase of 530 g C m^{-2} in the top 0-10 cm layer 4 years after planting. In contrast to our chemical fractionation results, the distribution of course, fine and aggregate particulates were similar between all species and the polyculture. Here we showed that using chemical fractionation, the organic C content of fraction 1 was significantly lower in soils under *A. glutinosa* than in *F. sylvatica* in the 0-10 cm soil layer (Fig. 3). We propose two mechanisms to explain our observed differences in fraction 1 C content. First, relative to *F. sylvatica*, *A. glutinosa* is poor at translocating nutrients and carbohydrates during senescence (Lecerf and Chauvet, 2008), and subsequently the senesced litter of *A. glutinosa* is considered to be of high quality due to thick, mesophyll rich, leaves with a low C:N ratio. These traits, which are favourable to grazing by soil organisms and microbial decomposition (Kazakou et al., 2009), probably resulted in a rapid removal of fraction 1. The influence of litter quality on decomposition processes was also supported by significantly faster litter mass loss of *A. glutinosa* than *F. sylvatica* during the first three weeks (Table 2). Second, during organic matter mineralization and microbial turnover, C not respired as CO_2 is 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

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

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

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732

733 **Figure Legends**

734

735 **Fig. 1** Plot positions overlayed 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 (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.*

sylvatica and grassland. Bars show means ±SE (n=3). Bars not followed by similar indices are statistically significant (P<0.05).

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

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